

# Diagnostic accuracy of toluidine blue in early detection of oral squamous cell carcinoma

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## ABSTRACT

**Background:** Indian sub-continent (India, Pakistan, and Bangladesh) is one of the high-risk populations for oral cancer cases. Intraoral screening is critical to diagnosis and treating oral cancer at an early stage for a better prognosis. Among the diagnostic adjuncts, toluidine blue staining is considered a simplistic, cost-effective, and highly sensitive method. The objective of the current study was to find out the diagnostic accuracy of toluidine blue in the early detection of oral squamous cell carcinoma.

**Patients and methods:** This prospective observational study was undertaken at the Department of Oral and Maxillofacial Surgery, Institute of Dentistry, CMH Lahore Medical College from 15-09-2019 to 15-03-2020 after getting approval from IRB. Based on inclusion criteria, a sample size of 100 was calculated and enrolled in the study. Non-probability convenient sampling technique was utilized. Oral staining of 100 patients was done with 1% toluidine blue on an OPD basis, and incisional biopsies were then performed. Staining pattern and histopathology reports of patients were evaluated to assess the diagnostic accuracy.

**Results:** The study results revealed the sensitivity of Toluidine Blue as 89.87%, and specificity of toluidine blue was found as 76.19%. Positive and negative predictive values of Toluidine Blue remained 93.42% and 66.67%, respectively.

**Conclusion:** Toluidine blue has good diagnostic accuracy for early detection of oral squamous cell carcinoma (SCC).

### Keywords:

Oral Squamous cell carcinoma, Pre-malignant lesion, Diagnostic accuracy, Toluidine blue.

## INTRODUCTION

Indian sub-continent (India, Pakistan, and Bangladesh) is one of the high-risk populations for oral cancer cases and a leading cause of mortality, accounting for nearly one-third of all cancers.<sup>1,2</sup>

Oral squamous cell carcinoma (OSCC) is the commonest type accounting for 90-95% of all oral cancer cases.<sup>3,4</sup> Clinically, OSCC may present as a persistent ulcer, leukoplakia, erythroplakia, a lump, or a nodule.<sup>5</sup> The most commonly involved sites include the tongue, lip, cheek mucosa, and floor of the mouth.<sup>6,7</sup> Oral cancer prognosis is directly linked with the stage of the tumor at diagnosis.<sup>8-10</sup> Oral Cancer detection at an earlier stage is of utmost significance as survival rate significantly improves for early-stage OSCC.<sup>11-13</sup> Periodic intraoral screening is critical to early diagnosis.<sup>14,15</sup> In-vivo staining is considered as one of the simplistic, cost-effective, and highly sensitive

diagnostic adjuncts.<sup>16</sup> The utility of toluidine blue has been reported for staining of potential malignant and pre-malignant lesions.<sup>10</sup> The sensitivity of toluidine blue used in-vivo for squamous cell carcinoma to stain positive has been shown somewhat 93.5% to 97.8%, and that of specificity was reported from 73.3% to 92.9%.<sup>10</sup> Another study reported sensitivity of 79.5% and specificity around 62% with pre-cancerous lesions.<sup>2</sup> Moreover, sensitivity and specificity of toluidine blue has been reported more than 90% used in vivo for pre-malignant and malignant lesions for OSCC.<sup>17,18</sup> Authors of a study demonstrated reduction of the false positive rate by 55.26% while maintained a 100% negative predictive value (NPV) and suggested more clinical trials in different populations.<sup>10</sup>

The study aimed to determine the diagnostic accuracy of toluidine blue staining to detect pre-malignant lesion with high suspicion of OSCC, thus the results of the study may be exploited to guide the clinicians for early detection of the potential malignant oral cavity lesions by utilizing a simple, non-invasive technique with good diagnostic accuracy.

**Conflict of Interest:** The authors declared no conflict of interest exists.

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## PATIENTS AND METHODS

This prospective observational study was undertaken at the Department of Oral & Maxillofacial Surgery, Institute of Dentistry, CMH Lahore Medical College from 15-09-2019 to 15-03-2020 after approval from Institutional Review Board.

