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Assessing the Sensitivity and Specificity of Toluidine Blue Staining in Oral Cancer Screening Among Inmates: A Prison-Based Study

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Abstract

Background: *In the confined environment of correctional facilities, access to healthcare resources can be limited, making it crucial to identify early signs of oral diseases especially oral premalignant lesions and conditions. This study presents the results of an oral health screening camp conducted for jail inmates in Gautam Buddha Nagar (GB Nagar), Uttar Pradesh, India aimed at detecting potentially malignant lesions (PMLs) using Toluidine Blue staining. PMLs are precursor lesions that have the potential to transform into malignancies if left untreated.* ***Objective:*** *This study's major goal was to find out whether applying Toluidine Blue (TB) staining would help with the diagnosis of suspected premalignant lesions and oral cancer & to use it as a routine chair side investigation and also to calculate specificity and sensitivity of TB staining in potentially malignant lesions (PMLs).* ***Materials and Methods:*** *This cross-sectional study was conducted in the prison; toluidine blue staining was performed on Eighty-four patients between the ages of 22 and 65 years made up the study for inmates at the G.B. Nagar jail. Males made up all eighty-four patients. The study included suspicious-appearing red and white lesions with & without any tobacco use history.* ***Results:*** *In this study, eighty-four patients with malignant and potentially malignant lesions were studied, TB Stain showed 76.19% sensitivity and 47.62% specificity.* ***Conclusion:*** *TB staining conducted for jail inmates in GB Nagar demonstrated its potential as a cost-effective and efficient method for identifying Potentially Malignant Lesions. The results underscore the significance of proactive healthcare measures within correctional facilities and emphasize the need for comprehensive oral health programs targeting vulnerable populations. Further research and larger-scale implementation of such camps are recommended to better understand the implications and benefits of early detection and intervention.*

Keywords: *Toluidine Blue staining, Red and White Lesions, Potentially Malignant Lesions (PMLs), Vital staining, Oral cancer*

1. Introduction

Toluidine blue, commonly known by the term Tolonium Chloride, is a specialized metachromatic dye. It is highly efficient at selectively staining acidic tissue components which include sulphates, carboxylates, and phosphate radicals. This unique property allows it to securely hold on to tissues rich in DNA and RNA, precisely labelling them. William Henry Perkin is credited with the discovery of

Toluidine Blue in 1856 (1) & Toluidine Blue found its inaugural application in 1963 when Richart employed it for the staining of uterine cervical carcinoma in situ and dysplasia.

Toluidine blue staining is considered a highly sensitive adjunctive method for the detection of early-stage oral squamous cell carcinoma (SCC) and high-grade dysplasia. This staining technique is invaluable in clinical practice for its ability to enhance the identification of these conditions. Nevertheless, the The application of Toluidine blue staining. in identifying low-grade (mild to moderate) oral dysplasia remains a subject of debate, as a notable proportion of such lesions do not display staining with this technique. (2) Across several research studies, the effectiveness of Toluidine blue staining has produced mixed results. Sensitivity has been documented to vary between 93.5% and 97.8%, while specificity ranges from 73.3% to 92.9%. However, when specifically assessing its sensitivity in identifying oral dysplasia, the numbers have been notably lower, with values fluctuating between 42% and 87%. Consequently, the majority of these investigations have consistently highlighted the staining method's high sensitivity but lower specificity. (3)

Toluidine blue, which falls under the category of cationic dyes, plays a crucial role in visualizing proteoglycans within tissues because of its strong affinity for sulfate groups present in these molecules. It exhibits a fascinating property known as metachromasia, where it interacts with anionic polymerized intermolecular bonds characterized by a high charge density. This interaction results in significant shifts in the spectral properties of the dye. The magnitude of the metachromatic color shift is determined by the increasing presence of anionic radicals, typically following a sequence that encompasses carboxylate, phosphate, and sulfate groups. This metachromatic phenomenon becomes especially pronounced when the distance between intermolecular bonds is minimized. (4)

