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The diagnostic value of the native fluorescence visualization device for early detection of premalignant/malignant lesions of the oral cavity

Maryam Amirchaghmaghi[a], Nooshin Mohtasham[a], Zahra Delavarian[a], Mohammad Taghi Shakeri[b], Masoud Hatami[c,*], Pegah Mosannen Mozafari[a]

[a] Oral & Maxillofacial Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Social Determinants of Health Research Center, Mashhad University o Medical Sciences, Mashhad, Iran
[c] Department of Oral and Maxillofacial Diseases, School of Dentistry, Lorestan University of Medical Sciences, Khorramabad, Iran

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ABSTRACT

Purpose: The present study aimed to determine the diagnostic value of a native fluorescence visualization device in the identification of oral malignant/dysplastic lesions.

Methods: This study involved 45 patients who had oral lesions that were suspected to be malignant, potentially malignant, or benign. The patients visited the Oral Medicine Department of the Mashhad Dental School. The sensitivity, specificity, positive and negative predictive values, and likelihood ratio of this device were determined.

Results: The histopathological assessment of samples showed 9 cases of oral squamous cell carcinoma and 12 lesions with dysplasia. Ten samples of dysplastic lesions and all malignant lesions appeared dark or red/orange when examined with the native fluorescence visualization device. In 90% of the dysplastic/malignant lesions, the label-free fluorescence results were positive. The sensitivity, specificity, and positive and negative predictive values of this device were 90%, 15%, 40%, and 71%, respectively.

Conclusions: The native fluorescence visualization device can be used in specialized centers as an adjunctive device to increase the sensitivity of a clinical examination, but is not capable of distinguishing benign lesions from malignant and dysplastic ones due to its low specificity.

1. Introduction

Oral cancer is one of the major issues in the field of health and is the tenth cause of death worldwide. Among all cancers, oral cancer is fourteenth in terms of the annual incidence rate and mortality [1]. Oral cancer is the sixth most common cancer in Asian countries. In Iran in 2008, it was the twelfth and fifteenth most common malignancy among men and women, respectively [2,3].

Oral squamous cell carcinoma (OSCC) is the main malignancy of the oral cavity; it makes up 90% of the malignancies in the upper aerodigestive tract mucosa and 94% of all oral malignant tumors [4,5]. Despite the ease of an oral examination, only 40% of the cases are diagnosed in the early stages, while 60% are diagnosed in the advanced stages (stages III-IV). Unfortunately, the disease does not have a desirable prognosis to date [6–11] and the survival rate of patients with OSCC has not improved in 50 years [12–15]. However, with early detection, the 5-year survival rate increases to 83%, while it drops to less than 30% with a delayed diagnosis and the occurrence of metastasis [12,16]. Since 50% of OSCCs arise from potentially malignant lesions, the detection of cancer in this stage can improve the survival rate (10). If the disease is diagnosed at this stage, the patient has the best prognosis [17].

The conventional oral examination (COE) has been used to screen and diagnose oral cavity lesions [18,19]. The method has certain limitations and disadvantages in the early detection of lesions because it is not possible to distinguish benign lesions from potentially malignant ones [10,20]. To compensate for the disadvantages of COE, adjunctive diagnostic methods, especially for high-risk patients, are used. Several adjunctive methods have been introduced in the screening of oral cancer, including live tissue staining with toluidine blue, cytology study with special brushes (oral CDx), and optical methods, such as the VELscope, ViziLite, and narrow band imaging. Each adjunctive method has a role in the diagnosis of potentially malignant lesions that depends on the circumstances [21].

In recent years, researchers have focused on optical biopsy methods. This technique gathers diagnostic information in situ, real time, and in

* Corresponding author.
E-mail address: hatami.m@lums.ac.ir (M. Hatami).

