Sri Lanka Journal of Medicine **SLJM**

Perspective

Citation: Gayathry VA, Jayasinghe RD, Hettiarachchi PVKS, et al. 2024. VELscope vs TBT: A Comparison Study. Sri Lanka Journal of Medicine, pp 42-48.
DOI: https://doi.org/10.4038/sljm.v33i3.508

Visually Enhanced Lesion Scope Versus Toluidine Blue Test for Early Diagnosis of Oral Cancer and Oral Potentially Malignant Disorders: A Prospective, Diagnostic Accuracy Comparison Study

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ABSTRACT

Introduction: Early identification of dysplasia is crucial in managing oral potentially malignant disorders (OPMD). Adjunct techniques facilitate the oral examination (OE) to diagnose early oral squamous cell carcinoma (OSCC) and high-risk OPMDs. Our study aims to compare the efficacy of VELscope with Toluidine blue test (TBT) in the early detection of oral dysplasia (OD). **Materials and Methods:** A total of 51 patients clinically diagnosed with OPMD and early oral cancer were subjected to VELscope examination and TBT followed by histopathological investigation. Lesions with loss of auto-fluorescence and royal blue staining were considered as positive for VELscope and TBT respectively. Sensitivity, specificity, positive predictive (PPV), and negative predictive values (NPV) were obtained for each test and combined tests. **Results:** In 38 OPMDs, VELScope and TBT detected 12 and 10 cases as true positives and 4 and 9 cases as true negatives respectively. Out of 12 OSCCs, VELscope and TBT detected 10 and 9 cases as true positives respectively. The overall efficacies of VELscope and TBT in the diagnosis of both OPMDs and malignant lesions were as follows: 66.6% and 57.6% sensitivity, 22.2% and 50% specificity, 61% and 67.9% PPV, 26% and 39% NPV respectively. The sensitivity, specificity, PPV, and NPV of combined tests with the diagnosis of dysplasia and OSCC were 78.8%, 22.2%, 65%, and 36.4% respectively. **Conclusion:** Combined tests have more sensitivity than individual tests. Concerning the individual tests, TBT results are more reliable than the VELscope test.

Keywords: dysplasia; oral cancer; toluidine blue test; VELscope.

Received: 2024-04-30 **Accepted revised version: 2024-12-08** **Published: 13-12-2024**

INTRODUCTION

Oral cancer is defined as, cancer that forms in the tissues of the oral cavity. It is the 16th most common cancer in the world (1) and oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy. The prognosis for patients with early-stage OSCC is excellent compared to late stages. The five-year survival rate is about 80% for early-stage OSCC. Despite the advances in treatment, the prognosis for late-stage OSCC remains less than 50% (2).

OSCC is usually preceded by oral potentially malignant disorders (OPMD) such as erythroplakia, leukoplakia, and oral sub-mucous fibrosis. However, OSCC can also arise from clinically normal mucosa. Therefore, early diagnosis of OPMD and oral cancer is the key to improving the overall prognosis of OSCC. The presence and degree of dysplasia are the markers for the malignant transformation of OPMD. Dysplasia is defined as disordered cellular development which is characterized by architectural and cytological changes. Hence, histopathology investigation from a tissue obtained via an incisional biopsy is the gold standard for accurate diagnosis of dysplastic changes in the oral mucosa (3). Being an invasive procedure, most people are reluctant to undergo a biopsy. The easiest way of early detection of such lesions is conventional oral examination (COE) consisting of visual and tactile examination. However, it is challenging to identify high-risk OPMD lesions and early OSCC only by COE. In spite of the clinical experience, it is difficult to differentiate dysplastic changes from benign inflammatory changes by COE.

Approximately two-thirds of patients with squamous cell carcinoma of the head and neck presented at late stages of the disease (4). Despite the advancement in cancer therapy, late presentation of the patient and failure to detect early dysplastic changes by COE are the main reasons for the overall poor prognosis of OSCC in the last 30 years. A cross-sectional study conducted in Sri Lanka has revealed that the annual cost for treatment of stage III or IV OSCC is five times higher than for early stages (5). Consequently, early detection of oral epithelial dysplasia may help to reduce the morbidity, mortality rate, and the cost of treatment of OSCC.

On the basis of the available literature COE of oral lesions is not predictive of histologic diagnosis. A meta-analysis in 2012 reported that COE had 93% sensitivity and 31% specificity in the diagnosis of dysplasia and carcinoma (6). Therefore, adjunctive methods are necessary to facilitate COE to detect early stages of OSCC and high-risk OPMD. In the last years, many diagnostic methods have been suggested to facilitate the detection of OSCC and OPMD such as vital tissue staining with Toluidine blue, Vizilite, Visualization adjuncts tissue autofluorescence, and saliva-based oral cancer diagnostics. Tissue autofluorescence can be assessed by visually enhanced lesion scope (VELscope) and InVivo confocal microscopy (7).

