Diagnostic value of objective VELscope fluorescence methods in distinguishing oral cancer from oral potentially malignant disorders (OPMDs)

Caijiao Wang[1,2], Xiangmin Qi[1], Xiaofang Zhou[1], Hongrui Liu[1,2], Minqi Li[1,2]

[1]Department of Bone Metabolism, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University & Shandong Key Laboratory of Oral Tissue Regeneration & Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Jinan, China; [2]Center of Osteoporosis and Bone Mineral Research, Shandong University, Jinan, China

Contributions: (I) Conception and design: C Wang, M Li, H Liu; (II) Administrative support: X Qi, M Li; (III) Provision of study materials or patients: X Qi; (IV) Collection and assembly of data: X Zhou, C Wang; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Minqi Li; Hongrui Liu. Wenhua West Road 44-1, Jinan 250012, China. Email: liminqi@sdu.edu.cn; yf1blhr@126.com.

Background: The VELscope fluorescence method has been applied to the identification and detection of oral potentially malignant disorders, but autofluorescence visualization lacks objectivity and its diagnostic value varies greatly. The effectiveness of VELscope in detection of the cancer risk in oral potentially malignant disorders at different lesion sites remains unclear, given that only a few studies have investigated the value of VELscope for detecting high- and low-risk lesions in oral potentially malignant disorders. This study used the objective VELscope fluorescence method based on quantitative analysis to investigate its value in oral potentially malignant disorders for: (I) detecting oral cancer; (II) distinguishing high-risk lesions from low-risk lesions; and (III) measuring differences in oral cancer diagnostic accuracy between different sites.

Methods: Conventional oral examination and subjective and objective VELscope examinations were performed on 59 oral potentially malignant disorders; autofluorescence results were compared with histopathological diagnosis.

Results: The sensitivity of subjective and objective VELscope fluorescence methods for detecting oral cancer in oral potentially malignant disorders was 76.9% and 65.4%, respectively; specificity for distinguishing high-risk from low-risk lesions in oral potentially malignant disorders was 50.0% and 82.1%, respectively; and sensitivity for detecting oral cancer in oral potentially malignant disorders of lining mucosa was 81.0%, higher than that of the masticatory mucosa.

Conclusions: The identification ability for low-risk lesions can be improved by combining it with objective autofluorescence. Autofluorescence has different screening abilities in different parts of the oral mucosa.

Keywords: VELscope; autofluorescence; oral potentially malignant disorders (OPMDs); oral epithelial dysplasia (OED); oral cancer

Introduction

Oral potentially malignant disorders (OPMDs) refer to oral clinical manifestations having a risk of malignant transformation, including oral erythroplakia, oral leukoplakia, oral lichen planus, discoid lupus erythematosus, and oral submucous fibrosis (1). OPMDs may be associated with different degrees of oral epithelial dysplasia (OED) (2). According to research, the overall malignant transformation rate (MTR) of OPMDs is approximately 7.9% and is different among different

diseases. The MTR of lichen was 1.4%, while that of leukoplakia with OED could be up to 10.5%. Additionally, the higher the degree of dysplasia, the higher the possibility of oral cancer (3,4). It is important to identify and diagnose oral mucosal diseases early and to monitor and intervene effectively for the prevention of oral cancer because of the prolonged course of OPMDs and risk of cancer. Comprehensive and systematic conventional oral examination (COE), timely surgical biopsy, and histopathological evaluation are the current gold standards for examining and evaluating any suspicious oral mucosal lesions (5,6). This entire process mainly relies on the clinicians' clinical experience and subjective judgment, which may lead to misdiagnosis or missed diagnosis. As mentioned in the review by Tomo *et al.*, this also leads to challenges in the prevention and early diagnosis of oral squamous cell carcinoma (OSCC) and OPMDs (7). Therefore, more objective and effective methods should be adopted to improve clinicians' diagnostic efficiency.

Endogenous autofluorescence substances (NADH, collagen, etc.) in the normal oral mucosa and submucosa emit light green autofluorescence when excited by a light source with an appropriate wavelength (400–460 nm) (8-10). VELscope® (LED Dental, Burnaby, British Columbia, Canada) was the first commercial autofocus-imaging device approved for oral use. Its principle is the tissue autofluorescence visualization technology and advantages include non-invasiveness and quick and convenient use. When it is used to detect oral mucosal lesions (11-13), the lesions appear as a black area on account of fluorescence visualization loss (FVL) owing to the destruction of autofluorescence substances (8).

Cicciù *et al.* (14) conducted a systematic review on the clinical efficiency of VELscope in the early detection of OPMDs and oral cancer; the diagnostic value varied widely, with the sensitivity and specificity ranging from 22% to 100% and from 8.4% to 100%, respectively, but the average sensitivity and specificity were 70.19% and 65.95%, respectively. One of the main reasons for these differences is the lack of objective autofluorescence visualization owing to the differences in operators' abilities and experiences, although autofluorescence may help improve the detection of non-specialists or non-experienced professionals in the diagnosis of oral high-risk lesions (15-17). Previous studies in patients with OPMDs and/or OSCC reported that the main reason for the limitations of VELscope diagnosis was its low specificity (18-22). Currently, most studies have investigated the value of VELscope in detecting oral cancer in OPMDs, while few have investigated the value of VELscope in detecting high- and low-risk lesions in OPMDs. OPMDs often occur in the masticatory mucosa and lining mucosa, but they have a high risk of malignancy when they occur on the tongue ventrum and on the edge of the soft palate (23). The efficiency of detecting the cancer risk in OPMDs at different lesion sites by VELscope is worth exploring; therefore, in this study, we used image analysis software to obtain a quantitative ratio to provide objective results for autofluorescence visualization, and analyze subjective and objective autofluorescence examinations with histopathological results.