An online sample size calculator (OpenEpi) was utilized to calculate the sample size of 100. Data were collected by using the non-probability sampling technique. Patients' age ranged from 24-65 as this is the most susceptible age group for potential squamous cell carcinoma due to habits, having non-healing ulcers, and with high risk or suspicion of malignancy (with history of betel nuts, Paan chewing, smoking, snuff dipping, and alcohol) diagnosed by taking a thorough history and clinical examination (so patients with non-healing oral ulcers for more than one month and habits were labeled as high-risk patients) and were included in the study). Patients who had clinically evident and histopathologically proven carcinoma or had lesions without risk or suspicion of malignancy were excluded. Informed consent was taken from the patients. A predesigned Performa was used for data collection.

Oral lesions of included patients were swabbed with 1% flavored acetic acid solution for one minute to remove any debris from the lesion. Then a pre-soaked swab was used to apply the 1% toluidine blue prepared by the pharmacy for one minute. Finally, 1% acetic acid was utilized to remove any excessive toluidine. The staining pattern was recorded either as negative or positive. Lesions that retained toluidine blue after applying 1% acetic acid were considered positive, while lesions that could not retain toluidine blue dye when 1% acetic acid was applied were considered negative. Incisional biopsy of the lesion was taken under LA and sent to the histopathology laboratory of the Hospital. This whole procedure was performed on a dental chair on an OPD basis by the principal investigators. All the data, including the staining pattern and result of the biopsy report were recorded.

Data analysis was performed by utilizing SPSS version 25.0. Frequencies and percentages were used to describe the qualitative variables like gender. Quantitative variables like age were described in the form of mean $\pm$  Standard Deviation (SD). A contingency table of 2x2 was formulated for positive predictive value, negative predictive value, sensitivity, specificity, and accuracy of toluidine blue staining to detect oral malignant lesions while taking histopathology as a gold standard.

Table 1. Distribution of site of lesion in relation to the gender of patients.

| Site of lesion | Gender        |                 | Total<br>n (%) |
|----------------|---------------|-----------------|----------------|
|                | Male<br>n (%) | Female<br>n (%) |                |
| Alveolus       | 7 (12.3)      | 1 (2.3)         | 8 (8)          |
| Buccal mucosa  | 22 (22)       | 16 (37.2)       | 38 (38)        |
| Lip            | 6 (10.5)      | 5 (11.6)        | 11 (11)        |
| Tongue         | 22 (38.6)     | 21 (48.8)       | 43 (43)        |
| Total          | 57            | 43              | 100            |

Table 2. Diagnostic accuracy of toluidine blue in the detection of oral malignancies.

| Diagnostic accuracy       | Biopsy                   |                         | Total<br>n (%) |
|---------------------------|--------------------------|-------------------------|----------------|
|                           | Malignant<br>n (%)       | Benign<br>n (%)         |                |
| Positive                  | 71 (89.8) <sup>TP</sup>  | 5 (23.8) <sup>FP</sup>  | 76 (76)        |
| Negative                  | 8 (10.1) <sup>FN</sup>   | 16 (76.2) <sup>TN</sup> | 24 (24)        |
| Total                     | 79                       | 21                      | 100            |
| Sensitivity               | 89.9% (81.02% to 95.53%) |                         |                |
| Specificity               | 76.2% (52.83% to 91.78%) |                         |                |
| Positive predictive value | 93.4% (86.81% to 96.84%) |                         |                |
| Negative predictive value | 66.7% (49.85% to 80.10%) |                         |                |
| Diagnostic accuracy       | 87.0% (78.80% to 92.89%) |                         |                |

## RESULTS

A total of 100 patients were enrolled in the study with slight male predominance; 57 (57%) males and 43 (43%) female patients (male to female ratio being 1.32:1). Mean age was 44.01 $\pm$ 12.8 years (range, 24 to 65 years). The mean ages of male and female patients were 44.42 $\pm$ 12.92 and 43.46 $\pm$ 12.76 years, respectively. Concerning the site of a highly suspicious lesion, 8 (8%) patients had lesions involving alveolus, 38 (38%) patients had lesions in Buccal Mucosa, 11 (11%) patients had lesions on their lips, and 43 (43%) patients had lesions on the tongue (Table1).

The sensitivity of toluidine blue was noted at 89.87%, i.e., it accurately detected 71 patients who had malignant lesions. The specificity of toluidine blue was 76.19%, i.e., it accurately detected 16 patients who did not have malignant lesions. The positive predictive and negative predictive value of toluidine blue was 93.42% and 66.67%, respectively (Table2).

