INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 24, no. 2 (S), 121-128 (2011)

DIRECT VISUALIZATION OF ORAL-CAVITY TISSUE FLUORESCENCE AS NOVEL AID FOR EARLY ORAL CANCER DIAGNOSIS AND POTENTIALLY MALIGNANT DISORDERS MONITORING

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Direct visualization of the oral tissue autofluorescence has been recently reviewed in several studies as a possible adjunctive tool for early recognition and diagnosis of potentially malignant and malignant oral disorders. The aims of this study were to assess: a) the value of a simple handheld device for tissue auto-fluorescence visualization of potentially malignant oral lesions; and b) the sensitivity, specificity and diagnostic accuracy of tested device, using histological examination as the gold standard. 175 consecutive patients, with at least one clinical oral lesion, were enrolled in the study. Clinical conventional inspections were performed for each patient by two blind operators. Then, oral biopsy and histological examination were performed. Pathologist was blind with respect to the autofluorescence results. The 175 histological assessments revealed no dysplasia, mild dysplasia, moderate/severe dysplasia and OSCC, in the 67.4%, 8.6%, 8%, 16% of cases, respectively. Oral lesions diagnosed as OSCC were found as positive under fluorescent light in the 96.4% of cases. Statistically significant correlation was observed between oral dysplastic lesions and the loss of tissue fluorescence (p-value=0.001). Low sensitivity values (60% and 71%) were recorded about the ability of the device in differentiating mild dysplasia vs. lack of dysplasia and moderate/severe dysplasia vs absence of dysplasia, respectively. The device tested in our study was found to not replace the histopathology procedure. However, we assessed its usefulness for oral tissue examination, especially within an oral medicine secondary care facility, before performing a biopsy and in monitoring oral lesions.

Oral Squamous Cell Carcinoma (OSCC) is the most common cancer of the oral cavity and it is the 6th most common malignancy with more than 275,000 new cases and 127,000 deaths worldwide in 2002 (1). Despite oral cavity is a readily accessible region for visual examination and cancer risk factors are well-known, a significant amount of OSCCs are still diagnosed late (Stage III or IV), reducing the 5-year survival rate and worsening morbidity and mortality related to the disease (2). Furthermore, OSCC is believed to arise from Oral Potentially Malignant Disorders (OPMDs) in the 50 % of cases, coming from hyperplasia, mild, moderate, and severe dysplasia to carcinoma *in situ*, and finally invasive OSCC from stages I to IV (3).

Conventional Oral Examination (COE) using incandescent white light (WL) is still the most widely used approach for OSCC and OPMD diagnosis and follow-up. The criteria used in COE for suspicion of an

Key words: oral cancer, autofluorescence, leukoplakia, oral lichen planus, epithelial dysplasia

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0394-6320 (2011)

OPMD or OSCC include changes in surface texture, loss of mucosal integrity, color, size, boundary features and tissue consistence and mobility. However, the COE of the oral soft tissue is routinely performed in dental practice by general dentists not trained in performing a differential diagnosis of OPMDs and early OSSC. Indeed, even oral medicine practitioners could find difficult to differentiate between benign and early malignant changes on high risk patients by performing COE alone (4). There is an increasing awareness that many OPMDs and early OSCC are clinically occult and not visible after WL examination (5); when visible, they could represent the "tip of the iceberg", signaling the presence of multiple or widespread subclinical changes to the tissue around.

Together, these considerations explain why oral biopsy represents today "*gold standard*" for the early detection and diagnosis of OPMDs and OSCC in terms of nature and degree of dysplasia/invasion (6). However, oral biopsy is a surgical approach and it is also important to highlight that often patients are reluctant to undergo one or multiple oral biopsies. For these reasons, many adjunctive non invasive tools have been proposed to detect early OPMDs and OSCCs, overcoming the limitations of COE, including the use of toluidine blue, brush cytology, reflectance visualization after acetic application, illumination with a chemiluminescent light source, and direct fluorescence visualization (7). Recently, several studies have shown that autofluorescence imaging may improve the ability to distinguish normal from premalignant and malignant oral tissue (5).

The aims of present study were to assess: a) the additional value of a simple handheld device for tissue auto-fluorescence visualization in evaluating clinical features of OPMDs and OSCC, previously detected by COE; and b) the sensitivity, specificity and diagnostic accuracy of the device tested in discriminating "*low risk*" *vs.* "*high risk*" OPMDs, using histological examination as the gold standard.