Vital tissue staining defines the staining of vital cells or tissues in the living state. It can be done either as intra-vital staining which is in the living body or supra-vital staining which is used for slide preparation of detached cells. The presence of dysplasia can be detected by vital tissue staining with Toluidine blue. Toluidine blue (TB) is an acidophilic metachromatic dye. It selectively stains acidic tissue components has an affinity for nucleic acids and binds to the nuclear material of tissues which has a high content of DNA and RNA. A Dysplastic cell has a high nuclear/cytoplasm ratio and nuclear hyperchromatism. Therefore, TB can be used to stain dysplastic cell nuclei. TB was first applied by Reichart in 1963 for vital staining of uterine cervical carcinoma in situ (8).

Autofluorescence is the emission of light by biological structures when they have absorbed light. There are naturally occurring fluorochromes in the oral epithelium and submucosa (e.g., Collagen and elastin) which are irradiated with different excitation wavelengths. VELscope is a hand-held device that uses blue spectrum light (400-460nm) for direct visualization of autofluorescence in the oral cavity. Tissue fluorescence is variable and it is affected by structural changes, the presence of hemoglobin, vascular dilation, and inflammation. The use of blue-spectrum light may maximize the differential profile in areas undergoing malignant changes and result in loss of fluorescence (9).

The aim of our study was to compare the efficacy of VELscope with Toluidine blue test (TBT) in the early

detection of oral dysplasia in clinically suspicious lesions.

MATERIAL & METHODS

The study was carried out at the Oral Medicine Clinic of the University Dental Hospital, Peradeniya, Sri Lanka. The study protocol was approved by the Ethics Review Committee of the Faculty of Dental Sciences, University of Peradeniya (ERC/FDS/UOP/E/2020/05).

The sample size for the study was calculated by using Burderer's formula. A total of 51 patients of age over 18 years with clinically suspicious lesions of either oral potentially malignant disorders or oral cancer were included in this study from 2019 to 2021. The entire study protocol was explained to each patient at the beginning of the study and informed written consent was obtained. Patients who have not given consent for incisional biopsy or study, patients previously diagnosed or have undergone treatment for OSCC or OPMD were excluded.

Demographic data and data regarding the details of the lesion (Site, size, shape, color, margin, and induration), habits, medical disorders, and allergies were collected. Photographic records of the suspicious lesions were obtained with patient consent. Autofluorescence of the lesions was assessed by using VELscope device. The assessment results were recorded according as: fluorescence visualization loss (FVL), fluorescence visualization retained (FVR), and photodocumentation.

After the VELscope assessment, TB staining test was performed for lesions. The test was done with following steps: First, asked the patient to rinse the mouth with water for 20s, then with 1% acetic acid for 20s, application of 1% TB solution for 20s with cotton swab. Then, asked the patient to rinse again the mouth with 1% acetic acid for 20s and then with water to remove the mechanically retained stain. Then the lesions were examined by the expert and interpreted for findings. To address the intra-examiner variability, the Calibration of 5 cases was done at different time intervals by the principal investigator under the guidance of the chief investigator.

Lesions stained with royal blue were considered as positive whereas lesions with pale blue or no stain were considered as negative. Meanwhile photo documentation of the lesions was done.

Finally, histopathological assessment of the lesions was done with an incisional biopsy. Biopsy sites were selected according to the positive results of the VELscope (FVL) and TB staining tests. Data were collected and entered into an Excel sheet and statistical analysis was done using SPSS 20 (statistical package for social sciences). Patient's flowchart is illustrated in Figure 1.