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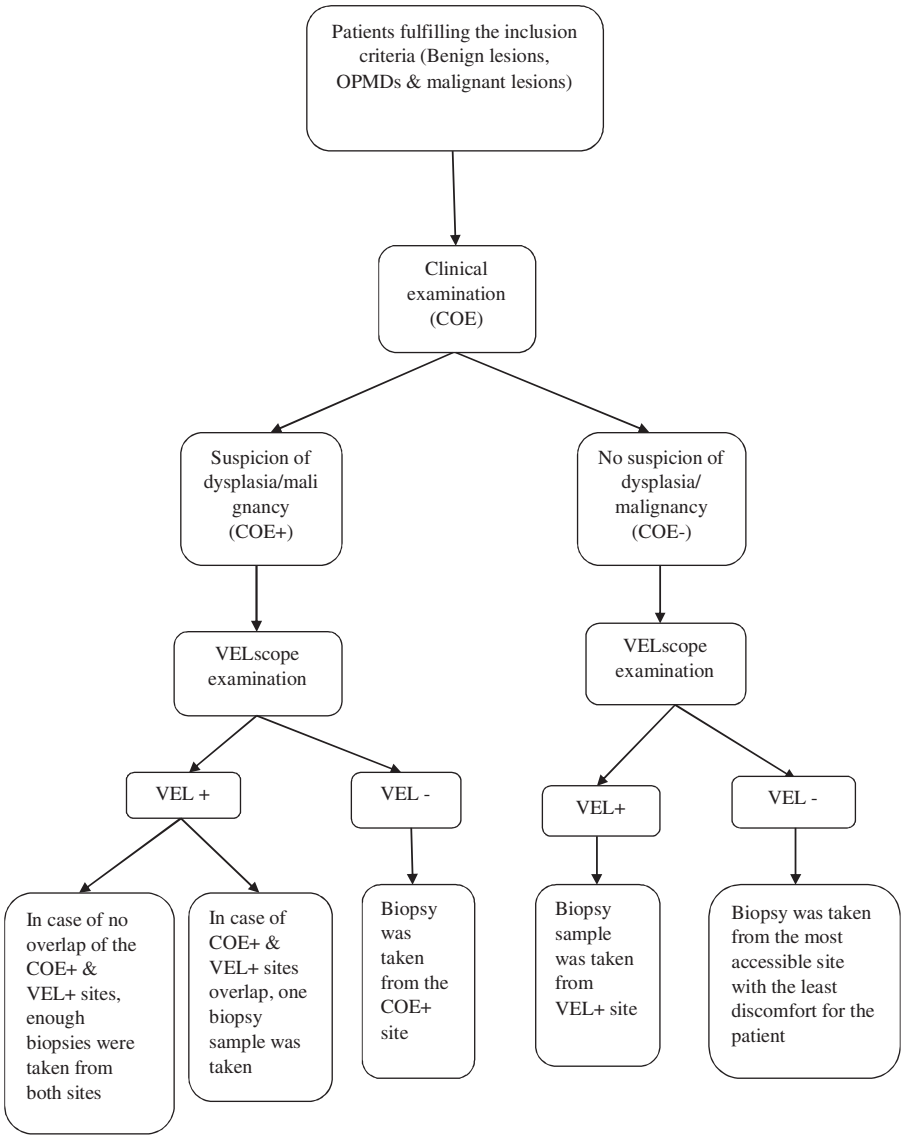

Fig. 1. The COE, VELscope examination results, histopathological study of patients, and the four possible modes for biopsy site determination are displayed.

a minimally invasive manner without tissue excision and histopathological assessments [22,23]. The term “optical biopsy” was first introduced by Alfano et al. in 1987. They employed native fluorescence for the detection of cancer in animals and later in humans using ex vivo tissues [24]. Optical biopsy methods are based on the optical spectroscopic characteristics of the target tissue at the time of measurement, and can be applied to detect precancerous and cancerous lesions quickly and reliably [22,23]. Several optical biopsy methods have been developed, including tissue fluorescence spectroscopy, Raman spectroscopy, elastic scattering spectroscopy, nuclear magnetic resonance spectroscopy, confocal reflectance microscopy, and optical coherence tomography [25].

In fluorescence spectroscopy, tissues are exposed to various excitation wavelengths and the emitted light spectrum is then measured. The differences between the ratio of excitation/emission wavelengths from normal and abnormal tissues is used to detect cellular alterations, such as cancer [26]. The history of using fluorescence spectroscopy to detect cancer goes back to 1924 [27]. When Policard discovered that a rat sarcoma emitted red fluorescence, he hypothesized that the source of this light was the porphyrin that was present in the bacteria on the surface of the sarcoma. Seven years later, the same results were identified in breast cancer; although the possible role of bacteria was excluded, porphyrin was confirmed as a useful fluorophore in native fluorescence imaging [28].

Since 1950, in vivo studies have showed that there are qualitative and quantitative differences in cellular fluorophores, which can be used to distinguish normal cells from malignant lesions. To increase the sensitivity and specificity in the diagnosis of cancer, native cellular fluorophores (NCF) have been used to distinguish normal tissues from neoplastic tissues; numerous cellular fluorophores that are capable of emitting light at specified wavelengths have been recognized [29]. During the carcinogenesis process, the concentrations of fluorophores and NCFs change and these variations can be detected using fluorescence imaging techniques [30,31].

In fluorescence imaging techniques, a specific wavelength of light excites a NCF, which fluoresces. By visualizing different colors in abnormal regions, fluorescence mapping of cancerous tissues can be achieved [26]. One of the most popular fluorescence imaging devices that uses native fluorescence in the oral cavity is the visually enhanced lesion scope (VELscope). After receiving approval from the U.S. Food and Drug Administration (FDA) and the Health Canada in 2006, this device was introduced to the commercial market as an adjunctive diagnostic tool in oral cancer screening [32]. It is a simple-to-use, non-invasive handheld scope that uses natural tissue fluorescence to enhance the visualization of oral mucosal abnormalities [33].

To date, a wide variety of results about the diagnostic value of the

VELscope has been reported by several studies. Its sensitivity and specificity in the diagnosis of malignant and dysplastic oral cavity lesions range from 30 to 100% and 15–92%, respectively [27,34]. Therefore, given the various discrepancies about the diagnostic value of the VELscope in the identification and diagnosis of malignant/dysplastic oral lesions, and the fact that the device is not used in Iran, this study aimed to determine the diagnostic value in terms of sensitivity, predictive value, and likelihood ratio indices.

2. Materials and methods

In this cross-sectional diagnostic study, 50 patients were enrolled in the study. The patients had benign, dysplastic, and malignant oral lesions, and had been referred to the Oral and Maxillofacial Medicine Department of the School of Dentistry, Mashhad University of medical sciences, between January and June 2015.

