

## SUPRAVITAL STAINING: IT'S ROLE IN DETECTING EARLY MALIGNANCIES

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**ABSTRACT:** *The efficacy of supravital staining in the detection of malignancies in oro and oropharyngeal lesions and its role in the detection of malignant changes in premalignant lesions were studied. This prospective study comprises 90 cases of clinically suspicious lesions and it was done over a period of 3 years. Most of the patients had multiple risk factors for the development of malignancy. All underwent staining with a modified solution of 1% toluidine blue (TB). In our study the overall sensitivity was 97.29% and the specificity was 62.5%.*

**Key Words:** *Supravital staining, toluidine blue, oral and oropharyngeal malignancy, premalignant conditions, cancer detection*

### INTRODUCTION

Malignancies of the upper aero digestive tract are one of the common malignancies in the world. Tumors arising from the oral and oropharyngeal malignancies are usually well advanced at the time of diagnosis. Oral cancer is usually first diagnosed when it becomes symptomatic. By this stage approximately 2/3<sup>rd</sup> of the patients will have already developed advanced disease with regional metastasis<sup>[1]</sup> (clinical or microscopic metastasis). The disease is life threatening, with high morbidity resulting from late treatment. However, if it is diagnosed at an early stage, oral cancer is often curable and inexpensive to treat.

It is the nightmare of any practitioner that an early lesion of the oral cavity may be missed due to their close resemblance to benign/premalignant lesions. The absence of a reliable method for early diagnosis in these lesions is the most important factor which is responsible for the delay in diagnosis and thus poorer prognosis.<sup>[2]</sup> Though clinical examination is one of the best modalities in suspecting the pathology, the biggest disadvantage in the diagnosis lies in deciding the site of biopsy in early lesions and sometimes whether or not a biopsy is required in these lesions. Early stages are difficult to detect as the lesion may not be palpable and colour changes are not very different from the colour of the surrounding mucosa. Thus identifying clinically suspicious/undetectable lesions has gained importance whereby diagnosis can be confirmed by biopsy at an earlier stage.

During the 1960s, the suggestion was made that TB may stain malignant epithelia of the mucous membranes in vivo, while normal tissues fail to retain the dye.<sup>[3]</sup> Supravital staining of the oral epithelium has been suggested as a means of surveillance in patients who are at risk of developing oral cancer<sup>4</sup> and for those who have had a confirmed neoplasm in other parts of the aero digestive tract. This study is mainly designed to know the following:

1. The efficacy of TB in the detection of malignancies in oro and or pharyngeal lesions.
2. Its role in high risk groups.
3. Early detection of malignant changes in premalignant lesions.
4. As a method of follow-up.

### MATERIALS AND METHODS

This prospective study was done over a period of 3 years. It included 90 cases oral lesions or mucosal alterations suspicious of malignancy in the oral cavity and or pharynx. A detailed history was taken in all our patients, with emphasis on a personal history to evaluate possible predisposing factors such as smoking, alcohol and paan chewing.

A thorough clinical examination of the oral cavity and or pharynx was done in all our cases. Routine blood and urine investigations were carried out in all the patients. A supravital staining procedure using modified Toluidine Blue (TB) was

done. The dye was either directly applied on the lesion or used as a mouth rinse. A biopsy was taken in all our cases and sent for histopathological examination. Patients with doubtful lesions after the staining were reevaluated after 14 days, after a course of antibiotics and anti inflammatory drugs. The staining procedure was repeated again and both positive and faint positive stained lesions were taken as positive. The biopsy site(s) was selected on the basis of clinical appearance and dye retention. Depending on the results (from both staining and histopathology) false positives, false negatives, sensitivity and specificity were calculated for supravital staining in detecting malignancies.

### Composition of the Modified Toluidine Blue stain

100 c.c of the 1% TB solution: this solution as described by Mashberg 5 consists of 1 gm of TB powder, 10 ml of 1% acetic acid, 4.19 ml of absolute alcohol and 86ml of distilled water to make up 100 ml of a 1% solution of TB. The ph of the solution is adjusted to 4.5.

