Influence of fluorescence on screening decisions for oral mucosal lesions in community dental practices

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BACKGROUND: Quality of oral screening examinations is dependent upon the experience of the clinician and can vary widely. Deciding when a patient needs to be referred is a critical and difficult decision for general practice clinicians. A device to aid in this decision would be beneficial.

The objective of this study was to examine the utility of direct fluorescence visualization (FV) by dental practitioners as an aid in decision-making during screening for cancer and other oral lesions.

METHODS: Dentists were trained to use a stepwise protocol for evaluation of the oral mucosa: medical history, head, neck and oral exam, and fluorescent visualization exam. They were asked to use clinical features to categorize lesions as low (LR), intermediate (IR), or high (HR) risk and then to determine FV status of these lesions. Clinicians made the decision of which lesions to reassess in 3 weeks and based on this reassessment, to refer forward.

RESULTS: Of 2404 patients screened over 11 months, 357 initially had lesions with 325 (15%) identified as LR, 16 (4.5%) IR, and 16 (4.5%) HR. Lesions assessed initially as IR and HR had a 2.7-fold increased risk of FV loss persisting to the reassessment appointment versus the LR lesions. The most predictive model for lesion persistence included both FV status and lesion risk assessment.

CONCLUSION: A protocol for screening (assess risk, reassess, and refer) is recommended for the screening of abnormal intraoral lesions. Integrating FV into a process of assessing and reassessing lesions significantly improved this model.

Keywords: early detection of cancer/methods; fluorescence visualization; mouth neoplasms/prevention and control; oral cancer screening; pre-cancerous conditions/diagnosis; referral and consultation

Introduction

The advent of adjunct tools for use as part of the conventional oral examination has been a driving force for change in the screening activity in community practices. Such devices presently include toluidine blue, brush cytology, reflectance visualization and, more recently, autofluorescence imaging. However, validation of these tools has been mainly restricted to high-risk referral clinic settings with use by experienced personnel, with little work carried out in community settings.

In this paper, we examined one such tool, a handheld device used to measure alterations in tissue fluorescence. Loss of tissue autofluorescence has been associated with cancer and pre-malignant lesions at several sites, including the lung, cervix, and oral cavity. Current evidence for utility of this device in identifying dysplastic lesions has found fluorescence visualization (FV) to be sensitive in the detection of high-grade dysplasias in the oral cavity (1), pre-cancerous occult lesions (2) and in enhancing delineation of surgical margins (3, 4); however, these studies have all been carried out in high-risk referral clinic settings with experienced personnel. There are various reports of confounding factors such as inflammation, infection, or highly pigmented areas, which may cause a decrease in the FV signal and hence affect specificity (5–8).

This study evaluates the use of FV by community practitioners as an adjunct to clinical evaluation following a conventional examination. Our goal was to determine whether FV added any value to white light oral cancer screening as introduced in the Guidelines for the Early Detection of Oral Cancer in British Columbia 2008 (9). The intent was to use this information to determine the value of
this new technology in community dental practices and if suitable to develop a framework for knowledge translation and to test the hypothesis that FV is useful in facilitating the clinical decision to refer forward suspect lesions.

Materials and methods

Study participants

This study was approved by the British Columbia Cancer Agency and the Simon Fraser University Research Ethics Board. Dental practitioners participating in this study were recruited using a notice in a Greater Vancouver dental association publication that described the study and requested volunteers. Each of these practices was contacted by telephone (DML) and was given a more in-depth description of the project and its timelines. A total of 18 dentists participated from 15 offices (two offices had two dentists participating), with each dentist signing informed consent.

Data collection

The study included a 1-day workshop to orient dental participants to the study protocols and subsequent follow-up of screening activities in each dental office, with facilitation and referral to dysplasia clinics for patients requiring further assessment.

Description of workshop

The workshop was comprised of three parts. Firstly, before the start of the workshop, two short self-administered questionnaires, adapted from Yellowitz et al. (10) and Horowitz et al. (11), were completed to assess knowledge of oral cancer risk factors and to collect personal demographics on the participating dentists and information on their current screening activities. These findings will be reported elsewhere.

