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SHORT COMMUNICATION

ORAL DISEASES | WILEY

Histopathological determinants of autofluorescence patterns in oral carcinoma

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Abstract

Biological tissues (including oral mucosa) can absorb and re-emit specific light wavelengths, detectable through spectrophotometric devices. Such a phenomenon is known as "autofluorescence" (AF). Several devices evaluating tissue AF have been developed and commercialized in the last two decades. Among these, the VELscope® system has been proposed as a visual diagnostic aid for potentially malignant disorders and malignant lesions of the oral mucosa. In the present pilot study, we investigated which are the main histopathological features possibly related to variations in AF patterns in a set of 20 oral squamous cell verrucous carcinoma. Among all the histological features investigated, only the mean width of keratin was significantly different between hypofluorescent and hyperfluorescent carcinomas. The results of the present study demonstrate that AF features of oral malignant lesions are significantly associated with the width of their keratin layer.

KEYWORDS

autofluorescence, early diagnosis, fluorophores, oral carcinoma

1 | INTRODUCTION

The current gold standard for oral squamous cell carcinoma (OSCC) and potentially malignant disorder (PMD) diagnosis is histopathological analysis, usually performed on preoperative incisional biopsies. Several tools have been proposed in order to improve the diagnostic accuracy and to possibly help the identification of most suspicious areas of dysplastic or neoplastic alterations (Holmstrup, Vedtofte, Reibel, & Stoltze, 2007). However, the use of such diagnostic aids is associated with low accuracy (Giovannacci, Vescovi, Manfredi, & Meleti, 2016).

Biological tissues (including oral mucosa) can absorb and re-emit specific light wavelengths, detectable through spectrophotometric devices. Such a phenomenon is known as "autofluorescence" (AF) (Croce & Bottiroli, 2014).

Several devices evaluating tissue AF have been developed and commercialized in the last two decades (Jayanthi et al., 2009; Kikuta et al., 2018; Lane et al., 2006; Nazeer et al., 2014). Among these, the VELscope® system has been proposed as a visual diagnostic aid for PMDs and malignant lesions of the oral mucosa (Morikawa, Kosugi, & Shibahara, 2019).

Clinically healthy oral mucosa, evaluated through the VELscope® system, appears bright green (normofluorescent). By contrast, specific alterations in the mucosal architecture (including the presence of malignant lesions) may display a wide range of AF patterns, varying from hypofluorescent (dark) to hyperfluorescent (very bright) (Cicciù et al., 2017; Yamamoto et al., 2017). However, the usefulness of VELscope® might be somewhat questionable, as the loss of AF is not restricted to neoplastic lesions (e.g., inflammatory disorders, benign ulcers) (Morikawa et al., 2019). Furthermore, contrary to expectations, some malignant lesions may have a hyperfluorescent pattern. A lack of knowledge on the specific histopathological features associated with AF variations is therefore evident.

In the present pilot study, we investigated which are the main histopathological features possibly related to variations in AF patterns in a set of OSCC and verrucous carcinoma (VC).

2 | MATERIALS AND METHODS

The study was approved by the Ethical Committee of the Academic Hospital of Parma (No. 46556/17).

Twenty oral lesions (2 VC and 18 SCC) from 15 patients (males: 5; females: 10; mean age: 69—min: 39, max: 90) were included. All lesions were evaluated with regard to AF features, through the VELscope® system, before excisional or incisional biopsy. Lesions were classified as normofluorescent, hypofluorescent, or hyperfluorescent (Table 1).

