Toludine Blue a Easy Screening Procedure for Detection of Epithelial Dysplasia & Detection of Biopsy Sites

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Abstract

The incidence of oral cancer in world has increased over the last 50 years. It is one of the most mutilating disease and sixth most common cancer worldwide and represents about 5.5% of all malignancies. Over 90% of all oral cancers are squamous cell carcinomas arising from lining mucosa. Oral squamous cell carcinoma is most commonly seen in males. Screening of suspicious oral lesions can help in the early detection of oral cancer and can help in reducing the morbidity associated with this condition. Incisional biopsy of the suspicious lesion remains a gold standard for diagnosis of oral premalignancy though the question of when to perform the biopsy and which site to choose remains debatable. There is always a question of inter observer variability that comes in our mind

Aims & Objective: To assess the reliability of toluidine blue as clinical screening test, for identifying the appropriate site for incisional biopsy for identification of epithelial dysplasia.

Study Design: Study Group consisted of 30 patients with leukoplakia. Biopsy specimens were taken from two different sites of same lesion taking in consideration intensity of staining. Then the results were critically analyzed by different observers.

Result: It is seen that there was 100% agreement between the three observers for clinically toluidine blue positive site being dysplastic histologically. Inter observe variability group A = 1; $p<0.001$ and for group B = 0.42; $p=0.002$.

Key Words: Toludine Blue, Oral Premalignant Lesions, Epithelial Dysplasia.

Introduction

How can dentists and doctors tell by sight which abnormalities to biopsy? There is a pressing need of a screening test. The pre requisite for good screening test are it should be simple, safe and acceptable to the public. To detect pathological conditions as soon as possible. Test should have a high positive predictive value and low false negatives (high sensitivity). The presence of dysplasia, the current gold standard, is a good predictor of high-grade lesions but has only a limited capacity to predict outcome for lesions with minimal or no dysplasia, which constitute the majority of OPLs.[1]

Vital tissue staining has been identified as an adjunct to the early recognition of malignant lesions. Toluidine blue {TB} (tolonium chloride) is a member of thiazine group of metachromatic dyes, and is partially soluble both in water and in alcohol. TB staining may appear as a Dark Royal Blue or a Pale Royal Blue color.[2]

Purpose of the study was to assess the reliability of toluidine blue as clinical screening test, for detection of epithelial dysplasia & its role in identifying the appropriate site for incisional biopsy.

Materials and Method

The following prospective study was conducted in a private clinic in Lucknow. The study group consisted of 18 patients with homogenous and non-homogenous leukoplakia. The exclusion criteria included patients with systemic disorders, patients not willing for biopsy, patients with lesions at sites where application of toloudine blue or biopsy was not possible, oral submucous fibrosis patients with severe fibrosis or trismus. Subjects were selected from both sexes and all age groups. Informed consent was taken from patients before application of the vital stain and biopsy.

Composition of Toluidine Blue Stain

100 c.c of the 1% TB solution: this solution as described by Mashberg, consists of 1 gm of TB powder, 10 ml of 1% acetic acid, 4ml 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.[3]

Technique of Staining

Patient is asked to rinse the mouth with for 20 seconds. 1% acetic acid was applied for 20 seconds to remove any saliva and organic debris left using cotton. After complete cleaning of oral mucosa 1% Toulidine Blue solution is then applied for 20 seconds with cotton Swab. Again 1% acetic acid again on mucosa attained by toluidine blue by cotton swab to remove nonspecific mechanically retained stain from the lesion. Finally patient was asked to rinse mouth with water and then toluidine blue staining was assessed.[3]

Two biopsy were taken from intensely stained area(Group A) and one from periphery or unstained area(Group B), within the boundary of the lesion. (Fig:1). All specimens were subjected to routine fixation staining. The histological sections was assessed microscopically and graded for epithelial dysplasia as dysplastic & non dysplastic lesion (Fig 2 & 3). The findings of the two biopsy sites were compared & analyzed statistically. Another group consisting of 18 successive patients of leukoplakia, in whom incisional biopsy had been done without any adjunct for biopsy site, was taken from our records and graded histopathologically in a manner as above. The findings of this group were than compared with our study group.

Interpretation

The clinical uptake of stain was always evaluated by the same examiner and the cases were divided into two groups dysplastic and non dysplastic. They were than observed by 3 observers and were scored on basis of architectural and cytological changes.