### Technique of staining

Initially the patient is asked to rinse his mouth twice with water for 20 seconds to remove the debris. Next 1% acetic acid is given for 20 seconds to remove any ropey saliva. 1% TB solution is then applied for 20 seconds either with cotton swab when a mucosal lesion was seen [Figure 1] or given as a rinse when no obvious lesion was detected. A second rinse with 1% acetic acid was given to reduce the extent of mechanically retained stain. Finally the mouth is rinsed with water.

### Interpretation

A dark blue (royal or navy) stain is considered positive [Figure 2] if either the entire lesion being stained or a portion of it is stained or stippled. A light blue staining is considered doubtful. If there is no colour absorbed by the lesion, it is taken as a negative stain.

The nucleated scales covering the papillae on the dorsum of the tongue as well as the pores of seromucinous glands in the hard palate frequently take up TB. This distribution of the stain is not likely to cause confusion once one becomes familiar with the staining characteristics of these areas. Trauma causing a breach in the mucosa also takes up TB.

### RESULTS AND OBSERVATIONS

Most of our patients (60%) are in the age group of 41-60 years with a mean age of 53.07 years. In our study a male preponderance was seen in the cases with a male female ratio of 5.6:1. Majority of our patients had more than one risk factor (they include smoking, alcohol, quid/ tobacco chewing, paan and spicy food). Our patients with various symptoms, the most

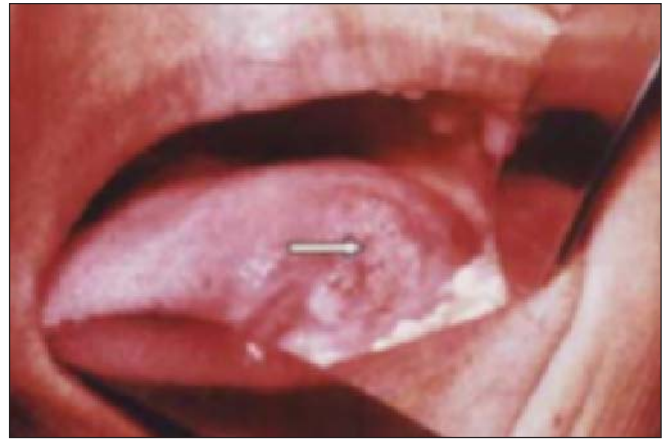


Figure 1: Lesion over the lateral border of tongue



Figure 2: Lesion after staining (dark blue) with 1% toluidine blue

common being pain/burning sensation in the throat in 74 cases (82.2%), ulcer/ swelling seen in 63 cases (70%), odynophagia seen in 28 cases (31.1%) and dysphagia seen in 22 cases (24.4%) in the decreasing order of frequency.

The most common site of lesion was seen to be on the anterior 2/3 of the tongue seen in 40 cases (44.4%) followed by the buccal mucosa in 16 cases (17.8%). Results of TB staining and histopathology are shown in [Table 1]. Among the 90 cases enrolled, 78 lesions (86.66%) were found to be malignant and 16 cases (17.7%) were benign. Of the 4 premalignant lesions found in our study 2 were erythroplakia and next half was leukoplakia.

Overall 78 cases stained positive. Of these cases when subjected to biopsy, 72 (92.3%) were positive for malignancy and 6 (7.69%) were benign histologically [Table 2]. Two of the negatively stained cases (16.67%) were positive for malignancy when subjected to biopsy. This gave a sensitivity of 97.29% and a specificity of 62.5%.