Eight histological categories were investigated, in hematoxylin-and-eosin-stained sections, in order to identify which histopathological features are possibly related to the pattern of AF: (a) mean width of the entire epithelium (MWE); (b) mean width of the keratin layer (MWK); (c) mean width of the epithelium without taking into account the overlying keratin; (d) overall area of the epithelium (OAE); (e) mean depth of inflammatory infiltrate (MDI). For each specimen, severity of inflammatory infiltrate was taken into account (classified as "mild," "moderate," and "severe"); (f) overall area of blood vessels (OAV); (g) mean area of blood vessels (MAV); and (h) mean diameter of blood vessels (MDV) (Figures 1 and 2). Evaluations were performed through the Nikon NIS-Elements software (version 3.06). All measures were expressed in μm and taken at 4× magnification except for OAV, MAV, and MDV which were taken at 10× magnification.

Both parametric tests (Student's *t* test) and non-parametric tests (Mann–Whitney *U* test) were used for group comparisons between the continuous variables. Binary logistic regression was used to test

TABLE 1 Clinical, histopathological, and AF features of 20 cases of SCC and VC

Specimen	Oral site	Clinical presentation	AF pattern[a]	Histopathological diagnosis
1	Cheek	Non-homogeneous white and red, harden	Hypofluorescent	VC
2	Cheek	Non-homogeneous red, harden	Hypofluorescent	In situ OSCC
3	Tongue	Non-homogeneous red, harden	Hypofluorescent	In situ OSCC
4	Tongue	Non-homogeneous red, ulcerated	Hypofluorescent	Infiltrative OSCC
5	Tongue	Homogeneous white, harden	Hyperfluorescent	In situ OSCC
6	Gingiva	Non-homogeneous white and red, harden	Hypofluorescent	In situ OSCC
7	Tongue	Non-homogeneous white and red, harden	Hyperfluorescent	Infiltrative OSCC
8	Tongue	Non-homogeneous red, harden, ulcerated	Hypofluorescent	In situ OSCC
9	Hard palate	Homogeneous red	Hypofluorescent	In situ OSCC
10	Gingiva	Non-homogeneous white and red, harden	Hypofluorescent	Infiltrative OSCC
11	Tongue	Non-homogeneous red, ulcerated	Hypofluorescent	Infiltrative OSCC
12	Tongue	Non-homogeneous red, ulcerated	Hypofluorescent	In situ OSCC
13	Tongue	Non-homogeneous white, ulcerated	Hyperfluorescent	VC
14	Tongue	Non-homogeneous white and red, harden, ulcerated	Hyperfluorescent	Infiltrative OSCC
15	Tongue	Non-homogeneous white and red, harden, ulcerated	Hypofluorescent	In situ OSCC
16	Tongue	Non-homogeneous red, harden, ulcerated	Hypofluorescent	Infiltrative OSCC
17	Tongue	Non-homogeneous white, harden	Hyperfluorescent	In situ OSCC
18	Gingiva	Non-homogeneous white and red, harden, ulcerated	Hyperfluorescent	Microinvasive OSCC
19	Soft palate	Non-homogeneous white and red, harden, ulcerated	Hyperfluorescent	In situ OSCC
20	Hard palate	Non-homogeneous white and red, harden, ulcerated	Hyperfluorescent	In situ OSCC

Abbreviations: OSCC, oral squamous cell carcinoma; VC, verrucous carcinoma.

[a]AF pattern is specifically referred to the site where the biopsy was taken.