Secondly, a presentation was given, including a short review of oral cancer statistics, etiological factors, clinical risk factors, and oral histopathology. An introduction to fluorescence visualization (FV), as an adjunctive device for lesion examination (1, 2), was also given followed by a presentation of the step-by-step protocol for clinical assessment of patients, including extraoral and intraoral examination as described in Williams and co-workers (12). Finally, the referral pathway for suspicious lesions and follow-up procedures was described.

Thirdly, the workshop concluded with a hands-on clinical session where each participant observed and performed an oral cancer screening examination of patients with active disease under both white light and FV conditions.

Assessment of oral cancer screening activities during follow-up of dental practices

After completion of the workshop, participants were asked to screen all patients over age 21 for the period from November 2007 to September 2008. Each practice was loaned a VELScope for the duration of the study (LED Dental, Inc., Burnaby, BC, Canada). The study community facilitator (DML) then contacted each dental practice monthly for data acquisition and to address any questions on the study protocol. Participating offices were contacted regularly via email for the duration of the study. Each patient screened was given a unique identifier which was also used at the follow-up clinic and on further diagnostic reports, if required.

The dental offices were asked to complete the screening at each new patient or recall examination. The protocol included the following:

Step 1. Patient History: This step involved recording the patient’s age, gender, personal and family history of oral cancer, tobacco use, and alcohol consumption. The study questionnaire was developed as an adjunct to the dental offices’ own medical history with the intent to minimize overlap and time spent by the clinician.

Step 2. Visual Screening Examination: This step involved both an extraoral and intraoral examination. The extraoral examination included inspection and palpation of the head and neck region, focusing on asymmetry, and swelling or tenderness. Participants were asked to refer to a medical doctor any patient with fixed, firm, or unexplained lymph nodes or asymmetries. An intraoral exam under incandescent (white light) conditions was then undertaken. If an anomaly was present, the site, color, texture, and appearance of the lesion were documented by checking off the appropriate boxes on a screening form and drawing the anomaly’s location on an oral cavity diagram (Fig. 1). Benign common mucosal changes not to be recorded included amalgam tattoos, Fordyce’s granules, vascularities, and pigmentation due to skin color.

Step 3. Lesion Assessment: This step involved assessing the risk of an anomaly. Low-risk lesions (LR) included obvious trauma, aphthous lesions, melanotic macules, candidiasis (including median rhomboid glossitis), and geographic tongue. Anomalies without apparent cause, non-healing ulcers, red or white patches, and lichenoid lesions were considered high-risk lesions (HR). Lichenoid lesions were later reclassified as intermediate-risk lesions (IR) because lichenoid lesions have a variation in clinical presentation from faint white striae to red and erosive and some may have increased cancer risk. Lesions in this latter group require further follow-up for clinical management.

Step 4. Direct FV: The FV component was the final step in the oral screening protocol. The FV examination followed the same methodical examination of all oral mucosa tissue as the conventional exam; however, it was performed under reduced room lighting whenever possible and with a handheld autofluorescence imaging device, marketed as the Velscope™ (LED Dental, Inc.). This device uses a blue/violet light (400–460 nm wavelength) to illuminate oral tissue, with long-pass and notch filters to allow clinicians to directly view fluorescence (1, 13). Lesions that retained the normal green autofluorescence under FV were classified as FV negative (FV−); those that showed a reduction in the normal pale green, appearing as dark patches, were classified as FV positive (FV+). Where the clinician was unsure of FV loss, these lesions were classified as FV equivocal (FVE).

Participating clinicians were further asked to document sites which appeared clinically normal but had a loss of FV (FV+).
Lesion follow-up
Patients with low-risk (LR) lesions without an obvious cause or with intermediate-risk (IR) and high-risk (HR) lesions were asked to return for reassessment in 3 weeks. If the lesion was still present after 3 weeks, the dental practice was requested to notify the study’s community facilitator (DML) who reassessed the patient’s lesion, both clinically and with FV, at the dental office. The community facilitator then referred any suspicious lesions to an oral medicine disease (OMD) clinic. In some cases, the dental offices directly referred patients to the OMD clinic. Oral medicine specialists at the OMD clinic determined if a biopsy or further follow-up was warranted.

