CASE REPORT

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DIRECT FLUORESCENCE VISUALIZATION OF CLINICALLY OCCULT HIGH-RISK ORAL PREMALIGNANT DISEASE USING A SIMPLE HAND-HELD DEVICE

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Abstract: *Background.* A considerable proportion of oral cancer and precancer is not clinically apparent and could contribute significantly to the late diagnosis and high mortality of oral cancer. A simple method to identify such occult change is needed.

Methods. Patients in the Oral Dysplasia Clinics at British Columbia are currently being examined with a simple hand-held device that permits the direct visualization of alterations to autofluorescence in the oral cavity. Tissue showing loss of autofluorescence is biopsied.

Results. We present 3 representative cases in which occult lesions were identified with fluorescence visualization during longitudinal follow-up, resulting in the diagnosis of a primary dysplasia in case 1, a second primary cancer in case 2, and cancer recurrence in case 3.

Conclusions. This is the first report of the diagnosis of occult oral disease using a simple noninvasive device. These early examples indicate the potential value of this technology to guide the management of patients with oral lesions, facilitating the detection of high-risk changes not apparent with white-light visualization. © 2006 Wiley Periodicals, Inc. *Head Neck* **29:** 71–76, 2007

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A consensus is gradually developing that there is a need to broaden the focus in patients with oral cancer and precancer beyond the clinical lesion, placing greater emphasis on such patients as having a high-risk condition or disease. Clinically visible oral lesions may represent the "tip of the iceberg," signaling the presence of multiple or widespread subclinical changes to the tissue.[1] New technology needs to be developed to identify such subclinical changes early to improve prognosis.

Direct fluorescence visualization is 1 potentially powerful approach that may be used routinely by clinicians in the future to facilitate the visualization and management of nonapparent

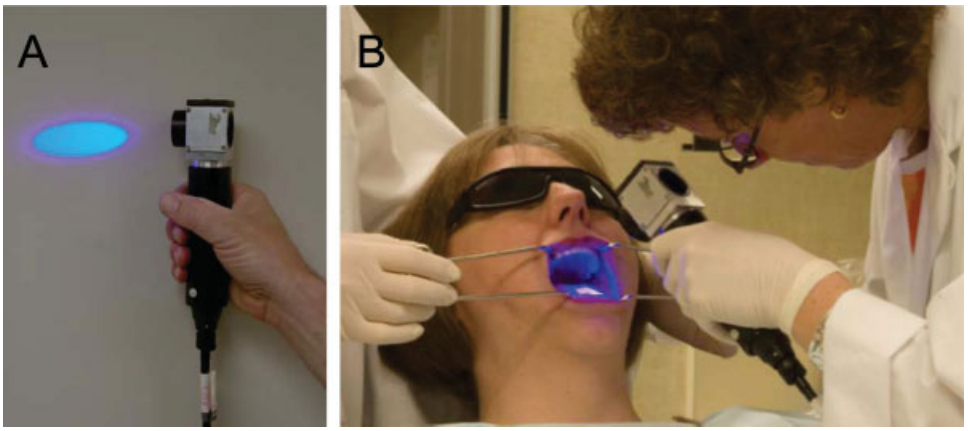

FIGURE 1. Illustration of direct fluorescence visualization technique. The procedure uses a hand-held viewing instrument (**A**) connected to a light source, which is used to illuminate the target tissue with an excitation light (extraoral light source) that is blue in color (400–460 nm). The target tissue fluoresces (green-red) under the excitation light, and the light produced by the tissue is called autofluorescence. An emission filter blocks the blue excitation and ensures that only green and red light is transmitted for direct visualization to the operator (**B**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

lesions. Ultraviolet light has been used for disease detection since the 1950s.[2] The goal of these devices has always been to enhance the visualization process. Visualization under standard room light can only perceive a fraction of the spectral differences that exist between diseased and normal tissue that optical techniques, especially those based on fluorescence imaging, may reveal.[3] Data are accumulating from both laboratory and clinical studies that suggest that changes in natural fluorescence reflect biochemical and morphological alterations to tissues that could serve as noninvasive indicators of high-risk lesions.[4–9]

In this study, we present early research done using a simple hand-held device (Figure 1) for the direct visualization of tissue fluorescence alteration in the oral cavity. This device illuminates the oral mucosa, exciting natural fluorophores in the tissue and causing them to emit fluorescence that is visualized directly by a human observer.[3,10] In a pilot study of 44 patients, we have shown that the device achieved a sensitivity of 98% and specificity of 100% when discriminating normal mucosa from severe dysplasia/carcinoma in situ (CIS) or invasive carcinoma.[3] In this study, we report on our experience using this device to assess patients entering longitudinal follow-up at the Oral Dysplasia Clinics in British Columbia. We present 3 representative cases in which occult lesions were identified with fluorescence visualization, resulting in the diagnosis of a primary dysplasia in a noncancer patient (case 1), a second primary cancer in a patient with a history of oral cancer (case 2), and cancer recurrence in the third patient (case 3).