## DISCUSSION

Oral squamous cell carcinoma cases are on the rise in Southeast Asian region.<sup>1</sup> Those with habits of using different forms of tobacco, smoking, drinking and betel nut chewing have a higher risk of having oral cancer. Screening for early diagnosis of oral malignant lesions is of utmost importance in this part of the world. Toluidine blue can be utilized as a diagnostic adjunct to detect oral malignant and pre-malignant lesions. Its higher efficacy has been reported with encouraging results.<sup>10</sup> Onofre and colleagues reported the sensitivity of 100% to detect the carcinoma-in-situ and invasive

carcinoma, and there were no false-negative results noted. They also observed false-negative results as the dysplastic lesions did not retain toluidine blue stain while false positivity rate remained for lesions with no dysplasia or showing atypical cells. The toluidine blue specificity was noted around 65%.<sup>19</sup>

In a study by Hegde and coworkers, the sensitivity and specificity were 97.29% and 62.5%, respectively, for lesions as non-healing ulcers with suspicion of malignancies. In comparison, they reported false-positivity and false-negativity as 7.69% and 16.67%, respectively. They proposed that the specificity in their study was reduced because it was retained in some of the benign lesions.<sup>20</sup> As far as the current study is concerned, it also had false positives, and some benign lesions also retained stain may be due to inflammation in the area. Gupta and coauthors noticed a sensitivity of toluidine blue as 96.9% and specificity of 86% in the detection of oral malignant lesions. They also noted 64% sensitivity and 86% specificity for pre-malignant lesions.<sup>21</sup> In the current study, the sensitivity of Toluidine Blue remained 89.87% which is closer to those of Hegde and associates, and Gupta and colleagues reported sensitivities of toluidine blue.<sup>20,21</sup>

False-positive has been described with the variable frequency with regard to retention of Toluidine Blue for non-dysplastic lesions. Epstein and coauthors reported toluidine blue sensitivity as 92.5 % and specificity as 63.2%, which are in accordance with the results of the current study where we reported the specificity around 76%.<sup>10</sup> In current study enrolled patients with oral lesion and with high suspicion or risk of malignancy (with history of betel nuts, Paan chewing, smoking, snuff dipping and alcohol) diagnosed by thorough history and examination whereas done by Epstein and coworkers who included 84 patients with 97 suspicious lesions.<sup>10</sup> This study shows that oral pre-malignant lesions are almost equally common in both genders in the suspected high-risk population in our setting. As far as the lesion site is concerned, malignancies were most common on the tongue's lateral border, followed by buccal mucosa and lower lip, while alveolus and palate were at the bottom. In the study by Hodge and coauthors, 2/3<sup>rd</sup> of patients ( $n=38$ ) presented with a history of betel quid chewing, and 52 patients presented a history of cigarette smoking. The sites of involvement of suspected lesions were noted as; on the buccal mucosa ( $n=25$ ), tongue ( $n=16$ ), gingivae ( $n=9$ ), lip ( $n=3$ ), palate ( $n=2$ ), the floor of the mouth ( $n=2$ ), and retromolar region ( $n=1$ ).<sup>20</sup> In their study buccal mucosa

was involved more than other sites which seems comparable to the current study.

In the current study, toluidine blue's sensitivity was 89.87%, which accurately detected 71 patients with a malignant lesion. Specificity of Toluidine Blue was noted 76.19% that means it accurately detected 16 patients who did not have malignant lesions. These results are well in line with the figures described in earlier studies.<sup>10,21</sup> Current study, reported 23.8% false-positive and 10.1% as false-negative whereas Hegde and coauthors reported false-positivity and false negativity of 7.69% and 16.67% respectively.<sup>20</sup> Gupta and colleagues reported false-positive rate of 14%.<sup>21</sup>

In the current study, positive and negative predictive values of toluidine blue have been reported as 93.42% and 66.67%, respectively, while a previous study described 100% negative predictive value.<sup>10</sup>

The use of toluidine blue has previously been reported in various studies as a valuable adjunct to the clinical judgment which can assist in choosing the site of the biopsy; a valuable adjunct in the diagnostic process, and it can preferably be used as a prime aid in early detection of oral pre-malignant and malignant lesions.<sup>22-25</sup>

## CONCLUSIONS

Toluidine blue is one of the diagnostic adjuncts available that can be utilized for the early detection of oral squamous cell carcinoma. The method is simple, non-invasive, and can be used as a chair-side technique with good diagnostic accuracy.

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