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ORIGINAL ARTICLE

Comparative evaluation of autofluorescence imaging and histopathological investigation for oral potentially malignant disorders in Taiwan

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Abstract

Objectives Autofluorescence imaging is gaining popularity as an adjunctive test for oral potentially malignant disorders (OPMD). This study evaluated the efficacy of autofluorescence imaging based on the current standard oral mucosal disorder checklist in Taiwan.

Materials and methods In total, 126 patients suspected to have mucosal disorders at the Division of Oral and Maxillofacial Surgery, Tri-Service General Hospital, Taipei, Taiwan, were enrolled. Following a conventional oral examination by using the oral mucosal disorder checklist and an autofluorescence imaging examination, all participants underwent histopathological examination to access epithelial dysplasia.

Results Among 126 patients, 68 patients were diagnosis as having an OPMD and 63 having epithelial dysplasia. Autofluorescence imaging exhibited a sensitivity, specificity, positivity predictive value (PPV), negative predictive value (NPV), and accuracy of 77.94%, 35.42%, 63.10%, 53.13%, and 60.34%, respectively, for OPMD and of 88.89%, 43.86%, 63.64%, 78.13%, and 67.50%, respectively, for epithelial dysplasia. After the exclusion of 48 non-OPMD cases according to the checklist, the sensitivity, specificity, PPV, NPV, and accuracy of autofluorescence imaging became 87.50%, 72.73%, 94.23%, 53.33%, and 85.07%, respectively, for epithelial dysplasia.

Conclusion The efficacy of epithelial dysplasia identification and OPMD risk assessment can be increased after the exclusion of the non-OPMD cases through autofluorescence imaging.

Clinical relevance Autofluorescence imaging is a useful adjunct that can assist specialists in assessing OPMD patients prone to dysplasia without compromising patient care.

Keywords Autofluorescence · Oral potentially malignant disorders · Sensitivity and specificity

Introduction

The concept of oral potentially malignant disorders (OPMD) was established by Warnakulasuriya et al., who stated that they are a family of morphological alterations among which some may have an increased potential for malignant transformation. It is also thought to be an indicator of risk for future malignancies elsewhere in the oral mucosa (appearing clinically normal) [1]. In Taiwan, leukoplakia, erythroplakia, and submucous fibrosis are the most frequently observed OPMD in addition to others such as lichen planus [2–4]. Although the prevalence of OPMD in the general population is debatable, 1–5% has been commonly accepted as the prevalence in the Western countries [5]. Because of cultural differences and the habitual use of carcinogenic products, the prevalence of OPMD can reach 24.4% in certain areas of Taiwan [6].

The current standard for the detection of OPMD, which mainly comprises leukoplakia, erythroplakia, and erythroleukoplakia, is a conventional oral examination (COE) [7]. Although it is insufficient for differentiating

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categories of OPMD, epithelial dysplasia is often observed in the tissue of patients with OPMDs [8]. Furthermore, compared to more mild histopathological findings of hyperkeratosis or epithelial hyperplasia, epithelial dysplasia is a more conclusive determinant of malignant potential [9, 10], which the malignant transformation is at a rate of 2.2–38.1% [11, 12]. Evidence of dysplasia and even microinvasive carcinoma has been missed when performing a COE [13]. Therefore, epithelial dysplasia after OPMD diagnosis is an important risk factor for poor prognosis and transformation [14], and the precise detection not only in OPMD but also in epithelial dysplasia prior to management is crucial and may prevent advancement in disease progression [15].

In this study, we used a noninvasive, handheld camera device designed to visualize early mucosal changes using the principles of tissue autofluorescence. The autofluorescence camera utilizes blue light in the spectrum of 400–460 nm to detect the difference in luminance between healthy tissue and diseased tissue. Excited fluorophores intrinsic in the oral mucosa result in pale green autofluorescence, indicating normal tissue, whereas abnormal tissue is associated with the loss of autofluorescence, which may be caused by structural changes. For example, the thickening of the epithelium, hyperchromatism, increased cellular or nuclear pleomorphism, and increased microvascularity all lead to the increased absorption or scattering of light; thus, abnormal tissues with such structural changes appear dark in contrast [16, 17]. Because of this loss of autofluorescence in abnormal tissues, autofluorescence imaging is used as an adjunct tool to assess the risk of potentially malignant disorders and select optimal biopsy sites [18–20].

