USAGE OF TOLUIDINE BLUE IN EARLY DETECTION OF MALIGNANT AND PREMALIGNANT LESIONS

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ABSTRACT

Objective: *To Test the utility of toluidine blue staining in the early diagnosis of malignant and premalignant lesions.*

Materials and Methods: *This was a Randomized control clinical trial, carried out at Oral and Maxillofacial Department Gajju Khan Medical College, Bacha Khan Medical Complex, Swabi from 15th April 2019 to 15th April 2020. Twenty two patients with the clinical suspicious lesions were selected. Oral rise was done with 1% acetic acid and later Toluidine blue (1%W/W) was applied with sterile swab stick. Again oral rise was done with normal saline. Colour of the lesion was noted. Later incisional biopsy was taken as gold standard.*

Results: *Total of 22 cases the males were 14(63.6%) and females were 8(36.4%). The sensitivity and specificity of toluidine blue against gold standard of histopathology were 88.89% and 61.54% % respectively. The diagnostic accuracy of toluidine blue was 72.73%.*

Conclusion: *Toluidine blue is very good, effective and simple staining procedure. The staining method is more tolerable by the patient. It may help not only in detecting early dysplastic and malignant lesions but will also help in determining the site of biopsy to be taken.*

Keywords: *Toluidine Blue, Histopathology, Blue staining*

INTRODUCTION

Oral cancer is a global problem nowadays, more common in males and most prevalent in lips and bucaal mucosa.[1] Oral malignancy usually presents itself in the form of non healing ulcer, white patch or red lesion. Red lesions are more suspicious for malignancy being more notorious for the cancer conversion. People usually ignore these warning signs and gets neglected which cause delays in the diagnosis with progression of disease. Due to similarity to the benign lesions and delay in seeking medical care these lesions get aggregated to the palliative treatment.[2]

The difficulty in differentiating clinically between benign, reactive, and inflammatory and pre malignant lesion a major problem arises that when and from where biopsy should be taken for the confirmatory diagnosis. Early diagnosis of the oral cancer is the key toward treatment and prognosis. The diagnosis of oral cancer is very difficult unless it becomes symptomatic.

As in advance disease, more aggressive treatment is needed which in turn gives more morbidity to

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the patient. Therefore early detection of premalignant and malignant lesions improves quality of life of the patient. Sometimes clinician is itself in doubt when to follow the lesion or to take the biopsy and when to intervene.[3]

Several adjuncts and diagnostic tests are available commercially for the diagnosis of these early malignancies. In terms of efficacy none of these tests and tools proved helpful in the diagnosis of these early lesions to be used in hospital setting.[4] Toluidine blue being soluble in both alcohol and water is a member of thiazide group. It is metachromatic acidophilic dye has the tendency to bind with nucleic acid, DNA and RNA. It is used for the vital staining of the tissues block in histological examination owing to its metachromtic properties. Being metachromitc and the affinity for the tissues suggest that it can be used for the early lesion and disease surveillance in head and neck cancers. It imparts dark blue or pale blue colour to the tissues applied, therefore can be used for the missed lesion clinically.[5]

In present study we used toluidene staining for the diagnosis early detection of oral pre malignant and malignant lesions and compared it with histological evaluation.

MATERIALS AND METHODS

This comparative cross sectional study was carried at Bacha khan medical complex / Gajju khan Medical College, Shah Mansoor swabi. Ethical approval letter was obtained from the hospital ethical committee. Total of 22 patients were included in the study, being 9 female with 13 males with age range from 40 to 80 years, both genders and Pakistani national. Patients having bleeding tendency, uncontrolled systemic disease, sensitivity to Toluidine blue and previous radiotherapy or chemotherapy were excluded from the study.

Clinical examination was done by examiners total of 22 patients and on inspection lesions include were 2 on palate, 3 in the floor of mouth, 2 on alveolar mucosa, 1 in retromolar trigone, 9 on buccal mucosa and 5 on the tongue. Informed consent was taken for biopsy and toluidene blue stain application. Patients were asked to rinse with 1% acetic acid for 20 seconds. Then Toluidine blue with 1% (W/W) concentration was applied with swab stick. Again patients were asked to re rinse again for 20 seconds with Acetic acid.6 The lesion having dark blue colour were labeled as malignant or premalignant positive while lesions having no color or light blue colour were labeled as benign. Later again rinsing oral cavity with normal punch biopsy was done under local anesthesia for the histological reporting. All data were documented. The range of histological report was defined as benign, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ, and invasive carcinoma were labeled malignant or pre malignant lesions.

The data was analyzed in SPSS version 22.0. Mean and standard deviation was calculated for age. Frequency and percentages were calculated for age groups, gender and site of pre-malignant lesions. Cross tabulation was done for toluidine blue against gold standard of histopathology. This cross tabulation was put in online calculator (https://www.medcalc.org/calc/diagnostic_test.php) to calculate sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, negative predictive value and diagnostic accuracy along with their 95% confident interval. The analysis was for overall sample and for each gender separately.

RESULTS

The mean age of the study was 61.77±12.11 years with range from 41 to 80 years. the males were 14(63.6%) and females were 8(36.4%). The most common age category was 40 to 70 years. The most common site of pre-malignant lesions was buccal mucosa (n=9, 40.9%) followed by floor of the mouth (n=3, 13.6%). The details are shown in table1. Cross tabulation of Toluidine blue versus histopathology of pre-malignant lesions showed that true negative were 8(36.36%), false positive were 5(22.73%), false negative were 1(4.54%) and true positive were 8(36.36%). (Table 2)

The sensitivity and specificity of toluidine blue against gold standard of histopathology were 88.89% (95% CI=51.75 to 99.72%) and 61.54% (95% CI=31.58 to 86.14%) respectively. Both these values were statistically significant. The diagnostic accuracy of toluidine blue was 72.73% (95 % CI=49.78 to 89.27%). Positive likelihood ratio, negative likelihood ratio, positive predictive value, and negative predictive value are shown in table 3. Cross tabulation of Toluidine blue versus histopathology of pre-malignant lesions of both genders is shown in Table 4.

