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Fluorescence influence on screening decisions for oral malignant lesions

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Abstract

Objectives: The aim of the present study was to assess the capability of the low-cost VELscope device to visualize the tissue auto-fluorescence of potentially malignant oral lesions and to establish the diagnostic accuracy, sensitivity, and specificity of this method when validating the retrieved data through the gold standard, *i.e.*, histological examination. *Patients, Materials and Methods*: Eighteen patients were evaluated by conventional oral examination (COE) followed by direct visual fluorescence evaluation (DVFE) using VELscope. Areas clinically suspicious detected by COE or with positive DVFE (visual fluorescence loss) were further investigated using surgical biopsy. *Results*: Eight positive biopsies for malignant lesions were detected by COE and DVFE. Only one positive biopsy for a premalignant lesion was not in accordance with COE and DVFE. One lesion identified on the VELscope and COE as a non-malignant lesion was confirmed by the biopsy. Therefore, the VELscope system had a sensitivity of 94.44% and a specificity of 100% in discriminating *in situ* normal mucosa from carcinoma or from invasive carcinoma, compared with histology. The predictive positive value was 100% and the negative predictive value was 50%, with a 95% confidence interval (CI). *Conclusions*: DVFE allows for a simple and cost-effective margin determination, in order to perform the detection and screening of oral precancerous and early cancerous disorders. It was found that the VELscope system could not fully replace the histopathology procedure. Nonetheless, the study demonstrated its usefulness for clinical examination, monitoring oral lesions, and guiding the biopsy. Therefore, this method may add sensitivity to the oral tissue examination and be an effective adjunct for high-risk patients.

Keywords: non-invasive diagnosis, oral cancer, fluorescence, VELscope Vx, histopathology.

Introduction

Optical instruments for diagnosis are based on the light interaction with tissue. The physical characteristics of light onto tissue can be optimized with regard to several parameters, including location of illumination relative to detection, light wavelength, polarization state, duration, and angles of illumination. The chemistry, morphology, and structure of the tissue interact with the light revealing for example epithelial thickness, cellular density, nuclear/cytoplasmic ratios blood vessels, and collagen matrix.

This interaction of light with tissue is done through absorption, scattering, or absorption with re-admission. Due to the strong interaction of light with tissue, the penetration depth of visible light inside the tissue is small; it is therefore helpful only for assessing changes situated mainly in the thin epithelial layer of tissues with risks of malignant conversion. On the other hand, the absorption of high-energy photons by molecules in the tissue makes the light to be re-emitted in the form of lower energy photons that generate tissue fluorescence. For example, the blue light absorbed by collagen cross-links is being re-emitted as auto-fluorescent green light. Basically, fluorescence is the process of detecting wavelength-shifted light.

Tissue auto-fluorescence (AF) technology has been generated for localizing diseases and for helping with the detection of lesions that require biopsy. AF is now an accepted clinical technique for detecting cancer and/or premalignant diseases of the colon, cervix, esophagus, and also of the oral cavity [1–3].

The mechanism beneath tissue fluorescence visualization (FV) is the combination of native fluorescence and tissue morphology. Naturally occurring fluorophores in the stroma and epithelium produces the intrinsic fluorescence; they become excited when specific light wavelengths are absorbed, re-emitting light of different wavelengths. The fluorescence is modified during carcinogenesis, because of the direct alterations of the fluorophores themselves or by changes in the tissue morphology, changes that affect the light scattering and absorption. The most relevant endogenous fluorophores for optical diagnosis and screening of pre-malignant and malignant lesions are those that are excited in the violet-blue part of the visible spectrum (400–450 nm) up to the ultraviolet A (315–400 nm). The properties of these fluorophores

have been spectroscopically correlated with disease progression [4, 5]. When considering visible light, most of the fluorescence originates in the collagen cross-links that bind together collagen fibrils, generating fibers in the stroma (collagen matrix). A small portion of fluorescence originates in the oxidized form of flavin adenine dinucleotide (FAD) and the reduced form of nicotinamide adenine dinucleotide (NADH). These represent important fluorophores that are excited at those wavelengths intervals in the epithelium cells.

