Adjunctive Utility of Toluidine Blue in Detecting Dysplastic Cells in Oral Mucosal Lesions in Comparison with Histopathology

K.M. Chandrani Somaratne[1,*], S.A.K.J. Kumara[2], R.M.N.D. Ratnayake[3], Priyantha Liyanage[4] and N.A.A.P.D. Gunasekera[5]

[1]*Department of Histopathology, District General Hospital, Gampaha, Sri Lanka*

[2]*Department of Oral and Maxillofacial Surgery, District General Hospital, Gampaha, Sri Lanka*

[3]*Department of Histopathology, District General Hospital, Kegalle, Sri Lanka*

[4]*Directorate of Health care Quality and Safety, Ministry of Health, Colombo, Sri Lanka*

[5]*Department of Histopathology, District General Hospital, Gampaha, Sri Lanka*

Abstract: *Introduction*: Oral cancer is one of the most common cancers globally and in Sri Lanka, which follows premalignant lesions. It is curable if it is detected early. Several adjunctive methods to diagnose premalignant lesions early are available. Among these, Toluidine blue staining method before a biopsy is currently receiving much attention.

Method: This is a prospective study done by studying 103 patients presented to the Oral and Maxillofacial Surgery Unit, District General Hospital, Gampaha, Sri Lanka. The oral lesions of all the patients are categorized as benign, premalignant, and malignant by clinical examination. Toluidine Blue mouth wash is introduced to all the patients, followed by biopsy from the stained sites and the clinically decided sites in non-stained lesions. Histopathological diagnosis was obtained for all cases. The accuracy of diagnosis of premalignant, malignant, and benign cases by clinical assessment and by using Toluidine blue was assessed and compared statistically in relation to sensitivity, specificity, positive predictive and negative predictive values, and likelihood ratios (LR).

Results: Toluidine blue has no added advantage over clinical examination in our setup even though it might be helpful in screening. However, it has an added value to confirm clinically benign cases as benign.

Conclusion: Toluidine Blue can be used as an adjunct in screening and to confirm clinically benign cases so that those can be followed up in clinics without doing unnecessary biopsies.

Keywords: Toluidine blue, Oral squamous cell carcinoma, Epithelial hyperplasia, dysplasia, biopsy.

INTRODUCTION

Oral cancer is one of the most common cancers globally, with marked geographic variance in incidence of occurrence. In Sri Lanka, Oropharyngeal cancers account for 9.7% of total malignancies and are the most common malignancy among males and are the 9th among females [1]. According to the National Cancer Control Program statistics in Sri Lanka, 2015, the age standardized incidence rate of lip, tongue, and mouth cancers was 15.8% in males and 4.1% in females. According to the Globocan statistics, in 2021 March, the age-standardized incidence rate is 9.7 (16.5 in males and 3.8 in females), and the mortality rate is 4.5 per 100 000 populations. The range of frequency of oral cancer varies from negligible in Japan to 45% in Sri Lanka and other Asian countries [2].

Oral cancer is associated with genetic and many environmental factors, and among those, beetle chewing and alcohol consumption are important, which are higher in Southeast Asian countries and Sri Lanka.

Oral cancer often occurs following premalignant lesions, sometimes progressing to cancer. Oral cancer is curable if detected early. The early detection of oral mucosal epithelial dysplasia could sometimes halt the progression of the lesions into malignant transformation.

The clinicians rely on clinical examination to decide when and where the biopsies are to be taken from suspected oral lesions. However, sometimes the oral premalignant lesions may be asymptomatic or display a benign clinical appearance making it difficult for the clinicians to differentiate. In some instances, repeated biopsies are required when the clinical picture is not compatible with the histology report. At the same time, the biopsy site selection may be difficult in patients who have undergone previous surgery and or radiation therapy to the site.

Several adjunctive diagnostic agents are available. Toluidine blue, a basic metachromatic dye, as a mouth wash, is currently receiving much attention and is the most comprehensively studied method. Toluidine blue binds preferentially to DNA and RNA rich cells

*Address correspondence to this author at the Department of Histopathology, District General Hospital, Gampaha, Sri Lanka;
E-mail: chandranisomaratne@gmail.com

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undergoing rapid cell division such as inflammatory, degenerative, and neoplastic tissues [3].