Given the multiple reports on differences in the diagnostic value of VELscope, this study adopted the quantitative VELscope fluorescence method to evaluate its applicability in detecting oral cancer, distinguishing between high-risk and low-risk lesions and measuring the difference in oral cancer diagnostic accuracy among different sites in OPMDs. We present the following article in accordance with the STARD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2804/rc).

Methods

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Stomatological Hospital, School of Stomatology, Cheeloo College of Medicine, Shandong University (approval No. 20170502). All subjects signed an informed consent form for participation in the study.

Sample collection and patient information

We identified 59 oral lesions in 54 patients between May 2017 and May 2021, of which 51 occurred in the lining mucosa and 8 occurred in the masticatory mucosa. The inclusion criteria were as follows: (I) lesions with a definitive clinical diagnosis of oral leukoplakia or oral lichen planus, or (II) ulcers or erosive lesions that had not healed after a follow-up period of 2–4 weeks, despite eliminating the cause of the acute inflammation (sharp teeth, insufficient edge of filling material, poor wearing of dentures, etc.). Patients were excluded if they had a history of malignant tumor in the head and neck.

Research process

First, a senior specialist dentist collected personal information and performed COE on the subjects and then took color photographs of the lesions with a Canon camera EOS 5D under white light. VELscope® was used to perform autofluorescence examination of the patients under dim indoor light; fluorescence photos were taken, and suspicious sites were identified. Both the patients and the examiner wore protective goggles during the entire process. Then, objective VELscope fluorescence examination with quantitative analysis was performed. Based on the results of COE and fluorescence examination, a biopsy was performed for all lesions by a senior specialist dentist with 20 years of clinical experience in treating mucosal diseases and oral cancer. The samples were stained with routine hematoxylin and eosin for morphological diagnosis by an experienced oral pathologist. The pathologist was blinded to the clinical examination results of the VELscope®. Finally, the research results were statistically analyzed and discussed.

Judgment criteria

A. Subjective autofluorescence examination

The results of the autofluorescence examination were determined based on the manufacturer's protocol, namely FVL, fluorescence visualization retained (FVR), and fluorescence visualization increased (FVI). The lesions were divided into two groups. For subjective autofluorescence examination, the first group (A1) included lesions showing complete FVL, considered as positive (FVL group); the second group (A2) included lesions showing autofluorescence results other than complete FVL and was considered as negative (FVR group). They were further divided into four subgroups according to their autofluorescence patterns: Group A2a (lesions showing FVR), Group A2b (lesions showing a combination of FVL and FVR), Group A2c (lesions showing FVI), and Group A2d (lesions showing a combination of FVI and FVL) (24).

B. Histopathological examination

The lesions were divided into two groups based on their histopathological diagnosis. For the first group (Group B1), on histopathology, lesions diagnosed as oral cancer were considered positive, while those diagnosed as OPMDs were considered negative. For the second group (Group B2), on histopathology, lesions diagnosed as high-risk lesions were considered positive, while those diagnosed as low-risk lesions were considered negative. Low-risk lesions included no dysplasia, mild dysplasia, or moderate dysplasia. High-risk lesions included severe dysplasia, proliferative verrucous leukoplakia (PVL), carcinoma in situ, and OSCC (including verrucous carcinoma). The definitive diagnosis of PVL was based on clinical and histopathological correlations (25). The histopathologic diagnosis criteria conform to the World Health Organization criteria and the new diagnostic criteria for oral lichen planus proposed by the American Academy of Oral and Maxillofacial Pathology in 2016 (1,26).

C. Objective autofluorescence examination

Image analysis software (Image J 1.8.0; National Institutes of Health, Germany) was used to analyze the subjective autofluorescence of the VELscope images and calculate the average fluorescence intensity ratio. As per the procedure (27), the image was converted to a gray image. Subsequently, FVL areas and the surrounding healthy mucosal area were used to calculate their average fluorescence intensity and the ratio between the two was then calculated. The FVL areas were the lesion areas, and the surrounding healthy mucosa area was the normal area. Average fluorescence intensity ratio = average fluorescence intensity of normal area/average fluorescence intensity of lesion areas. Statistical software (SPSS 20.0; IBM, Armonk, New York, USA) was used to create the receiver operating characteristic (ROC) curve, and the cut-off value was calculated; the criterion for judging the presence of objective FVL. When the average fluorescence intensity ratio was higher than the cut-off value, the lesion was considered as a positive objective autofluorescence examination. When the average fluorescence intensity ratio was lower than the cut-off value, the lesion was considered as a negative objective autofluorescence examination.

Statistical analysis

All data were analyzed using the SPSS 20.0. The accordance rates of subjective and objective autofluorescence examinations with histopathological examinations were calculated. Sensitivity = number of true positives/(number of true positives + number of false negatives), specificity = number of true negatives/(number of true negatives + number of false positives).

