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Widefield fluorescence imaging as an auxiliary tool to select the biopsy site for actinic cheilitis diagnosis

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Abstract

Actinic cheilitis (AC) is considered a potentially malignant disorder that mainly affects the lower lip, and it is caused by prolonged sun exposure. Clinical diagnosis relies on visual inspection by a trained clinician, when suspected of dysplasia changes, a biopsy is required. The heteregenous characteristics of the AC, makes the choice of the biopsy site a difficult task. Fluorescence detection has been presented as a useful tool to to detect biochemical and morphological tissue features related to cancer diagnosis, but still its effectiveness to discriminate premalignant lesion is not completely defined. In this clinical study, 57 AC patients were investigated using widefield fluorescence imaging (WFI) to evaluate the efficacy of this technique as an auxiliary tool to biopsy site location. A handheld fluorescence system based on 400-450 nm LED illumination Distinct trained clinicians evaluate the patient either with the conventional examination or the WFI, and were blinded to the other evaluation. A biopsy site was chosen based on the clinical examination, and another site was chosen using the fluorescence visualization. A total of 114 punch biopsies were performed, and 93% of the tissue samples presented epithelial dysplasia. The majority of the sites that presented moderate or severe dysplasia were sites chosen by WFI, showing its efficiency to improve the diagnosis of AC.

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I. INTRODUCTION

Actinic cheilitis (AC) is an ultraviolet radiation induced lesion that mainly affects the lips. The continuous and prolonged sun exposure, as in skin cancer etiology, produces DNA damages, resulting in epithelial cells alterations, so AC is considered a potentially malignant disorder. If the lesion is not properly treated, AC can progress to lip squamous cell carcinoma. The incidence is higher in middle-aged or older outdoors workers with types of skin complexions I or II [1].

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The most incident site is the lower lip, and the lesion is highly heterogenous, affecting all lip extension. Clinical features related to this diffuse lesion are: dryness, redness, ulceration, epithelial atrophy, edema, white plaques, loss of vermilion border delimitation, transverse fissures, and crusting [2,3].

Histopathology of an AC lip can vary from a hyperkeratosis, inflammatory conditions, until a SCC [2,3]. Distinct epithelial dysplasia levels may be present, making the diagnosis an important task. Due to the heterogenous clinical and histological patterns of this potential malignant disorder, the choose of the biopsy site is not simple and is highly depend on the clinician skills and experience on detecting and relating clinical aspects to the severe epithelial dysplasia and *in situ* SCC. The rate of AC malignant transformation is not clearly defined [4], but it is known that the lip SCC presents a higher metastatic rate when compared to the skin SCC [5,6]. In this sense, detecting the epithelial dysplasia degree in an AC lip is of great relevance.

Treatment planning must be focused on preventing lip SCC. AC usually affects the whole lower lip, and indication of a large excision is not usual for the moderate and severe dysplasia. Conventional treatments are based on topical chemical application, or localized surgery and posterior pathology review of the resected tissue margins. Photodynamic therapy has been presented as an alternative treatment for AC, with good cosmetic results, even though the current protocols require several sessions. Early diagnostics of higher degrees of epithelial dysplasia and *in situ* SCC shows strong effect on treatment planning and patient prognosis, especially considering the quality of life after the mutilation of a lip resection.

Several groups have already demonstrated the high resolution of the optical techniques for diagnostics of oral cancer [7-16], including fluorescence spectroscopy, widefield fluorescence imaging, and fluorescence microscopy. The efficacy of optical techniques on the discrimination of malignant versus premalignant lesions shows lower sensitivity and specificity rates [17-18]. Fluorescence based optical techniques are attractive as clinical instruments since their equipments are cost-effective, and this light-tissue interaction can provide information of tissue biochemical composition and architecture, through a non-invasive and *in situ* measurement.

Our main aim in this clinical study is the evaluation of the widefield fluorescence imaging as an auxiliary tool to select the biopsy site for the AC diagnostics. Nova Friburgo is a small town in Rio de Janeiro state (Brazil) that has its population based on Swiss and German descendants. The agriculture is one of the major economy sectors. These characteristics of Nova Friburgo result in a high incidence of AC among its habitants.

II. MATERIALS AND METHOD

Fifty seven AC patients of the Stomatology Clinic of the School of Dentistry of Fluminense Federal University at Nova Friburgo were enrolled in this study. Twenty seven men and 30 women with skin phototypes I, II, or III and clinical diagnostics of lower lip AC signed the inform consent form. The investigation protocol was approved by the Internal Review Board of the Medical School, Antonio Pedro Hospital, Fluminense Federal University.

First, a clinical exam under white light and with palpation was perform by two oral medicine specialists, and a chart was filled to indicate the level (none, mild, moderate, or important) of the following features: dryness, blurred vermilion border delimitation, ulceration, erosion, pigmented and pallor regions, swelling, crusts, vertical fissures, hardness, and red and white areas. The lower lip was divided in 14 areas to a better data correlation. The indicated biopsy sites were selected taking the ulceration, erosion, crust, and the red and white regions as the main clinical features potentially related to malignant transformation.