According to literature, Toluidine 1% allows the clinicians to select the specific sites to be biopsied more reliably. So, it increases the positivity rate of dysplasia or malignancy in histopathology reports. The gold standard for diagnosis of dysplasia is histopathological examination. Therefore, routine Toluidine blue mouth wash will facilitate early and easy detection of premalignant lesions.

Several studies have been carried out to establish the diagnostic role of Toluidine blue in detecting oral premalignant and malignant lesions. They have recorded a varying sensitivity and specificity in 93.5 – 97.8% and 73.3 – 92.9%, respectively [4-7].

Our study aimed to determine the diagnostic efficacy of Toluidine blue in selecting benign and premalignant or malignant lesions in the routine outpatient setup. In addition, we aimed to assess the efficacy of toluidine blue to decide the most representative biopsy site in larger lesions and in patients who have undergone previous surgery and radiotherapy where the clinical appearance is different.

In the meantime, we assessed the sociodemographic characteristics of the study population and the influence of some of the other pathological processes that give Toluidine blue staining in oral lesions.

MATERIALS AND METHOD

The study group comprised 103 patients above 18 years who visited the Oral and Maxillofacial Surgery Clinic at District General Hospital, Gampaha, Sri Lanka, for eleven months with clinically visible lesions in the oral cavity. Pregnant, lactating, and mentally subnormal patients and oral lesions following trauma were excluded from the study.

All the patients were registered in the clinic and examined by the consultant Oral and Maxillofacial Surgeon. The patients were grouped as benign, premalignant, and malignant and recorded. A biopsy was planned under local anesthesia.

Before the biopsy, with the informed written consent of the patients, all the patients were given 1% Acetic acid mouth wash for 20 seconds after rinsing the mouth with water. After that 1%, Toluidine blue is applied to the lesion with a cotton swab followed by Acetic acid mouth wash to remove the extra stain. The staining is recorded as positive or negative irrespective of the intensity of the stain, and the biopsy was taken from the stained sites. In non-stained lesions, the site of the biopsy was decided clinically. All the biopsies were sent to the Department of Histopathology and were assessed by two Histopathologists independently. All the results were grouped into the same clinical categories as above. The presence of additional histopathological features such as epithelial hyperplasia and heavy inflammation were recorded.

As in past studies, we also experienced that Toluidine blue mouthwash is harmless and did not develop allergic or any other reactions in the study group.

The data were analyzed by the Statistical package for social sciences (SPSS) method.

RESULTS

The sample consisted of 103 patients, and most patients, 45 (44%), were between the age of 61-80 years, and 75 (73%) patients were males.

Table 1: Distribution of the Sample According to Some Sociodemographic Characteristics

Age group	Total number	Percentage	Male	Female
<20	2	1.9%	0	2
20-40	8	7.7%	5	3
41-50	17	16.5%	10	7
51-60	30	29%	22	8
61-80	45	44%	37	8
>80	1	0.9%	1	0
Total	103	100%	75	28

Table 2: Distribution of the Sample According to the Histological Diagnosis

Histological diagnosis	Total	Percentage
Malignant	36	35%
Premalignant	19	18%
Benign	48	47%
Total	103	100%

(35%) of all the samples were histologically diagnosed as malignant, while 47% of biopsied samples were histologically benign.

Table 3: Accuracy of Clinical Diagnosis in Benign (Negative) and Premalignant or Malignant (Positive) Lesions

	Histological Diagnosis		
Clinical Diagnosis	True positives 50	False positives 23	Total 73
	False negatives 5	True negatives 25	30
	Total 55	48	103

This table shows the accuracy of clinical diagnosis in malignant and benign lesions in a sample of test results of 103 patients. The sensitivity of the test is 93%, and the specificity is 50%. The positive predictive value is 68%, and the negative predictive value is 86%. There is a 1.86 times higher chance of a patient with positive results having the disease than patients with negative test results (Positive likelihood ratio = 1.86). The chance of a person with negative results having the disease (negative likelihood ratio) is 0.14. (-LR = 0.14)