Materials and methods

Patient recruitment

A total of 150 consecutive patients with mucosal disorders at the Division of Oral and Maxillofacial Surgery, Tri-Service General Hospital (TSGH) of National Defense Medical Center, Taipei, Taiwan, were invited to participate in this study. The inclusion criteria were age more than 20 years and a history of alcohol, tobacco, or betel quid use; ten patients were excluded, because they refused to receive biopsy and requested other treatments, eight were excluded, because racial protection laws require a separate institutional review board for those of aboriginal race, and six were excluded, because the location of the lesion (oropharynx) would compromised the assessment of autofluorescence imaging. This study was approved by the Institutional Review Board of TSGH (approval no. 1-107-05-010).

Questionnaire

An oral mucosal disorder checklist developed by the Health Promotion Administration at the Ministry of Health and Welfare is commonly used for oral mucosal screening in Taiwan. The checklist collects basic background information, including age, gender, and contact information. A COE with location of the lesion recorded was conducted by a certified specialist to clinically diagnose nonhomogenous leukoplakia, homogenous thick leukoplakia, leukoplakia, erythroplakia, erythroleukoplakia, verrucous hyperplasia, submucosal fibrosis, lichen planus, and suspected oral cancer according to the checklist. Biopsy was performed to yield a histopathological diagnosis of mild-, moderate-, high-grade dysplasia or oral cancer, among others.

Histopathological assessment

Patients signed a standard informed consent form that is typically used in TSGH. A biopsy was performed for histopathological assessment, and the biopsy site selected was any area at the abnormal mucosa with autofluorescence loss or retained. The presence or absence of dysplasia or oral cancer in the biopsy specimen was recorded in the report from the pathology department at TSGH and approved by a certified pathologist.

Device

An autofluorescence examination was performed using the Horus UOC 100™ digital autofluorescence camera (Medimaging Integrated Solution Inc., Hsinchu, Taiwan). The device is a noninvasive, manually focused, light-adjustable, handheld 1920 × 1080-pixel camera designed to display mucosal changes on a 3.5-in. full-color TFT–LCD screen; image data are stored in a micro-SD card. The Horus UOC 100™ emits light in the 400–460-nm spectrum, and fluorophores intrinsic in the oral mucosa result in pale green autofluorescence. The examiners were trained through a thorough instructional course taught by an experienced professional recommended by the manufacturer. Possible outcomes of the autofluorescence examination were determined according to the manufacturer's instructions. The findings were listed as fluorescence visualization loss (FVL) or fluorescence visualization retained (FVR) (Fig. 1).

Clinical examination procedure

Patients were examined by COE through an oral mucosal disorder checklist, autofluorescence imaging, and pathological investigation protocol designed specifically for this study (Fig. 2). Using a Canon A2200 camera, a