MATERIALS AND METHODS

Patients recruitment

Among all patients consecutively referred to Unit of Oral Medicine of the Department of Oral Sciences (University of Palermo), a total of 175 subjects, with 18 years old and/or older and with at least one oral lesions, as detected during COE under WL and compatible with the clinical suspicion of OPMD or OSCC, were included in the study. Patients who did not have a lesion clinically diagnosed were excluded from trial to minimize patient burden.

Study design

After written informed consent, all patients underwent: 1) COE under WL; 2) clinical examination by using VELscope® (Visual Enhanced Lesion scope; LED Dental Inc., Burnaby, BC, Canada) device in order to assess any tissue fluorescence change; 3) incisional scalpel or punch biopsy to obtain a definitive histopathological diagnosis. All OPMDs visualized were recorded either as VELscope Positive (VsP) or as VELscope Negative (VsN). According to manufacturer's instructions, VsP lesions were considered those areas which appeared abnormally dark on fluorescence visualization in the body or boundary of the lesion (Fig. 1). VsN lesions were defined as sites that appeared as an apple-green glow after fluorescence examination. Furthermore, all VsP lesions, in which we obtained an apple-green appearance after compression, were reclassified as VsN.

Examination

All COEs were performed by two blind investigators (C.P. and D.C.), with experience in oral medicine and previously trained to use the device, under routine incandescent operatory WL by using dental mirrors and gauzes for digital palpation. Suspicious lesions were first identified with a COE under WL and features including the location of the lesion, the color of lesion and the presence of single or multiple lesions were identified and recorded. Then, room lights were dimmed, the oral cavity was examined and each clinically identified lesion was evaluated by using VELscope® device. All lesions, both under WL and fluorescent light, were photographed by means of a Nikon D100 digital SLR camera (Nikon Inc., Melville, NY, USA). Based on photographic aspect, the investigators, again in blindness, reported their subjective assessment of the impact of fluorescence light upon lesions features of brightness, sharpness, size and edge, by using a four-point Likert scale (decreased, no change, slight improvement, marked improvement) (8).

Oral biopsy

All visualized lesions were biopsied using scalpel or punch technique. In the case of VsP lesions, biopsy was performed on the area that appeared dark under fluorescent light, while in case of VsN lesions, it was carried out close to the lesion boundary with the clinically healthy tissue. Histological diagnoses were performed by a pathologist (V.R.) dedicated to oral tissues, who was blind with respect to the VELscope® results and clinical features. He was asked to define for each case the following characteristics: no dysplasia, mild, moderate/severe dysplasia, carcinoma in situ or invasive carcinoma, using the definition of these entities according to the WHO classification for grading oral epithelial dysplasia (9). In addition to that, the pathologist assigned the cases as "*low-risk*" or "*high-risk*" lesions, according to the binary system proposed by Kujan et al. (10). Finally, the association between the fluorescence changes in the site of OPMD or OSCC and the histological diagnoses was evaluated.

Statistical analysis

Data were analyzed by means of StatView for Windows (SAS Inc. v. 5.0.1, Cary, NC, USA). Descriptive statistics were used to illustrate the patients' variables. The chi-squared test for trend was calculated to assess the ability of the tested device in discriminating OPMDs with dysplasia *vs.* non dysplastic OPMDs and "*high risk*" *vs* "*low risk*" lesions. In all evaluations, p-values < 0.05 were considered statistically

significant. The STARD checklist for studies on the diagnostic accuracy of tests was performed (11). The sensitivity, specificity and diagnostic accuracy values of the diagnostic procedures examined were calculated by standard statistical methods (12). Positive and Negative Predictive Values (PPV and NPV) were also calculated. The concordance rate of the clinical examination under fluorescent light between the two observers was evaluated using Cohen's kappa statistic, as measuring agreement beyond that expected by chance (expressed as a coefficient ranging from 0 to 1.00) (13).

