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ORIGINAL ARTICLE

Lugol's iodine and methylene blue as an adjunctive tool for early diagnosis of premalignant oral lesions.

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ABSTRACT… Objective: To compare Lugol's Iodine and Methylene blue as an adjunctive tool for early diagnosis of premalignant oral lesions by taking histopathology as the gold standard. **Study Design:** Cross-sectional descriptive. **Setting:** Outpatient Department of Isra Dental College Hospital, Isra University Hyderabad. **Period:** January 2021 to July 2021. **Material & Methods:** All patients with oral premalignant lesions were selected. Methylene blue and iodine staining were used in the lesion area. The dye was applied directly with a cotton bud for 10–20 seconds, and the mouth was rinsed with tap water. The dye retention pattern was evaluated by the stain retention intensity on the lesion. Incisional biopsy was performed simultaneously at that site as a gold standard. **Results:** Out of 60 cases, males were 49 (81.6%) and females were 11(18.3%). The mean age of males and females was 41.910.7 years and 39.87.74 years, respectively. Out of all patients, 65.0% had more than one addictive habit of mainpuri, gutka, or supari. The commonest region of oral lesions was the buccal mucosa among 61.6% of patients, followed by the alveolus in 16.6%, lips 8.3%, tongue 6.6%, and retro molar area 3.33%, while the palate and floor of the mouth were involved in 1.66% of patients. According to the diagnostic accuracy of methylene blue, the sensitivity was 89.4% and the specificity was 66.6%. According to the Lugol's, iodine sensitivity was 83.3% and specificity was 50%. **Conclusion:** It has been determined that methylene blue and lugols iodine staining are the best, easy, and noninvasive screening tools to detect the early diagnosis of malignancy.

Key words: Lugol's Iodine, Methylene Blue, Premalignant and Malignant Lesions.

INTRODUCTION

Oral carcinoma is the most frequently detected malignancy throughout the world and shows noticeable geographical differences in its occurrence. It is very common for bidi, smoking, alcohol consumption, chewing habits of betel quid and a high consumption rate of tobacco. Consequently, oral cancer is often observed among Southeast Asia's population, where >100,000 new patients are reported per year, and also in Pakistan, is creating an alarming condition.[2] The prevalence of oral cavity malignant neoplasms has been increasing with a lower rate of survival in recent years, affecting approximately 50% of the best treatment centers.[1] Despite the fact that the oral depression is a potentially receptive site for diagnosis, 50% of oral malignant growths are not discovered until the illness has progressed significantly.[1,3] Though oral squamous cell carcinoma may develop from several conditions and different premalignant lesions of the oral cavity, a wide exhibit of conditions have been involved in the oral cancer advancement, including leukoplakia, palatal lesion of reverse cigar smoking, oral sub-mucosal fibrosis, erythroplakia, discoid lupus erythematosus, and genetic disorders.[4,5] There are many diagnostic tests for carcinoma of oral cavity, with variable limitations, implications and the indications.[6] Yet the gold standard diagnosis for the oral carcinoma is the surgical biopsy. But not all suspicious lesions should be biopsied surgically.[6] Many reports have showed that the oral cancer related mortality can be decreased by diagnosis and treatment at early stage of oral cancer.[1,7] Similarly, it is equally important to

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diagnoses and potentially treat cancer lesions as their progression to the advanced malignancy can be prevented.[1,8]

In diagnosis, Methylene Blue method's precision is used to identify dysplasia, carcinoma or intestinal metaplasia. One study has as well examined its validity in detecting oral carcinoma and precancerous lesions.[9]

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. Considering the low price and low toxicity, Methylene blue can possibly be easily used instead of large scale investigation among high risk cases. 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.[9]

Wide-ranging literature is present about lugol's iodine consumption as an aid to identify neoplasia of esophageal squamous epithelium.[10,11]

Lugol's iodine imagining of dysplastic mucosa is used to manage esophageal disorder. Though, lugol's iodine staining relies upon the glycogen contents of normal epithelium as well as this selective characteristic of staining assists in outlining the carcinomatous or inflammatory epithelium from usual epithelium at the site of low glycogen content. Though a much limited literature is present that describes the application of lugol's iodine in managing of neck and head mucosal neoplasia.[10,11]

This study is intended to critically evaluate these non-invasive methods and to exhibit the accessible body of evidence regarding their effectiveness in early diagnosis and detection of oral precancerous lesions. Clinicians can enhance survival rates of patients if a malignant lesion is spotted at an initial stage or when a precancerous lesion (dysplasia) is detected and managed before malignant development. One such method is the staining of these suspected lesions by different staining methods.