Results

Patient information

Fifty-four patients [21 men (38.9%) and 33 women (61.1%)] aged 32–80 years (average: 58.1±12.6 years) were included.
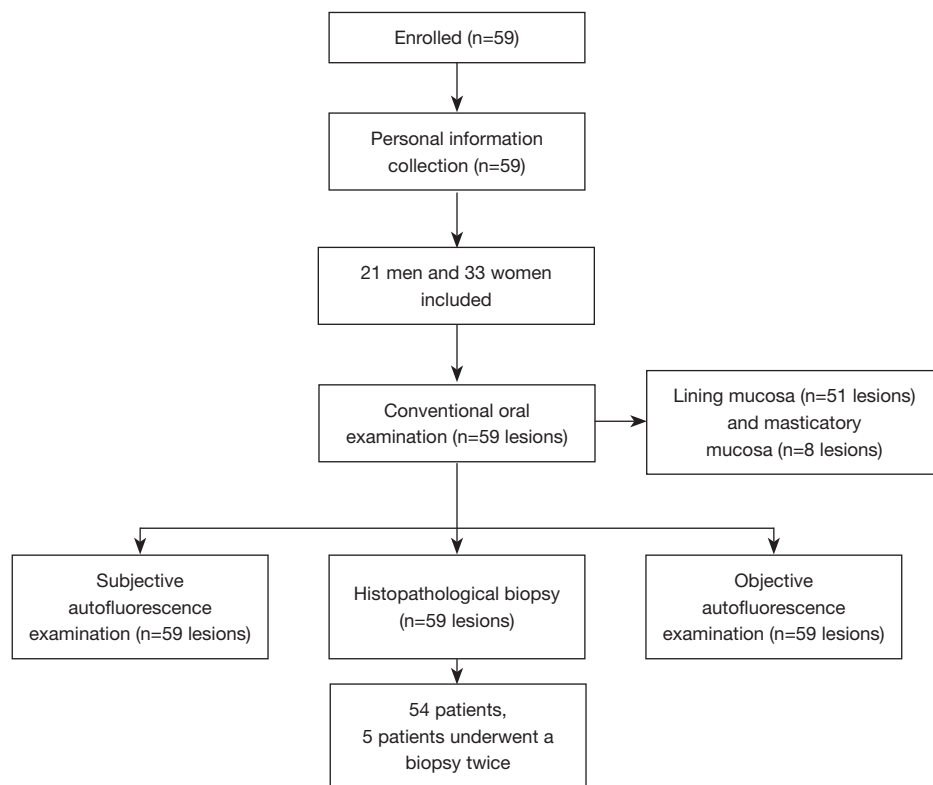
Figure 1 Flowchart of the study process.

Among them, five patients underwent a biopsy twice; 51 (86.4%) and 8 (13.6%) lesions occurred in the lining mucosa and masticatory mucosa, respectively (*Figure 1*). Subjective autofluorescence examination revealed 37 lesions with complete FVL. Demographic and clinicopathological characteristics were recorded for each patient (*Tables 1,2*).

Detection of oral cancer from OPMDs

Among the 26 lesions histopathologically diagnosed as oral cancer, the numbers of positive and negative samples, as revealed by subjective autofluorescence examination were 20 and 6, respectively. Among the 33 lesions pathologically diagnosed as OPMDs, the numbers of positive and negative samples, as revealed by subjective autofluorescence were 17 and 16, respectively (*Table 3*, Group B1). Consequently, there were 20, 17, 16, and 6 cases of true positives (*Figure 2A-2C*), false positives (*Figure 2D-2F*), true negatives (*Figure 2G-2I*), and false negatives (*Figure 2J-2L*), respectively, based on a combination of COE, subjective VELscope fluorescence examination, and histopathological examination.

The ROC curve was established and the area under

the curve was 0.716, with medium accuracy, and the 95% confidence interval (CI) was 0.579–0.852. The cut-off value was 1.40845, and 24 samples were positive in objective autofluorescence examination, of which 17 samples were diagnosed as oral cancer and 7 as OPMDs by histopathology; 35 samples were negative in objective autofluorescence examination, of which 9 were diagnosed as oral cancer and 26 as OPMDs by histopathology (*Table 3*, Group B1).

The accuracy of subjective autofluorescence examination and objective autofluorescence examination in the diagnosis of oral cancer were as follows: sensitivity (76.9% and 65.4%, respectively); specificity (48.5% and 78.8%, respectively); positive predictive values (PPV) (54.1% and 70.8%, respectively); negative predictive values (NPV) (72.7% and 74.3%, respectively); Jordan index (25.4% and 44.2%, respectively). The accuracy rates were 61.0% and 72.9%, respectively (*Table 3*, Group B1).