The widefield fluorescence imaging was performed in a dark room by a third oral medicine specialist, blinded to the clinical examination results. The fluorescence image was taken using a handheld homemade device. The fluorescence device is basically composed by a LED emitting centered at 405 nm and bandwidth of 40 nm (Edison, EDEV-3LA1), dichroic mirror (Semrock, NY, USA), and optical filters (Proteon, SP, Brazil). The autofluorescence features visualized at the handheld device were indicated at the lip chart as: increased or decreased green fluorescence, and red fluorescence. The decreased fluorescence was taken as the major feature to indicate the biopsy site.

After the indication of the biopsy sites by the clinical and fluorescence examination, 5 mm punch biopsies were taken. If the sites were coincident, only one tissue site was taken. Tissue specimens were processed for histopathological diagnosis. Three oral pathologists examined the HE slides and graded as normal lip epithelium, or mild, moderate or severe epithelial dysplasia, according to the World Health Organization classification.

III. RESULTS AND DISCUSSION

The AC lip presents a more heterogenous fluorescence when compared to the normal lip [19]. This characteristic is due to the diffuse and also heterogenous AC lesion. The AC lower lip can present several distinct lesions affecting the whole lip. The normal lip usually shows a medium green fluorescence pattern, intensity between the higher pattern from the skin and the lower one from the oral mucosa.

The higher intensity areas could be related to the more keratinized, dried, hypopigmented or whitish areas. The regions showing decreased green fluorescence were from areas of erosion or ulceration, red macule, fissures, or hyperpigmentation.

One hundred and thirteen biopsies were performed, with an average of 1.98 biopsies per patient, depending on the site indication of the clinical examination and widefield fluorescence imaging. Clinical assessment was used as a single clinical diagnostic technique in 19.5% of the cases, since the autofluorescence visualization did not show any region of relevant decreased fluorescence. The widefield fluorescence was used alone in 39.8% of the indications, when no clinical features of ulceration, erosion, crust or red/white lesions were observed. In the other 40.7% of the cases both diagnostic modalities were used.

Epithelial dysplasia was present in 105 biopsies (93%), being 55 mild, 41 moderate, and 9 severe levels. The combination of both techniques resulted in the indication of 45.4% and 41.5% of the mild and moderate dysplasia, respectively. The widefield fluorescence visualization when used alone was the most effective technique to indicate the sites with severe dysplasia, corresponding to 55.6% of the cases. The eight biopsies that did not show epithelial dysplasia was indicated by both modalities in 3, 1 by the clinical assessment alone, and 4 by the autofluorescence alone.

These results show that both investigated diagnostic modalities were efficient to select regions with epithelial dysplasia at the AC lower lip, and when comparing them, the autofluorescence visualization was more efficient on indicating sites with severe dysplasia. On the other hand, the fluorescence indication showed a higher number of false positive, no epithelial dysplasia was found in 4 cases.

The fluorescence features used as indicative of potential malignant change was the decreased green fluorescence. This feature was present at the sites considered critical as of ulceration, erosion and reddish areas, but it was also related to hyperpigmented innocuous areas. If the fluorescence data was correlated with the clinical examination, to discriminate these low fluorescent hyperpigmented regions, we believe the diagnostic resolution could be higher.

At a clinical setting, widefield fluorescence visualization is commercially available for oral cancer, as VELscope® (LED Dental Inc. White Rock, BC, Canada), and Identafi® (DentalEZ Group (USA). The application as a biopsy site indicator for AC is an attractive auxiliary tool. Other fluorescence technologies as spectroscopy and microscopy have also been developed to enhance diagnostic resolution. One interesting device is a portable fiberoptic microscopy that uses the acriflavine dye, targeting cell nuclei, and provides a real-time *in situ* image of the epithelial cells.

The fluorescence data has higher biochemical information compared to the white reflectance visualization. Detecting the severe epithelial dysplasia and SCC at initial stages results in improved AC treatment and patient prognosis.

CONCLUSION

The widefield fluorescence imaging is a potential auxiliary tool for diagnosing AC, especially driven to discriminate epithelial dysplasia and indicate the biopsy site. Our results showed that the autofluorescence visualization alone was the most effective to indicate the biopsy sites with severe dysplasia (55.6% of the cases), but this diagnostic modality resulted in a higher false positive cases (non-dysplastic epithelium).

Other information

The same fluorescence handheld device was used in the public campaign for Oral Cancer Prevention in the main downtown square at Nova Friburgo, Rio de Janeiro, Brazil. At this one-day campaign, 4 oral medicine specialists performed oral examination, and one of them did also fluorescence investigation. In order to avoid the sunlight exposure, a small dark fabric was placed in a rigid support attached to a chair, over the volunteer's head. The macro lens of the digital camera attached to the fluorescence handheld device was placed through a hole in the fabric. Fifty-five volunteers were examined, and 14 of them were scheduled for the Stomatology Clinic, all with suspected oral or lip lesions.

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