



## BIOPSY CONFIRMATION OF MALIGNANCY IN ORAL AND OROPHARYNGEAL LESIONS SCREENED BY TOLUIDINE BLUE

**Dr Jasmine Khader**

Assistant Professor, Pathology Karuna Medical College Vilayodi, Chittur Palakkad

**Dr Amrita S Kumar**

Assistant Professor, Biochemistry Karuna Medical College Vilayodi, Chittur Palakkad

**Dr Santha Sadasivan**

Retd Professor, Pathology Government Medical College, Thiruvananthapuram

**ABSTRACT** Oral cancer, a prevalent malignancy in India, exhibits higher curability when detected early. Unfortunately, many patients present at later stages, necessitating the development of an efficient early detection tool. Toluidine blue (TB), a cost-effective and user-friendly screening tool, was employed in a prospective study involving 31 ENT Department patients. Among them, 27 displayed positive staining, with 25 confirmed as malignant (SCC) through histopathological examination, while the remaining 2 showed no malignancy. The 6 negatively stained punch biopsies were also histopathologically negative. In this study, TB demonstrated 100% sensitivity, 67% specificity, and 94% accuracy as a diagnostic aid for oral/oropharyngeal malignancies. TB stands out as an affordable, straightforward, and non-invasive early diagnostic tool for oral cancer detection

**KEYWORDS :** oral cancer, toluidine blue, screening tool, histopathology

### INTRODUCTION

Oral cancer is a widespread global issue. Early diagnosis directly influences treatment outcomes. Detecting oral cancer is often challenging due to lesion location and appearance, leading to advanced diagnosis stages. Clinicians typically rely on visual cues for biopsy decisions. New objective methods alongside clinical judgment improve oral cancer management.

Toluidine blue (TB) at 1% concentration enhances high-risk site evaluation in the oral cavity. TB, an acidophilic metachromatic dye, selectively stains acidic tissue components, including DNA and RNA. It's used in vivo as dysplastic cells contain more nucleic acids, and malignant epithelium may have wider intracellular canals, aiding dye penetration<sup>1,2</sup>. This study aims to assess TB's effectiveness as an oral cancer screening tool.

### Aim Of The Study

Evaluating Toluidine Blue staining's sensitivity, specificity, and accuracy in detecting malignancy in oral/oropharyngeal lesions, compared to histopathological results.

### MATERIALS AND METHODS

#### Study Design

Diagnostic test evaluation

#### Sample Size

31

Sample size (N) =  $Z\alpha 2pq/d^2$

p = prevalence of positives in the test (TB staining 98%)

q = 100 - p (2%)

d = precision (Assuming to be 5%)

$N = (1.96 * 1.96) * 98 * 2 / (5 * 5)$

N = 31

#### Duration Of Study

January 2010 to December 2010.

#### Setting

A diagnostic test evaluation study was conducted on individuals with oral lesions or suspicious changes in the oral and oropharyngeal areas, who visited the ENT outpatient department at Government Medical College, Trivandrum. In all cases, biopsies were performed and sent for histopathological analysis to the Pathology Department at the same institution.

#### Inclusion Criteria

All patients with concerning oral and oropharyngeal issues who visited Trivandrum Medical College's ENT outpatient department.

#### Exclusion Criteria

1. Individuals with a prior diagnosis of oral and oropharyngeal cancer.
2. Biopsy samples unsuitable for analysis

### METHODOLOGY

#### Toluidine Blue Stain Composition

The Modified Toluidine Blue stain includes 1% Toluidine Blue solution, consisting of 1 gm of TB powder, 10 ml of 1% acetic acid, 4.19 ml of absolute alcohol, and 86 ml of distilled water, with pH adjusted to 4.5.

#### Topical Application Of TB

Suspicious lesions were stained with Toluidine blue following the Mashberg methodology<sup>3</sup>. Initially, patients rinsed their mouth twice with water for 20 seconds to clear debris. Then, 1% acetic acid was applied for 20 seconds to remove saliva. Subsequently, 1% TB solution was applied for 20 seconds using a cotton swab for mucosal lesions or as a rinse. A second rinse with 1% acetic acid reduced mechanically retained stain. The mouth was finally rinsed with water. A positive result was indicated by a dark blue (royal or navy) stain covering the entire lesion, a portion of it, or stippling, while a negative stain indicated no colour absorbed by the lesion.

