Analysis of fluorescence visualization in oral precancerous and cancerous lesions

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Various specialty clinics and research centers have conducted studies of direct tissue fluorescence visualization as a screening technique for oral precancerous and early cancerous lesions. The effectiveness of the VELscope is still not well known. The objective of this study was to analyze specificity of the VELscope system for the detection of precancerous and early cancerous lesions. We studied the use of this simple handheld device that facilitates the direct visualization of oral-cavity fluorescence for the detection of precancerous and early cancerous lesions. Blue excitation light (400 to 460 nm) is employed to excite green-red fluorescence from fluorophores in the oral mucosa. Tissue fluorescence is viewed directly along an optical axis collinear with the axis of excitation to reduce inter- and intraoperator variability. This robust, field-of-view device enables the direct visualization of fluorescence in the context of surrounding normal tissue. Results from 17 patients are presented. Histological examination of the lesions showed that the VELscope system has a sensitivity of 95% and specificity of 100% in discriminating normal mucosa from severe dysplasia/ carcinoma in situ (CIS) or invasive carcinoma. We envisage this device as a suitable adjunct for oral cancer screening. (J Osaka Dent Univ 2011; 45: 221–225)

Key words: Precancerous lesions; Early cancerous lesions; VELscope; Fluorescence visualization

INTRODUCTION

Early detection of precancerous and cancerous lesions is desirable because an early diagnosis and appropriate treatment reduces patient morbidity and improves survival.¹ The clinical signs of precancerous and early cancerous lesions are varied and may be misdiagnosed as other conditions, including mucosal inflammation, hyperkeratosis, or traumatic ulceration. Screening for oral precancerous and cancerous lesions may reduce the incidence and mortality rates associated with oral cancer,² indicating that screening programmes could be associated with a reduction in public health costs.³ On the other hand, screening can be associated with problems related to false positives, including psychological trauma, over diagnosis and overtreatment.

Recently, fluorescence imaging studies for the early detection of oral malignancies have employed indirect visualization using either photographic film or a sensitive or intensified CCD camera. De Veld et al. provide a good review of in vivo autofluorescence spectroscopy and imaging for oral oncology.⁴ Onizawa et al.⁵,⁶ used a custom UV-flash photography system to record porphyrin-like fluorescence in the oral cavity. Fluorescence was excited by the 360-nm spectral peak of the flash lamp and fluorescence was recorded on photographic film using a 480-nm long-pass filter. The authors reported 91% sensitivity and 84% specificity for discriminating benign from malignant lesions. More recently, Svistun et al. reported a system for the direct visualization
of oral cavity fluorescence. In their device, excitation light is provided by a handheld illuminator and tissue fluorescence is observed along an axis slightly inclined from the illumination axis using special glasses. Perceived tumor margins, as determined from the fluorescence images and not observed directly through the viewing glasses, were correlated with histopathology. The sensitivity and specificity were 91 and 86% for the discrimination of normal tissue from neoplasia at the best excitation wavelength.

Therefore, new diagnostic techniques and instruments have been developed for use in routine examinations, including the VELscope, mucosa visualization system. This device emits a particular wavelength and intensity of light that illuminates the oral mucosa and excites the natural fluorophores in the tissue. The tissues emit fluorescence that is visualized through a filter by a human observer.

We investigated the value of this device to delineate field change in autofluorescence around cancers by determining and comparing the histopathologic changes of margins that retained normal fluorescence visualization (FV) with those margins that showed a loss of FV.

**MATERIALS AND METHODS**

**Patients**

Seventeen patients with biopsy-confirmed primary cancer of the oral cavity were selected for this accrued to the study. Eligibility criteria included the presence of early stage disease (T0–T2) scheduled for surgical excision with intent to cure. Tumor staging of surgical specimens determined that 3 were carcinomas in situ (CIS, stage 0), 13 were stage I invasive SCC and 1 was stage II invasive SCC. The majority of the tumors were from the tongue (13 of 17, 76%), while 1 case was from the floor of the mouth, 1 from the gingiva, and 2 from the buccal mucosa.

**VELscope**

The VELscope system 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. The VELscope integrates four key elements: illumination, sophisticated filtering, natural tissue fluorophores, and the power of human optical and neural physiology. The VELscope has the potential to overcome many of the obstacles presented by conventional methods for screening, and aid in the detection of mucosal abnormalities including precancerous and cancerous lesions.

The VELscope illuminates tissue with specific wavelengths that interact with and provide metabolic and biochemical information about the cells at and just beneath the surface. This gives clinicians the ability to see early biochemical changes before they become obvious, and therefore detect lesions earlier in the disease process. It consists of a source of light that emits a wave length of 400 to 460 nm and a manual unit for direct visualization. Under this light, normal oral mucosa emits a green auto-fluorescence, whereas abnormal mucosa absorb the fluorescent light and appear dark. Hence, early biochemical changes are detected before their more evident appearance, permitting the early detection of pathological lesions. Reported sensitivity values ranged from 97% to 98% and specificity from 94% to 100%.

Under direct FV, the normal oral mucosa emits various shades of pale green autofluorescence. Clinical lesions that retained the normal green auto-fluorescence under FV were defined as FV retained (FVR). Tissue that showed a reduction in the normal pale green and appeared as dark patches were classified as FV loss (FVL). This distinction involved a comparison of the lesion site with both adjacent tissue and, as an anatomic control, with tissue on the contralateral side. Photographs of tissue fluorescence were acquired using illumination from the FV device and a digital single lens reflex camera with a long-pass filter. The camera was equipped with a macro lens and a ring flash for white-light images.
Surgical field assessment of FV status
Our protocol involved examination of the surgical site of each patient under both regular operating room illumination and with direct FV. Each step was photographed for documentation. The steps included an initial assessment under regular operating room light and demarcation of the boundary of the clinical tumor using a blue marker, followed by assessment of the site for altered fluorescence using direct FV. After turning off the room light, the oral cavity was viewed with direct FV. The operator would then decide whether the oral lesions required biopsy based on standard clinical features (clinical appearance and iodine solution staining results) and not based on the direct FV examination. Areas showing loss of normal green fluorescence were outlined, demarcating FVL boundaries.

