Aim of the study: Oral squamous cell carcinoma (OSCC) is one of the tenth most common cancers in the world. The primary sites for occurrence include buccal mucosa, tongue, alveolus, gingiva, palate, lip and floor of the mouth. It is also a common cause for mortality and morbidity around the world. A significant proportion of oral squamous cell carcinomas are preceded by potentially malignant lesions like leukoplakia and oral submucous fibrosis which are relied on clinical and laboratory based investigations.

Methods: In this cross-sectional study clinically diagnosed 25 patients of potential/precursor malignant lesions and 25 patients of oral squamous cell carcinoma were selected from OPD. After taking written consent Toluidine blue application was done for all the patients, scalpel biopsy was taken for all of them. Clinico-pathological comparison was done and results were analyzed by chi-square test, assessed for sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV).

Results: Potentially malignant and malignant lesions showed different sensitivity and specificity reaction with difference in positive and negative predictive values. However there was statistical significant difference among PM lesions and OSCC.

Conclusion: Toluidine blue staining has more sensitivity hence with highest PPV for malignant lesions, however it was almost equal sensitive and specific for PM lesions. Toluidine blue staining in OSCC and PM lesions is definitely helpful in many ways, but results should be carefully interpreted and one should not solely rely on it.

KEYWORDS: biopsy, oral cancer, toluidine blue.

Introduction
Oral squamous cell carcinoma (OSCC) is one of the tenth most common cancers in the world. The primary sites for occurrence include buccal mucosa, tongue, alveolus, gingiva, palate, lip and floor of the mouth. It is also a common cause for mortality and morbidity around the world. A significant proportion of oral squamous cell carcinomas are preceded by potentially malignant lesions like leukoplakia and oral submucous fibrosis which are relied on clinical and laboratory based investigations.

Techniques that are assessed to improve early detection and diagnosis of oral malignancy include Toluidine blue staining (TB), ViziLite Plus with TB, ViziLite, Microlux DL, Orascopic DK, VELscope, and Oral CDx brush biopsy. These adjunctive techniques are promoted to facilitate the detection of premalignant disease. It is demonstrated that if a premalignant lesion is detected and treated, the lesion may not progress to cancer.

TB (also known as toluidine chloride) which is a metachromatic dye containing zinc chloride double salt of amino dimethyl amino-tolu-phenathiazonium chloride, which stains mitochondrial DNA, cells with greater-than-normal DNA content or altered DNA in dysplastic and malignant cells. It is a member of the thiazine group and is soluble both in water and alcohol. It can be used as 1% or 2% oral rinse or an application either in aqueous form or as weak acid solution or of undefined formulation. TB application helps for detection of lesions and can be for selection of Biopsy sites. TB application has been used in many investigations.

Materials and methods
Fifty patients with clinically detectable oral potentially malignant lesions and malignant lesions were selected. A written consent was taken from all patients before the application of the TB. A clinical history of the lesion was taken from all patients, and was submitted to a systematic oral examination. The criteria for clinical and histopathological diagnosis were based on WHO guidelines.

The criteria for clinical diagnosis were (1) homogenous leukoplakia: a predominantly white, uniform, and flat lesion, not able to be scraped, with a smooth, wrinkled, or corrugated surface that may exhibit shallow cracks, (2) nonhomogenous leukoplakia: a predominantly white or red and white lesion with an irregular, nodular, or exophytic surface; (3) erythroplakia: a velvety red lesion with imprecise borders that could not be diagnosed as any other lesion; (4) reticular lichen planus: a predominantly white lesion with intertwining lines or striae that confer a lacy or annular appearance; (5) erosive/ulcerated lichen planus: a predominantly red, irregular erosion or ulceration associated with a reticular form, especially in the peripheral region of the lesion and with pseudomembranes covering the ulcerated areas; (6) superficial ulcerations suspicious of malignancy: localized, superficial lesions without invasion or loss of mobility of neighboring chronic tissues that do not heal after local treatment.

The lesions were histologically classified as (1) squamous cell carcinoma: severe dysplasias involving the entire extent of the epithelium without invasion of the connective tissue (in situ carcinoma) or with invasion of connective tissue (frank invasive carcinoma) or limited to the juxtaepithelial area (initial invasive carcinoma); (2) epithelial dysplasia: the parameters recombine in the WHO13 were used; dysplasias were classified as mild, moderate, and severe; (3) keratosis: the presence of various degrees of keratosis and/or acanthosis with or without epithelial dysplasia or atypical cells; (4) lichen planus: liquifying degeneration of basal cells, band-shaped lymphocyte infiltrate in the connective epithelial tissue, with or without saw-toothed projections, hyperkeratosis, the presence of Civatte bodies, and separation between the epithelium and connective tissue.