Punch biopsies, chosen based on clinical findings and dye retention, were processed for histopathological examination. Each Formalin-fixed, paraffin-embedded tissue block provided a 5-micron section stained with hematoxylin & eosin (H&E) for analysis.

#### Histopathological Evaluation

Histopathological examination categorized punch biopsy specimens into malignant and non-malignant groups.

Non-malignant cases were further divided into dysplastic and benign. Malignant lesions were primarily squamous cell carcinoma, an invasive malignancy originating from keratinocytes in stratified squamous epithelium that invades stroma.

Squamous cell carcinoma grading utilized the 4-grade Broders scale, based on well-keratinized cell percentage<sup>4,5</sup>. Dysplasia, histopathologically, denotes keratinocyte histomorphological changes affecting epithelial thickness without invasion evidence<sup>6</sup>.

#### Limitations and Ethical Issues

This study commenced with ethical approval and patient consent. A limited sample size was a study constraint. The study did not provide details about lesion appearance or define "suspicious." Nonetheless, Toluidine Blue was a cost-effective, safe, and straightforward method

### OBSERVATIONS AND RESULTS

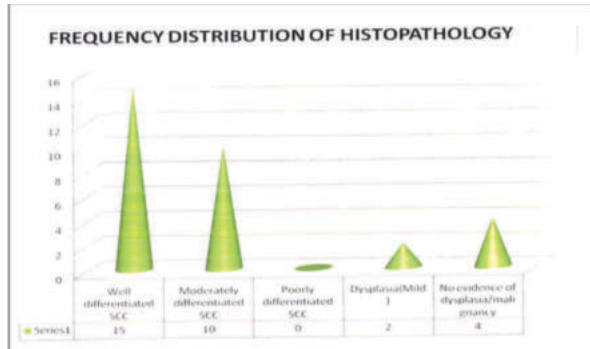
Out of 31 patients, most (54.8%) were aged 50-70, with a mean age of 59.3 and a male-to-female ratio of 5.25:1. Among 31 lesions, 27 stained blue, while 4 did not. Of the positively stained lesions, 25 were confirmed malignant (SCC) histopathologically. The other 2 had no

malignancy/dysplasia(Table 1).

Sensitivity, specificity, and accuracy of Toluidine Blue as a diagnostic aid were 100%, 67%, and 94%, respectively. All 25 malignant cases were squamous cell carcinoma (15 well-differentiated, 10 moderately differentiated). There were no poorly differentiated cases. Among the 6 non-malignant cases, 2 were mild dysplasia, and 4 were benign lesions (Figure 1)

**Table 1 : Correlation Between TB Staining And Histopathology**

STAIN	HISTOPATHOLOGY	
	MALIGNANT	NONMALIGNANT
POSITIVE (27)	25(100%)	2(33.3%)
NEGATIVE (4)	0(0%)	4(66.7%)



**Figure 1**

**DISCUSSION**

In this study, among 31 lesions, 27 were TB-positive and 4 were TB-negative, results similar to Reddy et al's and Shedd et al's studies<sup>7</sup>. The most common lesions found were squamous cell carcinoma (78.1%), consistent with earlier research, like Bayat and Tabesh's findings<sup>8,9</sup>.

The study demonstrated a highly significant sensitivity of 100% for Toluidine blue in detecting malignant lesions, aligning with previous research by Niebel, Chornet, Warnakulasuriya, Epstein, Scully, and Barbosa J<sup>10,11</sup>. Mashberg's work had similar results of 98% sensitivity and 91% specificity with direct application (Table 2).

The study's specificity was 67%, resembling studies by Silverman, Migliorati, Barbosa J, and Epstein JB, Scully C, Spinelli JJ, which reported specificities of 70%, 69%, and 63%. TB's lower specificity may be attributed to its uptake by benign factors like food debris, bacteria, and purulent exudates<sup>12</sup>.

Specificity can also be influenced by the experience of the clinicians and nurses performing the procedures. Some false-positive TB results may represent true molecularly positive lesions<sup>13</sup>.