Cancer is a condition characterized by the unchecked growth of cells, leading to their invasion and disruption of nearby tissues. When this occurs in the oral region, it is referred to as oral cancer. Typically, oral cancer presents as a small, unexplained growth or ulcer in areas such as the lips, cheeks, sinuses, tongue, and both the hard and soft palates. In some cases, it can even extend to affect the base of the mouth and the oropharynx. Globally, oral cancer is recognized as the sixth most prevalent type of cancer. Remarkably, India carries the highest burden of oral cancer cases, accounting for one-third of all cases worldwide. The prevalence of oral cancer represents a significant healthcare challenge, particularly for countries undergoing economic transitions and transformations. (5)

Oral cancer exerts a noteworthy economic strain on both the national and individual levels. In 2020, India grappled with an estimated 135,929 cases of oral cavity cancer, a number anticipated to surge by around 26% by 2030. What's particularly concerning is that a substantial portion, ranging from 60% to 80%, of these cases are diagnosed in advanced disease stages. When we calculate the average cost per unit for treating both early and advanced cancer cases, it becomes evident that India spent approximately USD 322 million exclusively on oral cancer treatment in the year 2020. This underscores the significant financial impact of this condition. (6)

Early diagnosis is a key to early prevention of oral cancer. Utilization of readily available chair side investigation tools like vital staining when used in potentially malignant lesions can aid in early management of such cases and prevent them converting into fully blown malignancy.

2. Materials And Methods

Study Design
Cross-sectional study Design was used.

Study Setting
This Cross-sectional study conducted in a prison, toluidine blue staining was performed on 84 inmates with potentially malignant lesions at a Central Jail in G.B. Nagar District, Uttar Pradesh, India during the month of May 2023. The Jail Authorities provided 3 hours' time for screening of the inmates.

Participants Selection Procedure
Due to limited time provided by the administrative authorities, 84 participants registered during this period. This investigation encompassed the participation of Randomly male inmates with age group of 22 to 65 years with clinically suspicious potentially malignant and malignant lesions of the oral cavity,

irrespective of site and stage. In our investigation, the mean age of clinically suspect oral premalignant lesions was 40.35. All the participants filled the detailed questionnaire regarding their habit. (Type, frequency, duration etc.) Each patient was examined thoroughly, and a differential diagnosis was built.

Toluidine Blue Staining Procedure

Following dental instruments and equipment were required to perform thorough examination - Mouth mirror, dental explorer, William's probe, CPITN probe, gauze & cotton rolls, disposable gloves, tongue depressor, dental operatory light, and data forms, alcohol swabs (for instrument sterilization), toluidine blue dye (CHEM TOLUIDINE BLUE), and 1% acetic acid solution (OXFORD LAB FINE CHEM LLP). Ethical approval from the concerned prison authorities (Prison superintendent) and from the Institute was taken for conducting this screening camp.

In the TB staining procedure, we applied a 1% aqueous toluidine blue dye for around 30 seconds. Afterward, we thoroughly rinsed the sample with distilled water and gently dabbed it using a 1% acetic acid solution**.** These solutions were applied meticulously with precision using cotton-tipped applicators. Any observable dye uptake was methodically recorded through photographic documentation. In this study, eighty-four patients were screened for malignant and potentially malignant lesions and TB Stain showed 76.19% sensitivity and 47.62% specificity.

Statistical Analysis

All the data was tabulated in excel format. Confusion matrix was calculated along with sensitivity and specificity of the TB stain using SPSS software. (IBM SPSS Version 28.0)

Principle of Tb Staining

TB uses the metachromasia principle to stain tissues. (7) In contact with tissues, the dye reveals an intriguing ability to manifest a different color than its original hue. In this context, metachromasia alludes to the phenomena that occurs when a dye can absorb light at various wavelengths depending on variables such as concentration and surrounding environment. Interestingly, it may change colors without suffering any alterations. During this process, a pigment possesses the ability to absorb light across a spectrum of wavelengths, dependent on its concentration and the surrounding circumstances. This enables it to alter its color without changing its chemical structure. These shifts in color stem from a unique and orderly form of pigment aggregation. To facilitate this phenomenon, it's essential to have accessible negatively charged entities on the surface. The accumulation of positive pigment ions in areas with a high density of negatively charged groups within the material is the primary cause of this remarkable color transformation. (7) In this given context, the stacking process leads to a hypochromic shift, effectively reducing the wavelength at which the maximum absorption occurs. This alteration results in an extension of the longest wavelength within the transmitted light spectrum, thereby changing the observed color in tissues from blue to red (1). The main mechanism by which the dye is held together to create dimers, trimers, or polymers is van der Waals forces. In addition to van der Waals forces, hydrogen and hydrophobic bonding are two other bonding types that exert a comparatively smaller influence. The dye can exist in an array of configurations, ranging from its typical monomeric state, known as orthochromatic, to a possible polymeric state known as metachromatic. The interaction between the positively charged polar groups on the dye and the negatively charged chromotropes leads to a particular and well-structured clustering of dye molecules, ultimately leading to the development of a polymeric structure. Metachromasia manifests itself in three distinct forms: alpha, beta, and gamma, each of which has the ability to develop a color spectrum. The specific spectrum of colors produced can vary based on the dye's properties and the conditions in which metachromasia occurs. (7)