Architectural criteria included, irregular stratification, loss of polarity, basal cell hyperplasia, drop shaped rete ridges, dyskeratosis, keratin pearls and mitosis.[4] All except mitosis were scored as 0, 1, 2 and 3 on basis of -no dysplastic change, dysplastic changes at focal areas, Intermediate dysplastic changes and generalized dysplastic changes respectively. Mitosis were scored as 0, 1, 2 and 3 where 0-1 (mitosis per high power field), 1-2 (mitosis per high power field), 2-3 (mitosis per high power field) and above 3 (above 3 mitosis per high power field atypical mitosis).

Cytological criteria included cellular and nuclear pleomorphism, increase nuclear cytoplasmic ratio, increased number and size of nucleoli and hyperchromatism. [4] They were scored as 0, 1, 2 and 3 same criteria as above.

Result

Most of the patients were in the age group of 30 - 60 years with a mean age of 45 years. In our study a male preponderance was seen and majority of our patients gave history of smoking, quid/ tobacco chewing and alcohol consumption. Buccal mucosa was the most common site of lesion followed by vestibule and commisure of lip.

We found that all 18 cases of toludine blue positive sites of homogenous and non leukoplakia clinically (Group A) were dysplastic when analyzed histologically by the three observers. Hence giving 100% result, as shown in graph1. Next we analyzed toludine blue negative sites of the same lesions (Group B) histologically. We found that all the three observers gave variable diagnosis related to dysplasia. Observer 1 found 7 out of 18 cases to be nondysplastic whereas observer 2 and 3 found 6 and 5 cases nondysplastic respectively. Graph 3 shows inter observer variability. It is seen that there was 100% agreement between the three observers for clinically toludine blue positive site being dysplastic histologically. Whereas only 42% agreement was there between observers regarding toludine blue negative site clinically, as non dysplastic histologically. Inter observe variability group A = 1; $p<0.001$ and for group B = 0.42; $p=0.002$. Graph 4 shows increase sensitivity in detecting epithelial dysplasia using toludine blue as adjunct. ($df = 1$, $p = 0.003$). Toludine blue stained 18 lesions when compared to our previous records (biopsy done without application of toludine blue) we found that all 18 sites of the lesion positive clinically for toludine blue were dysplastic histologically. Whereas when we analyzed our records we observed that out of 18 only 11 cases were diagnosed dysplastic and 7 as nondysplastic.

Discussion

Toludine blue is an acidophilic dye 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 quatitatively 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.[5]

As shown in our results of graph 2, even though the site of lesion did not take up stain or was weekly stained they were diagnosed non dysplastic histologically giving false results. This indicated that if on general assumption biopsy is done than there are chances that surgeon may take up biopsy form nondysplastic area of the lesion though the lesion may be dysplastic and may miss the correct dysplastic area hence giving wrong results. Our results also show high chances of interobserver variability for biopsy taken from toludine blue positive area as seen in graph 3. We can conclude from graph 4 that 7 out of 18 cases from our record without toludine blue stain would have been dysplastic, but biopsy site chosen may not be appropriate and hence may be giving false results.

As explained by Epstein et al. when a biopsy is performed, site selection is critical, as the histologic features may vary in non-uniform lesions. If only areas of less severe cellular change are sampled, the less severe cellular pattern observed may be interpreted as representative of the lesion as a whole (even if there are other areas of more severe cellular change), and appropriate treatment may not be given. Similarly, histologic interpretation is itself a subjective science and interpretation varies among pathologists; this variability can also lead to inappropriate diagnoses and treatment. However, widespread application of toludine blue should be undertaken with caution, as there are no studies assessing its use in nonspecialty centers or assessing the practices of individuals with less experience in interpreting results. If this dye is felt to be appropriate as an adjunct to visual examination, especially for patients with suspicious lesions, referral to a centre or individual with extensive experience in head and neck cancer is recommended. Toludine blue has also been reported as an aid in selecting biopsy sites and in delineating the margins of lesions.[6]

Draw backs in our study included confusion over inclusion of equivocal (pale) staining as positive or negative. Sample size was small. No cases of non dysplastic white lesion in study as it was prospective study done in 18 successive patients. Toludine blue staining in inflammatory mucosal conditions have not been studied.