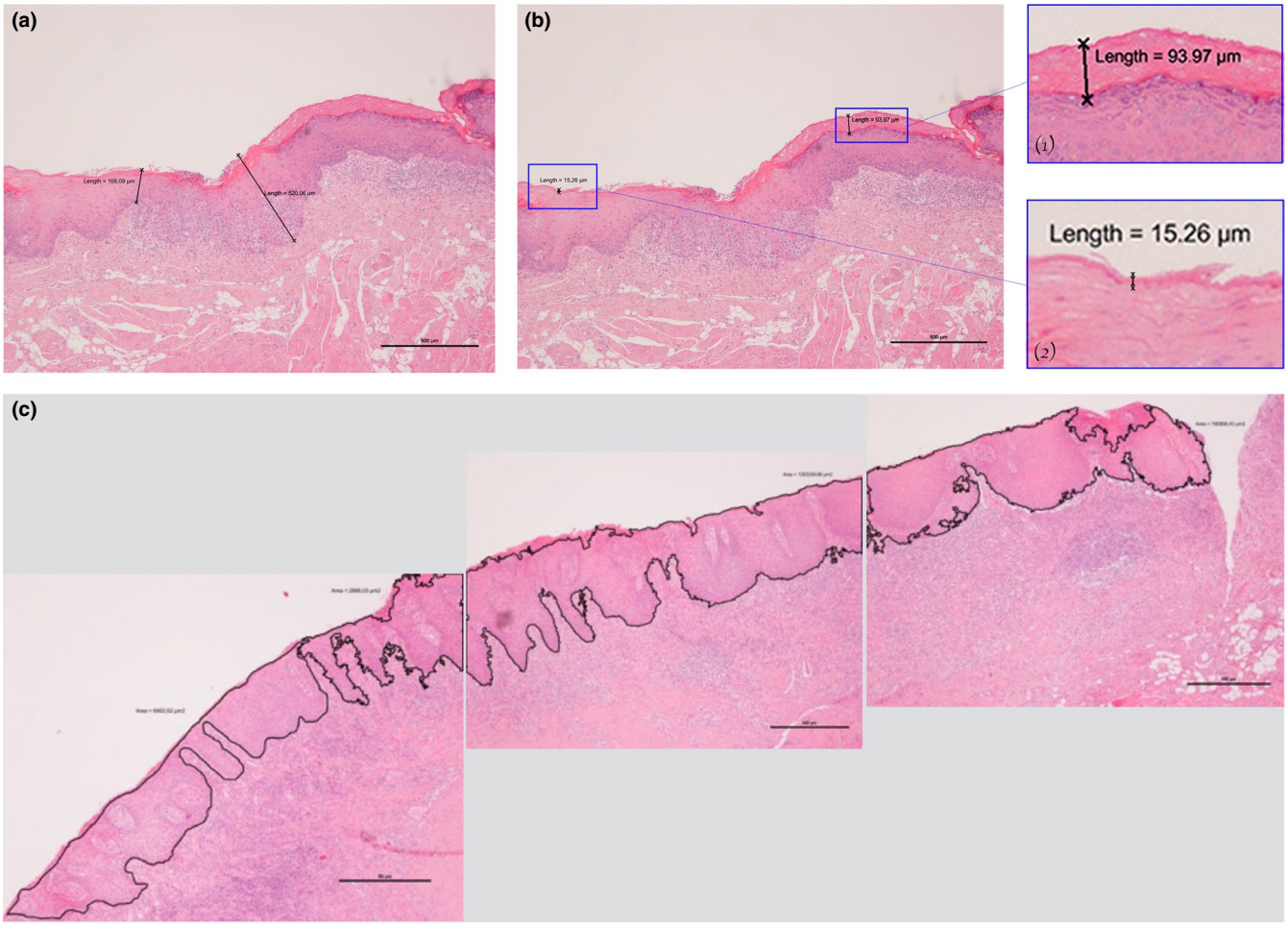

FIGURE 1 Examples of evaluation of width of the epithelium and keratin and area of the epithelium: (a) maximum and minimum length of the epithelium; (b) maximum and minimum length of the keratin layer; and (c) overall area of the epithelium in 3 consecutive fields (H&E staining, 4× magnification) [Colour figure can be viewed at wileyonlinelibrary.com]