The study protocol was fully explained to each patient and an informed consent was obtained from each participant prior to enrollment in the study. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (approval No: 930651). All patients over 18 years of age with oral soft tissue lesions who required incisional or excisional biopsy for further diagnosis were included in the study.

Patients with contraindications for biopsy sampling, such as hemorrhagic diseases or uncontrolled systemic diseases, or patients with a confirmed diagnosis of dysplasia or malignancy in a previous biopsy were excluded from the study. Data sheets were used to collect demographic data, medical or drug history, history of use of tobacco products or alcohol, clinical characteristics of the lesions, including history, location, size, clinical appearance, and initial clinical diagnosis, and the appearance of the lesion during a native fluorescence examination. The study stages are described below (Fig. 1).

2.1. Conventional oral cavity examination (COE)

The oral cavity of each patient was examined for premalignant lesions/conditions, benign, or malignant lesions by an oral and maxillofacial medicine specialist using a dental unit with a 15-W incandescent lamp. A positive COE was described as a lesion with one of the two following criteria. The first criterion was that the clinical probability of malignancy in a lesion could be determined according to the following signs: the lesion was exophytic (mass-forming polypoid or verruciform and papillary lesions); it was endophytic (crater-like and destructive ulcers); it had leukoplakia-like features (white plaques); it had erythroplakia-like features (red patches); or it had erythroleukoplakia-like features (combination of red and white components) [35].

The second criterion was the presence of dysplastic premalignant lesions/conditions. In addition to being diagnosed in the premalignant group, the lesion should have one or more of the following dysplasia criteria: stiffness and induration on palpation, an erythematous area in white lesions, surface roughness, exophytic growth, and a progressive course of growth [36]. Premalignant lesions, such as leukoplakia, erythroplakia, and oral submucosal fibrosis, and premalignant conditions, such as oral lichen planus and lichenoid reactions, were considered to be signs of a potentially malignant disease [35].

In this study, COE was regarded as negative for any premalignant lesions/conditions that did not have a clinical suspicion of dysplasia and for benign lesions of the soft tissue of the oral cavity. According to the history, course of growth, and clinical appearance, the probability of dysplasia and malignancy in these lesions were considered to be zero. Thus, all inflammatory fibrous hyperplasia, such as irritation fibroma, peripheral giant cell granuloma, epulis fissuratum, and vesiculobullous lesions (i.e., pemphigus vulgaris and mucous membrane pemphigoid), had negative COE results [2,35,37].

2.2. Native fluorescence visualization of the oral cavity with the VELscope

Native fluorescence visualization was performed using a VELscope® (LED Dental Inc., White Rock, BC, Canada) under dimmed room light with protective eye wear worn by the patient throughout the procedure. The instrument excites a wide field of blue light (400–460 nm) into the mouth to trigger a green–red light emission (approximately 500–700 nm with a proprietary stop band around 600 nm) from the native fluorophores of the oral mucosa, such as the flavin–adenine dinucleotide (FAD) and collagen and elastin cross-linkages. The VELscope interrogates the FAD by excitation at 450 nm and its fluorescence occurs at an emission wavelength of 515 nm. Collagen has significant fluorescence when excited between 410 and 470 nm. In this range, the collagen emission shifts to the red spectrum between 475 and 540 nm (38). The direct visualization of the native oral fluorescence is provided by emission filters within the VELscope that allows passage of the green–red light and blocks the blue light [39].

The possible outcome of the NCF examination was determined according to the manufacture's guideline. Some changes, such as the degradation of the collagen and elastin cross-linkages, an increase in the number of inflammatory cells in the connective tissue, an increased nucleus-to-cytoplasm ratio, hyperplasia of the epithelium layer, and increased concentrations of oxy- and deoxyhemoglobin in the connective tissue, can lead to the loss of fluorescence (LOF), which is appears dark. Some of these changes occur in malignant and premalignant lesions [15,38,40].

Initially, the oral cavity was fully examined, and the location and approximate LOF areas and regions irradiating red/orange light were drawn as a schematic illustration with different colors. An SLR digital camera (Canon 550 EOS, Tokyo, Japan) was used to take photos of the autofluorescent view of the lesions though the VELscope eye lens. Regions with LOF or that were seen as red/orange were considered to be suspicious sites (positive VEL).

2.3. Histopathological study and biopsy

Incisional biopsies were taken from areas in the malignant lesions that had maximum infiltration and from the regions that had characteristics of dysplasia in the premalignant lesions/conditions. However, the initial diagnoses of the lesions and the selection of biopsy sites were done after the consensus of two specialists in oral and maxillofacial medicine.

For premalignant lesions/conditions without any evidence of dysplasia (COE negative), the biopsy sample was taken from locations that are surgically accessible. Benign lesions also underwent incisional or excisional biopsy based on the size of the lesion. To determine the sensitivity and specificity of the VELscope, attempts were made to have the biopsy site cover both the COE and VEL positive regions.

