



Comparative Study on the Efficacy of Tissue Autofluorescence (Visually Enhanced Lesion Scope) and Toluidine Blue as a Screening Method in Oral Potentially Malignant and Malignant Lesions

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Objective and Study Design: Early identification of high-risk disease could greatly reduce both mortality and morbidity due to oral cancer. Hence, screening of such lesions and their early detection could improve prognosis. Metachromatic dye toluidine blue, which stains nuclear material in dysplastic epithelium, is widely used for screening of potentially malignant lesions. Visually enhanced lesion scope (VELscope) that identifies reduction in tissue fluorescence in dysplasia can also prove to be effective in screening for potentially malignant lesions. Hence, this study was done to compare the reliability of VELscope and toluidine blue as a screening tool in the diagnosis of malignant and potentially malignant lesions as compared to histopathological diagnosis which is gold standard. **Materials and Methods:** 90 patients with tobacco associated hyperkeratotic red and white lesion, ulcerative lesion, and frank malignancy included in study. Patients were subject to Tissue Autofluorescence (Visually Enhanced Lesion Scope) and Toluidine Blue stain tests. Nature of Autofluorescence and Toluidine Blue staining of tissue was recorded. Based on findings tissue was biopsied and examined under microscope from confirmatory diagnosis. **Results:** In this study, ninety patients with malignant and potentially malignant lesions were studied, VELscope showed 85.36% sensitivity and 75% specificity as compared to toluidine blue which showed 83.13% sensitivity and 87.5% specificity. The results were statistically significant ($P < 0.001$) with a mean deviation of ± 3 . **Conclusion:** From the study, we concluded that VELscope was more effective as a screening tool for the detection of oral malignant lesions as compared to toluidine blue.

Key words: Visually enhanced lesion scope, toluidine blue, dysplasia, potentially malignant and malignant lesions

INTRODUCTION

Oral malignant lesion has been found to be the most common head and neck malignancy in India. It is observed that the 5-year survival rate of oral squamous cell carcinoma patients has remained relatively low, only approximately 50%–60%, and the rate is even lower when the patients are diagnosed at the later stages of the disease.¹ Hence, screening of individuals at risk for malignant lesion and its precursor has potential for early detection and treatment, thereby improving survival.

Previously clinicians relied basically on case history, visual examination, and their experience in diagnosing oral

pre-malignancies and malignancies. Scientific advances have taken place and a number of screening aids are now available, namely, toluidine blue, Lugol's iodine, exfoliative cytology, tissue autofluorescence spectroscopy, chemiluminescence, and visually enhancing lesion scope (VELscope).

Toluidine blue has been successfully tried out as a screening agent in detecting dysplastic changes in various lesions. VELscope which has been recently introduced utilizes the property of autofluorescence to help in the diagnosis of dysplastic changes. The purpose of this study was to compare

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Interpretation of toluidine blue staining

the efficacy of toluidine blue and VELscope in the detection of early dysplastic lesions using biopsy and histopathology as “gold standard.”

Principle of working

Tissue autofluorescence (VELscope) imaging involves the exposure of tissue to a rather specific wavelength of light through multiuse device with handheld scope by the clinician which results in autofluorescence of cellular fluorophores after excitation. The presence of cellular alteration changes the concentration of fluorophores which will affect the scattering and absorption of light in the tissue, thus resulting in change in color that can be observed visually. It detects the loss of fluorescence in visible and nonvisible high-risk oral lesions by applying direct fluorescence.² It consists of a source of light that emits a wavelength of 400–460 nm and a manual unit for direct visualization. Under the intense blue excitation, normal oral mucosa emits a pale green autofluorescence in contrast to abnormal or suspicious tissue that exhibits decreased level of normal autofluorescence and appears dark in comparison to the surrounding healthy tissue.^{2–4} Hence, early biochemical changes are detected before they are evident clinically, permitting the early detection of pathological lesions.²

Toluidine blue is a metachromatic vital dye that may bind preferentially to tissues undergoing rapid cell division (such as inflammatory, regenerative, and neoplastic tissue); to the sites of DNA change associated with oral premalignant lesion or both. The binding results in the staining of abnormal tissue in contrast to adjacent normal mucosa.^{5–7}

The basic aim of this study was to compare the reliability of VELscope and toluidine blue as a screening tool in the diagnosis of malignant and potentially malignant lesion as compared to histopathological diagnosis which is gold standard.