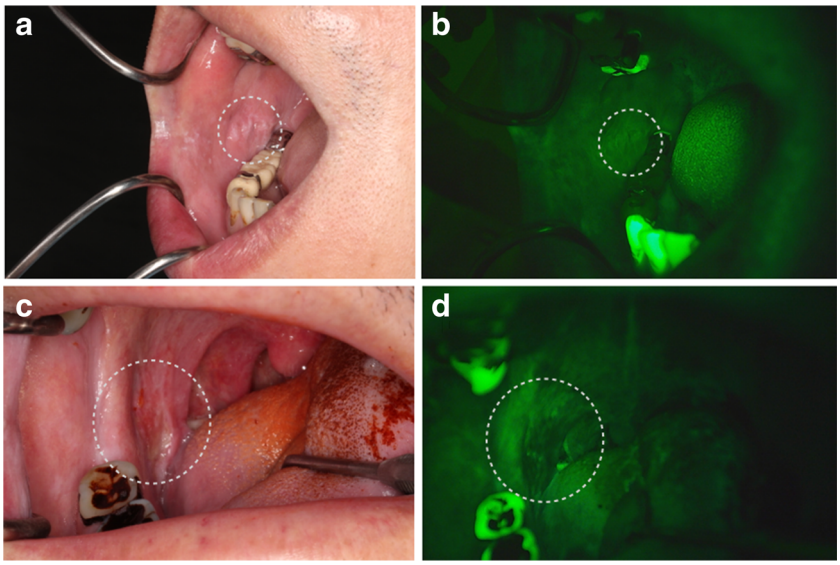

Fig. 1 Clinical and autofluorescence images of FVL and FVR. **a** Clinical photograph of OPMD revealing epithelial hyperplasia. **b** FVR as captured by the autofluorescence camera. **c** Clinical photograph of OPMD revealing high-grade dysplasia. **d** FVL as captured by the autofluorescence camera

specialist performed the COE diagnosis and imaging of OPMD. The noted lesion area was then imaged for autofluorescence by using the UOC 100™ under dimmed light, and protective eyewear was worn by the patient throughout the procedure. Depending on the location of the lesion within the oral cavity, the focus and modulation of the light along three scales could be adjusted. The image was displayed on the screen, and the biopsy is taken for histopathological examination.

Statistical analysis

All statistical analyses were performed using SPSS statistical software (version 22.0.0, IBM Corp., Armonk, NY, USA). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy (versus clinical diagnosis by a specialist and dysplasia grading from biopsy) of the autofluorescence test were calculated. A *P* value lower than 0.05 was considered statistically significant.