RESULTS

The average patient age was 60.38 (±12.26; range: 23-83) and male patients represented the 45.14% (79/175) of the sample. 61/175 patients (34.85%) were current smokers (more than 10 cigarettes/day) and 36/175 patients (20.57%) were former smokers (ex-smokers for less than 15 years). The 12.57% (22/175) of the sample referred to consume alcohol on a regular basis (more than 1 alcoholic units per day). The biopsies of 175 identified lesions revealed no dysplasia in the 67.42% (118/175) of the sample, mild dysplasia in 8.57% (15/175), moderate/severe dysplasia in the 8% (14/175) and OSCC in the 16% of cases (28/175). Table I shows the biopsy-confirmed histological diagnoses, the site and clinical features, and their appearance under fluorescent light.

There was a substantial agreement (κ=0.87) between the two observers after clinical oral examination by using VELscope® device. Oral lesions diagnosed as OSCC were found as VsP in the 96.42% of cases (27/28); thus, it was detected only 1 false negative for the diagnosis of the OSCC; whereas, 118 lesions with no dysplasia were highlighted 115 as VsN and 3 as VsP, suggesting the presence of 2.60% (3/118) of false positives. The 71.42% (10/14) of the lesion with moderate-severe dysplasia was observed as VsP lesions whereas the 37.5% (6/15) of the lesion with mild dysplasia were VsN. Table II shows statistical results for sensitivity, specificity and diagnostic accuracy of VELscope® device in discriminating OPMDs with dysplasia (mild and moderate/severe) *vs* OPMDs without dysplasia. Statistically significant correlation was found (p-value<0.0001) between the presence of dysplasia and the loss of tissue fluorescence. Low sensitivity values (60% and 71%) were recorded in differentiating mild dysplasia *vs* lack of dysplasia and moderate/severe dysplasia *vs.* absence of dysplasia, respectively. Specificity and diagnostic accuracy values in discriminating all OPMDs with dysplasia *vs.* non-dysplastic lesions were 97.4% and 91.1%, respectively.

In order to evaluate the ability of VELscope® device in discriminating "*low risk*" *vs* "*high risk*" OPMDs, all histological samples of OPMDs (147/175), excluding those of OSCCs (28/175), were revaluated according to the binary system proposed by Kujan et al. (10), distinguishing "*low risk*" (131/147; 89.11%) and "*high risk*" lesions (16/147; 10.88%) (Table III). VELscope® visualization revealed 12/16 VsP reports among "*high risk*" lesions (75%). The 25% (4/15) of OPMDs identified as high risk, therefore, were identified as VsN under VELscope® light. Oral lesions classified as "*low risk*" were negative in the 92.36% (121/131) of cases using the VELscope®, with false positive reports in 7.63% (10/131) of reported cases. Sensitivity, specificity and diagnostic accuracy values in discriminating "*high risk*" *vs* "*low*