3. Results and Discussion

The study comprised eighty-four participants in the age group of 22–65 years. All eighty-four participants were males. This research also included suspicious looking red and white lesions with no history of tobacco use. Out of the 84 individuals, 23 participants were on current habit of consuming tobacco, 29 had previous habit of tobacco consumption & 32 participants had no history of tobacco consumption. (Figure 1) Among the study population, 62 % were Tobacco Consumers. (Figure 2).

Among the participants with current habits of tobacco consumption, 17 participants had red and white lesions stained positive with Toluidine Blue Staining while six of the participants had no lesions and no stain retention was seen.

While considering participants with prior tobacco habits, it was observed that 15 patients exhibited red and white lesions that tested positive with Toluidine Blue Staining. In contrast, 14 participants showed no lesions, and there was no evidence of stain retention. Among the 32 patients with no history of tobacco use, no lesions were detected.

Buccal mucosa was the most common site being involved, 19 cases (53%) followed by 7 (22 %) cases in lower labial mucosa and 3 cases (9 %) in Dorsal Surface of Tongue, and 3 (9%) cases had a generalized involvement of the oral cavity. (Figure 3)

Out of 32 potentially malignant lesions, 4 were diagnosed clinically as speckled leukoplakia, 17 were homogeneous leukoplakia, 2 had an appearance of verrucous leukoplakia ,5 was OSMF with widespread fibrosis of the oral cavity, 2 cases revealed signs and symptoms of Tobacco Pouch Keratosis & 2 cases of Smokers Melanosis were found. The most prevalent presenting symptom among clinically suspected OPMLs was a whitish and reddish patch (Figure 4).

Out of 84 cases, TB stain retained on 55 cases (65.47%) and non-retention (34.53%) of stain was seen in 29 cases. (Figure 5) 32 cases were stained true positive, (participants having habit history with OPML's and a positive retention of the TB stain) 20 as true negative (participants without any tobacco history and without any OPML's and no retention of the TB stain), 22 as False Positive (participants without any tobacco history and OPML's but tissues retained TB stain) & 10 cases were False Negative (patients had a tobacco history with presence of OPML's but tissues failed to retain the TB stain) (Table 1).

The Sensitivity and Specificity of TB staining are calculated as under -:

1. Sensitivity (True Positive Rate):

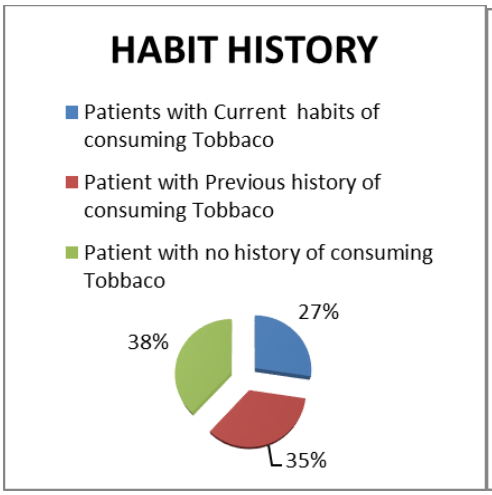
Sensitivity = TP / (TP + FN) = 32 / (32 + 10) = 0.7619 or 76.19%

2 Specificity (True Negative Rate):

Specificity = TN / (TN + FP) = 20 / (20 + 22) = 0.4762 or 47.62%

Table 1: Confusion Matrix Table of The Above Data

		Disease	
		+	-
Test	+	**True Positive** (Tp) (32)	**False Positive** (Fp) (22)
	-	**False Negative** (Fn) (10)	**True Negative** (Tn) (20)
		All With Disease (42)	**All Without Disease** (42)