The 6 cases which stained positive but were negative on biopsy

**Table 1: Results of TB staining and histology**

Clinical diagnosis	Cases n = 90	Staining		Histology	
		Positive %	Negative %	Malignant %	Benign %
Suspicious lesions	86	76 (88.37)	10 (11.63)	72 (83.72)	14 (16.28)
Premalignant lesions	4	2 (50)	2 (50)	2 (50)	2 (50)

were reported as non-specific inflammation, constituting false positives (7.69%). The 2 cases which were stained negative and biopsy positive were that of adenocarcinoma, constituting false negative cases (16.67%).

## DISCUSSION

Toluidine blue is an acidophilic dye of the thiazine group that selectively stains acidic tissue components (carboxylates, sulfates and phosphate radicals) such as DNA and RNA. Its use in vivo is based on the fact that dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissues. In addition, malignant epithelium may contain intracellular canals that are wider than normal epithelium; this is a factor that would enhance penetration of the dye.<sup>[7]</sup>

Clinical series have revealed false positive and false negative rates [Table 3] that vary depending on whether malignant or premalignant conditions are being evaluated. On staining both our cases of erythroplakia were positive, which was confirmed by histopathological examination. However both our cases of leukoplakia were negative both on staining and biopsy. This suggests that 1% TB is an effective means in picking up malignant changes in premalignant lesions.

**Table 2: Correlation between staining and histopathology**

Cases	Histology	
	Malignant (%)	Non malignant (%)
Stain positive (78)	72 (92.31)	6 (7.69)
Stain negative (12)	2 (16.67)	10 (83.33)

**Table 3: Results from different studies about the efficacy of TB staining**

	Ours	Warnaku- lasuriya	Arthur Mashberg	Epstein JB
No. of patients	120	145	235	59%
Sensitivity	97.29%	100%	98%	92.50%
Specificity	63%	62%	92%	63.20%
False positive	7.69%	-	8.50%	-
False negative	16.67%	20.50%	6.70%	-

Warnakulasuriya and Newell Johnson<sup>[8]</sup> studied 145 suspicious lesions using 1% modified TB stain. Results attained were sensitivity of 100% and specificity of 62%. They suggest that TB staining is an invaluable option in the surveillance of high risk subjects and in addition it has remarkable sensitivity in the detection of invasive carcinoma. Our results are in agreement with this.

Mashberg<sup>5</sup> modified the stain composition by mixing TB with acetic acid, absolute alcohol and distilled water to decrease the incidence of false positive rates. He also standardized the staining techniques to decrease both false positive and false negative rates. He studied a series of 235 lesions the results of which were notable [Table 3]. A false positive rate of 8.5% and false negative rate of 6.7% is observed. He further showed that a waiting period of 10-14 days would be useful in eliminating the false positives in inflammatory lesions. He concluded that persistent lesions which stain with TB should be considered malignant unless proven otherwise by biopsy.

Epstein JB et al<sup>[9]</sup> did a study of 59 patients to detect malignancy in the oral cavity using in vivo staining using either Toluidine blue or Lugol's iodine or both. They found that sensitivity is high with Toluidine blue alone when compared with the other two alternatives. But to increase the specificity, staining and interpretation with both compounds is required. Hence they recommended usage of the stain, not as a screening method in the general population but as a method which could be used with high sensitivity in doubtful lesions.

Specificity was reduced because of the retention of dye in some benign lesions. The technique we adopted is effective in detecting positive cases. Previous techniques which did not include the use of acetic acid had a much higher false positive response. In a clinical setting the false positives are of course, of less concern than false negative results because any positive should lead to confirmation on biopsy.

## CONCLUSION

Early detection and timely intervention is the essence of any cancer treatment protocol. Supravital staining with 1% toluidine blue is useful in the early detection of malignancies. TB stain is of value due to its high sensitivity but is reduced

in specificity due to the potential of false positive results in benign lesions. It is useful in high risk populations to enable earlier detection. It assists in selecting the best site for biopsy. It is very useful in the developing countries like India because of the cost effectiveness and easy technique. The test is sensitive, simple, noninvasive and highly cost effective procedure.

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