The first commercial AF imaging device approved for intra-oral use has been the VELscope® (LED Dental Inc., White Rock, BC, Canada). This low-cost device can identify clinically occult and high-risk oral lesions by offering health care professionals the possibility to visualize and map them. This approach has been improving overall survival through decreased rates of loco-regional recurrence [6]. There are proofs that the visual examination as part of a screening program decreases the mortality rate of oral cancer in high-risk population [7–10]. Direct visualization of the oral tissue AF has been reviewed in multiple studies as a possible adjunctive tool for early recognition and diagnosis of potentially malignant and malignant oral disorders [11–13].

Taking into account the importance and the impact of these aspects, as documented above, the scopes of this study were to assess: (*i*) the capability of the VELscope to visualize the tissue AF of potentially malignant oral lesions; (*ii*) the diagnostic accuracy, sensitivity, and specificity of the method, by validating the data obtained through histological examination – which is the gold standard.

⊟ Patients, Materials and Methods

VELscope® is a handheld device that uses a blue/violet light (400–460 nm) to illuminate the oral tissue. Using a selective long-pass filter, the observer can directly visualize the pale green auto-fluorescence that is emitted by the normal tissue [14]. Abnormal or suspicious tissue gives off decreased levels of AF, exposing a dark brown to black region when compared to the surrounding healthy tissue (Figure 1).

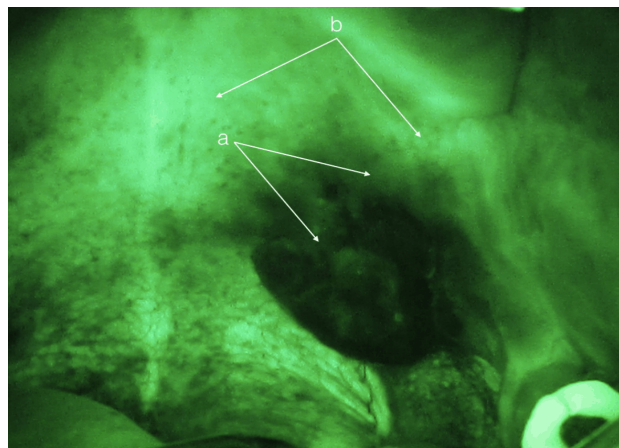In the present study, 18 patients were evaluated by conventional oral examination (COE) followed by direct visual fluorescence evaluation (DVFE) using VELscope. Areas clinically suspicious by COE or with positive DVFE [visual fluorescence loss (VFL)] underwent surgical biopsy. Association between COE and DVFE was assessed and compared with histopathology.

Throughout the study, all visual and tactile intraoral examinations followed by the fluorescence examinations were conducted by the same clinician. All subjects received an inspection of the following: lips; buccal and labial mucosa; dorsal, ventral and lateral sides of the tongue; floor of the mouth; hard and soft palate; uvula and oropharynx. All evaluations took place in a single visit at the Maxillofacial Surgery Hospital in Timişoara, Timiş County, Romania. All participants received oral cancer screening information and signed an informed consent. The local Ethical Committee approved the study.

The aim was to see if objective discrimination criteria could be obtained with this device when observing oral mucosal lesions. Lesions were examined under the conventional overhead light and then examined using this system. Each examination was recorded with a digital camera provided by the Velscope Vx system. For the fluorescence photography, the settings of the camera included 2× digital zoom, minimal optical zoom, 7 mm focal length, and a 3264×2448 pixels image size. It was found that several conditions and sites, such as keratinization and the degree of inflammatory cell infiltration were associated with the detection sensitivity using VELscope (Figure 2).

The pathologist has not been informed regarding the AF results.

⊟ Results

All 18 patients included in the study underwent COE and DVFE, the latter with VELscope, followed by biopsies. Biopsy results that showed invasive malignancy were considered positive. Sixteen positive biopsies for malignant lesions were detected by COE and DVFE (true positive) – as presented for example in Figure 3.

