

# Methylene Blue and Lugol's Iodine as an Adjunctive Tool for Early Diagnosis of Premalignant Oral Lesions

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## Abstract

**Objective:** To compare Methylene blue and Lugol's Iodine as an adjunctive tool for early diagnosis of premalignant oral lesions by taking histopathology as gold standard.

**Methods:** In this cross-sectional study patients of oral premalignant lesions were selected. Methylene blue and lugols iodine staining was used at lesion's area. Mucosa of target site was dried gently by gauze and power air spray with triple syringe to make sure that the lesion is not being contaminated with saliva. The dye was applied directly with a cotton bud for 10-20 seconds and was decolorized. The dye retention pattern was evaluated by stain retention's intensity on the lesion. Incisional biopsy was performed simultaneously from that site as gold standard.

**Results:** Out of 60 cases, males were 81.6%. Majority (65.0%) had more than one addicting habit of mainpuri, gutka and supari. The commonest region of oral lesions was the buccal mucosa (61.6%). According to the diagnostic accuracy of methylene blue the sensitivity was 89.4% and specificity was 66.6%. According to the Lugol's Iodine sensitivity was 83.3% and specificity was 50%.

**Conclusion:** Methylene blue and lugols iodine staining are an easy and non-invasive screening tools for the early diagnosis of malignancy.

**Key Words:** Premalignant and malignant lesions, Methylene blue, Lugol's Iodine, Oral Cavity

## Introduction

Oral cavity represents nearly 3% of overall cancers. Its annual incidence is approximately 0.5 million new cases per year.<sup>1</sup> Despite of incredible developments in therapeutic modalities, the survival rate of 5-year hasn't significantly enhanced over the previous many decades as well as yet drifts at nearly 50% to 60%.<sup>2,3</sup> Oral squamous cell carcinoma (OSCC) is placed in 8<sup>th</sup> level in cancer prevalence globally.<sup>3</sup> Several OSCCs emerge from premalignant lesions and oral cavity

disorders.<sup>4</sup> Tobacco usage has been found as a substantial risk for the occurrence of OSCC and premalignant lesions. Around 80% of cases with oral squamous cell carcinoma have utilized tobacco products.<sup>5</sup> Alcohol consumption has as well been considered as risk factor for developing oral squamous cell carcinoma and premalignant lesions. Studies have exhibited that heavy to moderate drinkers have 3 to 9 fold higher risk of acquiring malignancy. Heavy consumption of tobacco and alcohol in combination can possibly carry risk 100-folds higher than the general populace.<sup>5</sup> A variety of conditions are involved in occurrence of oral malignancy, including erythroplakia, oral submucous fibrosis, leukoplakia, palatal lesion of reverse cigar smoking, discoid lupus erythematosus, and hereditary conditions for instance epidermolysis bullosa and dyskeratosis congenital.<sup>7,8</sup> To avoid malignant metamorphosis of these predecessor lesions, multiple detection and screening methods have been established.<sup>9</sup>

Biopsy of oral cavity with histological appraisal is a benchmark for oral premalignant disorders. Yet, being invasive, a few patients can possibly not afford this investigation, particularly if lesion seems "normal". The outcomes can possibly be influenced by depth and size of biopsy, specimen quality, freezing & fixation methods, and experience of pathologist.<sup>10</sup> Techniques of detection, which are non-invasive, are of help for oral premalignant disorders. In previous few decades, wide-ranging non-invasive methods have emerged for oral premalignant disorders detection.<sup>10</sup>

In diagnosis, Methylene Blue method's precision is used to identify dysplasia, carcinoma or intestinal metaplasia.<sup>11</sup> The exact process for Methylene blue's uptake in epithelial tissues can possibly be similar to that of Toluidine blue in acidophilic characteristics of cells with uncharacteristic concentrations of nucleic acid, leading to differential uptakes amid benign/normal and highly malignant/dysplastic cells. Methylene blue is frequently applied stain that comforts in seeing microscopic organisms in vivid colors. The Methylene blue dye exhibits the deepest color of blue, representing a potential

temptation to acids together with DNA.<sup>11</sup> The structures of mucosal surface of oropharynx and mouth are much like the proximal esophagus and neoplastic transformational risk factors at these areas are alike. Lugol's iodine imaging of dysplastic mucosa is used to manage esophageal disorder. Though, lugol's iodine staining relies upon the glycogen contents existing within normal epithelium as well as this selective characteristic of staining assists in outlining the carcinomatous or inflammatory epithelium from typical epithelium where content of glycogen is low.<sup>15,16</sup>