Following the biopsy, the tissue sample was fixed in 10% formalin; it was then stained with hematoxylin and eosin and examined under a light microscope by an experienced specialist in oral and maxillofacial pathology. It is worth noting that in our study, the "gold standard" for the diagnosis of malignant lesions and the presence of dysplasia was a biopsy and a histopathological study. When epithelial dysplasia was present, the pathologist provided a description relating to its severity. Mild epithelial dysplasia referred to alterations that were limited to the basal and parabasal layers, while moderate epithelial dysplasia showed involvement from the basal layer to the midportion of the spinous layer and severe epithelial dysplasia demonstrated alterations from the basal layer to the level above the midpoint of the epithelium [35].

To calculate the sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), the results of both the COE and VELscope were compared with the histopathological results. In the mixed method, the COE and VELscope technique were used in parallel to identify dysplasia/malignancy. The diagnostic value of the mixed method was based on a comparison of the results of the COE and

the VELscope with the histopathological findings.

2.4. Statistical analyses

The statistical evaluation was performed using SPSS software (version 16.0, SPSS Inc., Chicago, IL). The sensitivity, specificity, PPV, NPV, positive likelihood ratio (PLR), and negative likelihood ratio (NLR) for the native fluorescence test results were calculated. Moreover, a clinical diagnosis by a specialist was compared with the histopathological findings. To assess the diagnostic parameters with a 95% Confidence interval, NCSS software (version 2007, NCSS, Kaysville, UT) was used; for other statistical analyses, SPSS software was used. The graphs were plotted using Excel software (version 2010). The chi-square test and Fisher's exact test (with significance set at $\ll 0.05$) were used to calculate the differences and agreement between the COE and VELscope examinations.

3. Results

In this study, 50 patients were given a COE and an examination using the VELscope. Biopsies and histopathological evaluations were not performed on the oral lesions of five patients because the patients did not visit the clinic. Eventually, 45 patients (21 males and 24 females) were enrolled in the study and given both a COE and a VELscope examination (Fig. 2). Among the 54 lesions examined in the 45 patients, 28 (52%) lesions were in males and 26 (48%) lesions were in females. The mean age of the patients was 52.3 ± 14.8 years (52.9 ± 16 years in males and 51.8 ± 14 years in females).

Most of the biopsies of the lesions (19 cases, 29.6%) were taken from the buccal mucosa, while 15 cases (27.7%) were taken from the labial mucosa and 10 cases (18.5%) were taken from the lateral border of the tongue. Only 1 lesion (1.8%) was found in the palatal mucosa.

A total of 37% of the oral lesions showed white features similar to leukoplakia. A red feature, such as erythroplakia, was found in 11.11% of the lesions. Red and white lesions were found in 5.56% of the lesions. An ulcerative aspect was described in 16.67% of the cases, while an exophytic aspect was found in 29.63% of the cases.

Various features were observed in the benign lesions, including ulcers (pemphigus vulgaris) and exophytic lesions (irritation fibroma, epulis fissuratum, pyogenic granuloma, and peripheral odontogenic fibroma). Premalignant lesions mostly had a white (leukoplakia) or red and white (lichen planus and lichenoid reaction) appearance. Various features were seen in the malignant lesions (OSCC), including exophytic lesions, a red and white appearance with or without superficial changes and solitary ulcers.

The clinical diagnosis was oral lichen planus in 16 cases, lichenoid reaction and erythroplakia lesions in 2 cases each, leukoplakia in 7 cases, and keratosis due to use of smokeless tobacco in 3 cases. Pooling