Distinguishing high-risk lesions from low-risk lesions of OPMDs

Among 31 lesions histopathologically diagnosed as high-

Table 1 Demographic and clinicopathological characteristics of patients

Characteristics	Number (%)
Age, mean ± SD (years)	58.1±12.6
Gender	
Male	21 (38.9)
Female	33 (61.1)
Risk factors	
Smoking status	
Never	37 (68.5)
Used to smoke	13 (24.1)
Currently smoking	4 (7.4)
Alcohol drinking history	
Never	36 (66.7)
Used to drink	10 (18.5)
Currently drinking	8 (14.8)
History of eating spicy food	
No	51 (94.4)
Yes	3 (5.6)
Pathological changes (conventional oral examination)	
Site	
Lining mucosa	51 (86.4)
Buccal mucosa	9 (15.3)
Tongue ventrum	42 (71.2)
Masticatory mucosa	8 (13.6)
Palate	4 (6.8)
Gingiva	4 (6.8)
Biopsy	
Histopathologic diagnosis	
Oral potentially malignant disorder	33 (55.9)
Oral lichen planus	5 (8.5)
Oral leukoplakia, with simple epithelial hyperplasia	5 (8.5)
Oral leukoplakia, with mild epithelial dysplasia	13 (22.0)
Oral leukoplakia, with moderate epithelial dysplasia	5 (8.5)
Oral leukoplakia, with severe epithelial dysplasia	2 (3.4)

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	Number (%)
Proliferative verrucous leukoplakia	3 (5.1)
Oral cancer	26 (44.1)
Oral squamous cell carcinoma	25 (42.4)
Verrucous carcinoma	1 (1.7)

SD, standard deviation.

risk, 23 positive and 8 negative samples were identified in subjective autofluorescence examination. Among 28 lesions histopathologically diagnosed as low risk, 14 positive and negative samples each were identified in subjective autofluorescence examination (*Table 3*, Group B2). Consequently, there were 23 cases of true positives (*Figure 3A-3C*), 14 false positives (*Figure 3D-3F*), 14 true negatives (*Figure 3G-3I*), and 8 false negatives (*Figure 3J-3L*), based on COE, subjective VELscope fluorescence examination, and histopathological examination, respectively.

The ROC curve was established, the area under the curve was 0.721, with medium accuracy, and the 95% CI was 0.588–0.854. The cut-off value was 1.4546. Of the samples, on objective autofluorescence examination, 22 were positive (17 high-risk lesions and 5 low-risk lesions per histopathological examination) and 37 were negative (14 high-risk lesions and 23 low-risk lesions per histopathological examination) (*Table 3*, Group B2).

The accuracy of subjective autofluorescence examination and objective autofluorescence examination in the diagnosis of high-risk and low-risk oral lesions were as follows: sensitivity (74.2% and 54.8%, respectively); specificity (50.0% and 82.1%, respectively); PPV (62.2% and 77.3%, respectively); NPV (63.6% and 62.2%, respectively); Jordan index (24.2% and 37.0%, respectively). The accuracy rates were 62.7% and 67.8%, respectively (*Table 3*, Group B2). It is noteworthy that the specificity of objective autofluorescence examination in distinguishing high-risk lesions from low-risk lesions in OPMDs was 82.1%, which was 32.1% higher than that of subjective autofluorescence examination.

Differences in oral cancer diagnostic accuracy between different sites in OPMDs

Twenty-one cases of histopathologically diagnosed oral cancer occurred in the lining mucosa, and five occurred in

Table 2 Comparison of subjective VELscope examination results with histopathology

Group	Number (%)	Histopathological diagnosis	Number (%)
A1 (FVL)	37 (62.7)	Oral lichen planus	3 (5.1)
		Oral leukoplakia, with simple epithelial hyperplasia	2 (3.4)
		Oral leukoplakia, with mild epithelial dysplasia	5 (8.5)
		Oral leukoplakia, with moderate epithelial dysplasia	4 (6.8)
		Oral leukoplakia with severe epithelial dysplasia	1 (1.7)
		Proliferative verrucous leukoplakia	2 (3.4)
		Oral squamous cell carcinoma	20 (33.9)
A2a (FVR)	5 (8.5)	Oral lichen planus	2 (3.4)
		Oral leukoplakia, with simple epithelial hyperplasia	1 (1.7)
		Oral leukoplakia with mild epithelial dysplasia	2 (3.4)
A2b (FVL + FVR)	6 (10.2)	Oral leukoplakia with simple epithelial hyperplasia	1 (1.7)
		Oral leukoplakia with mild epithelial dysplasia	2 (3.4)
		Oral squamous cell carcinoma	3 (5.1)
A2c (FVI)	5 (8.5)	Oral leukoplakia with mild epithelial dysplasia	1 (1.7)
		Proliferative verrucous leukoplakia	1 (1.7)
		Oral squamous cell carcinoma	2 (3.4)
		Verrucous carcinoma	1 (1.7)
A2d (FVI + FVL)	6 (10.2)	Oral leukoplakia with simple epithelial hyperplasia	1 (1.7)
		Oral leukoplakia with mild epithelial dysplasia	3 (5.1)
		Oral leukoplakia with moderate epithelial dysplasia	1 (1.7)
		Oral leukoplakia with severe epithelial dysplasia	1 (1.7)

FVI, fluorescence visualization increased; FVL, fluorescence visualization loss; FVR, fluorescence visualization retained.

the masticatory mucosa. According to subjective VELscope fluorescence examination, there were 17 cases of true positives (*Figure 3A-3C*), 15 false positives (*Figure 2D-2F*, *Figure 3D-3F*), 15 true negatives (*Figure 2G-2I*, *Figure 3G-3I*), and 4 false negatives (*Figure 2J-2L*, *Figure 3J-3O*) located in the lining mucosa. Meanwhile, there were three cases of true positives (*Figure 2A-2C*), two false positives, one true negative, and two false negatives (*Figure 2J-2L*) located in the masticatory mucosa. The sensitivity, specificity, and NPV of lesions located in the lining mucosa (51 lesions, 86.4%) were 81.0%, 50.0%, and 79.0%, respectively, which were higher than those of lesions located in the masticatory mucosa (8 lesions, 13.6%) (*Table 4*).