Conclusion

Toludine blue is a good for screening test as it is simple, safe and acceptable to the patient. Detect disease early in its natural history. Preferentially detect those lesions which are likely to progress and is helpful technique for determining the site of incisional biopsy.

References

References are available on demand at editor@healtalkht.com

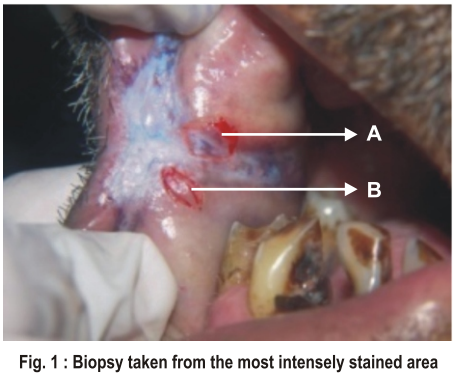


Fig. 1 : Biopsy taken from the most intensely stained area (A) & second from unstained/weakly stained area (B) within the boundary of the lesion

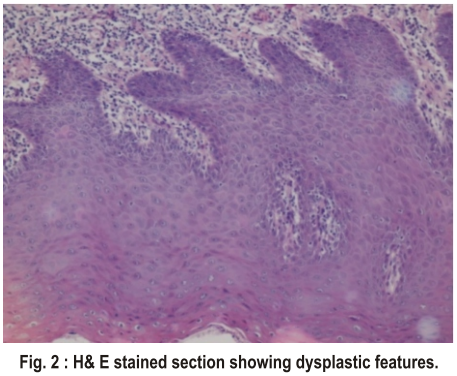


Fig. 2 : H& E stained section showing dysplastic features.

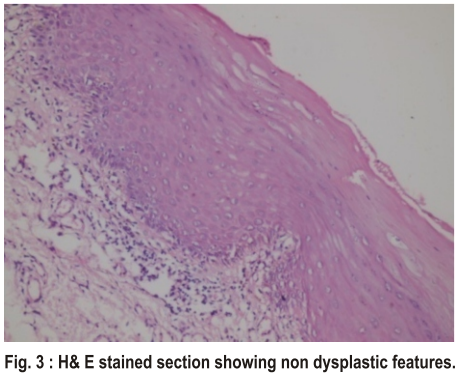
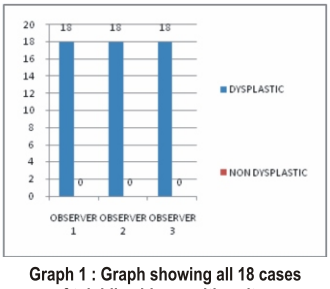
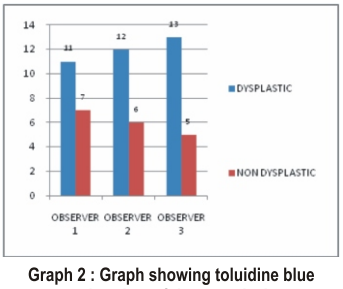


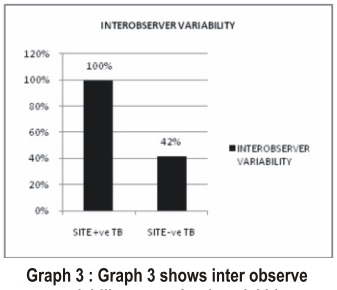
Fig. 3 : H& E stained section showing non dysplastic features.



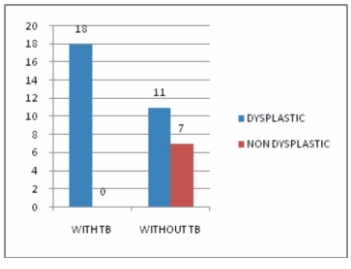
Graph 1 : Graph showing all 18 cases of toludine blue positive sites, were dysplastic giving 100% result.



Graph 2 : Graph showing toludine blue negative sites of the same lesions (Group B) histologically nondysplastic cases varied.



Graph 3 : Graph 3 shows inter observe variability group A = 1; $p<0.001$ and for group B = 0.42; $p=0.002$.



Graph 4 : Graph 4 shows increase sensitivity in detecting epithelial dysplasia using toludine blue as adjunct.