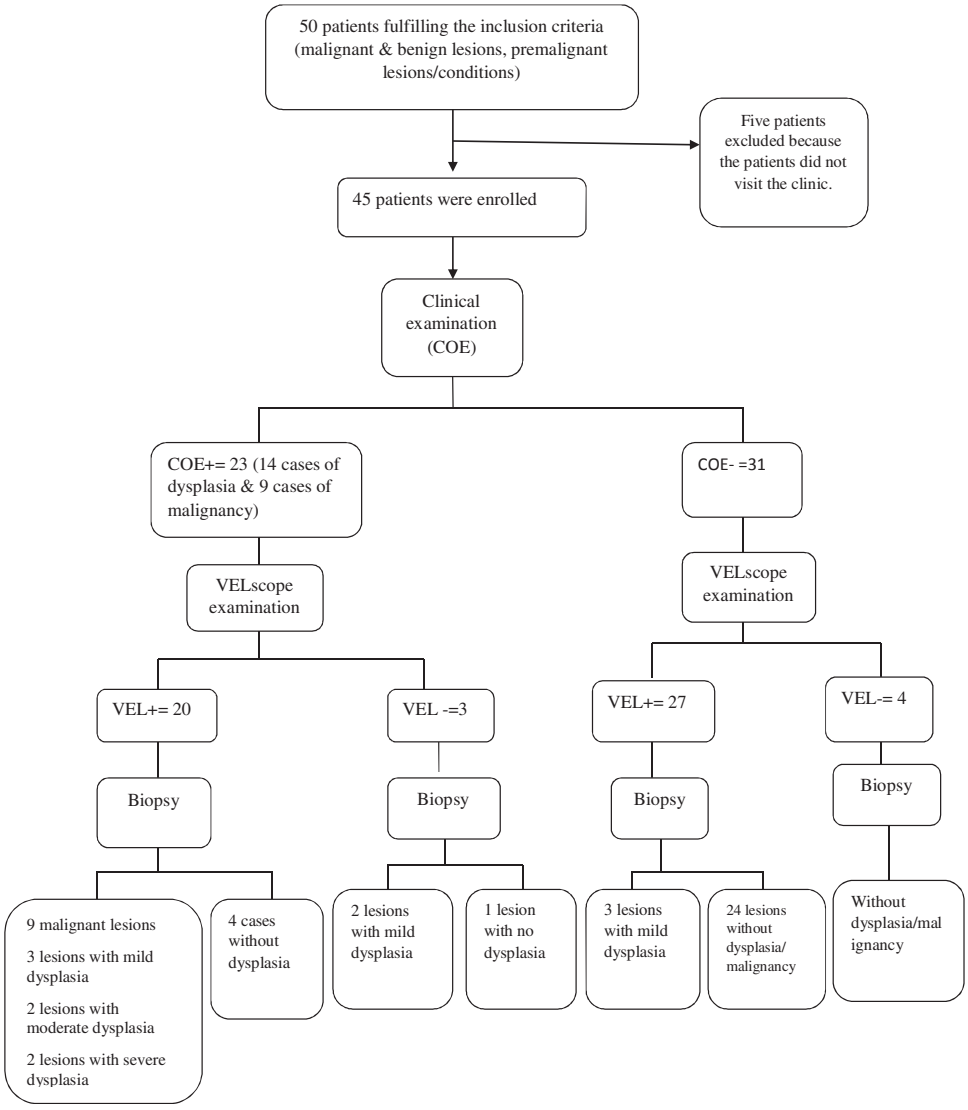

Fig. 2. Diagram of the study design.

Table 1
The demographic characteristics, risk factors, clinical appearance, and the COE and VELscope examination results of the studied cases.

Clinical & histopathological characteristics		Lesions' clinical classification				Histopathological results			
		Benign	Premalignant	Malignant	X2 test	Without dysplasia/ malignancy	With dysplasia	Insitu or malignant carcinoma	X2 test
		No (%)				No (%)			
Sex	M	7 (25)	20 (71.4)	1 (3.6)	0.018	20 (71.4)	7 (25)	1 (3.6)	0.027
	F	9 (34.6)	10 (38.5)	7 (27)		13 (50)	5 (19.2)	8 (30.8)	
Tobacco use	No use	12 (75)	20 (66.7)	7 (87.5)	0.638	22 (56.4)	9 (23)	8 (20.5)	0.637
	In the past	0 (0)	2 (6.7)	0 (0)		2 (100)	0 (0)	0 (0)	
	Active	4 (25)	8 (26.7)	1 (12.5)		9 (69.2)	3 (23.1)	1 (7.7)	
Lesions' clinical appearance	White	0 (0)	20 (66.7)	0 (0)	0.000	9 (45)	10 (50)	1 (5)	0.000
	Erythematous	1 (6.3)	5 (16.7)	0 (0)		6 (100)	0 (0)	0 (0)	
	Red & white	0 (0)	2 (6.7)	1 (12.5)		0 (0)	2 (66.7)	1 (33.3)	
	Ulcer	6 (37.5)	3 (10)	0(0)		9 (100)	0 (0)	0 (0)	
	exophitic	9 (56.3)	0 (0)	7 (43.8)		9 (56.3)	0 (0)	7 (43.8)	
Clinical suspicion for dysplasia/ malignancy	COE +	0 (0)	15 (65.2)	8 (34.8)	0.000	5 (21.7)	9 (39.1)	9 (39.1)	0.00
	COE −	16 (51.6)	15 (48.4)	0 (0)		28 (90.3)	3 (9.7)	0 (0)	
VELscope feature	VEL +	13 (27.7)	26 (55.3)	8 (17)	0.434	28 (59.6)	10 (21.3)	9 (19.1)	0.443
	VEL −	3 (42.9)	4 (57.1)	0 (0)		5 (71.4)	2 (28.6)	0 (0)	

the histopathological findings of dysplasia and malignancy, 5 (35.7%) lesions that had a clinical diagnosis of dysplasia were not dysplastic, while all clinical diagnoses of malignancy were also found to be malignant in the histopathological findings. In premalignant lesions/conditions, 12 (22.2%) lesions had dysplasia: 8 (14.8%) lesions had mild dysplasia, 2 (3.7%) lesions had moderate dysplasia, and 2 (3.7%) had severe dysplasia. None of the lesions with a benign clinical diagnosis had dysplasia in the histopathological studies. The demographic characteristics, risk factors, clinical features, and the COE and VELscope examination results are summarized in Table 1. The accuracy of the COE in identifying dysplasia or malignancy was evaluated by its sensitivity (86%) and specificity (85%) values. The PPV was calculated to be 78%, the NPV was 90%, and the PLR and NLR were 5.7 and 0.12, respectively.

The native fluorescence imaging analysis revealed native fluorescence extinction (VEL^+) in 47 (87%) lesions. However, the histopathological findings revealed that 83% of the dysplastic lesions and 100% of the malignant lesions were VEL^+. Cross table calculations showed a sensitivity of 90% and a specificity of 15% in identifying dysplasia and/or malignancy. The PPV was 40% and the NPV was 71%. The PLR and NLR were 1.06 and 0.63, respectively, for the VELscope examination.