CASE REPORT

Case 1 involved a 51-year-old white woman who had no history of tobacco use. In November 2003, a severe dysplasia was excised by laser from her left ventral tongue. In February 2005, oral examination at the Dysplasia Clinic in the British Columbia Cancer Agency (BCCA) showed no apparent oral lesion at the previous surgical site under white light examination (Figure 2A). Strikingly, fluorescence visualization examination detected a large area showing loss of autofluorescence (termed FVL for fluorescence visualization loss) posterior to the previous surgical site (Figure 2B). The FVL site appeared dark green to black. In contrast, the surrounding oral mucosa, as an internal anatomic control showed normal pale green autofluorescence (termed FVR for fluorescence visualization retained). A comparative follow-up biopsy of the FVL area showed a moderate epithelial dysplasia (Figure 2C).

Case 2 involved a 43-year-old white female smoker who had a CIS removed surgically from the left floor of mouth in October 2002. At the 1-year recall examination (October 2003), the operating ear-nose-throat (ENT) surgeon reported her examination unremarkable. The patient was also seen by the oral maxillofacial medicine specialists at the Oral Dysplasia Clinic the same day. Again, a scar at the former cancer site was observed but no apparent oral lesion under white light. In contrast, fluorescence visualization examination detected a well-demarcated area of FVL at the right lingual aspect of retromolar and soft palate region (Figure 2E). A mild erythematous area was then noted on reexamination (Figure 2D).