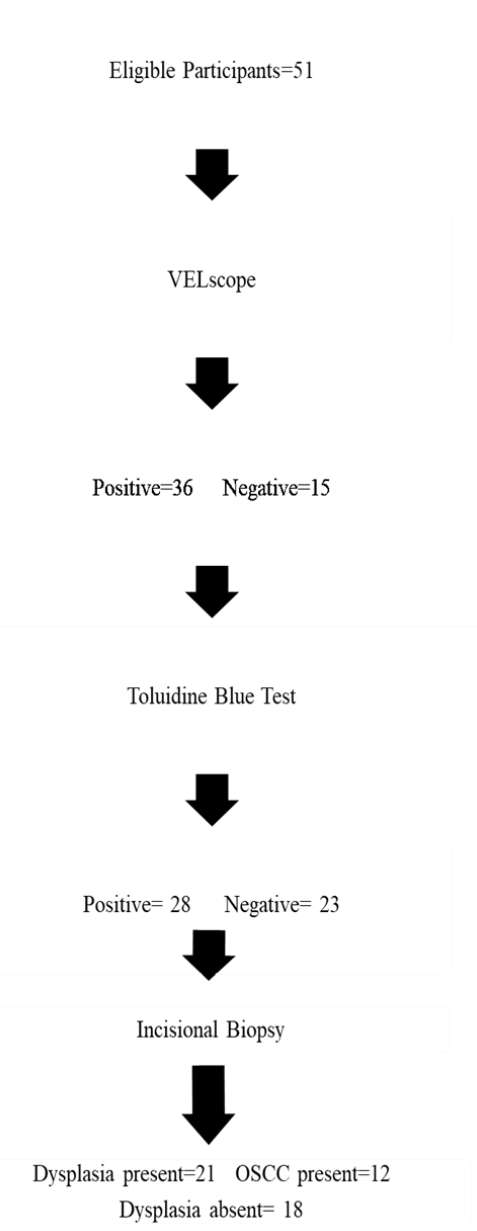

Figure 1: Patient's flowchart

RESULTS

Out of the 51 patients, 39 (76.4%) were males and 12 (23.6%) were females with the male: female of 3.25:1. Age range was 18 to 83 years. Three patients were less than 25 years and four were more than 75 years. 10 patients (20%) were in the age group of 61 to 70 years and 8 patients (16%) were in the age group of 51 to 60.

The commonest site was buccal mucosa (43%) followed by the tongue (19.6%), gingiva (7.8%), and floor of the mouth (5.8%). Histopathological diagnosis revealed 38 cases of OPMD, 12 cases of OSCC, and one case of plasma cell mucositis. In 38 cases of OPMD, dysplasia was present in 21 cases (Table 1).

Table 1: Cross-tabulation between clinical diagnosis and the results of VELscope, Toluidine Blue test and Histopathology report

Clinical Diagnosis	Number of patients	VELscope-Positive	Toluidine blue test-Positive	Dysplasia present
Leukoplakia (Homogenous)	22	13	12	16
Oral submucous fibrosis	9	8	6	3
Erythroleukopl akia	2	2	1	2
Oral lichen planus/ Lichenoid reactions	4	0	0	0
Candidal Leukoplakia	1	1	1	0

In our study, out of 38 OPMDs oral leukoplakia and OSF were diagnosed in 22 and 9 cases respectively (50% and 18.4%). Further, oral leukoplakia was most prevalent among 55-64-and 65-74-year-old age groups whereas OSF was mostly reported in 25-34- and 45–54-year-old age groups.

VELscope displayed true positives for 22 cases (12 OPMD and 10 OSCC) and true negatives for four cases. Further, VELscope displayed false positives and false negatives for 14 and 11 cases respectively. The sensitivity and specificity of VELscope were 66.6% and 22.2%. Positive predictive value and negative predictive value were 61% and 26%.

The toluidine blue test revealed true positives for 19 cases (10 OPMD and 9 OSCC) and true negatives for 9 cases. Further, the test showed false positives and false negatives for 9 and 14 cases respectively. The sensitivity and specificity of TB test were 57.6% and 50%. Positive and negative predictive values were 67.9% and 39%.

The sensitivity and specificity of the combined tests were 78.8% and 22.2%. Further, the positive and negative predictive values are 65.0% and 36.4%.

DISCUSSION

Oral cancer is considered one of the major public health problems in most of the South East Asian countries including Sri Lanka. OSCC is the most common oral cancer reported and mostly they proceed from OPMD. The global prevalence of OPMD is reported at 1-5%. However, varying prevalence has been reported in some Southeast Asian countries (Taiwan 12.7%) and Western Pacific countries (Papua New Guinea 11.7%) (10).

In Sri Lanka, oral leukoplakia and oral submucous fibrosis (OSF) are the most prevalent OPMDs and are reported as 26.2 and 4.0 per 1000 respectively (11). National oral health survey of Sri Lanka 2015/2016 reported that oral leukoplakia and OSF are most prevalent among 35-44- and 65-74-year-old age groups [3]. In contrast, our study revealed that the very young age group (25-34) also reported OSF.

Other OPMD which encountered in our study were oral lichen planus/lichenoid reaction, erythroplakia, and chronic hyperplastic candidiasis. According to the World Health Organization's classification of head and neck tumours-2017, chronic candidiasis was considered as OPMD. However, it remains controversial to confirm chronic hyperplastic candidiasis as OPMD and needs more scientific evidence (12).

