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Evaluation of the efficacy of toluidine blue as an adjunctive tool to clinical examination in early diagnosis of clinically suspicious oral premalignant and malignant lesions.

BY

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Abstract

Aim: *The aim of this investigation was to assess the effectiveness of using toluidine blue dye for vital staining in addition to a routine clinical examination to enable the early identification of malignant lesions in the oral cavity.*

Settings and Design: *Over the course of 18 months, 16 participants with clinically suspect premalignant or malignant lesions in the Department of Oral Medicine and Radiology at a hospital.*

Methods and Material: *Every lesion had a thorough clinical examination, toluidine blue staining, and picture documentation of dye retention.*

Statistical analysis used*: Data entries were done in Microsoft Office Excel 2010 and analyses of results was done using Statistical Product and Service Solution (SPSS) version 22 software. Descriptive statistics such as mean and standard deviation was calculated for quantitative variables. Qualitative statistics was expressed in percentages. The p-value was fixed at 0.05. Diagnostic tests were applied to observe efficacy of the test method versus the gold standard method.*

Results: *According to the clinical examination, sensitivity was 86.6 Specificity was 0.0% for the Diagnostic efficacy of screening test (Toluidine blue) versus histopathological examination (gold standard). The positive predictive value was shown to be 92.86 %*

Conclusion: *Toluidine blue staining and other screening techniques are reliable in detecting oral cancer, but more thorough research with larger study samples is required for a more precise evaluation.*

Keywords*: biopsy, cancer, efficacy, toluidine blue*

INTRODUCTION

Throughout the world, oral cancer ranks sixth in frequency, with Southeast Asia having the highest incidence. The incidence of oral cancer is very high in India accounting to about 30–40% of the cancer load and this malignancy poses a significant challenge to health services, both preventive and diagnostic. The increasing tendency of pan masala and gutkha chewing may be the reason behind the rise in the occurrence of oral premalignant lesions in the Indian population, particularly among the youth.(Varshitha, A. ,2015, Sankaranarayanan R et al ,1992)

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The main cause of the delay in diagnosis and consequently bad prognosis in these patients is the lack of a trustworthy technique for an early diagnosis. Many approaches have been developed to support clinical evaluation to enhance the early diagnosis of premalignant and malignant lesions in the mouth. (Nair, D. R et al, 2012)

The most popular and well-researched method is toluidine blue in vivo staining. Since its discovery by William Henry Perkin in 1856, toluidine blue (TB) has been known for a variety of medical purposes; nevertheless, its primary use thereafter has been by the dye industry. (Chhabra, Net al, 2015). Toluidine blue is a crucial stain for mucosal diseases and can be used to specifically stain specific components in tissue sections due to its metachromatic character. Toluidine blue has been used as an essential stain to highlight potentially malignant oral lesions and perhaps reveal early lesions that might be missed during a clinical examination. Toluidine blue may be useful in identifying premalignant and malignant lesions, according to available data. (Nair, D. R et al, 2012) Currently, biopsy with histopathological examination is considered the gold standard for diagnosis of oral cancer. The primary issue is determining the best time and location for the biopsy, which is dependent on the clinician's ability to distinguish reactive and inflammatory illnesses from premalignant and malignant tumours. Therefore, this study aims to evaluate the safety and efficacy of toluidine blue as an adjunctive tool to clinical examination in early diagnosis of clinically suspicious oral premalignant and malignant lesions. (Vijayakumar, V et al 2019)

Materials and Methods:

Armamentarium –

- Diagnostic set of instruments (Fig 1)
- Waste receiver
- Cotton swab

Material –

- 1% Toluidine blue solution (Fig 2)
- 1% Acetic acid
- Distilled water

Methodology - This hospital-based diagnostic test accuracy study was conducted over a period of 18 months after approval from the institutional ethics committee via (/SMBT/EC/2022/755). 16 subjects presenting to the OPD with suspicious oral lesions were enrolled after obtaining full informed written consent. From the selected subjects with oral lesions, Case 1 (Fig 3. a, 4. a) detailed history and exposure to possible carcinogenic substances was recorded, after obtaining informed consent. All underwent detailed head and neck examination and were subjected to staining with 1% solution of toluidine blue Case 2 (Fig 3. b, 4. b) and checked if the stain was retained. (Fig 3.c, 4.c). All the lesions' stains were biopsied under local anaesthesia by biopsy and sent for histopathological evaluation.