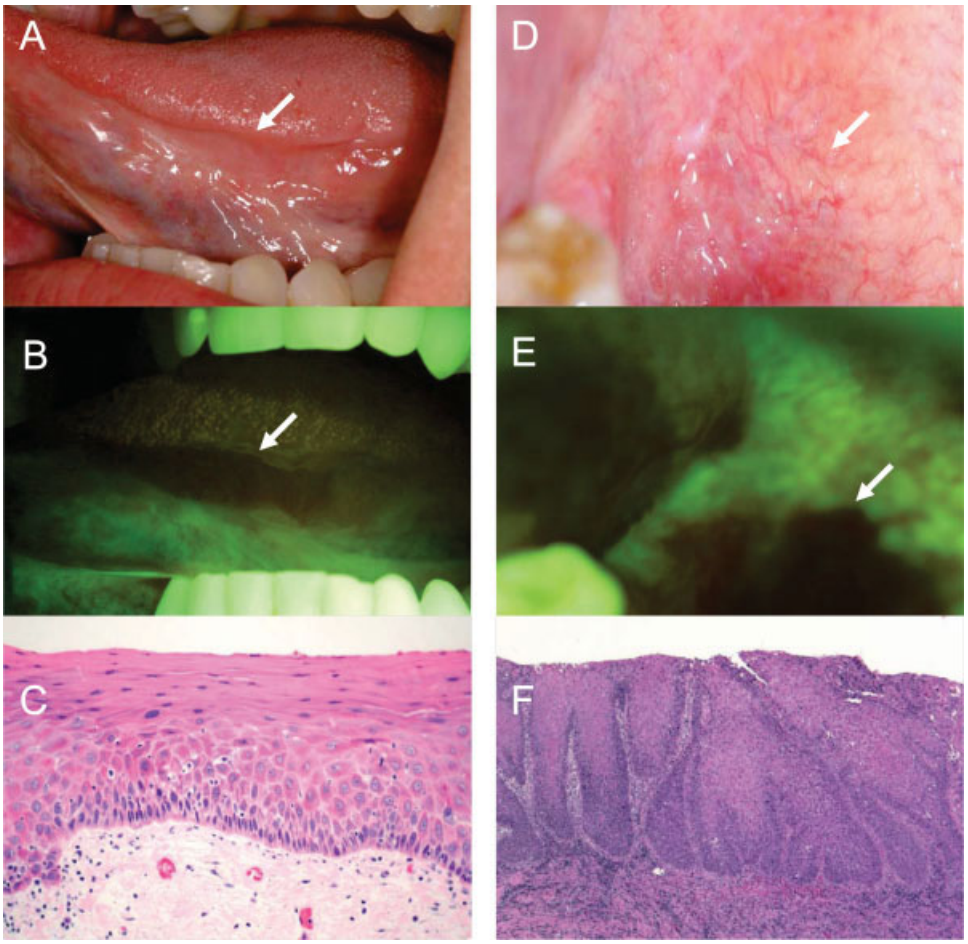

FIGURE 2. Detection of clinically occult disease in cases 1 (left panel) and 2 (right panel) using direct fluorescence visualization. Case 1 shows an occult lesion at the left ventral tongue under white light examination (**A**), which is identified by fluorescence visualization (**B**, arrow). The associated histology (**C**) shows moderate dysplasia with thickening of the epithelium and subepithelial connective tissue with a mild degree of inflammatory cell infiltration and increased capillaries. Case 2 shows a nonapparent carcinoma in situ (CIS) at right lingual retromolar and posterior soft palate region under white light (**D**), which was identified by fluorescence visualization (**E**, arrow). Biopsy revealed a CIS (**F**) with marked epithelial thickening with areas of atrophy. The connective tissue showed a heavy inflammation with increased vasculature (C and F: hematoxylin and eosin stain, original magnification: ×100).

This area had no previous biopsy or treatment. Incisional biopsy showed a CIS (Figure 2F), which was subsequently removed completely. Recurrence has not been seen at last follow-up 19 months later in May 2005.

Case 3 involved a 56-year-old Asian male smoker who had a CIS removed from his left ventral tongue by laser in July 2002. At the 18-month recall examination (January 2004), a slightly diffuse, erythematous change was noted at the previous surgical site (Figure 3A). Both the operating ENT surgeon and an oral maxillofacial medicine specialist at the Dysplasia Clinic regarded this as within the normal limit of postlaser treatment mucosal alteration. Fluorescence visualization examination, however, detected a very large, well-demarcated area of FVL, close to 4 cm in size (Figure 3B). Three punch biopsies were taken: 1 from the anterior orange-colored FVL area (arrow head) showing severe dysplasia (Figure 3D), a second from the posterior dark-colored FVL area (arrow) showing CIS (Figure 3E), and a final biopsy from an FVR area (asterisk) immediately anterior to the orange-colored FVL site, which was shown to be unremarkable histologically (Figure 3C).

There was an obvious layer of bacteria on the epithelium surface in D, which was probably responsible for the perceived orange fluorescence at this site. Studies have shown that orange auto-

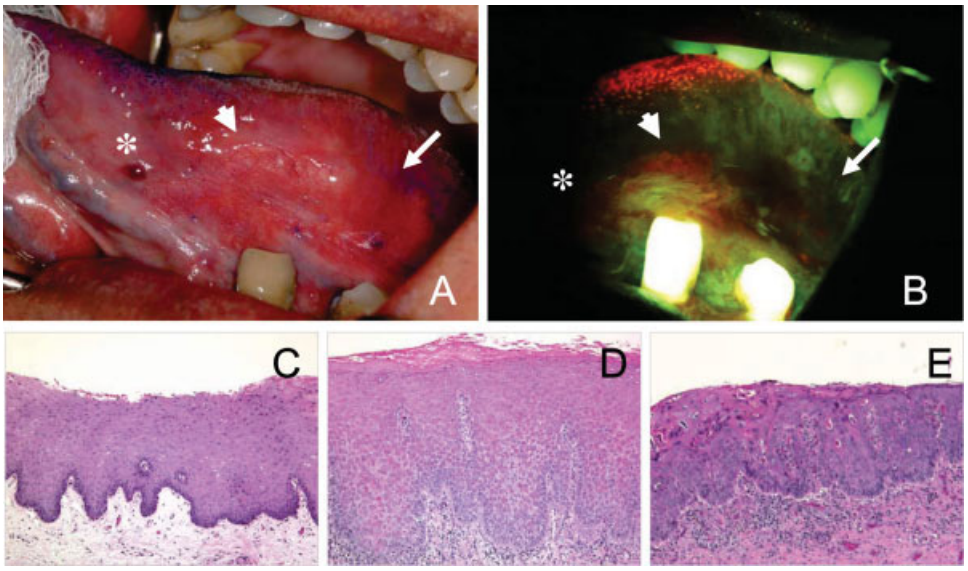

FIGURE 3. Case 3 demonstrates an occult recurrent lesion on the left ventral tongue under white light (**A**), which was identified by fluorescence visualization (**B**). The fluorescence visualization loss (FVL) is manifest by a visual perception of a change of green to orange color (B, arrowhead) in the anterior region and the usual dark shadow in the posterior region (B, arrow). Biopsy of the anterior FVL region showed severe dysplasia with marked thickening of the epithelium, while the biopsy from the posterior region showed a carcinoma in situ. The increased stroma from both biopsies showed inflammation (marked in **D** and moderate in **E**). In contrast, a biopsy from an adjacent clinically normal fluorescence visualization retained (FVR) area (A, asterisk) was unremarkable histologically (**C**). (C, D, and E: hematoxylin and eosin stain, original magnification: ×100).