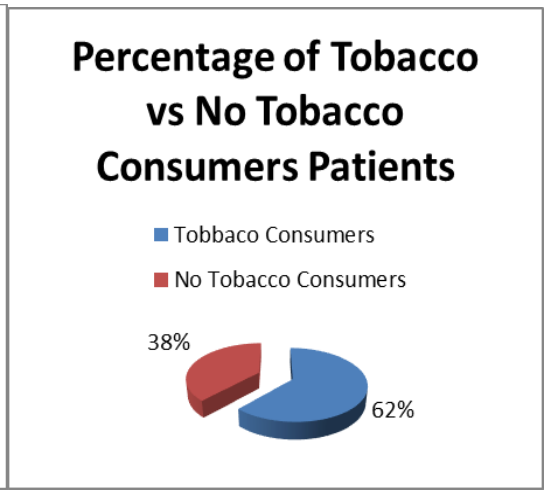
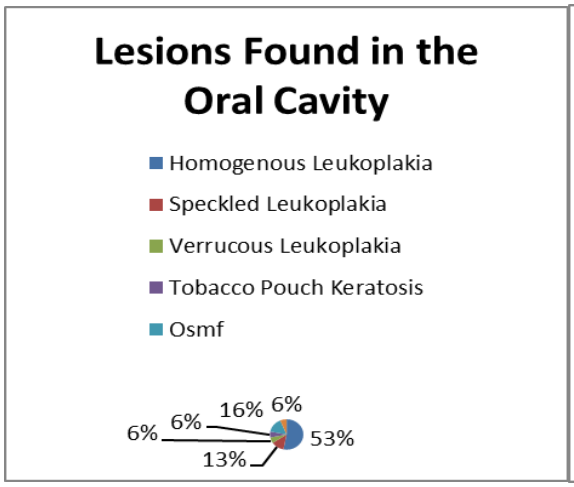


Figure 1: Habit history of the participants

Figure 2: Percentage of participants consuming Tobacco



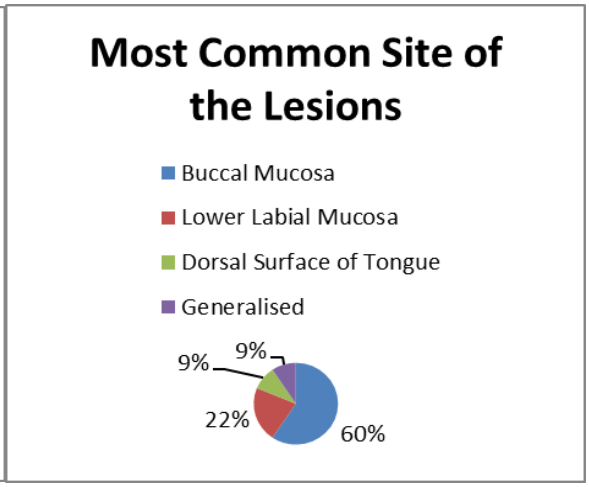


Figure 3: Lesions Found in the oral cavity in the participants.

Figure 4: Most Common sites of the lesions.