Tissue sampling and histological assessment
After resection, a total of 51 samples were taken from the tumor margins in each of three regions (anterior, posterior and medial). All samples were fixed in formalin and submitted for histopathological evaluation by pathologists who had no knowledge of the FV status.

RESULTS
Direct FV
Under direct FV, the normal oral mucosa emits various shades of pale green autofluorescence. Clinical lesions that retained normal green autofluorescence under direct FV were classified as lesions with FV retention (FVR). Tissue that showed a distinct reduction in the normal pale green and appeared as dark green to black was classified as FV loss (FVL) (Fig. 1). This assessment involved a comparison of the lesion site with both adjacent tissue and, as an anatomical control, with tissue on the contralateral side.

Pathology
As shown in Table 1, of the 51 samples, 9 were FVR, and 42 were FVL. None of the 7 samples with a histological diagnosis of normal showed loss of FV, whereas 92% of precancerous lesions (severe dysplasia and CIS) and 97% of invasive SCC showed loss of FV.

Comparison with another report
According to the BCCA, this system has a sensitivity of 98% and specificity of 100%. Poh et al. showed 97% sensitivity and 100% specificity. This was very similar to our results of 95% sensitivity.

Table 1 Correlation of direct FV results with lesion histopathology

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<tr>
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<th>Normal</th>
<th>Severe Dysplasia and CIS</th>
<th>Invasive SCC</th>
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<tbody>
<tr>
<td>Number of lesions</td>
<td>7</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>FVR</td>
<td>7(100%)</td>
<td>1(8%)</td>
<td>1(3%)</td>
</tr>
<tr>
<td>FVL</td>
<td>0(0%)</td>
<td>11(92%)</td>
<td>31(97%)</td>
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Fig. 1 An early SCC case. The margin was confirmed to be a severe dysplasia (A). Arrow indicates FVL (B).
and 100% specificity.

**DISCUSSION**

The current gold standard for diagnosis of oral precancerous and early cancerous lesions is the histopathology of a biopsy specimen. However, intraobserver findings vary among pathologists in the diagnosis of the mild and moderate dysplasia that comprise the largest proportion of precancerous lesions, and in determining early cancerous lesions. Furthermore, a diagnosis of oral SCC by means of histopathological examination depends on recognition and sufficient sampling of oral lesions because of variation in abnormal microscopic changes within the clinical lesion. In addition, histopathological changes may be present in areas in which there is no clinical evidence of an oral lesion on visual examination alone (for instance, “field cancerization”), and it now is known that molecular and genetic changes may precede both clinical and microscopic morphological changes and may be present in histologically benign tissue. Poh et al. used the VELscope system to detect field cancerization 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 heterozygosis. 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.

Usually, screening tools are highly sensitive but are not specific, resulting in high rates of false positive results. A false positive occurs when a clinical diagnosis of abnormality is downgraded to histologically normal by surgical biopsy. Therefore, a screening technique does not provide a definitive diagnosis. A surgical biopsy with microscopic examination by a pathologist remains the standard for diagnosing oral mucosal disease. The VELscope is a form of direct tissue fluorescence visualization that utilizes the loss of natural fluorescent characteristics of metabolic intermediaries to identify dysplastic and hypermetabolic activity. According to the BCCA, this system has a sensitivity of 98% and a specificity of 100% in discriminating between normal tissue and severe dysplasia, *in situ* carcinoma or invasive carcinoma. Because our results were almost the same with 95% sensitivity and 100% specificity, we concluded that the VELscope is useful in assessing lesion margins in patients with oral precancerous and early cancerous lesions. This makes it very useful in surgical management. However, false positives have been reported, for instance in cases of inflammation, and it does not detect all areas of dysplasia. Therefore, the VELscope cannot be used as a diagnostic tool, but rather is complementary to a thorough visual inspection and palpation.

Significant progress has been made in understanding the mechanisms responsible for endogenous fluorescence from epithelial tissues and how this fluorescence changes with dysplastic progression. The fluorophores of interest here are those that excite in the blue spectrum and have properties that have been spectroscopically correlated with dysplastic progression. The reduced form of nicotinamide adenine dinucleotide (NADH) and the oxidized form of flavin adenine dinucleotide (FAD) are important fluorophores that are good indicators of cellular metabolism. It has been shown that fluorescence intensity due to NADH increases

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<th>Author</th>
<th>Type of article</th>
<th>Sample</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>Poh et al.</td>
<td>Crosssectional study</td>
<td>20</td>
<td>97%</td>
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<td>Kois and Truelove</td>
<td>Case series</td>
<td>4</td>
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<tr>
<td>Blevi</td>
<td>Opinion article</td>
<td>–</td>
<td>98%</td>
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<tr>
<td>Ohnishi et al.</td>
<td></td>
<td>17</td>
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with dysplastic progression, and that of FAD decreases.\\n
Based on the present study of the origins of fluorescence and its change with dysplastic progression, we believe that FVL associated with dysplastic progression in the current device is primarily due to breakdown of the collagen matrix and increased hemoglobin absorption. Secondary to these effects is increased scattering in the epithelium, epithelial thickening, and a decrease in FAD concentration. These preliminary results suggest that this direct FV device has potential as a simple, cost-effective screening, biopsy guidance, and margin setting device for oral precancerous and early cancerous lesions.

REFERENCES