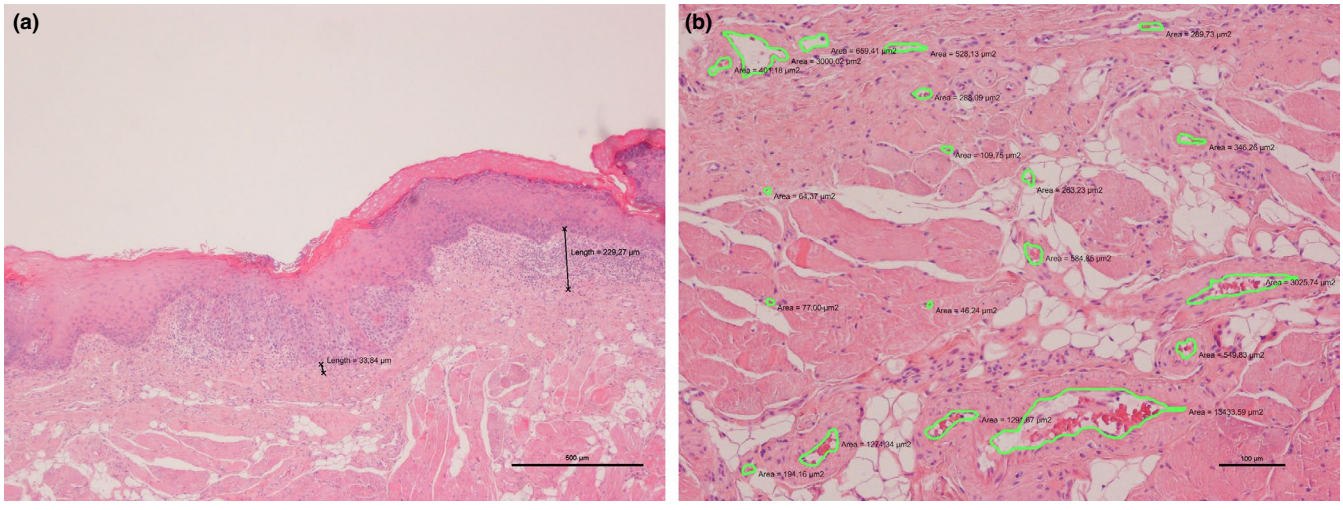

FIGURE 2 Examples of evaluation of the depth of inflammatory infiltration and area of blood vessels: (a) maximum and minimum depth of inflammatory infiltration (H&E staining, 4× magnification); and (b) overall area of blood vessel in one field of observation (H&E staining, 10× magnification) [Colour figure can be viewed at wileyonlinelibrary.com]

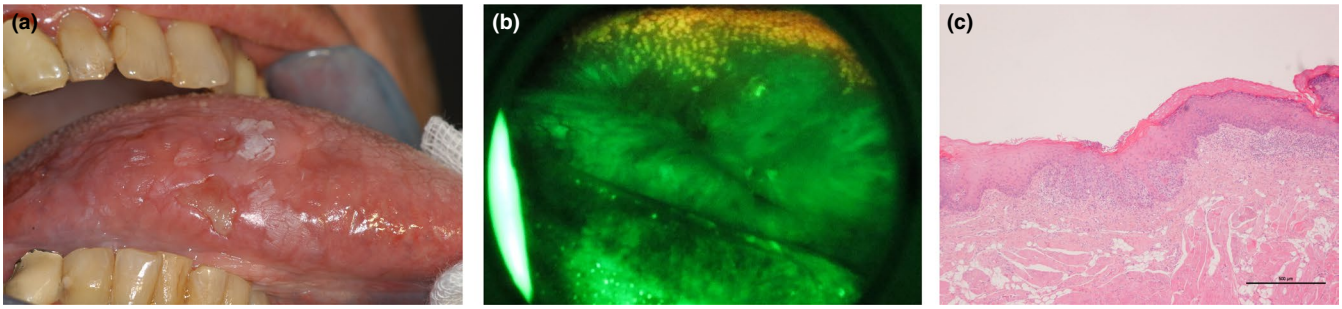

FIGURE 3 Example of clinicopathological correlation of AF alteration: (a) non-homogeneous lesion on the right side of the tongue; (b) AF inspection through the VELscope® system (hypofluorescent and hyperfluorescent patterns); and (c) histological features of OSCC (H&E staining, 4× magnification); incisional biopsy was performed in the hypofluorescent area [Colour figure can be viewed at wileyonlinelibrary.com]

the possible association between fluorescence expression (hyper, hypo) and the main covariates and factors.