One lesion identified on the VELscope and COE as a non-malignant lesion was confirmed by the biopsy (true negative) – Figure 4. Only one positive biopsy for carcinoma was not in accordance with COE and DVFE (false negative) – Figure 5.
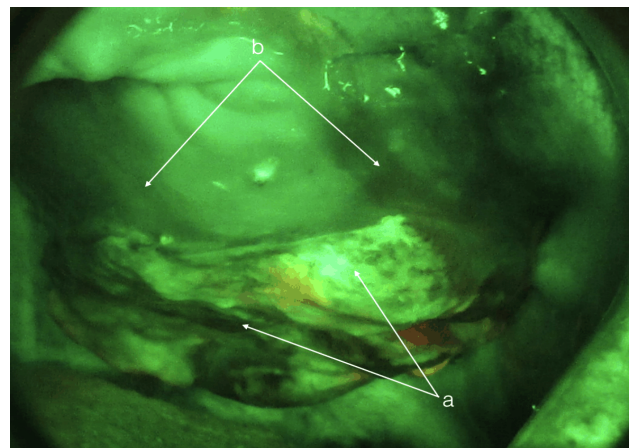

Figure 1 – *Characteristic dark aspect (a) of a malignant lesion in the oral cavity compared to the green AF (b) of the healthy surrounding tissues on the soft palate. AF: Autofluorescence.*

Figure 2 – *Areas of inflammatory cell infiltration (a) associated with keratinization area (b) in an oral squamous cell carcinoma on the hard palate.*
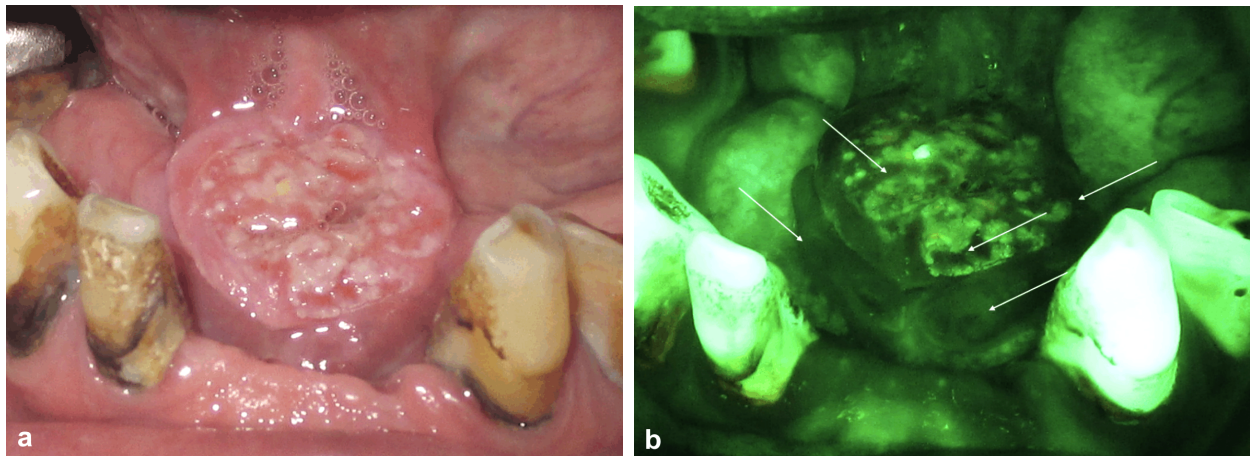
Figure 3 – *(a) Oral squamous cell carcinoma in the floor of the mouth – conventional white-light image; (b) Same lesion viewed through VELscope, showing a well-defined dark VFL area. The arrows indicate areas with VFL. VFL: Visual fluorescence loss.*
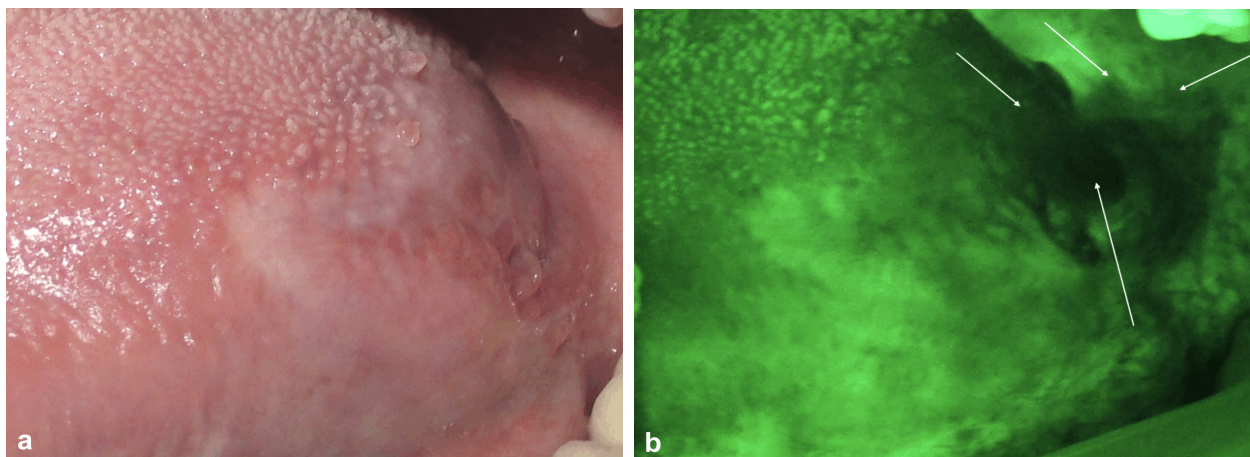