Inclusion criteria-

- Age more than 18 years and below 70 years.
- Lesions with leukoplakia, erythroplakia, erosive/ulcerated lichen planus, and superficial ulcerations - oral mucosal PML suggestive of malignancy and other suspected malignant lesions.

Exclusion criteria-

- Patients above the age of 70 years.
- Patients with any medical history or allergy to any specific drugs.
- Patients with any medical condition or debility.

Withdrawal criteria-

- Any subject meeting exclusion criteria during the course of study
- Any subject wishing to exit the study voluntarily
- Any subject with adverse effects if any

  If subject cannot adhere to the study timelines and appointments

Preparation of toluidine blue:

Toluidine Blue is prepared in 1% concentration for oral application a 100 ml of 1% toluidine blue solution was prepared by mixing 1 g toluidine blue powder, 10 ml 1% acetic acid, 4.19 ml absolute alcohol, and 86 ml distilled water with pH regulated at 4.5. 1% acetic acid solution was prepared by diluting 5% acetic acid. It was done by mixing 1 part of acetic acid with 4 parts of distilled water.(Sridharan, G.,2012)

Staining Protocol:

To remove particles, the application procedure entailed rinsing the mouth with water for 20 seconds. After that, ropey saliva was removed by applying 1% acetic acid for 20 seconds. After that, 1% toluidine blue was applied using a cotton swab for 20 seconds. To get rid of the discoloration that was mechanically maintained, 1% acetic acid rinse was used once more. At last, water was used to rinse the mouth. (Mashberg, A,1980).

Interpretation of Stain:

The interpretation was based on colour; dark blue (royal/-navy) stain was considered positive. Light blue staining or when no colour was observed it was interpreted as negative(Gandolfo, S, 2006)

The biopsies were performed under local anaesthesia, for each specimen the clinical examination and the result of the toluidine blue staining were reported.

The clinical or staining evaluation of every sample was not disclosed to the pathologist reviewing every biopsy. The following terms were used to describe histopathologic diagnoses: non-neoplastic (hyperkeratosis, hyper-para-keratoses, etc.); mild, moderate, or severe dysplasia; in situ carcinoma; and invasive cancer.

Data entries were done in Microsoft Office Excel 2010 and analyses of results was done using Statistical Product and Service Solution (SPSS) version 22 software. Descriptive statistics such as mean and standard deviation was calculated for quantitative variables. Qualitative statistics was expressed in percentages. The p-value was fixed at 0.05. Diagnostic tests were applied to observe efficacy of the test method versus the gold standard method.

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Results:

Most of the subjects in the study were above 40 years of age (81.25%) with mean age being 50.9 of which 56% were males. (Graph no 1) The buccal mucosa, was the commonest site; accounting for 62.5% (10/16). The next common site was the labial mucosa 18.75% (3/16) 87.5% (14/16) of the lesions were positively stained with toluidine blue and 15.5% (2/16) were stained negative. (Graph no 2). Following histological examination of the biopsy samples, 15 (93.75) were diagnosed as malignant i.e. Oral squamous cell carcinoma; of which 37.5% (6/16) were moderately differentiated; 50.0% (8/16) well differentiated; and 6.25% (1/16) were poorly differentiated. (Graph no 3)

Sensitivity and specificity were determined from true-positive and true-negative results. Positive predictive value was calculated as true positive/true positive + false positive and negative predictive value as true negative/false negative/true negative.

Toluidine blue stain retention was compared with histopathologic assessment as the gold standard for statistical analysis. To determine the test's diagnostic accuracy, we calculated the sensitivity, specificity, positive predictive value, negative predictive value, and true-positive, true-negative, false-positive, and false-negative outcomes.