3 | RESULTS

Twelve (60%) lesions were classified as hypofluorescent, and 8 (40%) were hyperfluorescent (Table 1).

Severity of inflammatory infiltrate was homogeneously distributed among hypofluorescent and hyperfluorescent cases ("mild": 3 in hypo and 3 in hyper; "moderate": 3 in hypo and 3 in hyper; "severe": 4 in hypo and 4 in hyper).

Among all the histological features, only MWK was significantly different using both tests (mean MWK in hypofluorescent: 41.3 μm (*SD*: 34.4; *SE*: 9.93); mean MWK in hyperfluorescent: 197 μm (*SD*: 69.9; *SE*: 24.7); $p < .001$). Particularly, hypofluorescent lesions had a decreased width of the keratin layer (mean MWK: 41.3 μm—*SD*: 34.4; *SE*: 9.93) when compared to hyperfluorescent specimens (197 μm—*SD*: 69.9; *SE*: 24.7).

A mild trend toward significance was observed also for MAV ($p = .094$; Student's *t* test). In fact, mean MAV in hyperfluorescent lesions was 3,755 μm^2 (*SD*: 3,697; *SE*: 1,307), and it was 1,711 μm^2 (*SD*: 1,352, *SE*: 390) in those hypofluorescent. Even if not statistically significant (p: 0.328; 0.397; 0.792), also the MDV was smaller in hypofluorescent lesions (38.8 vs. 48.2 μm).

A tentative classification produced by a binomial logistic regression model including the predictors MWE, MWK, MAV, and OAV has shown a convergence to a solution only for a model with the variable MWK, with a prediction accuracy of 90% regarding the typology of fluorescence (sensitivity = 87.5%; and specificity = 91.7%). None of the other variables was statistically associated with AF.

4 | DISCUSSION

According to the results of the present study, when different epithelial compartments (cellular and keratin layers) are evaluated separately, keratin is, by far, the main portion of the whole oral epithelium that influences AF patterns. Epithelia from malignant lesions with thicker keratin layers are hyperfluorescent, whereas lesions with thinner keratin layers are dark hypofluorescent (Figure 3).

Taking into account that hyperplasia and increase in keratinization are more frequently observed in PMDs such as leukoplakia, in well-differentiated SCC (e.g., presence of keratin pearls) and in VC (e.g., presence of keratin plugs) it is possible to hypothesize that brighter lesions are presumptively at an early stage of the malignant developmental process. On the contrary, oral malignant lesions with thin epithelial and/or keratin layers (or without keratin at all) are supposedly composed of cells far from their original developmental line (e.g., undifferentiated SCC) and probably to a later stage of malignant development (Burian et al., 2017). Different AF patterns within the same lesion might guide the clinician about where to perform a biopsy. Moreover, as demonstrated on PMDs, AF may be useful in delineation of excision margins (Farah, Kordbacheh, John, Bennet, & Fox, 2018).

Blood vessels in hyperfluorescent lesions have a mean area greater than those in hypofluorescent ones. Taking into consideration possible bias due to the low sample size, it can be hypothesized that a higher quantity of relatively small blood vessels (e.g., phase of (neo) angiogenesis) can, in association with other factors, be associated with the loss of AF.

In conclusion, even with the possible limitations and bias of the present pilot study, it seems plausible that AF features of oral malignant lesions are significantly associated with the width of their keratin layer.

AUTHOR CONTRIBUTIONS

M. Meleti and I. Giovannacci Study design, analysis of data and writing of paper. G. Pedrazzi Statistical analysis. P. Govoni Histomorphometric measurements. P. Vescovi1 Clinical activity and selection of patients. C. Magnoni Supervision and editing.

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