Statistical analysis
Descriptive statistical analysis was used to describe data on knowledge and baseline screening behavior of participating dentists collected at the initial workshop and on the patient screening forms. These latter forms were imaged and then uploaded directly into a Microsoft Excel study database using Teleform (version 10.1; Vista, CA). Chi-squared tests were used to compare demographic and risk-habit variables (Table 1), logistic regression models were used for Tables 2, 3, and 4, and the ‘Akaike Information Criteria (AIC)’ was also used in Tables 4 and 5. Data analysis was performed with SPSS software, version 16.0 for Windows, 2007 (SPSS Inc., Chicago, IL).

Results
A total of 2404 patients received a white light and FV screening examination and 357 patients with lesions (15% of patients) were identified. Of these lesions, 192 were FV+(54%), 26 FVE (7%), and 139 FV− (39%).

Demographic and risk habits
Table 1 displays the demographic and risk-habit behavior of study patients, comparing patients with lesions to those without a lesion. Lesions were significantly more likely to be found in patients who were older (40 years and older), smoked, consumed alcohol, and attended dental practices with larger clinic volumes.

Of the 357 patients with a lesion, age, gender, tobacco consumption (either smoking or chewing), alcohol consumption, lesion appearance, and risk of site were not associated with FV+ status (Table 2). Only the presence of color and texture were associated with FV+ status. Red, or mixed red and white lesions had a 5.6-fold (95% CI: 3.5–10.4) increased risk of being FV+. Lesions that were brown, black, or purple (common confounders such as amalgam tattoos, melanotic macules, nevi, and vascularities) had a 2.8-fold (95% CI: 1.2–6.7) increased risk of being FV+. Many of these benign conditions are dark and hence will provide a positive FV result. This emphasizes the importance of training in the use of FV and awareness of possible confounders. Lesions with a rough [RR = 0.5 (95% CI: 0.2–0.9)] or ‘other’ [RR = 0.3 (95% CI: 0.1–0.8)] texture

Table 1 Demographic information and risk-habit behaviors associated with an oral lesion of 2404 patients

<table>
<thead>
<tr>
<th>Demographic Information</th>
<th>Lesion (%)</th>
<th>No lesion (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (N = 2390)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>165 (46)</td>
<td>857 (42)</td>
<td>0.164</td>
</tr>
<tr>
<td>Female</td>
<td>192 (54)</td>
<td>1176 (58)</td>
<td></td>
</tr>
<tr>
<td>Age at screening (years) (N = 2301)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>58 (17)</td>
<td>458 (23)</td>
<td>0.006</td>
</tr>
<tr>
<td>≥40</td>
<td>288 (83)</td>
<td>1497 (77)</td>
<td></td>
</tr>
<tr>
<td>Family history of oral cancer (N = 2342)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (4)</td>
<td>45 (2)</td>
<td>0.040</td>
</tr>
<tr>
<td>No</td>
<td>332 (96)</td>
<td>1950 (98)</td>
<td></td>
</tr>
<tr>
<td>History of smoking (N = 2343)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smokera</td>
<td>166 (47)</td>
<td>764 (38)</td>
<td>0.002</td>
</tr>
<tr>
<td>Never smoker</td>
<td>186 (53)</td>
<td>1227 (62)</td>
<td></td>
</tr>
<tr>
<td>History of chewing tobacco (N = 1824)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smokerb</td>
<td>9 (3)</td>
<td>32 (2)</td>
<td>0.277</td>
</tr>
<tr>
<td>Never smoker</td>
<td>278 (97)</td>
<td>1505 (98)</td>
<td></td>
</tr>
<tr>
<td>Clinic volume (no. of patients screened per clinic) (N = 2404)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤200</td>
<td>130 (36)</td>
<td>924 (45)</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt;200</td>
<td>227 (64)</td>
<td>1123 (55)</td>
<td></td>
</tr>
</tbody>
</table>

aEver smoker – smoked more than 100 cigarettes and longer than 1 year.
bEver drinker – drinks 2 or more alcohol drinks per week.
were found to be significantly less likely to be FV+ than smooth lesions.