Out of 14 toluidine blue positive lesions, 1 was diagnosed as benign/inflammatory lesions i.e. False Positive (FP). Out of the 2, toluidine blue negative lesions; none were histologically proved to be benign i.e. True Negative (TN). (Table No. 1)

According to the clinical examination, sensitivity was 86.6 Specificity was 0.0% for the Diagnostic efficacy of screening test (Toluidine blue) versus histopathological examination (gold standard). The positive predictive value was shown to be 92.86 % (Table no 2)

Discussion:

In the high-risk countries, oral cancer is the most common malignancy, accounting for over 25% of all new cases of cancer each year.[1] The incidence of oral cancer increases with age and is highest over 60 years, even though cases in people younger than 40 years are increasing. (Warnakulasuriya, S, 2009).

For a patient with cancer, prevention, early detection, and rapid referral for diagnosis offer the highest chance of a cure. Vital dyes or stains, as toluidine blue (Scully, C, 1986) Lugol's iodine, and crystal violet, have become popular diagnostic techniques in recent years for the early detection of malignant and potentially malignant tumours. This is a low-risk, low-cost, non-invasive technique that can be completed quickly as an outpatient operation.

1949 saw the Journal of the American Medical Association (JAMA) publish one of the earliest papers on toluidine blue. (Porter, S, 1998). It can identify early nuclear alterations of malignancy and selectively stains acidic tissue components (carboxylates, sulphates, and phosphate radicals), including DNA and RNA. (Martin, I. C, 1998)

In the present study, it was found that the most common site for the malignant lesion was buccal mucosa with a higher prevalence in males. A sensitivity of 86.6% and specificity of 0.0% was recorded. These values of sensitivity and specificity were considerably lower than those reported by Hegde et al. (Hedge, M. C, 2006) (97.29 and 62.5% respectively), E. Allegra, (Allegra, E, 2009) (96.2% and 86.6% respectively) and the sensitivity values were considerably higher than those reported by Ram and Siar in their study. (Ram, S et al, 2005) (70.3 and 25% respectively). The specificity values are in accordance with Warnakulasuriya KA, Johnson NW. et. al (Warnakulasuriya, K et al, 1996) (0.00% and 0.62% respectively)The differences in the outcomes could be attributed to the tendency of ulcerative and inflammatory lesions to hold onto the dye, leading to a higher rate of false positives. Moreover, dye penetration into the deeper epithelial layers of hyperkeratotic lesions is hindered, leading to erroneous negative results. Additionally, the staining methodology and procedure, the type of lesions, and interobserver variation may all affect the staining results.

Our positive predictive value was 92.86% which was higher than that reported by Onofre et al. (43.5%) (Onofre, M. A et al, 2001) and E. Allegra (86.6%) (Allegra, E, 2009) and Rodriguez et al. (35.2%) (Cancela-Rodríguez, P et al, 2011) but lower than that reported by Nagaraju et al (94.8%). (Nagaraju, K et al, 2010). This implies that in cases where the disease is more prevalent, positive results will verify its existence, whereas in cases where the disease is less prevalent, a positive result will prevent its confirmation.

Hence if toluidine blue dye test is used in high-risk population, diagnostic validity of the test increases. Conversely, in cases when the prevalence is minimal, a negative test result allows the condition to be safely ruled out.

Clinical significance of the study:

It is suggested that the technical modality of toluidine blue may be very useful since there is not as much subjective determination necessary with the stain. With the use of toluidine blue and well-defined visual criteria, combined with the ease of observation of oral structures, the use of cytology in intraoral cancer detection appears redundant. (Mashberg, A,1981).

Moreover, lesions positive for toluidine staining have recently been shown to exhibit genetic changes linked to several sites of heterozygosity loss, which are commonly implicated in the multistep process of head and neck carcinogenesis. Zhang et al. demonstrated the potential value of toluidine blue to detect precancerous and cancerous oral lesions with molecular features at high risk of clinical progression.

The limitation of our study:

The study's main drawback was that, because it was conducted in a hospital, it did not address the value of the toluidine blue dye test in primary care settings.