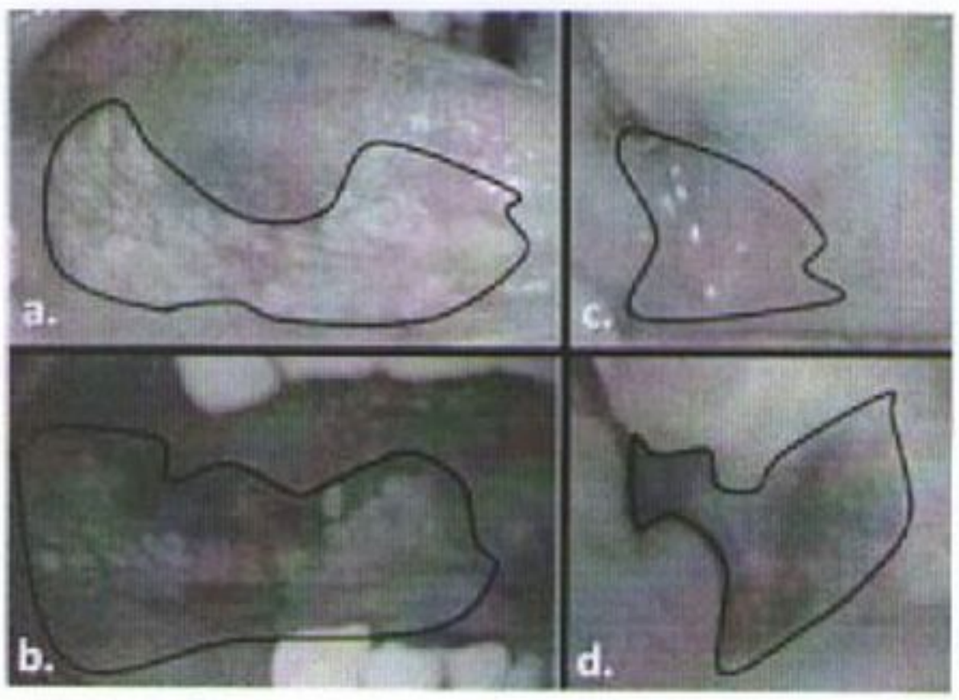

Fig. 1. *a) Non omogenoues speckled leukoplakia on right tongue border, histologically defined as "leukoeritroplakia with severe dysplasia" ("high risk" lesion, according to the binary system); b) the above described OPMD resulted VsP. Fluorescent light provides a marked improvement in visualization of brightness, size and boundary; c) erythroplakia on the right anterior pillar of the soft palate, histologically diagnosed as "infiltrating OSCC"; d) the clinical features of the above described lesions appears markedly enhanced after VELscope® examination*

Table I. *Biopsy-confirmed diagnoses with demographic, clinical and VELscope® examination features*

Biopsy-confirmed diagnoses	Patients (n=)	Clinical features (n=)	Sites of localization (n=)	VELscope® examination results (n=)	Smokers	Alcohol drinkers
OL with no dysplasia	50	Homogenous white patch (50)	Gingival m. (15) Buccal m. (11) Tongue dorsum (1) Tongue ventrum (9) Tongue border (4) Hard/Soft palate (5) Retrocommissural m. (5)	**VsP (1)** **VsN (49)**	24	8
OL with mild dysplasia	11	Homogenous white patch (10) Speckled OL (1)	Tongue dorsum (1) Gingival m. (1) Tongue border (2) Buccal m. (3) Tongue ventrum (4)	**VsP (6)** **VsN (5)**	1	None
OL with moderate/ severe dysplasia	5	Non homogenous OL (3) White patch (2)	Buccal m. (4) Tongue ventrum (1)	**VsP (4)** **VsN (1)**	1	1
OLP*	64	Atrophic red lesion (11) Reticular and atrophic-erosive (8) White patch (36) Reticular lesions (9)	Gingival m. (14) Buccal m. (35) Tongue dorsum (7) Tongue ventrum (3) Tongue border (5)	**VsP (2)** **VsN (62)**	19	6
OLL with mild dysplasia*	2	Atrophic-erosive red lesion (2)	Buccal m. (2)	**VsP (1)** **VsN (1)**	None	1
OLL with moderate dysplasia*	1	White patch lesion (1)	Tongue ventrum (2)	**VsN (1)**	1	None
VPL with moderate or severe dysplasia	3	Multiple verrucous white patch (3)	Gingival m. (1) Buccal m. (1) Tongue ventrum (1)	**VsP (2)** **VsN (2)**	2	None
VPL with mild dysplasia	2	Multiple verrucous white patch (2)	Gingival m. (1) Buccal m. (1)	**VsP (2)**	2	1
VPL with no displasia	3	Multiple verrucous white patch (3)	Gingival m. (2) Buccal m. (2)	**VsN (3)**	None	None
Leuko-erythroplakia with no dysplasia	1	1 White-red patch (1)	1 Buccal m. (1)	**VsN (1)**	1	None
Leuko-erythroplakia with moderate-severe dysplasia	5	White-red patch (5)	Gingival m. (1) Tongue border (1) Tongue dorsum (1) Tongue ventrum (2)	**VsP (4)** **VsN (1)**	1	None
OSCC	28	Exophytic lesion (14) Ulcerative lesion (4) Red lesion (2) Verrucous white (3) patch Ulcerative exophytic lesion (4) White-red lesion (1)	Hard palate (1) Gingival m. (4) Inferior lip m. (1) Tongue dorsum (2) Tongue border (7) Buccal m. (9) Tongue ventrum (3) Retrocommissural m. (1)	**VsP (27)** **VsN (1)**	10	5
TOTAL	175	175	175	**VsP (49)** **VsN (126)**	61	22

Abbreviations: m= mucosa; OL = Oral Leukoplakia; OLP = Oral Lichen Planus; OLL = Oral Lichenoid Lesions; VPL = Verrucous Proliferative Leukoplakia; OSCC = Oral Squamous Cell Carcinoma.