The area under the curve for lesions located in the lining mucosa was 0.725, with moderate accuracy, and 95% CI was 0.579–0.872. The area under the curve for lesions located in

the masticatory mucosa was 0.667, which had low accuracy, and a 95% CI of 0.265–1.000, P=0.456.

Discussion

In this study, 59 OPMDs were analyzed by COE, in addition to subjective and objective VELscope fluorescence examinations. We then compared the autofluorescence results of these lesions with the histopathological diagnosis and explored their diagnostic value in OPMDs for (I) detecting oral cancer; (II) distinguishing high-risk lesions from low-risk lesions; and (III) measuring differences in oral cancer diagnostic accuracy between different sites. This study is of great significance in preventing the progression of oral cancer and improving survival rates.
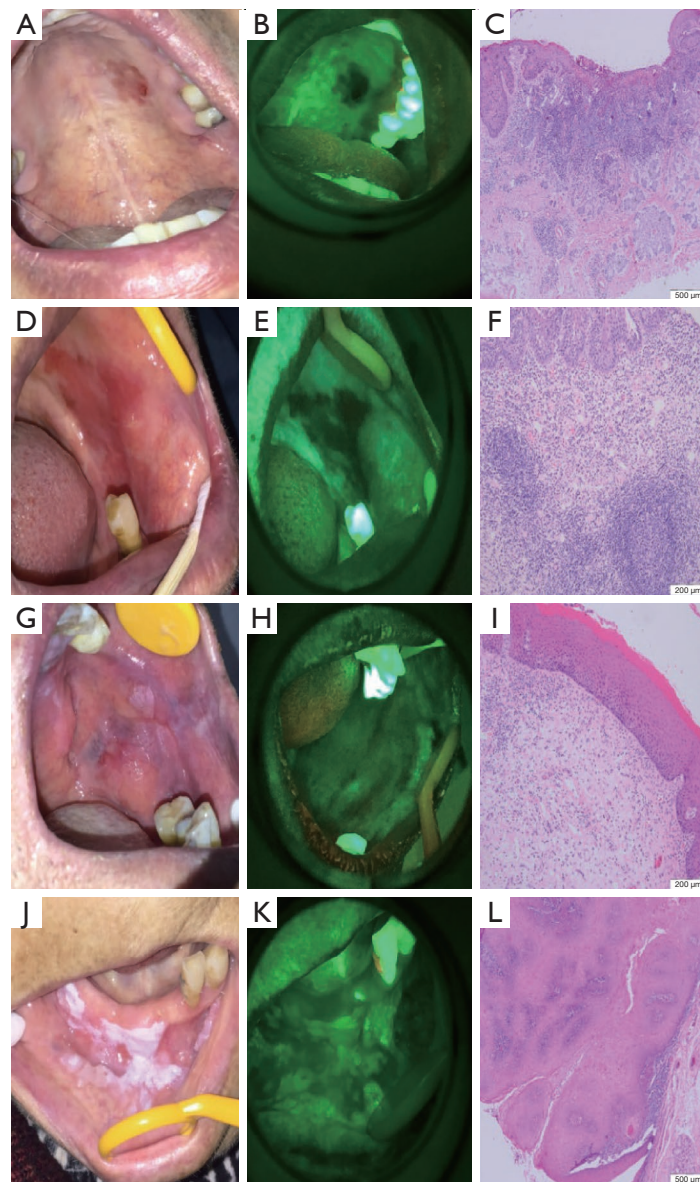
The value in detecting oral cancer from OPMDs

Figure 2 Representative true- and false-positive and true- and false-negative cases of oral cancer and OPMDs on subjective autofluorescence examination. (A-C) Case 1. True-positive case on subjective autofluorescence examination, histopathologically diagnosed as oral cancer: (A) clinical examination shows erythema and erosion of the mucosa on the left side of the palate; (B) VELscope examination of the lesion shows FVL; (C) moderately differentiated oral squamous cell carcinoma was diagnosed on H&E staining. Scale bar =500 μm. (D-F) Case 2. False-positive case on subjective autofluorescence examination, histopathologically diagnosed as OPMDs: (D) clinical examination shows erythema and ulceration from the posteroinferior part of the left buccal mucosal region to the sulcus vestibularis; (E) VELscope examination of the lesion shows FVL; (F) moderate epithelial dysplasia was diagnosed on H&E staining. Scale bar =200 μm. (G-I) Case 3. True-negative case on subjective autofluorescence examination, histopathologically diagnosed as OPMDs: (G) clinical examination shows extensive white plaques on the left buccal mucosa; (H)VELscope examination of the lesion shows a combination of FVL and FVR; (I) leukoplakia with mild epithelial dysplasia was diagnosed on H&E staining. Scale Bar =200 μm. (J-L) Case 4. False-negative case on subjective autofluorescence examination, histopathologically diagnosed as oral cancer: (J) clinical examination shows leukoplakia-like changes and raised folds in the mandibular gingiva, vestibule, and corresponding labial mucosa; (K) VELscope examination of the lesion areas show FVI; (L) verrucous carcinoma was diagnosed on H&E staining. Scale bar =500 μm. OPMDs, oral potentially malignant disorders; FVI, fluorescence visualization increased; FVL, fluorescence visualization loss; FVR, fluorescence visualization retained; H&E, hematoxylin and eosin.