It will not be correct to state that toluidine blue does not have any shortcomings but evaluating as a whole and compared

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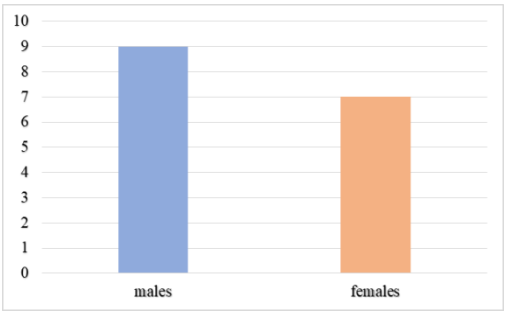
with other modalities it clearly emerges as a winner based on evidence.

Conclusion:

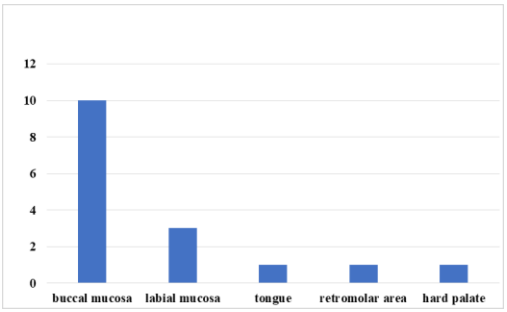
Toluidine blue seems to provide an instantaneous, workable diagnostic "control" on the physician's subjective assessment. Its benefits include preserving the clinical impression, managing clinical false negatives, lowering false positives, and maybe identifying additional lesions that were overlooked during the examination (by staining high-risk areas). There is no need for a middleman in the diagnosing process because it is a very quick and easy office procedure. It should be emphasised, nonetheless, that a biopsy is still required even if a lesion stains with toluidine blue and/or fits the visual criteria for early cancer.

While it would not be accurate to say that toluidine blue is flawless, when considering it as a whole and contrasting it with alternative modalities, the evidence plainly shows that it is superior. Toluidine blue staining and other screening techniques are reliable in detecting oral cancer, but more thorough research with larger study samples is required for a more precise evaluation.

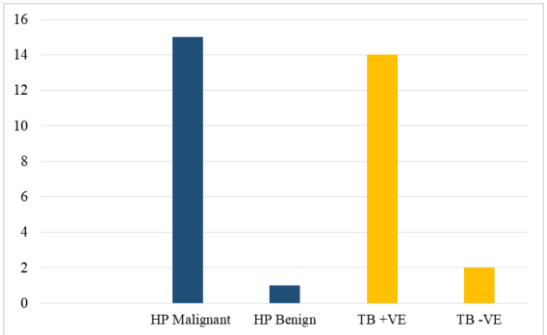
Tables:



Graph no 1 **Gender distribution**



Graph no 2 **Site distribution**



Graph no 3 **Histopathology results and toluidine blue retention**

HP Malignant – histopathological malignant
HP Benign – histopathological benign
TB +VE – Toluidine blue positive
TB - VE – Toluidine blue negative

Table 1: Effectiveness of toluidine blue as screening agent versus histopathological examination (gold standard)

HP EXAMINATION ⇒	PRESENT (n=15)	ABSENT (n=1)
TEST (TB STAINING) ⇩		
POSITIVE (N=14)	**13**	**1**
Negative (n=2)	**2**	**0**
TRUE POSITIVE	**13**	
FALSE POSITIVE	**1**	
FALSE NEGATIVE	**2**	
TRUE NEGATIVE	**0**	

Table 2: Diagnostic efficacy of screening test (Toluidine blue) versus histopathological examination (gold standard)

Statistic	Value	95% CI
Sensitivity	**86.67%**	**59.54% to 98.34%**
Specificity	**0.00%**	**0.00% to 97.50%**
Positive Likelihood Ratio	**0.87**	**0.71 to 1.06**
Negative Likelihood Ratio	**----**	**------**
Disease prevalence (*)	**93.75%**	**69.77% to 99.84%**
Positive Predictive Value (*)	**92.86%**	**91.42% to 94.07%**
Negative Predictive Value (*)	**----**	**------**
Accuracy (*)	**81.25%**	**54.35% to 95**