Table 4: Accuracy of Toluidine Blue Staining in Diagnosis of Benign (Negative) and Premalignant or Malignant (Positive) Lesions

	Histological Diagnosis		
Staining Diagnosis	True positives 51	False positives 24	Total 75
	False negatives 4	True negatives 24	28
	Total 55	48	103

This table shows the accuracy of staining in diagnosis in malignant and benign lesions in a sample of test results of 103patients. The sensitivity of the test is 91% and 52% specificity. The positive predictive value is 68%, and the negative predictive value is 86%. There is a 1.86 times higher chance of a patient with positive results having the disease than patients with negative test results (Positive likelihood ratio = 1.86). The probability of a person with negative results having the disease (negative likelihood ratio is 0.1703 (-LR = 0.1703).

Table 5: Accuracy of Clinical Diagnosis in Benign (Negative); Malignant and Premalignant (Positive) Lesions who had Undergone Previous Surgery and / or Radiotherapy

	Histological Diagnosis		
Clinical Diagnosis	True positives 10	False positives 5	Total 15
	False negatives 0	True negatives 0	0
	Total 10	5	15

This table shows the accuracy of clinical diagnosis in malignant lesions in a sample of test results of 14 patients who have undergone previous surgery and/or radiotherapy. The sensitivity of the test is 100%, and the positive predictive value is 67%.

Table 6: Accuracy of Toluidine Blue Staining in Diagnosing Malignant Lesions who had Undergone Previous Surgery and/ or Radiotherapy

	Histological Diagnosis		
Staining Diagnosis	True positives 10	False positives 2	Total 12
	False negatives 0	True negatives 3	3
	Total 10	5	15

This table shows the accuracy of Toluidine blue staining in diagnosing malignant lesions in a sample of test results of 14 patients. The sensitivity of the test is 100% and the specificity 60%. The positive predictive value is 83%, and the negative predictive value is 100%. There is 2.5 times higher chance of a patient with positive results having the disease than patients with negative test results (LR = 2.5).

DISCUSSION

As oral cancer is the most common malignancy in Sri Lankan males, early detection is paramount to a

better prognosis. Despite the availability of good clinical practice, small lesions go unrecognized, and 60% of the lesions are well advanced by the time of diagnosis [8]. The people's lack of awareness appears to play a significant role in this late presentation. In the meantime, despite the surgical techniques and adjunctive therapies, the prognosis of the patients with oral squamous cell carcinoma remains poor, with a five-year survival rate of 40 - 50%, which has not changed significantly over the past decades [6]. These factors hint at the necessity of diagnostic adjuncts to visual examination of oral lesions.

According to the literature, many studies are done to assess the diagnostic efficacy of Toluidine blue, Toluidine blue in combination with Lugols Iodine, and chemiluminescent methods as adjuncts to the clinical diagnosis of oral premalignant and malignant lesions.

Our study assessed the adjunctive utility of Toluidine blue to detect benign, premalignant, and malignant cases compared with the clinical assessment of visible oral lesions. We selected Toluidine blue, as it is a simple, cost-effective technique and safe to use.

Some studies have shown the additive value of Toluidine blue. Other studies show that the sensitivity of Toluidine blue is in the range of 80 - 100%, and the specificity is in the range of 44 - 100%, indicating that it has a good sensitivity with a very low false-negative rate. Toluidine blue has consistently shown low false-negative rates, which is important because false-negative results are critical in cancer management [9]. Same time, the studies show no added value of adjunctive methods to the clinical examination [4,10-12]. In some instances, it helped to detect clinically undetectable high-risk sites as well [13].

In our study, the sensitivity of the Toluidine blue test is 91%, and the specificity is 52% which is relatively low. The positive predictive value of the Toluidine blue test is 66%, but the negative predictive value is 83%. The above results indicate that the false positives are high. The test is suitable to detect dysplastic changes but not specific; and features other than dysplastic cells give staining with Toluidine blue.

Some studies showed that false positives using applications like Toluidine blue for asymptomatic lesions could be reduced to 8.5% by the second evaluation of the same lesions after about two weeks of the period. This reduction may be due to the healing of traumatic lesions and settling the inflammatory changes with time. Therefore, traumatic, and inflammatory lesions were among the exclusion criteria of some studies [14].