Figure 4 – *(a) Conventional white-light image showing an ill-defined slightly erythematous area; (b) Fluorescence image of a white benign lesion on the side of the tongue showing a slightly demarcated area of VFL area. The arrows indicate VFL areas with irregular margins, which extend toward the esophagus. VFL: Visual fluorescence loss.*
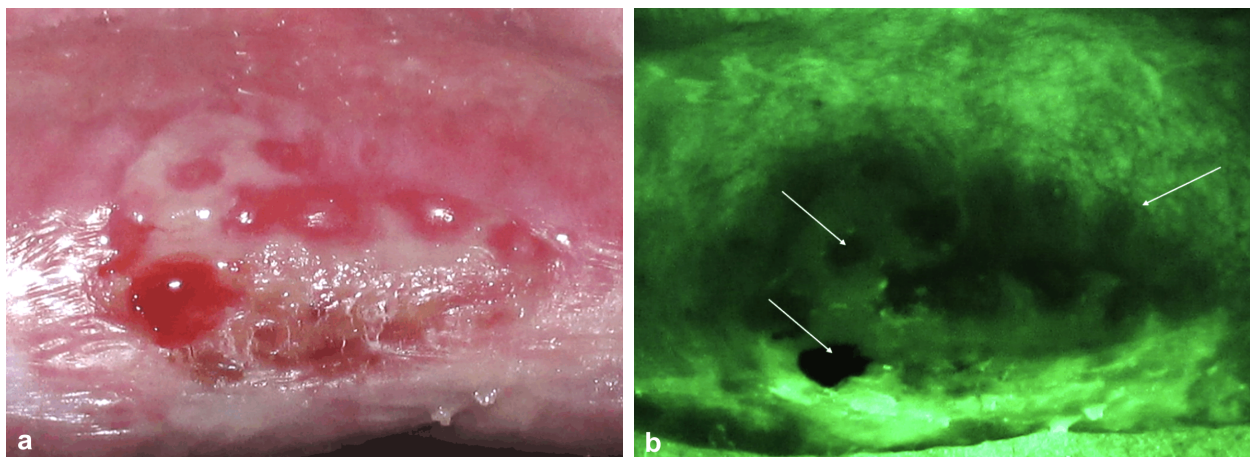

Figure 5 – *(a) Conventional white-light image showing an ill-defined erythematous area; (b) Fluorescence image of an ulcerative lesion on the lower lip showing a well-defined dark VFL area. The arrows indicate VFL areas with irregular margins. VFL: Visual fluorescence loss.*