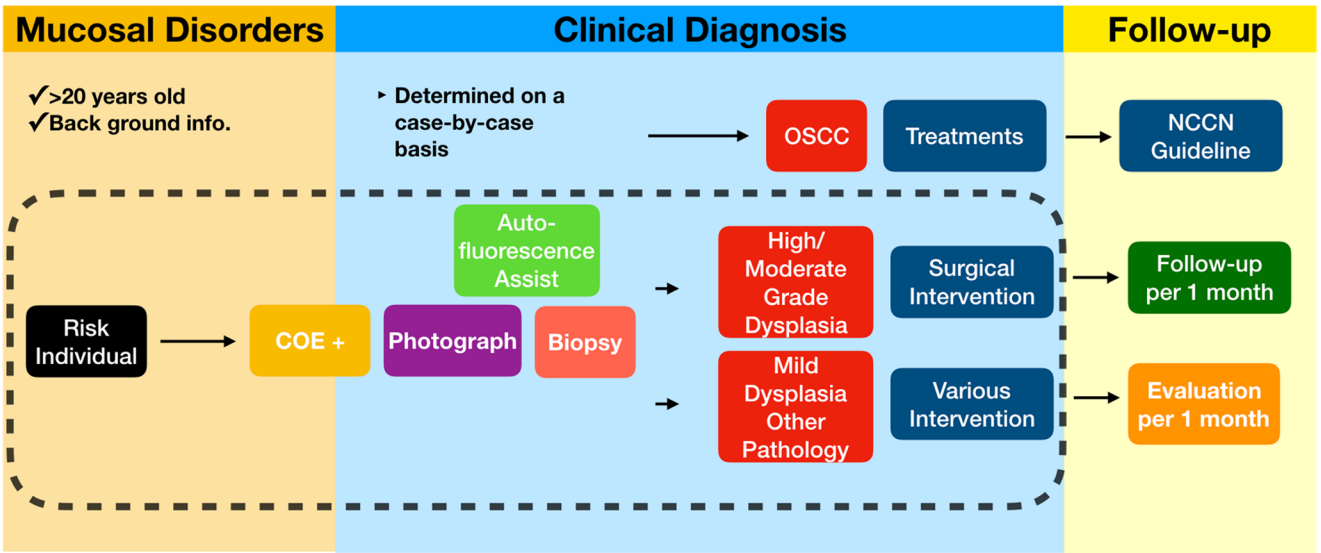

Fig. 2 Mucosal disorders and autofluorescence investigation protocol. Patients were referred from local health posts, clinics, and district health promotion programs. Background information and suspected lesion sites were obtained using the current mucosal disorder checklist. A certified experienced examiner specialized in oral and maxillofacial surgery used the checklist to confirm diagnoses of the lesions through COE. The locations of the lesions of morphologically altered mucosa found during the COE were photographed using a camera (Canon A2200). Clinical examinations were then repeated using the UOC 100™. All patients provided informed consent and agreed to undergo biopsy. The tissues were examined by the pathology department at TSGH and confirmed and graded by a certified pathologist. This included the final diagnosis and dysplasia grading. Related management or follow-up was indicated according to the nature of the lesions. The black dotted line indicates the focus of this study; oral cancer was excluded from statistical analysis

Results

Patient profiles are presented in Table 1. In 126 patients included in this study, most patients were men ($n = 110$), and the lesion sites were most frequently located on the buccal mucosa ($n = 71$).

Summary of OPMD diagnosis through COE and histopathology

In the COE, more than half ($n = 68$) were diagnosed as having an OPMD; ten patients were diagnosed as having cancer, and 48 lesions categorized as others (i.e., non-OPMD). The most frequent lesion sites for both OPMD and non-OPMD were located on the buccal mucosa ($n = 46$ and $n = 20$, respectively).

All 126 patients underwent incisional biopsy for histopathological assessment. The results confirmed epithelial dysplasia in 63 patients and cancer in six patients; moreover, 57 lesions were categorized as others (i.e., nondysplasia). Moreover, the most frequent lesion site for both epithelial dysplasia and nondysplasia was located on the buccal mucosa ($n = 44$ and $n = 25$, respectively).

Clinical OPMD diagnosis and histopathological description

The clinical diagnoses of OPMDs in Table 2 show that 38.1% of total identifications were benign lesions, including benign fractional keratosis, aphthous ulcer, and candidiasis thrush. In the study population, leukoplakia in 25.5% of patients, and erythroplakia or erythroleukoplakia, was identified in 19.9%. In the suspected oral cancer group, induration or unhealed ulceration for more than 2 weeks was considered symptomatic for early diagnosis. A COE diagnosis was made prior to histopathological examination.

Autofluorescence imaging in relation to OPMD and dysplasia

We calculated the efficacy of autofluorescence imaging after excluding cancer diagnoses made through COE and histopathology. Table 3 indicates that in the COE, both the OPMD and non-OPMD groups were prone to FVL; the same was observed in the dysplasia and nondysplasia groups. For both OPMD versus non-OPMD and dysplasia versus nondysplasia groups, autofluorescence imaging exhibited high sensitivity of 77.94% and 88.89%, respectively, but low specificity of 35.42% and 43.86%, respectively; in addition, the overall accuracy was 60.34% and 67.50%, respectively.

Autofluorescence imaging in relation to dysplasia among OPMD cases

To investigate the efficacy of autofluorescence imaging for dysplasia among OPMD cases, we excluded non-OPMD features found in COE from the oral mucosal disorder checklist and then ran the accuracy comparisons again; the results are listed in Table 4. The nondysplasia group revealed a pattern of higher FVR compared with FVL (in contrast to the pattern noted for the nondysplasia group in Table 3). The sensitivity and specificity of autofluorescence imaging were satisfactory: 87.50% and 72.73%, respectively. Nevertheless, after the exclusion of non-OPMD cases, regarded as having benign