MATERIALS AND METHODS

This study comprised ninety patients with tobacco-associated hyperkeratotic red and white lesion, ulcerative lesion, and frank malignancy. Suspicious-looking red and white lesions [Figure 1] without any history of tobacco were also included in this study. Patients who presented with hypermelanotic lesion associated with tobacco habit were excluded from the study. As it has been reported that hypermelanotic lesion interferes with autofluorescence and likely to give rise to false-positive result,⁸ patients presenting with hyperkeratotic red and white lesions without tobacco association and/or lesions that have little or no malignant potential were also excluded from the study. The selected patients were subjected to a thorough history with special stress on tobacco-associated habit. Clinical diagnosis of lesion was made which was confirmed with histopathological examination. Pretest photograph of lesion in all the patients was obtained. Patients were screened with VELscope, and the nature of fluorescence was noted down and photographically recorded.

Interpretation of visually enhanced lesion scope fluorescence

Greenish fluorescence emanating from site was considered as indicative of normal and healthy mucosa. Dark/brownish color fluorescence observed through VELscope was considered as positive for dysplastic changes within the mucosa [Figure 2]. Dysplastic tissues with significant keratinization (leukoplakia) can exhibit increased whiteness with loss of fluorescence (darkness) around the periphery of the lesion.^{9,10} All the cases which appeared dark or brownish under VELscope were subjected to blanch test which involves rubbing and pressing lesion with mirror end and rescrutinizing



Figure 1: Chronic ulcer at the right posterior buccal mucosa

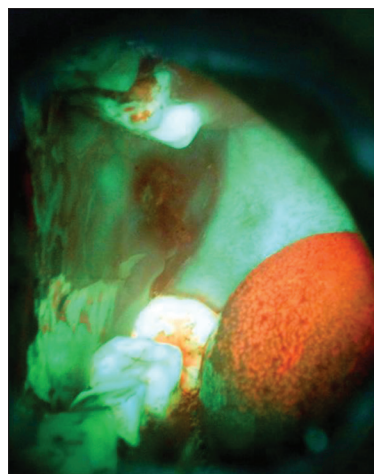


Figure 2: Lesion showing loss of autofluorescence

the lesion. If greenish fluorescence appears after this test, such lesion was considered normal or inflammatory and not associated with dysplasia. If the lesion appears brownish even after blanching test, it was so recorded and considered positive for dysplasia.

After completion of VELscope examination and photograph of fluorescence was recorded for documentation, the lesion was subjected to standard 1% toluidine blue staining. Dark blue (royal or navy) staining of lesion was considered positive for dysplasia [Figure 3]. No staining or light blue staining of lesion was considered as negative for dysplasia. After scrutinizing photographic evidence of VELscope finding and toluidine blue testing, sites for surgical biopsy were decided. Incisional biopsy was done, and biopsy sites were selected based on fluorescence loss and/or toluidine blue stain test. In case both the VELscope and toluidine blue test were negative, the site for biopsy was dictated by clinical examination. The dysplastic lesions were graded according to the number of dysplastic features exhibited into mild, moderate, and severe dysplasia [Figure 4].

Statistical analysis

All the observations obtained after applying both screening tests were drawn into tabulated form and subjected to statistical analysis using Chi-square test ($P < 0.05$), and the association of VELscope and toluidine blue with histopathological findings was established.

RESULTS

The study comprised ninety patients in the age group of 22–70 years. Among ninety patients, 75 (83.33%) were males and 15 (16.67%) were females. Among the study population, 41.11% were smokers and 58.88% were nonsmokers. Buccal

mucosa was the most common site, 70 cases (77.77%), followed by 7 (7.77%) cases in lateral border of tongue and 6 cases (6.66%) in labial mucosa, 4 cases in palatal mucosa, and 1 case was seen in the floor of mouth. The association of age ($P = 0.682$, $P = 0.1$), sex ($P = 0.364$, $P = 0.213$), and site of lesion ($P = 0.637$, $P = 0.133$) with VELscope and toluidine blue stain was statistically insignificant.

Out of 56 potentially malignant lesions, 34 were speckled leukoplakia, 18 were homogeneous leukoplakia, 3 were verrucous leukoplakia, and 1 was erythroplakia. Out of 34 malignant ulcers, 21 were ulcerative lesion, 5 were proliferative growth, and 7 were early cancer (erythematous nonhealing ulcer with granular surface). Out of 56 cases of clinically diagnosed potentially malignant lesions, VELscope detected overall 39 cases as true positive, 4 cases as true negative, 1 case as false positive, and 12 cases as false negative with sensitivity of 76.6% and specificity of 80% with positive predictive value (PPV) as 97.5% and negative predictive value (NPV) as 25%. Out of 34 clinically diagnosed malignant lesions, 31 were true positive, 2 were true negative, 1 was false positive, and none

Table 1: Summary of VELscope and histopathological findings in malignant and potentially malignant lesions

Clinical finding	VELscope/ Histopathology				Validity in %				Chi square test
	TP	TN	FP	FN	Se	Sp	PPV	NPV	
N ₁ =56	39	4	1	12	76.6	80	97.5	25	0.001*
N ₂ =34	31	2	1	0	100	67	96.8	100	
Total=90	70	6	2	12	85.36	75	97.22	37.33	