For discrimination criteria, we used four normal volunteers. Sites investigated in the oral cavity for this group included tongue, buccal mucosa, lip, soft and hard palate, gingiva, and floor of mouth. Fine vasculature was clearly identifiable in images acquired from the floor of mouth, hard and soft palate, lip, using white light, and

fluorescence techniques. Figure 6 shows an image of the lower lip of a normal volunteer under the two illumination conditions. The white light image shows microvasculature from a variety of depths beneath the epithelial surface (Figure 6a). The fluorescence image obtained with the Velscope Vx system shows only the superficial, fine

vasculature due to the reduced penetration of the wavelength (Figure 6b); vessel contrast is also increased because this wavelength matches the Soret absorption band of hemoglobin. Vasculature was not as apparent in the buccal mucosa and tongue. The hard and soft palate, as well as the buccal mucosa and floor of mouth provided a higher fluorescence signal than the tongue, gingiva, and lip. The midline of the hard palate was particularly bright. Teeth were highly fluorescent and tooth fluorescence could be seen through portions of the gingival mucosa. Blood vessels appeared dark under fluorescence mode compared to the surrounding tissue. Red fluorescence occasionally appeared on the dorsal tongue.

Figure 3 shows images from the floor of the mouth in a subject with histologically confirmed carcinoma. An ulcerative lesion is shown in the center of the field of view. In the fluorescence image, increased contrast is noted by arrows in the tissue surrounding the ulcer. Larger dark areas were noted in the ulcerative lesion and at its margins compared to the white light image. Following the imaging, a portion was resected and was determined by using histopathology to contain invasive squamous carcinoma centrally, with dysplasia near the margins of resection.

Figure 4 shows images acquired from a subtle lesion on the left lateral tongue. The clinical impression of the lesion was leukoplakia, but not overly suspicious for dysplasia or cancer. Following the imaging, the lesion was surgically resected and histopathology showed mild epithelial dysplasia. The standard white light image shows some patchy irregularities in the mucosal surface (Figure 4a). Using the fluorescence light an apparent increase in visual contrast was observed between the lesion and surrounding normal areas, as indicated by arrows in each case (Figure 4b). A decreased blue/green AF was observed in the area of the lesion. An image of a contralateral normal area is shown in Figure 7 for comparison.

Figure 5 shows images acquired from a lesion on the lip. The clinical impression was erythroplakia; a reddish lesion associated with a high risk of dysplasia or early carcinoma (Figure 5a). Histopathology from a biopsy of the lesion indicated squamous dysplasia with a focal ulceration and chronic inflammation. The fluorescence image showed an area of abnormality (appearing darker on the fluorescence image as indicated by arrows), which is more extensive in peripheral extent and has an increased contrast as viewed against the surrounding mucosa.

In Figures 2 and 8, an oral squamous cell carcinoma on the hard palate is presented, showing an extensive VFL due to the intense keratinization in the area associated with inflammatory cell infiltration.
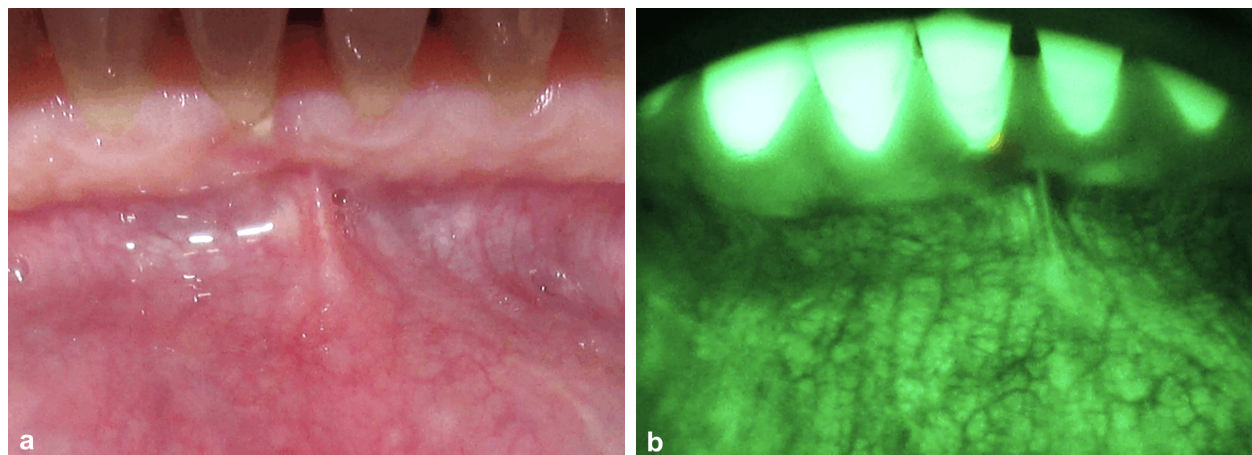