Transl Cancer Res 2022;11(6):1603-1615 | https://dx.doi.org/10.21037/tcr-21-2804

Table 3 Accuracy of subjective and objective autofluorescence tests for the diagnosis of oral cancer

Item	Histopathological diagnosis		Total number	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value	Youden index	Accuracy rate
	Positive (cancer)	Negative (OPMDs)							
Subjective autofluorescence examination for Group B1				76.9%	48.5%	54.1%	72.7%	25.4%	61.0%
Positive (FVL group)	True positive (n=20)	False positive (n=17)	37						
Negative (FVR group)	False negative (n=6)	True negative (n=16)	22						
Objective autofluorescence examination for Group B1				65.4%	78.8%	70.8%	74.3%	44.2%	72.9%
Positive	True positive (n=17)	False positive (n=7)	24						
Negative	False negative (n=9)	True negative (n=26)	35						
Subjective autofluorescence examination for Group B2				74.2%	50.0%	62.2%	63.6%	24.2%	62.7%
Positive (FVL group)	True positive (n=23)	False positive (n=14)	37						
Negative (FVR group)	False negative (n=8)	True negative (n=14)	22						
Objective autofluorescence examination for Group B2				54.8%	82.1%	77.3%	62.2%	37.0%	67.8%
Positive	True positive (n=17)	False positive (n=5)	22						
Negative	False negative (n=14)	True negative (n=23)	37						

OPMDs, oral potentially malignant disorders; FVL, fluorescence visualization loss; FVR, fluorescence visualization retained.

was as follows: the sensitivity, PPV, and NPV were moderate; the specificity of subjective autofluorescence examination was low (48.5%), and the specificity of objective autofluorescence examination was high (78.8%); the sensitivity of subjective autofluorescence examination was consistent with the results of a published systematic review (14). This study had a large number of false-negative cases, which affected sensitivity. This might be because lesions showing a combination of FVL and FVR and lesions showing a combination of FVI and FVL were classified as negative in subjective autofluorescence examination; this resulted in some true-positive cases being counted as false negatives, which reduced the sensitivity. Additionally, there were three categories of false-negative cases for subjective autofluorescence examinations: Group A2b (lesions showing the combination of FVL and FVR), Group A2c (lesions showing FVI), and Group A2d (lesions showing the combination of FVI and FVL) (*Figure 3M-3O*). Another reason is that false-negative cases manifesting as FVI have reduced sensitivity. FVI lesions are common, and histopathology revealed a thick keratinized layer. Meleti *et al.* (28) reported that the epithelium of malignant lesions with a relatively thick keratin layer showed high fluorescence and that the autofluorescence characteristics of oral malignant lesions seemed to be significantly correlated with the width of the keratin layer.

Keratin increases autofluorescence with a decrease in the wavelength (29). It was unsuitable for correct diagnosis of hyperkeratotic lesion areas because the established method considered fluorescence loss as a manifestation of the lesion. Consequently, false-negative results may appear in both subjective and objective autofluorescence examinations, suggesting that the lesions showing FVI limit the ability of VELscope to detect malignant changes. Furthermore, some patients may have had chronic OPMDs, which progresses from different degrees of hyperplasia to oral cancer (30-33). Therefore, with lesions exhibiting FVI, effective long-term supervision and intervention may play a positive role in preventing oral cancer.