Table 1 Patient characteristics

		COE				Histopathology			
	All *n* (%) *n* = 126	OPMD *n* (%) *n* = 68	Cancer *n* (%) *n* = 10	Others *n* (%) *n* = 48	*P* value	Dysplasia *n* (%) *n* = 63	Cancer *n* (%) *n* = 6	Others *n* (%) *n* = 57	*P* value
Age	55.97 ± 12.72	56.71 ± 10.54	61.11 ± 12.19	53.84 ± 15.25	0.203	57.68 ± 11.26	62.45 ± 13.07	53.39 ± 13.81	0.079
Gender					0.958				0.074
Female	16 (12.7)	9 (13.2)	1 (10)	6 (12.5)		12 (19)	1 (16.7)	3 (5.3)	
Male	110 (87.3)	59 (86.8)	9 (90)	42 (87.5)		51 (81)	5 (83.3)	54 (94.7)	
Lesion site					0.094				0.003
Lip	11 (8.7)	4 (5.9)	0 (0)	7 (14.6)		4 (6.3)	0 (0)	7 (12.3)	
Buccal mucosa	71 (56.3)	46 (67.6)	5 (50)	20 (41.7)		44 (69.8)	2 (33.3)	25 (43.9)	
Gingiva	19 (15.1)	9 (13.2)	2 (20)	8 (16.7)		4 (6.3)	1 (16.7)	14 (24.6)	
Tongue	13 (10.3)	5 (7.4)	2 (20)	6 (12.5)		4 (6.3)	2 (33.3)	7 (12.3)	
Floor of the mouth	2 (1.6)	1 (1.5)	1 (10)	0 (0)		1 (1.6)	1 (16.7)	0 (0)	
Palate	10 (7.9)	3 (4.4)	0 (0)	7 (14.6)		6 (9.5)	0 (0)	4 (7)	
Others	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	

Table 2 Description of all categories on oral mucosal disorder checklist

All *n* (%) *n* = 126	
Suspected oral cancer	10 (7.9)
OPMD	
Erythroplakia	5 (4)
Erythroleukoplakia	20 (15.9)
Nonhomogenous leukoplakia	7 (5.6)
Thick homogeneous leukoplakia	5 (4)
Thin homogeneous leukoplakia	20 (15.9)
Verrucous hyperplasia	6 (4.8)
Oral submucous fibrosis	3 (2.4)
Oral lichen planus	2 (1.6)
Others	48 (38.1)

conditions, as an adjunct to COE when performing biopsy, autofluorescence imaging yielded a high PPV and accuracy of 94.23% and 85.07%, respectively, in OPMD patients.

Discussion

In Taiwan, oral mucosal disorders are often neglected until the advanced stage and are then usually treated in a final referral medical center. The early diagnosis of OPMD may serve as a preventive measure in the high-risk population [21]. Reviews conducted in the Western countries reveal that approximately 80% of cases of leukoplakia display no evidence of dysplasia, but biopsy indicates the remaining 10–20% are either dysplastic or are already invasive carcinomas [22]. In Taiwan, the occurrence of OPMD correlates highly with the habit of chewing betel nuts [23] and malignant transformation [24]. Attention should specifically be focused on premalignant lesions to prevent disease progression [25]. However, the COE of OPMDs is limited as a diagnostic method for predicting pathological dysplasia [26].

Most adjuncts for assisting clinicians in daily OPMD- and cancer-related work are considered most suitable for use in secondary-care facilities, such as the current study site; however, the more effective purpose of these adjuncts is to assist the specialists in selecting biopsy sites for cancer and OPMD surveillance [19]. Autofluorescence imaging as an adjunctive tool has gained popularity as a modality because its physical effect is exerted without the need of other assisting agents [27]. When the oral mucosa is illuminated with blue excitation light with a wavelength of 400–460 nm, the targeted normal oral mucosa containing abundant endogenous autofluorescent substances, such as collagen and flavin adenine dinucleotide, emits green fluorescence with a wavelength of 515 nm [28]. Dysplasia is associated with alterations in the stromal architecture, which cause the loss of autofluorescence [29]. Abnormal tissue with the loss of autofluorescence appears dark in contrast; this may be because of lower levels of endogenous autofluorescent substances in the abnormal tissue than in the surrounding tissue [30]. This effect may be explained by a decrease in the main source of cellular fluorescence, namely flavin adenine dinucleotide, in tissues with dysplasia [31]. As the collagen cross-links and basal lamina are destroyed, glucose may be consumed in malignant tissue even in an aerobic environment; this is called the Warburg effect [32–34].