MATERIAL & METHODS

This descriptive study was held at the outpatient department of oral surgery, Isra Dental College, and the histopathological laboratory of Isra University, Hyderabad. The study lasted for six months, from January 2021 to July 2021. All the patients aged 20 to 70 years old with oral premalignant lesions, patients with habits of chewing betel quid, areca nuts, etc., and patients with the habit of tobacco consumption were included in the study. All subjects with an earlier history of radiotherapy, chemotherapy, or patients with a recurrence of oral SCC were excluded from the study. All the patients were divided equally into 2 groups.

After documenting the clinical photographs and features of a clinically suspicious lesion, the lesion sites were applied with 1% lactic acid by cotton bud for 20 seconds and additionally rinsed with water to eliminate excess saliva and food debris and provide a reliable oral setting. The mucosa of the targeted site was gently cleaned with gauze and power air sprayed with a triple syringe to make sure that the lesions were not infested with saliva.

The patients of group A underwent dyeing (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 photographs were taken.[11] The dye retention pattern was evaluated with intensity on the lesion. Local and deep blue stains were exhibited as positive (+) reactions. While shallow, wide, faint blue or no retention was exhibited as a 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 with a cotton bud for 20 to 30 seconds, and a photograph was taken. The dye retention pattern was evaluated by the stain's intensity on the lesion. Dark brown stains were exhibited as a

negative (-) reaction. While light brown, shallow, faint or no retention of brown stains were exhibited as positive (+) reactions. 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. All the samples were evaluated microscopically by pathologists who were blind to the outcomes of all the dyes.

The data was analysed via SPSS 22.0. The continuous variables were computed as the mean and standard deviation. Categorical variables were computed as frequency and percentage. ROC-curve analysis was applied for sensitivity and specificity.

RESULTS

A total of 60 patients were studied. The majority of patients were in their 4th or 5th decade of life and the most common age groups were 30-39 years and 40-49 years. Out of 60 study subjects, 49 (81.6%) were male and 11 (18.3%) were female. Most of the cases (65%) had habits of mainpuri+betel-nut+gutka, followed by mainpuri 8.3%, gutka 3.3%, betel nuts 13.3%, and 5% had a history of pan consumption and smoking respectively. Buccal mucosa was the commonest site of oral lesions among 61.6% of the cases, followed by alveolus in 16.6%, lips 8.4%, tongue 6.6%, retro molar area 3.4%, and palate and floor of mouth were involved in 1.46% of the cases. Table-I.

Homogenous leukoplakia was most common among 65.0% of cases, speckled leukoplakia in 16.7% of patients, epithelial hyperplasia was in 13.3% of cases, and erosive lichen planus was found only among 5.0% of patients. Table-II

Methylene blue showed 89.4% sensitivity and 66.6% specificity, while Lugol's Iodine showed 83.3% sensitivity and 50% specificity. Figure-1 and 2.

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-I. Basic information of patients (n=60)

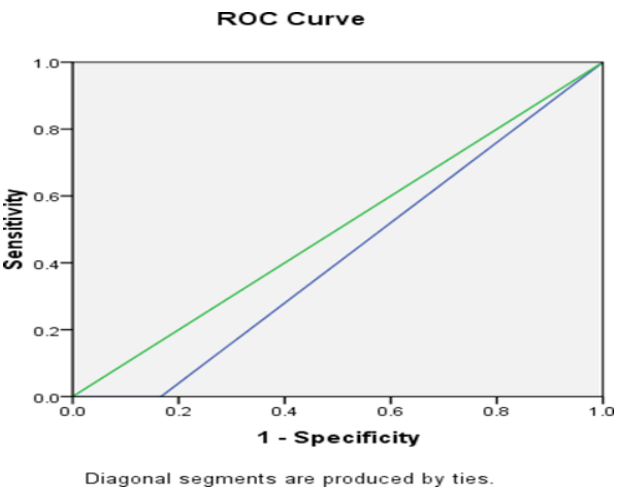


Figure-1. Specificity and Sensitivity of (MB) in diagnosis of malignantlesions by ROC curve analysis

(95% CI; = (0.163-0.670)
AUC (area under curve) = 0.417
Sensitivity: 89.4% Specificity: 66.6%

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%)

Table-II. Distribution of groups according to precancerous lesions (N=30)