In this study, the ratio of average fluorescence intensity between the lesion areas and healthy area was calculated by quantitative VELscope autofluorescence imaging, and the cut-off value was calculated by establishing the ROC curve to objectively evaluate the ability of autofluorescence visualization, to enable the naked eye to distinguish the color of the oral epithelium, and to reduce possible human bias and errors. It is worth noting that this method has solved the limitations of VELscope diagnosis caused by low specificity to a certain extent (18-24). In the present study, the specificity of objective autofluorescence examination in distinguishing high-risk lesions from low-risk lesions in OPMDs was 82.1%, which was 32.1% higher than that of
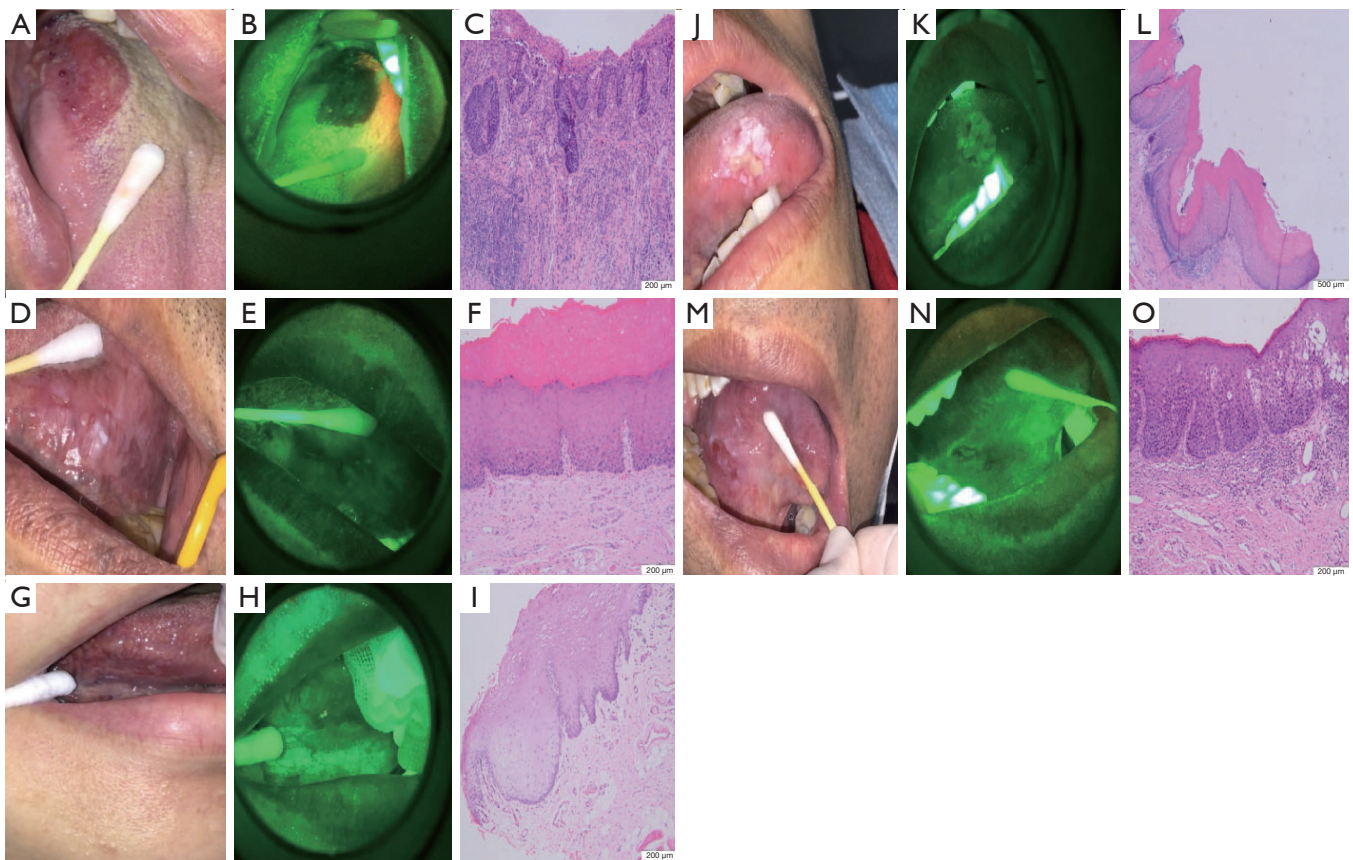
Figure 3 Representative true- and false-positive and true- and false-negative cases of low- and high-risk lesions on subjective autofluorescence examination. (A-C) Case 5. True-positive case on subjective autofluorescence examination, histopathologically diagnosed as a high-risk lesion: (A) clinical examination shows bright red erosion on the ventrum of the right-side of the tongue and a grayish-white plaque near the floor of the mouth. (B) VELscope examination of the lesion shows FVL; (C) carcinoma in situ and local early invasion of squamous cell carcinoma was diagnosed on H&E staining. Scale bar =200 μm. (D-F) Case 6. False-positive case on subjective autofluorescence examination, histopathologically diagnosed as a low-risk lesion: (D) clinical examination shows homogeneous leukoplakia-like changes on the left ventral aspect of the tongue; (E) VELscope examination of the lesion shows FVL; (F) leukoplakia with mild epithelial dysplasia was diagnosed on H&E staining. Scale bar =200 μm. (G-I) Case 7. True-negative case on subjective autofluorescence examination, histopathologically diagnosed as a low-risk lesion: (G) clinical examination shows a few faint white net-like striae on the right ventral aspect of the tongue with local hyperemia. (H) VELscope examination of the lesion shows FVR; (I) leukoplakia with simple epithelial hyperplasia was diagnosed on H&E staining. Scale bar =200 μm. (J-L) Case 8. False-negative case on subjective autofluorescence examination, histopathologically diagnosed as a high-risk lesion: (J) clinical examination shows raised white plaque on the left ventral aspect of the tongue with an ulcer in the center. (K) VELscope examination of the lesion shows FVI; (L) hyperplastic verrucous leukoplakia, and mild epithelial dysplasia was diagnosed on H&E staining. Scale bar =500 μm. (M-O) Case 9. False-negative case on subjective autofluorescence examination, histopathologically diagnosed as a high-risk lesion: (M) clinical examination shows white changes on the right side of the tongue ventrum with an erosion in the center; (N) VELscope examination of the lesion shows a combination of FVI and FVL; (O) severe epithelial dysplasia was diagnosed on H&E staining. Scale bar =200 μm. FVI, fluorescence visualization increased; FVL, fluorescence visualization loss; FVR, fluorescence visualization retained; H&E, hematoxylin and eosin.