### Patients and Methods

This descriptive study was held at outpatient Department Oral surgery, Isra Dental College and histopathological laboratory of Isra University, Hyderabad. Study duration was 6 months from Jan. 2017 to July 2017. All the patients aged from 20 to 70 years, clinical suspicion of oral squamous cell carcinoma and oral premalignant lesion, patients with habits of chewing betel quid, areca nuts etc and patients with the habit of tobacco consumption and/or smoking either of gender were included. Patients were distributed equally in 2 groups. Lesions sites were applied earlier with 1% lactic acid by cotton bud for 20 seconds and additionally rinsed with water to eliminate excess saliva and food debris and for providing a reliable oral setting. The mucosa of targeted site was gently cleaned with gauze and power air spray with triple syringe to make sure that the lesions are not being infested with saliva. The patients of group one were underwent dye (1%) methylene blue directly smeared with cotton bud for 10 to 20 seconds and were decolorized with 2% lactic acid with cotton bud for 20 to 30 seconds, and a photographs were taken.<sup>11</sup> The dye retention pattern was appraised with intensity on the lesion. Local, and deep blue stains were exhibited as positive (+) reaction, while shallow, wide, faint blue or no retention was exhibited as negative (-) reaction. In patients of Group B dye (1%) lugols iodine was directly applied with a cotton bud for 10 to 20 seconds and was decolorized with 2% acetic acid by cotton bud for 20 to 30 seconds, and a photograph was taken. The dye retention's pattern was evaluated by stain's intensity on lesion. Dark brown stains were exhibited as negative (-) reaction, while light brown, shallow, faint or no retention of brown stains were exhibited as positive (+) reaction. The outcomes of lugol's iodine dye and Methylene blue dye staining were noted with photos

and incisional biopsies were carried out simultaneously for suspected lesions to evaluate the diagnostic accuracy of lugol's iodine. The continuous variables were computed as mean and standard deviation. Categorical variable were computed as frequency and percentage. ROC-curve analysis was applied for sensitivity and specificity.

### Results

Majority of patients were in 4<sup>th</sup>-5<sup>th</sup> decade of life and mean age of males was 41.9±10.7 years and females 39.8±7.74 years. Most of the cases (65%) had more than one habit followed by Mainpuri 8.3% (Table 1). According to the pre-cancerous lesions homogenous leukoplakia was most common (65.0%)(Table 2). Methylene blue showed 89.4% sensitivity and 66.6% specificity, Lugol's Iodine showed 83.3% sensitivity and 50% specificity(Figure 1-4).

**Table 1: Basic information of patients (n=60)**

| Variables           | Frequency | %      |
|---------------------|-----------|--------|
| Age Groups          |           |        |
| 20-29               | 03        | 05.0%  |
| 30-39               | 19        | 31.6%  |
| 40-49               | 20        | 33.4%  |
| 50-59               | 15        | 25.0%  |
| >60                 | 03        | 05.0%  |
| Total               | 60        | 100.0% |
| Gender              |           |        |
| Male                | 48        | 80.0%  |
| Female              | 12        | 20.0%  |
| Total               | 60        | 100.0% |
| Habits              |           |        |
| Mainpuri            | 05        | 08.3 % |
| Ghutkha             | 02        | 03.3 % |
| Betel nut           | 08        | 13.4 % |
| Pan                 | 03        | 05.0 % |
| Smoking             | 03        | 05.0%  |
| More than one Habit | 39        | 65.0%  |
| Total               | 60        | 100.0% |
| Site involvement    |           |        |
| Buccal mucosa       | 37        | 61.6%  |
| Alveolus            | 10        | 16.6%  |
| Lip                 | 05        | 08.4%  |
| Tongue              | 04        | 06.6%  |
| Retromolar area     | 02        | 03.4%  |
| Palate              | 01        | 01.6%  |
| Floor of mouth      | 01        | 01.6%  |

**Table 2. Distribution of groups according to precancerous lesions (n=30)**

| Precancerous lesions   | Frequency | %      |
|------------------------|-----------|--------|
| Homogenous leukoplakia | 39        | 65.0%  |
| Speckled leukoplakia   | 10        | 16.7%  |
| Erosive lichen planus  | 03        | 05.0%  |
| Epithelial hyperplasia | 08        | 13.3%  |
| Total                  | 60        | 100.0% |