In this study, the Positive Predictive Value (PPV), Negative Predictive Value (NPV), and diagnostic accuracy were 93%, 100%, and 94%, respectively, akin to Silverman Jr S, Migliorati C, and Barbosa J's findings of 90% PPV, 92% NPV, and 90% diagnostic accuracy.

**Results From Different Studies About The Efficacy Of Tb Staining In Malignant Lesions**

Author	Year	Sensitivity (%)	Specificity (%)
Present Study	2010	100	67
Niebel and Chornet	1964	100	NR
Myers	1970	98	100
Rosen et al	1971	50	50
Rcddvel al	1973	99	83
Lundgren	1979	91	51
Mashberg	1981	98 (direct application) 94 (rinse)	91 (direct application) 93 (rinse)
Silverman	1984	98	70
Hpstein and Scully	1992	93	63
Silverman	1994	94	94
Warnakulasuriya	1996	100	62
Epstein and Scully	1997	100	52

NR- not reported

**Table 2 : Review Of Sensitivity And Specificity Of TB In Detection of SCC7**

The results of the present study in the case of premalignant cases cannot be compared with findings in the previous studies due to very few dysplastic lesions (2) in the samples studied.

**CONCLUSION**

Toluidine blue demonstrated 100% sensitivity, 67% specificity, and 94% accuracy for oral/oropharyngeal malignancy detection in this study. While staining can be inconsistent in early premalignant lesions, it remains a valuable diagnostic tool. It complements clinical examinations, aiding in carcinoma identification among high-risk patients and enhancing biopsy results. Future research with larger, longitudinal samples, studying stain retention patterns, histopathological and molecular changes, is recommended for more robust conclusions.

**REFERENCES**

- Epstein, J. B., Scully, C. & Spinelli, J. Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. *J Oral Pathol Med* 21, 160-163 (1992).
- Saku, T. & Okabe, H. Differential lectin-bindings in normal and precancerous epithelium and squamous cell carcinoma of the oral mucosa. *J Oral Pathol Med* 18, 438-445 (1989).
- Mashberg, A. Tolonium (toluidine blue) rinse-a screening method for recognition of squamous carcinoma. *Continuing study of oral cancer IV. JAMA* 245, 2408-2410 (1981).
- Broders AC. *Practical points on the microscopic grading of carcinoma.* NY State Med J 1932;32:667-671.
- Murphy GF, Elder DE. *Non-melanocytic tumors of the skin.* Atlas of tumor pathology. Vol 1. 3rd ed. Washington, DC: Armed Forces Institute of Pathology; 1991.
- Miller, S. J., & Moresi, S. J. (2003). Actinic keratosis, basal cell carcinoma, and squamous cell carcinoma. In J. L. Bologna, J. L. Jorizzo, & R. P. Rapini (Eds.), *Dermatology* (pp. 1677-1696). New York: Mosby.
- Johnson N (1998) diagnosing oral cancer : can TB mouth wash help. *FDI World Dental Federation*,2,22-26.
- Funk GF, Karnell LH , Kooinson RA, Zhen WK, Trask DK, Hoffman HT. Presentation, Treatment and Outcome of Oral Cavity Cancer: A National Cancer Data Base Report. *Head-Neck* 2002;24(2):165-180.
- Tabesh H. *Epidemiological Study of Oral and Perioral Cancer Patients Referred to Sayyedoshohada Hospital and Faculty of Dental Medicine in Isfahan from 1981 to 1993.* DDS thesis, Dental School, Isfahan University of Medical Sciences, 1995.
- Silverman S Jr, G. M., Lozada F. Oral leukoplakia and malignant transformation. *Cancer* 53, 8(1984).
- Mashberg A. (1981) Tolonium (Toluidine Blue) rinse-a screening method for recognition of squamous carcinoma. *Jama*, 245, 2408-10.
- Strong M.S., Vaughan, C.W., and Ince, J.S. (1968) Toluidine Blue in the management of carcinoma of the oral cavity. *Arch Otolaryng*, 87, 101-105.
- Zhang L, Williams M, Poh CF, et al. Toluidine blue staining identifies high-risk primary oral premalignant lesions with poor outcome. *Cancer Res* 2005; 65(17):8017-8021.