Photograph of each patient was taken before and after application of TB for the record purpose. Method of application of the 1% TB staining technique used in the study was according to Onofre et al12. The lesion was first wiped with H2O2 using cotton swab to remove any saliva, and other debris. Following this, 1% acetic acid was applied to the lesion & then cleaned with water jet. 1% aqueous solution of TB was applied with cotton tip and left for 30 seconds, followed by 1% acetic acid application to remove any excess stain. Photographs were taken of the stained site. The pattern of dye retention was assessed at sites with dark royal blue color as positive (Fig 1 A, B & Fig 2 A, B), and were biopsied. Areas which did not demonstrate any kind of blue staining were considered as having a negative staining pattern, and in such conditions clinical judgment directed the biopsy. The biopsy
specimens were submitted to routine procedures of hematoxylin-eosin staining and were examined by two pathologists for presence or absence of dysplasia and invasive carcinoma (Fig 3A, B). These examiners were unaware of clinical examination and TB staining to avoid any bias. The results of the clinical and histological diagnosis were compared to the staining results. Statistical analysis was done with chi-square test for TB staining and the sensitivity, specificity as well as the positive and negative predictive values (PPV and NPV) was calculated.

Results
Out of 50 clinically suspected lesions (both malignant & premalignant) 76% lesions showed positive reactions, amongst which 60% were oral potentially malignant lesion (OPML) and 92% were OSCC.

Chi-square test showed statistical significant difference between these two groups on clinical examination for TB staining (Table I). Histopathologically, 80% of OPML showed true positive and with OSCC 92% were true positive lesions. False positive lesions were highest in OPML than OSCC (Table II). The sensitivity and positive predictive value were higher with OPML lesions over OSCC (Table III).

Discussion
Morbidity and mortality rate of OSCC can be reduced by early detection of oral potentially malignant lesions. Apart from clinical examination, application of TB for recognition of mucosal malignant lesions and OPML is in practice from decades, however, the biopsy remains gold standard, but non invasive techniques are helpful in many ways. Pre malignant lesion cells are found to be associated with loss of heterozygosity, 1-3 and as well show faster advancement into invasive carcinoma. 4,5

In the present study, TB staining has revealed statistical significant difference for OPML versus invasive carcinoma. Our study finding is consistent with the study done by Gupta et al. where it was found that TB staining was more sensitive to malignant lesions than OPML lesions 6. Visual examination is dependent on the royal dark blue color appreciation; however, its uptake in tissues varies within cells from benign and malignant lesions 7-9. TB staining showed statistically significant difference for pre and post application in carcinoma and potentially malignant lesion of oropharyngeal lesions, thus helped in recognizing the clinically unnoticed lesions. 10-12 However, these results should be interpreted cautiously as TB staining is more sensitive to malignant than potentially malignant lesions. 10,11,13 Our finding have also shown similar results, i.e. TB staining is more sensitive and has high PPV in malignant lesions.

Some authors have found that, clinically, TB staining utility can be enhanced by addition with chemiluminescent light and exfoliative cytology.14-15. TB application followed by Lugol’s iodine application has increased the differentiation of inflammatory lesions from PM lesions/OSCC with high proliferative index.16. Many authors found its application useful in identification of lesion's extension or surgical borders for OSCC patients and even for follow up 16-18.

Within the limitation of this study, it can be concluded that staining with TB is highly reliable for the detection of in-situ carcinoma and invasive carcinoma. Few false positive results were seen with OPMLs. Hence, clinicians should be aware of false positive and false negative results of this technique. It acts as an adjunct to clinical judgment and not a substitute for biopsy. From patients view point this technique is compliable as it is easy to perform, economic and gives rapid results.

FIGURE LEGENDS

Figure 1: Lesion before (A) and after (B) application of TB in PM lesion.

Figure 2: Lesion before (A) and after (B) application of TB in carcinoma.

Figure 3: Photomicrograph of hematoxylin-eosin stained lesional tissue of PM lesion (A) and carcinoma (B) (both original magnification 40x).

Table I: Clinical diagnosis of oral lesion and reaction to toluidine blue staining.

<table>
<thead>
<tr>
<th>Clinical suspected lesion</th>
<th>Toluidine blue staining</th>
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<tbody>
<tr>
<td>OPML (n=25)</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Total (n=50)</td>
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Table II: Comparison with histopathological diagnosis and clinical staining of toluidine blue.

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Clinical result of toluidine blue staining</th>
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<tr>
<td>OPML with Dysplasia (n=17)</td>
<td>Positive 11/11 (65)</td>
</tr>
<tr>
<td></td>
<td>Negative 6/6 (40)</td>
</tr>
<tr>
<td>OPML without Dysplasia (n=8)</td>
<td>Positive 4/4 (50)</td>
</tr>
<tr>
<td></td>
<td>Negative 4/4 (50)</td>
</tr>
<tr>
<td>OSCC (invasive carcinoma)  (n=22)</td>
<td>Positive 21/21 (95)</td>
</tr>
<tr>
<td></td>
<td>Negative 1/1 (50)</td>
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</tbody>
</table>

Table III: Sensitivity, specificity, positive predictive value and negative predictive value for toluidine blue staining.

<table>
<thead>
<tr>
<th>OPML (%)</th>
<th>OSCC (%)</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>11/15 (73)</td>
</tr>
<tr>
<td>Specificity</td>
<td>4/10 (40)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>11/17 (65)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>4/8 (50)</td>
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</tbody>
</table>

Figure 1: Lesion before (A) and after (B) application of TB in PM lesion.

Figure 2: Lesion before (A) and after (B) application of TB in carcinoma.

Figure 3: Photomicrograph of hematoxylin-eosin stained lesional tissue of PM lesion (A) and carcinoma (B) (both original magnification 40x).
References