## Discussion

Ya-Wei Chen et al reported that the patients' ages (patient group) varied between 31 and 82 years ( $41 \pm 15$  years), with the ratio of male to female being 51:7.<sup>1</sup> In present study when habit profile of study population were seen more than 39(65%) patients had more than one habit. 5(8.3%) patients were used to take mainpuri while 2(3.3%) were in habit of eating gutka. 8(13.3%) were taking betel nuts and 3(5%) were using pan and were smokers. Similarly Ya-Wei Chen et al reported that 2/3<sup>rd</sup> of cases (n=38) were found with history of betel quid chewing, and 52 subjects had a history of cigarette smoking.<sup>1</sup> When habit profile in male and females were compared than it was found that males were more addicted as compared to females ( $p=0.001$ ). In majority (60%) of the patients, buccal mucosa was affected. In different studies it is reported that Leukoplakias on the floor of mouth, lateral tongue, and lower lip exhibit more dysplasia or malignant alteration.<sup>2,3</sup>

In this study Methylene blue showed best diagnostic efficacy as; sensitivity was 83.3% and specificity was 50.0%. Epstein et al conducted a comparative study, in which lugol's iodine and TB were applied both in blend and individually in 59 patients and the sensitivity and specificity of Lugol's iodine were 0.875 and 0.842, respectively.<sup>14</sup> The authors established that Lugol's iodine had less sensitivity in detecting oral malignant and dysplastic lesions however it had higher specificity. In another study, 30 subjects with clinically suspicious oral PMDs and 30 subjects with clinically suspicious malignant lesions were stained consecutively with lugol's iodine and TB, the general sensitivity to detect malignant lesions or dysplasia was 92.7% however the specificity was 60% reported by Nagaraju et al.<sup>15</sup> Majority of the studies accessed established that Lugol's iodine could possibly have high potential for oral PMDs and oral carcinomas screening. As well as in this study no significant difference was found between three groups of precancerous patients, positive reaction was found in 7 patients out of 10, in group 2 reaction was positive in 8 cases while in group 3 reaction was positive in 9 patients out of 10.

In this study Methylene blue showed best diagnostic efficacy as; sensitivity was 89.4% and specificity was 66.6%. Nagaraju K et al stated that general sensitivity of Lugol's iodine if used consecutively with toluidine blue in detecting dysplasia or malignant lesions was 92.7% however specificity was 60% and DA, NPV and PPV were 90%, 43% and 96% respectively.

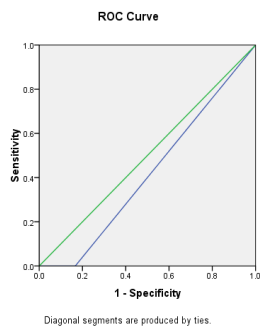


Figure 1: Specificity and Sensitivity of (MB) in diagnosis of malignant lesions by ROC curve analysis (95% CI; = (0.163-0.670); AUC (area under curve) = 0.417; Sensitivity: 89.4% Specificity: 66.6%

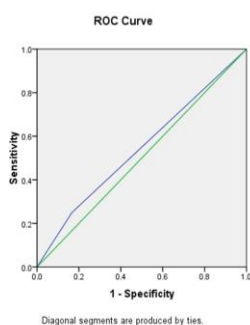


Figure 2: Specificity and sensitivity of (LI) in diagnosis of malignant lesions by ROC curve analysis; (95% CI; = (0.276-0.808); AUC (area under curve) = 0.542; Sensitivity: 83.3% ; Specificity: 50%

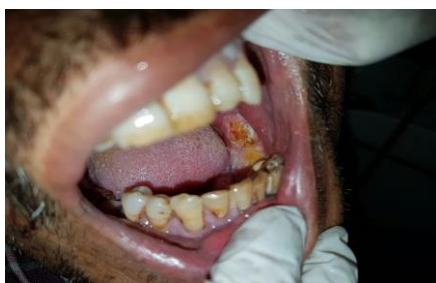


Figure 3. Photograph of a patient with positive lugols iodine staining (Printed with patient's consent)



Figure 4 Photo of a patient with positive methylene blue staining (Printed with patient's consent)

Similarly Riaz A et al reported that the sensitivity of methylene blue in determining dysplastic and carcinomatous changes was determined as 91.4% however the specificity was determined as 66.6%.<sup>21</sup> Other studies showed overall sensitivity of 95% (100% for malignancy and 92% for potentially malignant lesions) was reported, contrasted to the 72–100% sensitivity stated in earlier studies.<sup>22,23</sup>

## Conclusion

1. Methylene blue and Lugol's iodine should be considered as a non-invasive diagnostic tools for the early diagnosis of premalignant and malignant lesions of oral cavity with high sensitivity and specificity.
2. Early identification of these lesions is important for prevention and management of malignancy. Betel quid and smoking habits should be avoided

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