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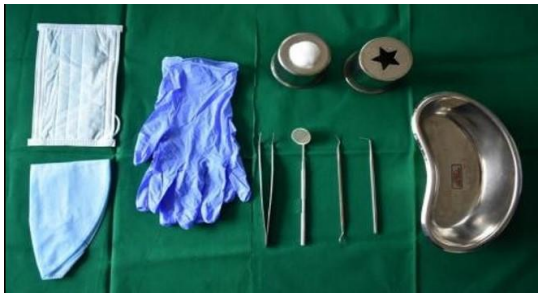

Fig 1 Armamentarium

Fig 2: Water, Acetic acid, and Toluidine blue stain

Case 1 Homogenous leukoplakia on Left buccal mucosa

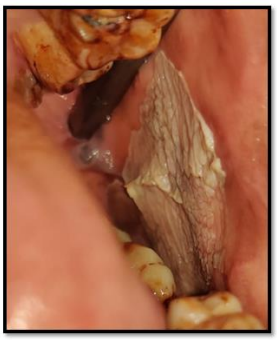

Fig 3.a

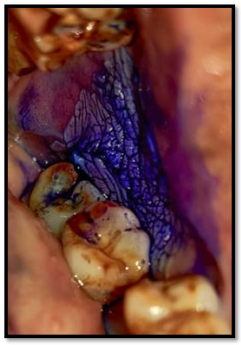

Fig 3. b

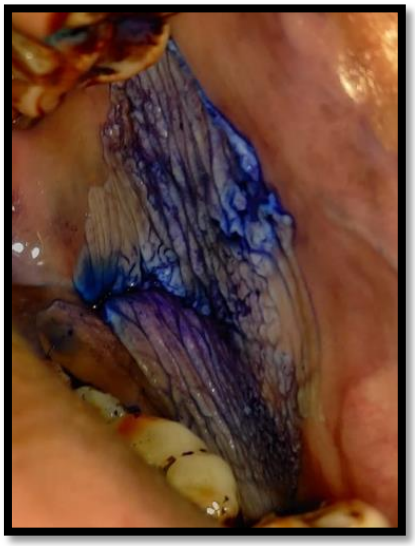

Fig 3. C

Fig 3.a: Homogenous leukoplakia on left buccal mucosa, Fig 3.b: Toluidine blue staining, Fig 3.c: Toluidine blue retention

Case 2 Malignant growth on left buccal mucosa

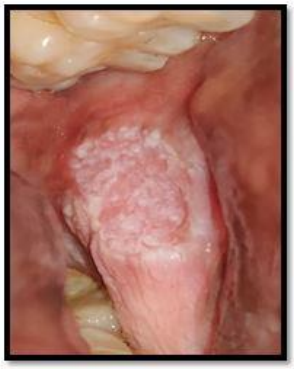

Fig 4.a

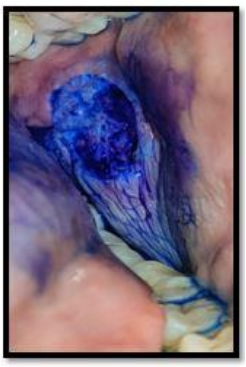

Fig 4.b

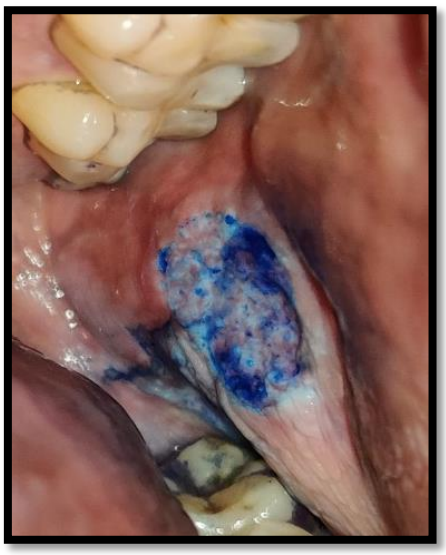

Fig 4.c

Fig 4.a: Malignant growth on left buccal mucosa, Fig 4.b: Toluidine blue staining, Fig 4.c: Toluidine blue retention

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