Figure 6 – *(a) Conventional white-light image showing microvasculature from a variety of depths beneath the epithelial surface; (b) The fluorescence image obtained with the Velscope Vx system showing only the superficial, fine vasculature due to the reduced penetration of the wavelength.*
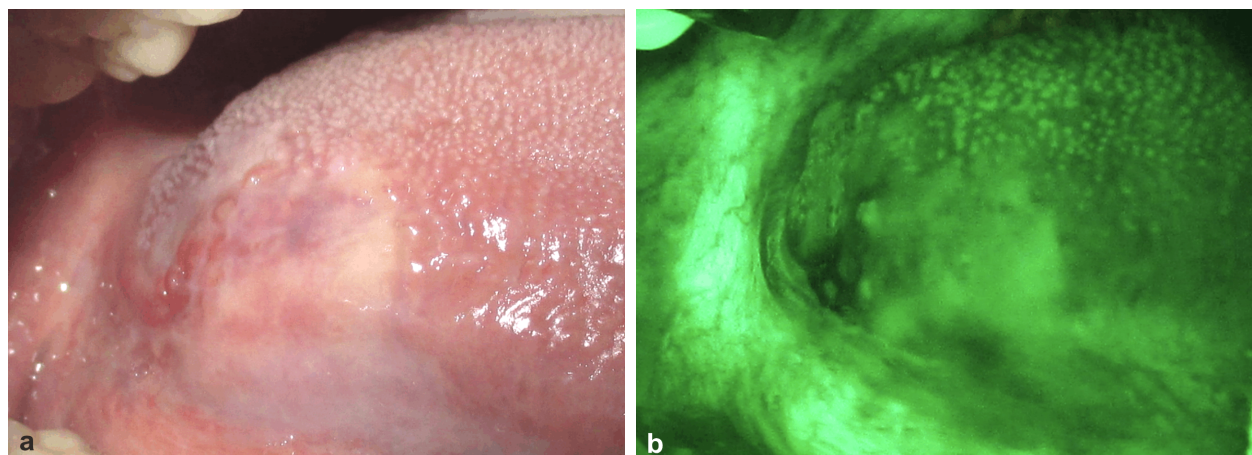

Figure 7 – *(a) Conventional white-light image; (b) Fluorescence image of the contralateral normal side of the tongue.*
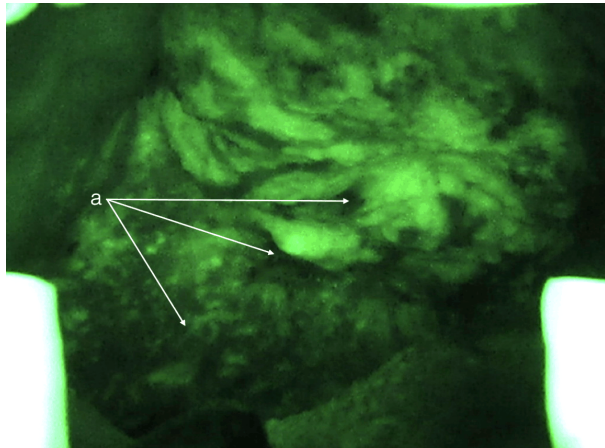
Figure 8 – *Area with an extensive VFL: an oral squamous cell carcinoma on the hard palate in final stages is pointed out by the arrows (a), having also an irregular development and extending invasively towards the esophagus. VFL: Visual fluorescence loss.*

In these representative examples, we observe one or more of the following features in areas histologically determined to be abnormal: a decrease of fluorescence, and an increase of contrast in highly vascular regions. Blood on the surface of the tissue also appeared dark compared to white light under blue illumination (Figure 5, a and b). Based on these results (Table 1) obtained under direct FV, various shades of pale green AF have been emitted by the normal oral mucosa. In the pre-malignant and early cancers, the lesion presents itself as a well-defined dark area with different degrees of VFL loss. The VELscope system had a sensitivity of 94.44% and a specificity of 100% in discriminating normal mucosa from carcinoma *in situ* (CIS) or invasive carcinoma, compared with histology as gold standard. The predictive positive value was 100% and the negative predictive value was 50% [95% CI (confidence interval)] respectively.