For the mixed method in which the results of the clinical examination and the native fluorescence visualization were combined, the sensitivity to identify dysplasia and malignancy was 100%. The specificity, PPV, and NPV were evaluated as 12%, 42%, and 100%, respectively. The PLR and the NLR of the mixed method were 1.14 and 0, respectively.

The above mentioned values were also determined separately for the premalignant lesions/conditions group and the malignant lesions and premalignant lesions/conditions group. Table 2 and Figs. 3–5 show the results of the COE, VELscope examinations, and histopathological evaluations for some of the cases. In Fig. 3, the suspicious area in the COE appears dark in the VELscope visualization (LOF). In Fig. 4, a red and white plaque (erythroleukoplakia), which is suspected to dysplasia because of its palpable stiffness, revealed a dark area in the VELscope examination. Further histopathological evaluations confirmed severe dysplasia. Fig. 5 shows a false positive result of the VELscope.

4. Discussion

The early detection of premalignant oral lesions/conditions is one of the most effective approaches to reduce the complications and related mortality of oral cancer. Various screening and adjunctive diagnostic methods have been introduced to improve the diagnostic power of the COE for the early identification of oral potentially malignant disorders (OPMDs). The VELscope is an adjunctive diagnostic tool that uses direct native fluorescence visualization during an oral examination; in recent years, this device has been highly researched. The VELscope allows us to view native fluorescence and has been developed for the early detection of OPMDs and malignant lesions.

Instead of using UV and near-UV (NUV) spectrum, the manufacturer of the VELscope has rationales for selecting visible excitation light in the range of 400–460 nm. The first rationale is that UV light has several biological hazards to the eye and skin tissues and the second one is that the peak intensity of the VELscope 10 cm from the light source on axis is about100 mW/cm^2. Therefore, if the manufacturer used UV or NUV, the minimal erythema dosage of 10 mW/cm^2 for 300 nm radiation, as set by the American Conference of Governmental Industrial Hygienists, would be exceeded [24,38].

Manufacturers of the VELscope claim that it is possible to notice the histological changes that occur during carcinogenesis. Some of these changes, such as the destruction of collagen and elastin, an increased nucleus-to-cell ratio, and an increased tissue blood flow, appear as a LOF or as a dark view during the VELscope examination. Although recent studies have investigated the diagnostic value of using the VELscope to discover OPMDs and oral cancer, the findings have been contradictory. In the current study, 54 lesions in 45 patients were examined. Lesions were clinically diagnosed as benign, malignant, or OPMD using the COE and the VELscope.

4.1. Sensitivity

In our study, the VELscope gave few false negative results and, thus, demonstrated high sensitivity (90%) in determining dysplasia/malignancy. Most previous research acknowledged the high sensitivity of the VELscope in identifying high-risk lesions [26,27,33,39,41,42]. However, other studies, such as Paderni et al., Farah et al., and Mehrotra et al., reported a low sensitivity for this device: 75%, 30%, and 50%, respectively [10,43,44]. One of the main reasons for the low sensitivity of the VELscope may be its low power in diagnosing white lesions compared with red lesions. For example, Paderni et al. found that white patches accounted for 66% of the lesions and the VELscope sensitivity was found to be 75%, which is relatively low [10]; however, in our

Table 2
A comparison of the diagnostic power of the COE and VELscope examination in dysplasia/malignancy identification.

Diagnostic value indices		Sensitivity	Specificity	Positive predictive value	Negative predictive value
Diagnostic tests in different lesions' groups					
COE	Premalignant lesions/conditions	75%	71%	64%	80%
	Premalignant lesions/conditions & malignant lesions	81%	67%	74%	80%
	Total	86%	85%	78%	90%
VELscope	Premalignant lesions/conditions	83%	12%	40%	50%
	Premalignant lesions/conditions & malignant lesions	90%	12%	56%	50%
	Total	90%	15%	40%	71%
Mixed method	Premalignant lesions/conditions	100%	11%	43%	100%
	Premalignant lesions/conditions & malignant lesions	100%	6%	57%	100%
	Total	100%	12%	42%	100%

study, white plaques/patches had a lower prevalence (38%) and, thus, a higher sensitivity of the VELscope was obtained (90%). In the studies by Farah et al. and Mehrotra et al., the clinical view of the lesions was not mentioned and, therefore, no interpretation can be made [43,44].

In studies in which the VELscope showed high sensitivity, there was a high prevalence of erythematous, red and white, and ulcerous lesions [39]. In our study, the prevalence of erythematous, red and white, and ulcerous lesions was 54% (29 lesions). In a study by Koch et al. in which the VELscope sensitivity for OSCC or dysplasia determination was reported to be 94%, the rate of erythroplakia, erythroleukoplakia, and ulcerous lesions was 79% (61 lesions) [39]. The reason for the high sensitivity of the VELscope in erythematous lesions is related to the biological mechanism that generates the LOF view. Several agents are responsible for the LOF view in a native fluorescence examination. Some of them are caused by the destruction of the light-producing fluorophores, such as the destruction of collagen cross-linkages and elastin degradation, while others are the result of increased absorption or the scattering of the fluorescence in the tissues. In inflammatory and erythematous tissues, the destruction of structural molecules is less common; however, two factors, an increased hemoglobin concentration due to increased circulation and an increased density of chronic inflammatory cells, such as lymphocytes, result in the increased absorption and dispersion of the fluorescence along with a darkening of the lesions (the LOF view), respectively [45].