Table 1: Frequency of age groups, site of lesion and genders

		Frequency	Percent
Age group	40-50	5	22.7
	51-60	5	22.7
	61-70	7	31.8
	71-80	5	22.7
Site	Alveolar mucosa	2	9.1
	Buccal mucosa	9	40.9
	Mouth floor	3	13.6
	Palate	2	9.1
	Retromolar trigone	1	4.5
	Tongue	5	22.7
Gender	Female	8	36.4
	Male	14	63.6
	Total	22	100

Table 2: Cross tabulation of Toluidine blue versus histopathology of lesions

		Toluidine blue	
		Negative	Positive
Biopsy	Negative	8(36.36%)	5(22.73%)
	Positive	1(4.54%)	8(36.36%)

Table 3: Overall diagnostic accuracy of Toluidine blue against gold standard of histopathology

Statistic	Value	95% CI
Sensitivity	88.89%	51.75% to 99.72%
Specificity	61.54%	31.58% to 86.14%
Positive Likelihood Ratio	2.31	1.12 to 4.77
Negative Likelihood Ratio	0.18	0.03 to 1.20
Positive Predictive Value (*)	61.54%	43.65% to 76.77%
Negative Predictive Value (*)	88.89%	54.54% to 98.16%
Diagnostic Accuracy (*)	72.73%	49.78% to 89.27%

DISCUSSION

The principle of using toluidine blue being multichromatic acidophilic dye selectively stains carboxylates, sulpahtes and phosphates of tissue components. DNA and RNA having more previous mentioned components have greater affinity for toluidine stain. Malignant lesions having more DNA and RNA components thus have more tendency to take up the stain. In addition to have more nucleic acid components, the epithelium have wider intracellular canals thus facilitate dye penetration.[7]

Table 4: Cross tabulation of Toluidine blue versus histopathology of pre-malignant lesions of both gender

			Toluidine blue	
			Negative	Positive
Female	Biopsy	Negative	3(13.63%)	1(4.54%)
		Positive	0(0%)	5(22.73%)
Male		Negative	5(22.73%)	4(18.18%)
		Positive	1(4.54%)	3(13.63%)

In our study males were 14 (63.6%) and female were 8 (36.4%). In a study conducted also documented more male (75%) as compared to the female (25%)[8] while in contrast to other study.[9] Male gender being more, one of the reason being more habitual use of tobacco products especially in third world country.

Buccal mucosa was the commonest site for the lesion being 40.9% followed by tongue 22.7% in our study which is in accordance with the previous study by Junaid et al[8] while in contrast to other study where commonest site was tongue (44.4%).[9]

The Study we conducted had toluidine blue sensitivity in detecting malignant and premalignant lesions was 88.89%. In accordance to the study conducted by Hegde et al[9] also shown 98% sensitivity. Few lesions in our study were endophytic lesions with intact mucosa with difficulty in staining may the reason of low sensitivity.

The specificity in our study was 61.54% which is in accordance with the previous studies conducted by Waruakularuriya (62 %)[10] Nagaraju et al[11] and in contrast to to Hegde et al (100%).[8]

In our study there was high negative prediction value as 88.89% which is in coherence with the previous study conducted by Cancela-Rodriguez[12] while in contrast to other study.[10] We shall be liable to say that toluidine blue may reduce the number of biopsies to almost half. It will not only reduce the morbidity of the patient but also will save the precious time of biopsy taking. We can say that no further biopsy may be need after application of toluidene blue thus saving resources and time.

The positive prediction value in our study was 61.54%.It is in contrast to the study conducted by deepti singh and R.K Shukla[7] reported positive prediction value as 100% while coherence to the study by Epstain et al.[13]

The diagnostic accuracy in our study is 72.73%. the sudy conducted by Epstain et al[13] shown 83%.in other study the diagnostic accuracy was almost 90%.[7]

In non uniform lesions site of biopsy is very critical. if biopsy is taken from less severe area ultimately the less severe interpretation will be documented by histopathologist (whole lesion will be considered same) though other areas may have more aggressive changes. As there is great variability in histopathological interpretation and operator dependent there are greater chances of miss diagnosis and wrong interpretation during histopathological assessment. In this regard Toluidine blue staining may be a helpful tool to decide the site from which biopsy may be considered. Totally depending on Toluidine blue may be discouraged specifically in centers where head and neck malignancies is not dealt, especially where individual having less experience is clinical suspension of malignancy. It is recommended to refer the patient to these centers where such cases are dealt.[13]

For a long time toluidene blue has been used for the early detection of malignant and premalignant lesions. It has good clinical outcome for the detection of potentially malignant when compared with visual examination.[14]

Drawbacks in our study were that we have taken small sample size and there was also confusion over inclusion of the pale staining being document as positive. There was more variability in rang of lesions as multiple inclusion criteria as ulcer, red, white and exophytic lesions were included. Also inflammatory lesion were not studied.

CONCLUSION

Toluidine blue is very good effective and simple staining procedure. The staining method is more tolerable by the patient. It may help not only in detecting early dysplastic lesions but will also help in determining the site of biopsy to be taken.

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