*Statistically significant values ($P < 0.05$) for Chi-squared test.
N₁=potential malignant lesion; N₂=malignant lesion; TP=true positive; TN=true negative; FP=false positive; FN=false negative; Se=sensitivity; Sp=specificity; PPV=positive predictive value; NPV=negative predictive value



Figure 3: Lesion showing positive staining of toluidine blue dye

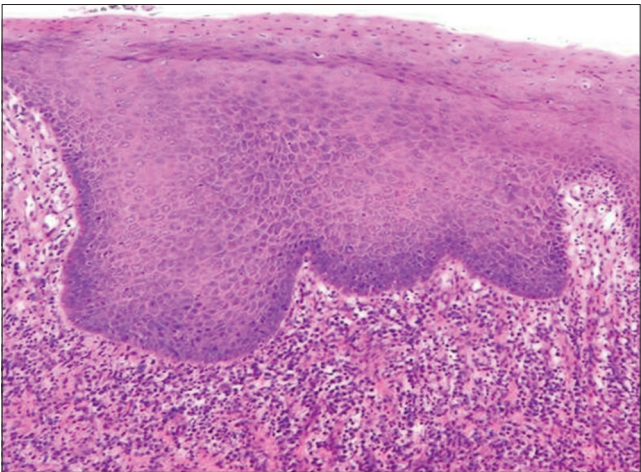


Figure 4: Regional tissue showing epithelial dysplasia

Interpretation of toluidine blue staining

was false negative with sensitivity of 100% and specificity of 67% and PPV of 96.8 and NPV of 100%. The overall efficacy of VELscope in both potentially malignant and malignant lesions was as follows: 85.36% sensitivity and 75% specificity with 97.22% PPV and 37.33% NPV. The results were statistically significant ($P < 0.001$) with mean deviation of ± 3 [Table 1].

Out of 56 cases of clinically diagnosed potentially malignant lesions, toluidine blue stain detected 39 cases as true positive, 5 cases as true negative, no case as false positive, and 12 cases as false negative. In potentially malignant lesions, toluidine blue showed sensitivity of 76.5% and specificity of 100% with 100% PPV and 45% NPV. Similarly, out of 34 cases of clinically diagnosed malignant lesions, 30 cases were true positive, 2 cases showed true negative, 1 case showed false positive, and 1 case showed false negative. In malignant lesions, toluidine blue showed sensitivity of 97% and specificity of 67% with 96.77% PPV and 66.66% NPV. Comparing the results of VELscope with toluidine blue as a screening tool in the diagnosis of potentially malignant and malignant lesions, it showed 85.36% of sensitivity and 75% of specificity as compared to toluidine blue which showed 83.13% of sensitivity and 87.5% of specificity. The results were statistically significant ($P < 0.001$) with a mean deviation of ± 3 [Table 2].

In this study, 76 out of 90 cases showed findings of VELscope and toluidine blue coinciding with each other. Keeping the above fact in mind, it was decided to tabulate the result of two screening modalities into a single table and the following results were observed. Out of 76 cases, 65 were true positive, none were false positive, 4 cases were true negative, and 7 cases were false negative. Overall sensitivity of this combination was 85.56% and specificity was 100% with 100% of PPV and 36.36% of NPV. The results were statistically significant in both potentially malignant lesions ($P = 0.046$) and malignant lesions ($P < 0.001$) with mean deviation of ± 3 [Table 3].

DISCUSSION

This study was conducted with the intention of ascertaining the efficacy of VELscope and toluidine blue as a screening tool in the diagnosis of potentially malignant and malignant lesion. With this aim, ninety cases of potentially malignant and malignant lesions having malignant potential were subjected to VELscope examination followed by toluidine blue stain test. After these two examinations, patients were subjected to histopathological examination (gold standard).

In our study, the overall sensitivity and specificity of VELscope in the screening of oral potentially malignant and malignant lesion were 85.36% and 75%, respectively. The PPV

Table 2: Summary of toluidine blue test and histopathological findings in potentially malignant and malignant lesions

Clinical finding	Toluidine blue/ Histopathology				Validity in %				Chi square test
	TP	TN	FP	FN	Se	Sp	PPV	NPV	
N ₁ =56	39	5	0	12	76.5	100	100	45	0.001*
N ₂ =34	30	2	1	1	97	67	96.77	66.66	
Total=90	69	7	1	13	83.13	87.5	98.57	35	

*Statistically significant values ($P < 0.05$) for Chi-squared test. N₁=potential malignant lesions; N₂=malignant lesions; TP=true positive; TN=true negative; FP=false positive; FN=false negative; Se=sensitivity; Sp=specificity; PPV=positive predictive value; NPV=negative predictive value