⊟ Discussions

The results of this study suggested a possible advantage of using DVFE to identify lesions that cannot be seen by clinical examination alone. This aspect is being explored within on-going studies nowadays among patients in follow-up for cancer recurrence, looking for the re-appearance of a clinical lesion in the treatment area that may or may not show AF loss; if such a loss appears the question is if it is with or without clinical change. In the latter case, elements being explored are changes to the intensity and size of the VFL fields and the persistence of areas showing VFL over time and subsequent evolution of clinical lesions – as shown for example, from the present study, in Figure 6.

Table 1 – *Centralization of data for the patients included in the study*

Patient No.	Gender	Age [years]	Localization of the lesion	Clinical changes	Intensity and size of the VFL	Histopathological results
1.	F	62	The posterior area of the soft palate, near the left posterior pillar (Figure 1)	Ill-defined mildly erythematous area (EA) with extensive proliferation	Well-defined dark VFL area	Oral squamous cell carcinoma, invasive
2.	M	60	Left hard and soft palate (Figure 2)	Ill-defined ulcerative area (UA)		
3.	M	61	Floor of the mouth (Figure 3)	Ill-defined mildly EA area		
4.	F	52	Left posterior dorsum of the tongue (Figure 4)	Ill-defined slightly EA	Slightly demarcated moderate VFL area	Mild epithelial dysplasia
5.	M	59	Lip (Figure 5)	Ill-defined EA		Oral squamous cell carcinoma, *in situ*
6.	M	65	Hard and soft palate (Figure 6)	Ill-defined mildly EA with extensive proliferation		
7.	M	55	The posterior area of the soft palate, near the left posterior pillar	Ill-defined mildly UA		
8.	M	68	Left lower gingiva	Ill-defined mildly UA		
9.	M	54	Upper right gingiva, hard and soft palate	Ill-defined mildly EA with extensive proliferation		
10.	F	68	Upper right gingiva	Ill-defined mildly EA		
11.	M	60	Right side of the tongue	Ill-defined mildly EA with extensive proliferation	Well-defined dark VFL area	Oral squamous cell carcinoma, invasive
12.	F	68	Left lower gingiva	Ill-defined mildly UA		
13.	M	68	Upper right gingiva	Ill-defined mildly EA		
14.	F	60	Left hard and soft palate	Ill-defined UA		
15.	M	52	Left posterior dorsum of the tongue	Ill-defined EA		
16.	M	69	Hard and soft palate	Ill-defined mildly EA with extensive proliferation		
17.	M	65	Floor of the mouth	Ill-defined mildly EA		
18.	F	60	Right side of the tongue	Ill-defined mildly EA with extensive proliferation		

F: Female; M: Male; EA: Erythematous area; UA: Ulcerative area; VFL: Visual fluorescence loss.

One of the biochemical transformations associated with an alteration of fluorescence during cancer evolution is the AF reduction from the collagen cross-links, probably due to the disruption of the extracellular matrix. This modification was considered to be generated by collagen remodeling helped by alterations to matrix metallo-

proteinases (MMPs) expression in the host stromal cells, together with stromal remodeling associated with angiogenesis [15, 16]. Although collagen alteration is considered an initial biochemical cause of AF change, other fluorophores are also modified. For example, changes in the metabolic activity with dysplasia are associated with alterations in the electron transport chain of NADH and FAD levels. FAD fluorescence intensity is reduced with dysplastic progression. Finally, during carcinogenesis a growth in microvasculature in the stroma can also result in a decrease in AF, due to the strong hemoglobin absorbance of the violet-blue excitation light, reducing the quantity that reaches the fluorophores. This process has been observed in its different stages for all the cases included in this research (Figures 1 and 7).