**According to Van der Meij et al., all clinically OLP-like lesions with histological evidence of epithelial dysplasia were classified as OLL, and not as OLP with dysplasia.*

Table II. *Classification of the biopsy-confirmed diagnoses according to Kujan et al. Demographic, clinical and VELscope® examination features.*

Biopsy-confirmed diagnoses	Patients (n=)	VELscope® examination Result (n=)	Smokers	Alcohol drinkers
High Risk OPMD	16	VsP (12) VsN (4)	6	2
Low Risk OPMD	131	VsP (10) VsN (121)	44	15
TOTAL	147	VsP (22) VsN (125)	50	17

Table III. *Statistical results. Sensitivity, specificity and diagnostic accuracy values of VELscope® device in discriminating high risk vs low risk lesions.*

	Biopsy-confirmed diagnoses			
p-value Chi-squared	Lesions with dysplasia (mild and moderate/severe) *vs* lesions without dysplasia	Lesions with mild dysplasia *vs* lesions without dysplasia	Lesions with moderate/ severe dysplasia *vs* lesions without dysplasia	*High risk* lesions *vs low risk* lesions
Sensitivity (%)	0.001	<0.0001	<0.0001	<0.0001
Specificity (%)	10.64	53.52	66.89	50.85
	65.5%	60%	71.4%	75%
	97.4%	97.4%	97.4%	92.3%
Diagnostic accuracy (%)	91.1%	93.2%	94.7%	90.5
PPV (%)	86.3%	75%	77%	54.5%
NPV (%)	92%	95%	96.6%	97%

Table IV. *Clinical features of the VsP lesions under fluorescent light, compared with COE under WL, using a four-point scale, according to Likert.*

Clinical Features	No Change		Slight Improvement		Marked Improvement		Decreased	
	n =	%	*n* =	%	*n* =	%	*n* =	%
Sharpness	24	49	14	28.6	8	16.3	3	6.1
Brightness	17	34.7	10	20.4	22	44.9	None	-
Edge	9	18.4	16	32.7	24	49	None	-
Size	16	32.7	11	22.4	22	44.9	None	-

Table V. *Results of the main published studies about direct tissue auto-fluorescence visualization, compared with our study*

Authors	Year	Study Design	N. of Patients (n=)	Aims of the study	Sensitivity (%)	Specificity (%)	Diagnostic accuracy	Main conclusions
Poh *et al.*	2006	Cross sectional study	20; 122 biopsies	Evaluation of the field changes around oral malignant lesions	97	94	Not calculated	Direct fluorescence visualization can identify subclinical high-risk fields. It may help to establish safer secure surgical margins in tumor excision
Poh *et al.*	2007	Case series	3	Detection of clinically occult high-risk oral premalignant disease	Not calculated	Not calculated	Not calculated	Ability of direct tissue fluorescence visualization to guide the management of patients with oral lesions, facilitating the detection of clinically occult high-risk changes
Lane *et al.*	2006	Cross-sectional study	44; 50 biopsies	Ability of discriminating normal mucosa from severe dysplasia/ carcinoma in situ (CIS)/invasive carcinoma	98	100	Not calculated	The device is a suitable adjunct for oral cancer screening, biopsy guidance, and margin delineation
Kois et al.	2006	Case series	4	Valuation of a new device for the direct tissue autofluoreseence visualization	Not calculated	Not calculated	Not calculated	VELscope® is an adjunctive tool for the detection of oral lesion not otherwise identified
Rohlyer *et al.*	2009	Case-control study	54 cases; 11 control	Discrimination between neoplastic and non neoplastic oral mucosa using autofluorescence imaging	95.9	96.2	Not calculated	Autofluorescence imaging coupled with objective image provides a sensitive and non invasive tool for the detection of oral neoplasia
Huber *et al.*	2009	Observational study	130	Assessment of the VELscope as an adjunctive examination tool	Not calculated	Not calculated	Not calculated	VELscope® interpretation did not enhance or otherwise alter the clinical management of the suspicious lesions
Jayaprakash *et al.*	2009	Cross-sectional study	60; 189 biopies	Detection of premalignant or cancerous lesions, compared to the conventional white-light examination	75% for low grade lesions; 100% for high grade lesions and oral cancer	38%	Not calculated	The addition of direct tissue fluorescence visualization to conventional visual exam improves the ability to detect high grade lesions or oral cancers
Moro *et al.*	2010	Prospective study	32	Autofluorescence as adjunctive tool for early oral cancer detection	100%	93%	Not calculated	Autofluorescence is a high-performing test for the individuation of oral cancer in populations at risk
Mehrotra *et al.*	2010	Cross sectional study	156 biopsies	Detection of clinically innocuous precancerous and cancerous oral lesions	50%	38.9%	Not calculated	The study results indicate that use VELscope® was not beneficial in identifying dysplasia or cancer
Koch *et al.*	2010	Prospective blinded clinical trial	78	Effectivenes to identify suspicious oral lesions	93 % for SCC; 94% for SCC/ dysplasia	15% for Scc; 16% for SCC/ dysplasia	Not calculated	Autofluorescence could help to identify any type of pathological oral lesions but is not able to differentiate between benign and malignant oral lesions