The risk of development or malignant transformation of OPMD depends on several factors. Although there is no consensus, several

literatures reported some clinical and histopathological factors as risk factors. Clinical factors are female gender, heavy alcohol/tobacco/areca nut consumption, genetic disorders, clinical type of OPMD (non-homogenous leukoplakia), site and size of the lesion (>200mm2), nutritional status, immune and oral hygiene status, microbes (Human papillomavirus, Epstein-Barr virus, and Candida) (13 14). Histopathological factors include type of OPMD, degree of dysplasia, and involved/close margin of the excisional biopsy (13).

There are different theories available that describe the association between candida infection and oral carcinogenesis (15). In the literature, candida infection was reported in 13.5% of oral leukoplakia cases (2). In this study, candida superinfection was confirmed in three oral leukoplakia cases (15.7%) and one case with oral lichen planus. In those leukoplakia cases, a moderate type of dysplasia was reported in two cases and one with severe dysplasia.

Since dysplasia is an independent predictor of malignant transformation of OPMD, it needs to be evaluated for OPMD management. Histopathological diagnosis is required for the definitive treatment. However, it may be difficult to judge a site for a biopsy of OPMD with conventional oral examination only.

Although there are several visual adjuncts assessed for the detection of OPMDs, there is limited evidence to recommend a single best visual adjunct for routine use. In this study, we assessed the accuracy of VELscope and TBT in the diagnosis of dysplastic changes of OPMD/ early OSCC.

VELscope is a non-invasive, wireless, and simple device that can be used to detect abnormal tissue changes. Several studies have been done to assess the efficacy of VELscope with varying results (15). According to a Meta-analysis done by Nallan et al., the sensitivity of VELscope varies from 30% to 100%. The specificity is ranging from 7% to 100% (16). In our study, the sensitivity is contradictory to the results of Rana et al., and Scheer et al. who found 100% sensitivity for autofluorescence examination [17 18]. Whereas, it was consistent with the studies done by Mc Namara et al., Paderni et al., and Bhatia et al where their reported sensitivities were 67%, 66%, and 64% respectively (19 20).

Regarding the specificity of the VELscope test, our results were contradictory with Lane et al., who found 100% specificity whereas coincides with Hanken et al., Felix et al., and Awan et al., who found very low specificity 33%, 16%, and 15% respectively (21 22 23 24). Although some studies showed very high sensitivity and high specificity for the VELscope test in diagnosing OPMD, according to our study, there is a higher probability of overtreating patients with OPMD than undertreatment (16).

With regard to the toluidine blue test, Rosenberg et al., have reported the sensitivity and specificity of TBT in their meta-analytic study as ranging from 97.8% to 93.5% and 92.9% to 73.3% respectively (25). According to Jayasinghe et al, the reported sensitivity and specificity of TBT were 68.3% and 63.1% respectively (26). Our results were more consistent with the later study (17). On the other hand, Parakh et al., have shown in their case-control study that TBT has higher sensitivity and specificity for malignant lesions than OPMD (27). Their study demonstrated that TBT has 92% sensitivity and 82% specificity for malignant lesions whereas 61% sensitivity and 80% specificity for OPMD. In contrast, our results revealed that TBT has a higher sensitivity for OPMD (90.9%) than malignant lesions (75%). According to our study, the sensitivity was higher when both tests were combined whereas the specificity was lower than the TBT alone.

CONCLUSION

From the results of our study VELscope and TBT tests can be useful as visual adjuncts for the conventional oral examination when both tests were combined. When comparing the individual tests, TBT is more reliable than VELscope. However, the histopathological investigation is the most reliable investigation for the diagnosis of dysplasia in OPMD and OSCC. Small sample size was a limitation factor in our study therefore, further comparative study is recommended to obtain a proper conclusion.

Author declaration

Acknowledgement
Dr. S. P. Abeysundara for carrying out the statistical analysis of the study.

Authors' contributions:
Conceptualization: RDJ; Data Collection: VAG, WMDLW; Data Analysis: VAG, RDJ, PVKSH, BSMSS; Writing—Original Draft Preparation: VAG, RDJ, PVKSH; Writing—Review and Editing: VAG, RDJ, PVKSH, BSMSS. Access to all of the raw data of the study: VAG, RDJ. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest:
The authors declare that there is no financial or non-financial conflict of interest.

Funding statement:
Self-funded.

Ethics statement:
The study protocol was approved by the Ethics Review Committee of the Faculty of Dental Sciences, University of Peradeniya (ERC/FDS/UOP/E/2020/05). Informed written consent was taken from the participants.

Statement on data availability:
Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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