On one hand, these two factors lead to a higher identification of erythematous lesions compared with white lesions, which produces fewer false negative results. On the other hand, this causes a higher rate of false positive cases and lower device specificity. However, in the study by Scheer et al., the VELscope sensitivity was high (100%) despite the low rate of erythematous lesions (48%), which may be due to the role of other factors [32].

4.2. Specificity

A wide range of specificity has been reported in different studies [45]. This range may be due to the variation in study samples. For example, Lane et al. reported 100% specificity in a very limited study in which all cases were dysplastic or malignant [38]. However, Awan et al. reported 15.3% specificity due to the few cases of dysplasia or malignancy in their study sample [27]. Our results also showed low specificity (15%) for the VELscope in determining dysplasia/malignancy. One of the main etiologies may be the inclusion of inflammatory and ulcerous lesions in our study. The low specificity, which is a major limitation of the VELscope, has been mentioned in a number of studies [27,32,39,41,43,44,46–48]. This low specificity is due to the high rate of false positive results and is actually a reflection of its weakness in distinguishing high-risk lesions (i.e., lesions with dysplasia and malignancy) from low-risk lesions (i.e., inflammatory and benign lesions without dysplasia). To overcome this problem, the lesions were followed up for 2 weeks in several studies; therefore, if the redness and inflammation were due to the inflammatory process, the lesion could be excluded, which would result in fewer false positive results [20,32,42,49–52]. Among the aforementioned studies, only Rana et al. determined the diagnostic value of the VELscope. Thus, it cannot be clarified whether the 2-week follow-up increased the specificity of the VELscope. In several other studies, a 2-week follow-up was not done because the researchers believed that with a clinical diagnosis of a high-risk lesion, the definite diagnosis (biopsy) should not be postponed [41,43,45,46,48–51,53].

Other efforts to increase the specificity of the VELscope include the use of red/orange light irradiation from tissues for identifying dysplasia and malignancy. The red/orange spectrum originates from a NCF called protoporphyrin IX (PpIX). Because this fluorophore increases in malignant and premalignant lesions, the differentiation of these tissues from normal tissues can be done using native fluorescence [54].

Despite the identification of the biological origin of the red/orange light spectrum, there is controversy about the use of the spectrum in the detection of malignancy and dysplasia. Opponents to the use of the red/orange light spectrum believe that certain fluorophores act as confounding agents and emit the red/orange fluorescence spectrum; an example of this type of fluorophore is porphyrin, which is present in the bacteria in OSCC wounds and on the dorsal surface of the tongue and

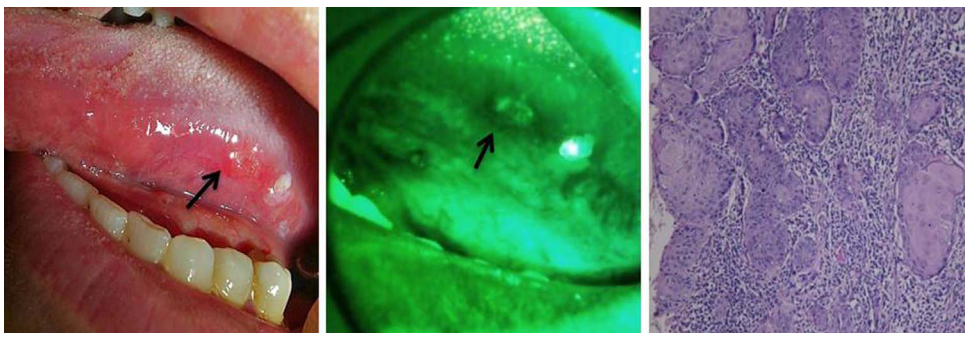

Fig. 3. Squamous cell carcinoma in lateral border of the tongue. The area with suspicious to malignancy in COE, appears dark in VEL scope imaging (LOF). (100X magnification − H&E staining).

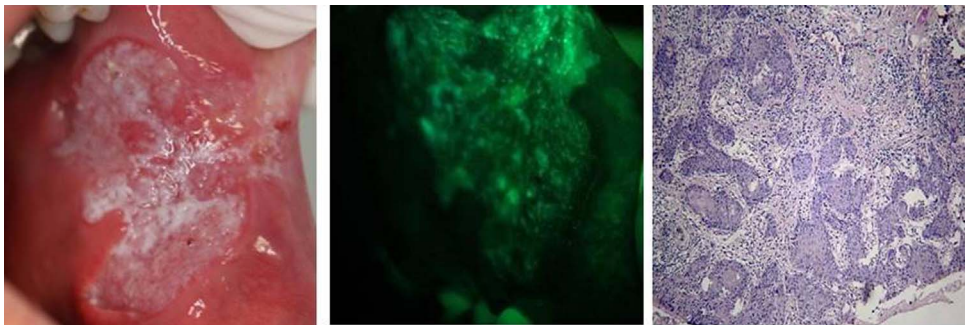

Fig. 4. A red and white plaque (erythroleukoplakia) in buccal mucosa and lip commissure. In clinical evaluation, dysplasia was proposed due to stiffness in palpation (COE +). VELscope revealed dark area and in histopathologic evaluation severe dysplasia reported. (100X magnification − H&E staining). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dental plaques. In these cases, the red/orange spectrum cannot exclusively represent dysplasia or malignancy [32,40]. Among the studies that determined the diagnostic value of the VELscope, only Koch et al. used the red/orange light spectrum to increase the specificity of the VELscope [39]; the red/orange light spectrum observed in the VELscope showed a high specificity (98%) for identifying malignancies. In the present study, in addition to LOF, we also introduced the red/orange light spectrum as a possible indicator of dysplasia or malignancy, and considered such cases to be positive (VEL^+).