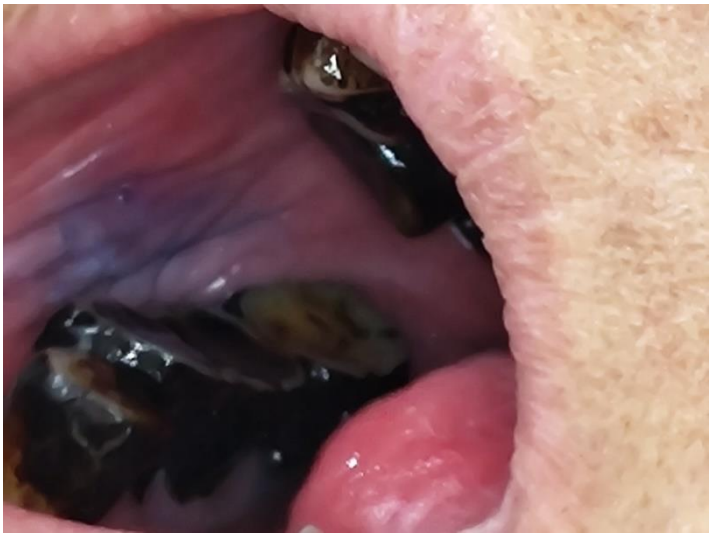


Figure 5: Toluidine Blue Staining done in right Buccal Mucosa

Our study was carried out in a closed environment in a confinement zone, when compared to similar studies it was found that it yielded approximately same results. In our study, the average age for those with clinically questionable oral premalignant lesions was 40.35 years. This conclusion is consistent with the findings of Bayad HC et al., who found a mean age of 43.86 years in a comparable study. (8) Pallagatti et al. reported that within their study group of 40 participants, 29 individuals tested positive for TB staining, representing a percentage of 72.5%. Interestingly, this finding bears similarity to our own study, in which out of 84 cases investigated, 55 cases (or 65.47%) were positive for TB staining. (9)

In Soni et al.'s study, it was disclosed that most patients exhibited lesions at buccal mucosa [109 cases] (59.6%)] followed by tongue [42] (23%)] and soft palate [11 cases] (6%) and lips 5 (2.87%) which is similar to our study having Buccal mucosa the most common site being involved, 19 cases (53%) followed by 7 (22 %) cases in lower labial mucosa and 3 cases (9 %) in dorsal surface of tongue, and 3 (9%) cases had a generalized involvement of the oral cavity .

In the study conducted by Allegra et al., involving 45 patients, a total of 26 patients tested positive for TB staining. This yielded a TB positivity rate of 57.7%. (10) On the other hand, the research conducted by Soni et al. included a larger patient group consisting of 183 individuals. Among these, a significant

146 patients were found to be positive for TB staining, resulting in a much higher percentage of TB positivity at 79.8%. This notable discrepancy in TB positivity rates between the Allegra et al. study and the study by Soni et al. might be attributed to differences in patient demographics, geographical locations, or perhaps variances in the sensitivity of the staining method used. (11) Pallegatti et al. also contributed to the discussion with their study involving 40 patients. Among this cohort, 29 individuals tested positive for TB, leading to a calculated TB positivity rate of 69.5%. This percentage lies between the rates observed in the Allegra et al. and Soni et al. studies, indicating a consistent trend of TB prevalence across these studies. (9) Comparing these three previous studies, it's evident that TB positivity rates can vary considerably based on the specific patient population, study methodology, and the region under investigation. The variations in the percentages could be attributed to differences in factors such as the prevalence of TB in the studied population, variations in diagnostic techniques, and differences in the quality of healthcare infrastructure. Table 2 describes different authors studies depicting the positivity rates of TB staining. In the present study, which includes 84 patients, 54 were identified as TB positive through staining. This results in a TB positivity rate of 64%. When compared to the previous studies, this rate falls between the rates observed in the studies by Allegra et al. and Soni et al., but it is higher than the rate reported by Pallegatti et al.

Lingen et al. reported that in their analysis, the sensitivity and specificity of TB for identifying oral cancer ranged from 78% to 100% and from 31% to 100%, respectively while the sensitivity of TB in our study is approximately 76.19%, and the specificity is approximately 47.62%. (12)

Table 2 shows the positive rate of TB staining with previous research and the present research. -

S. nos.	**Study**	**Total no. patients**	**T.B. positive**	**% of T.B. positive**
1.	Allegra et al. (2009)	45	26	57.7
2.	Soni et al. (2019)	183	146	79.8
3.	Pallegatti et al (2013)	40	29	72.5
4.	Present Study (2023)	84	54	64

4. Conclusion

TB staining can be utilized in all clinical settings since it is straightforward, affordable, generally accessible, noninvasive, and simple to apply, especially in underdeveloped nations where more sophisticated diagnostic techniques are not readily available. Toluidine Blue staining camp conducted for jail inmates in GB Nagar demonstrated its potential as a cost-effective and efficient method for identifying potentially malignant lesions. The results underscore the significance of proactive healthcare measures within correctional facilities and emphasize the need for comprehensive oral health programs targeting vulnerable populations. Further research and larger-scale implementation of such camps are recommended to better understand the implications and benefits of early detection and intervention.

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