risk" OPMDs were 75%, 92.3% and 90.5%, respectively.

As regards the aptitude of direct auto-fluorescence visualization (VELscope®) to enhance the evaluation of clinical features of OPMDs and OSCC, previously identified by COE, Velscope® device was shown to highly improve brightness (44.9%), size (49%) and visibility of the edges (44.9%) of VsP lesions, compared with COE under WL (Table IV).

DISCUSSION

It is well known that the reduction of mortality and morbidity for OSCC depends mainly on the recognition of OPMDs, in fact, although it is believed that about 50% of OSCC stems from OPMDs, not all OSCCs are preceded by OPMDs (4). The most common oral diseases classified as OPMD are leukoplakia, oral lichen planus, verrucous proliferative leukoplakia and erythroplakia, manifesting as white-, red-, white-red patches or spots (6,14, 15).

In recent years new diagnostic tools have been developed to support visual examination: brush biopsy, toluidine blue staining, Lugol's iodine solution staining, chemiluminescence, fluorescence imaging and spectroscopy.

In detail, the association between cancer development and loss of normal tissue fluorescence has been reported for a large number of tissues and organs (16). The mechanism behind tissue autofluorescence tissue has been extensively described elsewhere (17). Briefly, loss of autofluorescence is believed to reflect the complex and progressive morphological and biochemical changes, typical of squamous epithelial carcinogenesis. Some of these changes, as the breakdown of collagen matrix, the decrease of flavin adenine dinucleotide concentration, the hyperchromatic nuclei, the increased microcirculation involve changes in the distribution of tissutal fluorophores, thus leading to subclinical changes in the tissue autofluorescence features (18). When oral mucosa is excited by a beam of blue-violet light (400-460 nm), it has a distinctive green-apple color, and, if there was an alteration of the tissue fluorophores, mucosa exhibits a characteristic loss of fluorescence. Several studies have evaluated the possibility of using tissue autofluorescence to differentiate and early detect cancers of the oral cavity (19). Results reported in these studies are so extremely interesting, although there is still a limited evidence to conclude that the tissue autofluorescence can provide new aids for OSCC screening and early detection alone. Table V lists the main studies published in recent years about this issue.

Our study, similarly to most of those mentioned above, is a cross-sectional study but, differently, all patients were evaluated by two blind operators, trying to minimize possible assessment biases. We found a statistical significant correlation between the presence of dysplasia (mild and moderate/severe) and a VELscope® positive result and between the presence of "*high risk*" lesion *vs.* loss of fluorescence. However, our study reported relatively low values of sensitivity, indicating that VELscope® has a certain probability of giving false negatives. This is a great limitation in using this device as screening tool in "high risk" population, since it cannot always identify lesions with dysplasia, especially in mild dysplasia, who represents the first histological change that could be the first step of oral carcinogenesis. However, specificity values are high, suggesting that the VELscope® has a low probability to give false positives. The NPV reported values are extremely high, but they are affected by the lesion prevalence in a high-risk population, like our hospital base sample, where lesion frequency is higher than that in general population.

Our study shows that direct visualization of oral cavity tissue autofluorescence contributes to enhance the examination of some important clinical features of the oral lesions, such as brightness, size and margins, compared with COE under WL.

On the basis of the reported values of sensibility, specificity and diagnostic accuracy, the VELscope® device can not fully replace histopathology procedure, which still represents the gold standard for definite diagnosis of OPMD or OSCC. Rather, this non invasive tool should be seen as complementary device to both the conventional visual examination and the histopathological assessment during diagnosis and monitoring steps.

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