The main location of OPMD was (according to incidence) the buccal mucosa, followed by the gingiva, tongue, and floor of the mouth [35–37]. In our study, the location of the lesion was similar to conventional locations, which were mostly the buccal mucosa (67.6%), followed by the gingiva (13.2%) (Table 1). These data are comparable to the findings of a recent large population-based study in southern Taiwan [38]. We noted the highest occurring lesion was leukoplakia, and this is also similar to a recent large population study done in Taiwan [39]. Leukoplakia occurred in 25.5% of patients, which is a quarter of the study population, and erythroplakia and erythroleukoplakia occurred in 19.9% of patients. The group of "others" (Table 2), which were considered by the specialist in the COE to be benign, occurred in 38.1% of patients. Hyperkeratosis, hyperplasia, and inflammation were most reported by the pathologist.

Our comparative results showed a disappointing lack of specificity in the autofluorescence examination performed before excluding the non-OPMD features from the COE groups of the oral mucosal disorder checklist (Table 3). The specificity was 35.42% as the result because FVL was observed in the majority of the clinically diagnosed cases of non-OPMD. Low specificity was also observed in the study conducted by Awan et al.; they noted that the FVL findings were positive in the

Table 3 Autofluorescence imagining in relation to OPMD and dysplasia

Diagnosis	Case	Autofluorescence		Se	Sp	PPV	NPV	Accuracy
	(*n*)	FVR	FVL					
OPMD	68	15	53	77.94	35.42	63.10	53.13	60.34
Non-OPMD	48	17	31					
Dysplasia	63	7	56	88.89	43.86	63.64	78.13	67.50
Nondysplasia	57	25	32					

Table 4 Autofluorescence imagining in relation to dysplasia among OPMD

Diagnosis	Case	Autofluorescence		Se	Sp	PPV	NPV	Accuracy
	(*n*)	FVR	FVL					
Dysplasia	56	7	49	87.50	72.73	94.23	53.33	85.07
Nondysplasia	11	8	3					

majority of the benign cases that may be mistakenly diagnosed as OPMD by the nonspecialist [40]. This lack of specificity creates drawbacks and remains a constant problem to other studies[41–43]. In a recent meta-analysis, Luo et al. demonstrated an overall superiority in accuracy in detection of OPMD compared with other aerodigestive lesions using autofluorescence examinations. Additionally, approaching the diagnosis with algorithms could ensure the specificity in general practice [44].

Several studies utilizing autofluorescence examination for mucosal screening have found increased rates of detection of epithelial dysplasia in the high-risk group but with a substantial number of false positives [45–47]. Pigmented, vascular, and inflammatory lesions are especially likely to present with LAF [16]. In our study, the clinical diagnoses of "others" in OPMD (Table 2) show that 48 cases in 38.1% of total identifications were benign lesions, including benign fractional keratosis, aphthous ulcer, and candidiasis thrush. After the exclusion of these non-OPMD cases using the current standard oral mucosal disorder checklist in Taiwan along with autofluorescence imaging, the specificity for dysplasia increased to 72.73% (Table 4). Moreover, the overall accuracy for dysplasia in OPMD cases improved after using the current standard oral mucosal disorder checklist in Taiwan in combination with autofluorescence imaging. However, with the understanding of oral mucosal disorders, COE protocol can aid in differentiating benign lesions from OPMDs, and proficient autofluorescence examination is required for proper FVL assessment before biopsy to identify dysplasia.

Conclusion

Digital autofluorescence imaging can be used as an adjunct tool of clinical value, assisting clinical specialists in OPMD surveillance and biopsy. The results suggest that the autofluorescence imaging could be considered as an aid when targeting mucosal dysplasia in high-risk patients, such as those with OPMD. Proper examination protocol along with autofluorescence imaging should be considered for decision-making regarding the biopsy site or in repeat OPMD follow-ups. However, the device alone may not identify lesions without the standard COE and histopathological examination. Future large population–based trials to assess the benefits of autofluorescence technology are warranted.

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Compliance with ethical standards

This study was approved by the Institutional Review Board of TSGH (approval no. 1-107-05-010).

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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