Additionally to the diminishment of the intrinsic sources of tissue fluorescence, the alteration of nuclear morphology at the cellular level and epithelium thickness during the disease evolution has a significant impact on fluorescence through scattering of the excitation and emission light. For example, a reduction of the intensity of AF will be observed in the case of a thicker epithelium with increased nuclear scattering, which means that less excitation light will manage to get to the stroma where the fluorescence is generated.

The first reported utilization of VELscope® system involved a study of 44 patients within the *Oral Cancer Prediction Longitudinal Study* in Vancouver, BC, Canada. This included 33 patients with invasive squamous cell carcinoma and 11 with severe dysplasia/CIS. Six healthy patients were used as a control group. All cases were biopsy-confirmed. Data were promising, with a 98% sensitivity and a 100% specificity to distinguish dysplasia and cancers from healthy mucosa [14].

An intriguing early observation for the use of AF as a diagnostic tool for oral and cervical cavities has been the AF identification of lesions that under white light inspection were clinically occult [17]. This ability is yet to be entirely explored; however, it encourages three potential clinical directions for the use of this evaluation method: (*i*) early diagnosis of premalignant lesions and cancers that are clinically occult; (*ii*) early identification of recurrent disease, either as a second primary tumor situated elsewhere in the oral cavity or as a recurrence at treated lesion site; (*iii*) better delineation of the surgical margin in malignant lesions.

Support for the potential ability of FV to provide real-time guidance for intraoperative direct use comes from a study where FV was used to identify surgical tumor margins for oral cancer in the operating room [18]. The study included 20 consecutive patients undergoing surgical excision, documenting molecular and histological alterations within areas showing loss of AF in tumor margins. All cases except one demonstrated a loss of AF, which extended beyond the clinically visible tumor boundary that had different lengths (from 4 mm to 25 mm), having an extension unevenly distributed around the clinical apparent perimeter. Eighty-nine percent of margin biopsies from these areas showed either cancer or dysplasia. Molecular analysis of AF margins with lack or low-grade dysplasia in the study mentioned above suggests that FV is able to identify histologically low-grade margins with high-risk molecular clones. Sixty-three percent of such margins showed high-risk loss of heterozygosity (LOH) patterns. Even if their results are intriguing and even if data are promising, a larger sample group and an eventual movement to a clinical trial is a future direction of study in order to further confirm the utility of the VELscope.

Recent studies have also sustained the fact that by using FV as part of the surgical margin decision process, the rate of local recurrence in pre-invasive high-grade and early-stage oral malignancy was significantly reduced [6]. This is in accordance with the present study in which, in nine out of 10 cases VELscope was more reliable in detecting the lesions margins when compared to COE, suggesting that this method could be of great importance in the surgical phases of the treatment.

There are also studies in which VELscope was useful in confirming the presence of oral erythroplakia and leukoplakia and other oral mucosal lesions, but was unable to distinguish high-risk from low-risk disorders [19–21]. The same conclusion can be drawn from our results, due to the low specificity obtained after analyzing the degree of VFL for all the lesions included in this study.

Future work in our group comprises investigations of oral tissue using another non-invasive biomedical imaging technique, optical coherence tomography (OCT) [22, 23], with in-house developed systems and using custom-designed handheld probes [24] that we have already demonstrated to allow for investigations of hard tissue in the oral cavity [25, 26]. Also, microRNA investigations of the saliva are carried out in conjunction with both AF and OCT imaging.

⧉ Conclusions

The present study has investigated on patients the efficiency of the VELscope system to give the possibility for a simple, cost-effective margin determination, detection, and screening of oral precancerous and early cancerous disorders. We found that the direct DVFE system utilized in our study cannot fully replace the gold standard, *i.e.*, the histopathology procedure. Nonetheless, its usefulness for clinical examination, in monitoring oral lesions, and before performing a biopsy was fully demonstrated. This device may thus add sensitivity to the oral tissue examination and be an effective adjunct for high-risk patients.

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