fluorescence is associated with bacterial infection/host response as a result of bacterial porphyrins. High-risk lesions could demonstrate perceived orange autofluorescence, because the signal is a mixture of the tissue autofluorescence and the bacterial autofluorescence due to subclinical infection. However, the significance of orange autofluorescence should be assessed in conjunction with other clinical factors. If its presence could be explained by mucosa infection or its appearance is on dorsal tongue where there is frequent bacterial lodging, the orange fluorescence does not indicate risk.

The large field was removed again by laser. Recurrence was not seen at last follow-up 23 months later in December 2005.

DISCUSSION

There is a growing awareness that potentially malignant intraoral lesions and early cancers do not always manifest clinically. Our current inability to detect many of these early changes clinically could contribute significantly to the late diagnosis of this disease and its dismal prognosis, even though the oral cavity is a site that is readily available for assessment.

There is an increasing interest in using optical technology to provide a more complete picture of the alterations that occur during the development of cancer, identifying alterations to biochemistry and morphology that lie beyond the visual range. Autofluorescence detected at the tissue surface is determined by tissue morphology (both clinical and microscopic) and biochemistry. Cancer development in a number of tissues and organs has been characterized by a loss of normal autofluorescence. Such loss probably reflects multiple changes in the tissue (see discussion in ref. 3). The loss of autofluorescence in clinically occult high-risk oral lesions as shown in this study could reflect histomorphological changes (eg, dysplastic nuclei, thickening of the epithelium, and increased vascularization), and/or biochemical changes such as decreased density of collagen crosslinks (fluorophores), possibly owing to the breakdown of the extracellular matrix in response to the signals from the dysplastic cells and decreased flavin adenine dinucleotide concentration due to increased metabolic activity.

The promise of the optical technology has been shown by increasing numbers of studies, including those demonstrating alteration of autofluorescence in oral malignancies (reviewed in ref. 4). However, these studies have mostly

employed complex devices to measure spectroscopy indirectly using either photographic film or a sensitive or intensified charge-coupled device camera.[5,6,8] Furthermore, these studies have focused on clinically visible lesion areas, and have not screened the rest of the oral cavity of cancer/dysplasia patients.

This is the first study to report noninvasive screening of not only the visible oral lesions but also the whole oral cavity of high-risk patients using an inexpensive, simple, hand-held robust device. The 3 case reports here are representative of our early findings in using this device for detection of occult lesions. This device is currently being evaluated within a longitudinal study in British Columbia that is following 200 patients with treated oral cancer and 200 patients with primary dysplasia for a total of 8 years. Approximately half of these patients have FVL oral lesions, with a third of these FVL lesions either completely or partially clinically occult under white light.

Patients with a history of oral cancer are known to have increased cancer risk. However, despite vigorous follow-up, many second primary tumors and recurrences are still not caught at an early preinvasive stage, partly due to their occult presentation. As illustrated in cases 2 and 3, fluorescence visualization could detect such change early during the management of such cases, allowing the disease to be identified before it can progress into an invasive cancer, and thus reducing mortality and morbidity. Remarkably, occult disease is also apparent in patients with primary dysplasia and is detectable using direct fluorescence visualization, as exemplified by case 1. Optical methods based on fluorescence imaging have previously been shown to have great promise in detection and localization of precancers in lung, cervix, skin, and oral cavity.[11–19] This current report extends these studies, showing an additional role for the device in delineating occult disease present prior to invasion. As such, this technology may provide a mechanism by which the clinically nonapparent spread of abnormal cells might be monitored during studies of the natural history of the disease.

In summary, this early publication highlights a potentially significant contribution of fluorescence visualization to early detection of occult disease. Of interest, the device also appears to facilitate the visualization of high-grade occult disease at the margin of clinically apparent lesions, further supporting its use in detecting occult disease (unpublished data). The value of this device will have to be ascertained within longitudinal follow-up, but the data to date are encouraging.

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