Table 3: Summary of combine VELscope and toluidine blue test findings in potentially malignant and malignant lesions

	Histopathology		Total	Chi square test
	Positive	Negative		
VEL/Toluidine blue				
Positive	65	0	65	0.046*
Negative	7	4	11	
Total	72	4	76	
Se	-	-	85.56%	
Sp	-	-	100%	
PPV	-	-	100%	
NPV	-	-	36.36%	

*Statistically significant values ($P < 0.05$) for Chi-squared test

was 97.22% and NPV was 37.33%. The diagnostic accuracy of the test was 84.44%. The results of our study are in agreement with the results of a study done by Rehman and Ingole *et al.* 2010, Svistun *et al.* 2004, Awan *et al.* 2011¹³, Onizawa *et al.* 1999,¹⁴ and Kulapaditharom and Boonkitticharoen 2001 who also reported roughly similar sensitivity and specificity in their study.¹⁵

In our study, it was found that homogeneous leukoplakia having hyperkeratotic white lesion when examined under blue light of VELscope showed gain of fluorescence giving rise to dense whitish appearance. Such findings have also been reported by Truelove *et al.*¹⁰ and Scheer *et al.*¹⁶ in their studies. This could probably explain the comparatively lower sensitivity in our study. The low specificity in our study may be attributed to the fact that, during the VELscope examination, loss of fluorescence visualized as dark area is primarily due to epithelial change blocking the transmission of light. However, this can also be due to other factors such as high vascularization and high content of fibrous tissue. Such contingency can give rise to increased chances of positive VELscope test not substantiated on histopathology.⁸ Furthermore, there is

intra- and inter-operator variability in the actual assessment of VELscope findings and its ability to distinguish between oral malignant and other abnormal tissue. This can possibly explain the reason for comparatively high false-positive rate and low specificity with VELscope in this study.¹⁷

Overall sensitivity and specificity of toluidine blue in the screening of oral potentially malignant and malignant lesions were 83.13% and 87.5%, respectively. The PPV was 98.57% and NPV was 35%. The diagnostic accuracy of the test was 84.44%. Results of our study are in agreement with the results of the study by Rahman *et al.*, 2012 (sensitivity of 81.3% and specificity of 66.67%),¹⁸ Warnakulasuriya and Johnson 1996 (sensitivity of 79.5% and specificity of 62%),¹⁹ Onofre *et al.*, 2001 (sensitivity of 77% and specificity of 67%),²⁰ Epstein *et al.*, 1992 (sensitivity of 92% and specificity of 67%),²¹ Allegra *et al.*, 2009 (sensitivity of 96.2% and specificity of 77%),²² and Cancela-Rodríguez *et al.*, 2011 (sensitivity of 67% and specificity of 73.3%).²³

The reason for low sensitivity (76%) in our study could probably be explained by the fact that the homogeneous leukoplakias with thick hyperkeratotic patch result in decreased penetration of dye into the deeper layers of epithelium where dysplastic changes are more likely to be present. Therefore, this results in high false-negative rate, thereby low sensitivity. Similar findings were also reported by Mubeen 2011 (sensitivity of 50% and specificity of 83.3%)²⁴ and Miller *et al.*, 1988 (false negative rate of 27.8%) in their study.²⁵

When both screening test results were tabulated into a single table, it was found that the sensitivity of combined screening modality (85.56%) is more than VELscope (76.6%) and toluidine blue (76.5%) when used alone. The specificity of combined screening modality (100%) is higher than VELscope (75%) and toluidine blue (87.5%). The overall diagnostic accuracy of combined screening test is 82.2%, which is more compared to VELscope (77%) and toluidine blue (78.5%) when used alone.

Results of our study are in agreement with the findings of study by Nagaraju *et al.*²⁶ who found sensitivity to be 93%, the specificity to be 80%, and the PPV and NPV to be 98% and 50%, respectively. The overall diagnostic accuracy of Lugol's iodine when used consecutively with toluidine blue stain in distinguishing potentially malignant lesions and malignant lesions was 90%.

CONCLUSION

In this study, it was found that VELscope was more effective as a screening tool for the detection of oral malignant lesions as compared to toluidine blue. Accuracy of interpreting

this screening test can be enhanced by examining the patient in dark and this probably would limit the use of VELscope in clinical setting.

Toluidine blue was more effective in the detection of oral potentially malignant lesions compared to VELscope. The diagnostic accuracy of VELscope was comparable to toluidine blue and both were found to be effective screening tools in the diagnosis of potentially malignant and malignant lesions and will prove to be of positive benefit to the clinician faced with the task of diagnosing potentially malignant and malignant lesions.

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Conflicts of interest

There are no conflicts of interest.

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