A study done at the University of California has separately assessed the efficacy of Toluidine blue staining compared to microscopic diagnosis; it was 100% in dysplastic lesions, 96% in malignant lesions, and 30% in benign lesions. Some studies showed that Toluidine blue had a 30% significant false-positive rate and a 4% false-negative rate [15].

So, we considered the possible false positives and selected two histopathological features, epithelial hyperplasia and heavy inflammation that is commonly seen in the biopsies of oral lesions, to assess whether those are the possible reasons.

We removed the cases with both those features, and the remaining cases were reassessed as previously.

In the second assessment, the sensitivity of the clinical diagnosis is 100%, the specificity is 85.7%. The positive predictive value is 94.8, and the negative predictive value is 100%. The likelihood ratio of the clinical diagnosis is 7.04.

The sensitivity of the Toluidine blue test is 100%, and the specificity is 60%. The positive predictive value is 83%, and the negative predictive value is 100%. The likelihood ratio of the Toluidine blue test is 2.5, which is lower than the clinical assessment.

According to the likelihood ratios obtained in our study, there is no added value of Toluidine blue other than the clinical diagnosis. However, in both instances, assessing with or without additional histological features, the negative predictive value of the Toluidine blue test is high and is more than 80%. The false-negative rate is very low at 4.8%. So, it indicates the usefulness of Toluidine blue to assess and confirm the clinically decided benign cases rather than to identify premalignant and malignant cases.

The studies have suggested that although Toluidine blue's specificity is low, it can still be used as a diagnostic aid in the screening purposes where false-positive results are of less concern than false negatives and the positive findings can be confirmed by biopsy [16]. But a biopsy is an invasive, time-consuming, and costly procedure.

Studies show that Toluidine blue is useful in selecting the biopsy site in post-radiation patients and

improving the diagnostic yield [17-19]. At the same time, we studied the advantage of Toluidine blue in patients who have undergone previous surgery or radiotherapy, where the clinical assessment of the lesions is difficult. In our study, although we have separately assessed such patients, the number of these cases was limited, only fifteen, so it was impossible to get an inference.

Our setup in assessing the Toluidine blue test for premalignant and malignant lesions does not significantly differ from the clinical assessment. This similarity may be because the Consultant Oral and Maxillo Facial Surgeon examines all the cases. However, the clinical assessment is subjective and may differ from clinician to clinician depending on the clinical experience and capability. For screening purposes, most of the cases may be examined by junior medical officers, and the results will be different. However, the interpretation of Toluidine blue does not differ from case to case if there are well-defined criteria. Assessment of staining positivity or negativity does not need much clinical experience.

As mentioned in research done in India, the false positive rate was high. 70% showed positive results on the first staining, but re-staining after two weeks resulted in only 32% staining, indicating the false positives affecting the results. So, they thought that interpretation of staining is also subjective to a certain extent and well-defined criteria for staining had to be established [5].

So, we can consider Toluidine blue test as an important adjunct to confirm the clinically benign cases. Hence, those patients who do not need biopsy at that instant can be followed up in the clinics with repeated Toluidine blue tests which are harmless, cost-effective, and can be done as an outpatient procedure.

Some studies provide evidence that chemiluminescence is more sensitive in detecting suspicious lesions, but it is not readily available. Toluidine blue was 100% sensitive in detecting malignant lesions, so it is important to detect the most suspicious site in the lesions for biopsy. Therefore, we planned to assess this feature in our study as mentioned in the proposal, but the number of larger lesions was limited.

So, we would like to design a further assessment of larger lesions and take two biopsies when there is a discrepancy between the clinically decided site and the Toluidine blue stained site.

CONCLUSION

Toluidine blue is an important adjunct to screen the oral lesions as benign, premalignant, or malignant and decide when and where to biopsy. Even though it is not emphasized here that Toluidine blue is an important adjunct, it might be helpful when the clinical decision is indeterminate and also it has an additive value. Our study shows that Toluidine blue is beneficial more to confirm clinically benign cases thus reducing the number of biopsies; and such patients can be followed up in clinics.

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