Another approach that is used to improve the specificity is pressing tissues to blanch them and decreasing the blood flow. However, in a study by Farah et al., the pressure produced false negative results. Since the ideal pressure for blanching the oral mucosa has not been standardized, the results of studies that used this method were subjective [44,45].

4.3. Predictive values

In our study, the PPV and NPV for the VELscope diagnostic method in identifying dysplasia/malignancy were 40% and 71%, respectively. Therefore, if a physician suspect dysplasia/malignancy in a patient from a VELscope examination, there is a 40% possibility that the patient may have these two conditions; similarly, if the physician rules out dysplasia/malignancy using the device, the patient has a 71% chance of being normal. The PPV and NPV for the COE method were 78% and 90%, respectively. Therefore, the COE method has a higher predictive power compared with the VELscope for diagnosing a patient.

The PPV and NPV are strongly related to the disease prevalence in the community, unlike sensitivity and specificity; thus, higher disease prevalence results in a higher PPV and a lower NPV. Therefore, if we want to compare the predictive value amounts in this study with other studies, the disease (dysplasia/malignancy) prevalence in the community must be taken into consideration.

In our study, the PLRs of the VELscope, COE, and the mixed methods were 1.06%, 5.7%, and 1.14, respectively. The NLRs were 0.12, 0.63, and 0, respectively. Although none of the previous studies reported the likelihood ratio, it can be calculated using the sensitivity and specificity values and compared with the findings of the current study.

When comparing the PLR and NLR of the VELscope in this study with the PLR and NLR of studies that performed the oral CDx brush biopsy method, the latter method was superior in diagnosing dysplasia/malignancy. For example, Scheifele et al. found that the PLR and NLR of the brush biopsy method were 16.2% and 0.08%, respectively, while Delavarian et al. found that the PLR was infinite and the NLR was 0.11% [36,55]. Despite the fact that the brush biopsy has a higher diagnostic value compared with the VELscope, the benefits of the VELscope over the brush biopsy should not be overlooked. These benefits, which include its noninvasiveness, simple application, and more rapid access to results, have encouraged researchers to not dismiss the use of the VELscope in the identification of lesions.

In a systematic review on the optical detection systems in oral cancer, Rashid et al. discussed the advantages and limitations of the VELscope. They found that the device can help in decision-making for observable lesions, such as whether to biopsy or not, although using it as a screening device in apparently normal tissue is not advised [45]. Simonato et al. showed that fluorescence visualization can improve the specificity of COE from 8% to 10% in adept examiners in detecting epithelial dysplasia and OPMDs, although unskilled examiners had less of an advantage [56].

Comparing the results of these methods with the gold standard method (histopathology) showed that the sensitivity, specificity, PPV, NPV, PLR, and NLR for the VELscope to be 90%, 15%, 40%, 71%, 1.06, and 0.63, respectively. The same values were 86%, 85%, 78%, 90%, 5.7, and 0.12 for the COE method and 100%, 12%, 42%, 100%, 1.14, and 0 for the mixed method, respectively.

Similar to our study, Farah et al. used the mixed method for dysplasia and OSCC determination, which resulted in an increase in COE sensitivity from 25% to 46%. However, it was not clear whether the parallel method was used for determining the results of the mixed method [44].

Our study results showed that native fluorescence does not support the examiner in terms of further therapeutic decisions because it is not capable of distinguishing between benign mucosal lesions and malignant ones. However, based on the high sensitivity of the VELscope, when it is used as a noninvasive adjunct to a routine oral premalignant/malignant lesions examination, it has the potential to reduce the high mortality rate associated with oral malignancies.

Fig. 5. Pyogenic granuloma of tongue. In VELscope examination appears dark (false-positive VEL +). However histopathologic evaluation confirmed clinical diagnosis (pyogenic granuloma) without dysplasia. (100X magnification − H&E staining).

5. Conclusion

This study showed that the VELscope, as an adjunctive diagnostic device, is capable of increasing the sensitivity of the COE in identifying malignant or dysplastic lesions from 86% to 100%, which is a 14% increase. However, the low specificity of the VELscope (15%) in distinguishing high-risk lesions (dysplastic) from benign ones indicates that it cannot be used in the screening of dysplastic or malignant lesions in primary health care centers. In addition, due to the high probability of false positive results, it may lead to high referral rates or unnecessary biopsies in patients. However, the use of the VELscope, especially in specialized centers with well-trained specialists, reduces the number of false positive cases and makes it possible to accurately determine the biopsy site for dysplasia/malignancy identification. Nevertheless, due to the small study population and possible errors in interpreting the examination results, further research into the diagnostic value of the VELscope is highly recommended.

Conflict of interest

The authors of this manuscript certify that they have no conflict of interest.

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