**Impact of reassessment**

Demographic, risk habit, and clinical factors were also examined for an association with lesion persistence in the 141 patients called back for reassessment at 3 weeks. Six FV+ patients were referred directly to the oral medicine specialist without reassessment. Gender, age, tobacco consumption, alcohol consumption, risk of site, lesion appearance, and color were not found to be associated with lesion persistence in these patients. Only a rough lesion texture was associated with lesion persistence [RR = 3.7 (95% CI: 1.2–11.2)] (Table 3). Lesions that the dental professionals assessed as high risk (N = 16) at the initial visit were also more likely to still be present at the 3-week reassessment visit than lesions assessed as low risk (N = 121) [RR = 2.7 (95% CI: 1.4–5.1)].

To see if FV and lesion risk assessment have the potential to predict lesion persistence, four different prediction models were compared (Table 4). Model 1 included all variable except for FV and lesion risk assessment, model 2 included FV, model 3 included lesion risk assessment and model 4 included both FV and lesion risk assessment.
### Discussion

In this study, we began the process of evaluating new technology in community dental practices, to ensure that such a technology transfer would be integrated into the conventional oral examination. Practitioners were introduced to FV during the workshop and supplied with a device for use in their practice. They were instructed to conduct an FV examination at the completion of each conventional white light exam and to record observations made. As this was the first study to introduce FV technology into a community screening framework, our questions mainly focused on whether positive FV results were associated with persisting lesions identified through a step-by-step procedure as described in the oral cancer screening guidelines (9). With training and experience, we hypothesized that this device would add support to the reassessment and referral decision-making of community dental professionals.

The appearance of FV relies on three principles, the scattering of light as it interacts with tissue, the reflection of light from the tissue surface, and the absorption of the light by the tissue components and re-emission as fluorescing light. How the light is absorbed, re-emitted, or scattered depends on the biochemical composition of the tissue (14). Variation in light scattering may differ between individuals and by site (for example, the thicker epithelium of the buccal mucosa may reduce the back scattering of light as compared to the non-keratinized epithelium of the floor of the mouth) (12,15). Fluorophores are components of the tissue which absorb light at particular wavelengths and re-emit the light at longer wavelengths. They quickly become unstable and release the energy in the form of fluorescence, which is very sensitive to cellular and tissue changes. Three fluorophores that react to the wavelength of...
Increased thickening of the epithelium leads to less reflectance and back scatter.

While the use of FV has not, to date, validated itself within the general dental practice setting, it may increase the desire of oral health professionals to perform oral screening examinations and follow-up of patients with suspicious lesions.

There are several limitations of this study. Firstly, FV data were missing for the reassessment appointment. Clinicians may not have used FV at the reassessment appointment if the clinical lesion had resolved. Secondly, we were not able to biopsy all lesions for a definitive diagnosis due to ethical considerations. Thirdly, a clinical examination is subjective and varies with the experience of the clinician.

One of the most difficult decisions a clinician may face is when to refer a lesion for further investigation and biopsy. Recent evidence suggests high rates of clinical misdiagnosis by general oral health practitioners (22). For those clinicians in general practice without the experience and expertise of a specialist, an imaging device to aid in the decision to refer would be very helpful. At the community level, the critical decision is not whether or not the lesion is cancer but whether or not the lesion should be referred for further investigation. Reassessment at a 3-week follow-up appointment is critical to improving the specificity of the FV autofluorescence imaging device.

References


Table 6  Tissue and cellular alterations that influence FV during carcinogenesis (23)

| Increased breakdown of collagen cross-links and the basement membrane by MMPs, including collagenase, causing less collagen fluorescence |
| Increased nuclear scattering due to changes to the cell nuclei resulting in less back scatter |
| Increased metabolism alters FAD and hence less fluorescence intensity |
| Increased microvascularity leads to more absorption by hemoglobin |
| Increased thickening of the epithelium leads to less reflectance and back scatter |

Acknowledgements
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Conflict of interest
The project received 10 VELScope units as a donation from LED Dental Inc., Burnaby, British Columbia, Canada.