Table 4 Accuracy of subjective autofluorescence examination and objective autofluorescence examination in detecting oral cancer in OPMDs at different sites

Site	Autofluorescence examination	Group A1 (FVL)	Group A2 (FVR)	Statistics			
				Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
Lining mucosa	Subjective	True positive (n=17)	False negative (n=4)	81.0%	50.0%	53.1%	79.0%
		False positive (n=15)	True negative (n=15)				
	Objective	True positive (n=14)	False negative (n=7)	66.7%	83.3%	73.6%	78.1%
		False positive (n=5)	True negative (n=25)				
Masticatory mucosa	Subjective	True positive (n=3)	False negative (n=2)	60.0%	33.3%	60.0%	33.3%
		False positive (n=2)	True negative (n=1)				
	Objective	True positive (n=4)	False negative (n=1)	80.0%	66.7%	80.0%	66.7%
		False positive (n=1)	True negative (n=2)				

OPMDs, oral potentially malignant disorders; FVL, fluorescence visualization loss; FVR, fluorescence visualization retained.

subjective autofluorescence examination, indicating that objective autofluorescence examination is more conducive to identifying low-risk lesions from OPMDs. It is crucial to distinguish between high-risk and low-risk patients, as the treatment for the former often requires active interventions such as surgical resection and frequently subsequent treatment, while the latter generally only need a management plan for regular observation (34); thus, for the aforementioned reasons, we have classified the lesions as low and high risk. PVL, a rare oral leukoplakia, is regarded as a disease with invasive biological behavior because of its high recurrence probability and high MTR; usually higher than 70% (35). Therefore, it was classified as a high-risk lesion.

The number of lesions occurring in the lining mucosa (buccal and tongue ventrum) was significantly higher than that in the masticatory mucosa (palate and gingiva), and the sensitivity (81.0%) and specificity (50.0%) for lesions in the lining mucosa were higher than those for lesions in the masticatory mucosa when VELscope was used to detect oral cancer. Because objective fluorescence examination had low accuracy in the masticatory mucosa group, the results of subjective fluorescence examination were used for this comparison. This may occur because the palatal mucosa and gingival mucosa are thinner than the buccal mucosa and lingual mucosa. Therefore, it is necessary to further verify the influence of tissue thickness on autofluorescence. The multi-band light source for detecting autofluorescence in different tissues may help distinguish precancerous lesions

from oral cancer (36). When VELscope was used to detect oral cancer, the specificity of objective fluoroscopy was higher than that of subjective autofluorescence examination; the specificity of autofluorescence examination of lining mucosa was higher than that of masticatory mucosa. In future studies, we will attempt to improve the objective autofluorescence examination method and establish standards by increasing the sample size and categorizing the lesions according to sites.

Autofluorescence results should be interpreted with caution because OPMDs, oral cancer, and even some benign lesions may show similar autofluorescence results. Clinical examination of case 2 (*Figure 2*) showed erythema and ulcers from the posteroinferior part of the left buccal region to the sulcus vestibularis. Subjective VELscope examination showed a positive result, and the pathological diagnosis was moderate epithelial dysplasia. However, on histopathology, a large number of chronic inflammatory cells and blood vessels were found below the epithelium. This "false positive" result can be explained as follows: FVL resulted from increased absorption of light due to increased subepithelial blood flow and from reduced autofluorescence substances in the inflammatory mucosa due to enhanced metabolism (10). Case 6 (*Figure 3*) was a false positive case, positive on subjective autofluorescence examination but negative on objective autofluorescence examination. Similar findings were observed in three other cases that were histopathologically diagnosed as lichen planus.

This study has some limitations, such as the small

sample size and the patients being unrepresentative of all dental patients, which might have introduced a selection bias. The strength of this study is that it was carried out by stomatologists and specialist dentists with rich clinical experience, who had sufficient skills and experience in interpreting the results. As per the systematic review by Tiwari *et al.* (22), optical fluorescence imaging for oral cancer and OPMDs is mainly performed by specialists. An increasing number of auxiliary diagnostic equipment and methods have become available for the detection of oral mucosal lesions. To improve diagnostic efficiency, it is necessary to combine VELscope with other non-invasive techniques (e.g., exfoliative cytology combined with DNA quantitative analysis, toluidine blue staining, and fluorescent probe) (37-39), which is also a direction for our future research.

Since all possible findings may be observed through VELscope fluorescence examinations for OPMDs and oral cancer, we cannot rely solely on the VELscope fluorescence examination to interpret the oral mucosal lesions. Comprehensive and systematic COE, timely surgical biopsy, and histopathological evaluation are still the gold standards for the evaluation of suspicious oral mucosal lesions. Nevertheless, the use of autofluorescence examination can assist in screening oral cancer and high-risk lesions from OPMDs, and combined with objective autofluorescence examination, it can improve the recognition ability of low-risk lesions. Additionally, autofluorescence examination differs in its screening ability for different parts of the oral mucosa.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2804/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2804/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2804/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Stomatological Hospital, School of Stomatology, Cheeloo College of Medicine, Shandong University (approval No. 20170502). All subjects signed an informed consent form for participation in the study.

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