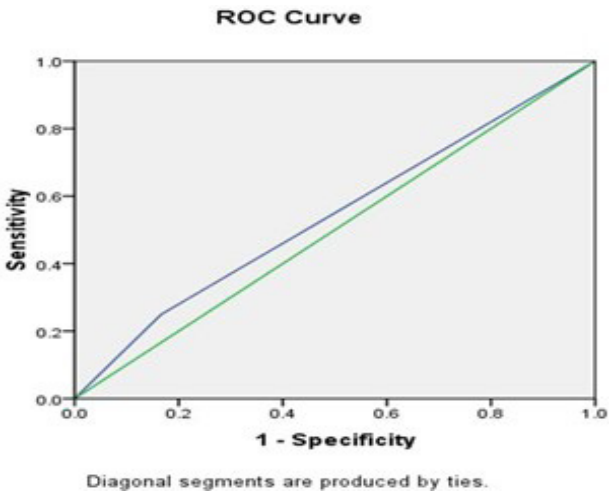
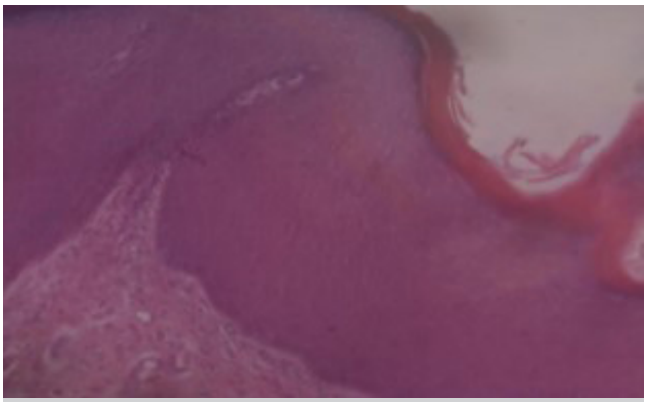


Figure-2. Specificity and sensitivity of (LI) in diagnosis of malignantlesions by ROC curve analysis

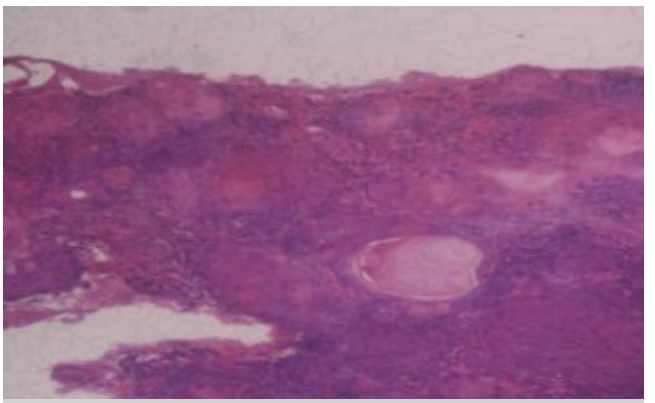
(95% CI; = (0.276-0.808)
AUC (area under curve) =0.542
Sensitivity: 83.3% Specificity: 50%

Figure-3. Photo of a patient with positive methylene blue staining (Printed with patient's consent)

Figure-4. Photo of a patient with positive lugols iodine staining (Printed with patient's consent)



Photomicrograph-1: Microscopic features showing Hyperkeratosis, acanthosis without dysplasia. H&E X 100



Photomicrograph-2: Microscopic features showing well differentiated squamous cell carcinoma H&E X100

DISCUSSION

Total 60 patients were studied their mean age was 41.58+6.23 yearsIn comparison to this study, Ya-Wei Chen et al. reported that the patients' age range was between 31 and 82 years, with a mean age of 41+15 years, and with the ratio of male to female being 51:7.[12] Similarly, in this study also, males were in the majority with 48(80%) and females had 12(80%). In this study, when

the habit profile of the study population was seen, 39(65%) patients had more than one habit, followed by 5(8.3%) patients who were used to taking mainpuri, while 2(3.3%) were in the 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.[12] reported that 2/3rd of cases (n = 38) were found with a history of betel quid chewing, and 52 subjects had a history of cigarette smoking. When habit profiles in males and females were compared, it was found that males were more addicted as compared to females (p = 0.001).

In this study, buccal mucosa was affected in 36(60.6%) patients. Alveolus was In 16.6%, lips were affected in 5 (8.3%), the tongue was affected in 4 (6.6%), the retro molar area was affected in 3 (5%), and in 1(1.6%) patient, the palate and floor of the mouth were affected. Similarly, in other studies, it is reported that leukoplakias on the floor of the mouth, lateral tongue, and lower lip exhibit more dysplasia or malignant alteration.[4,13]

In this study, Methylene blue showed the best diagnostic efficacy as sensitivity was 83.3% and specificity was 50.0%. Epstein et al.11 conducted a comparative study in which Lugol's iodine and TB were applied both in blend and individually to 59 patients, and the sensitivity and specificity of Lugol's iodine were 0.875 and 0.842, respectively. The `authors established that Lugol's iodine had less sensitivity in detecting oral malignant and dysplastic lesions, but it had higher specificity. Furthermore, in one more study, 30 subjects with clinically suspicious oral PMDs and 30 subjects with clinically suspicious malignant lesions.

According to Nagaraju et al., when malignant lesions were stained consecutively with lugol's iodine and TB, the general sensitivity to detect malignant lesions or dysplasia was 92.7%, but the specificity was 60%.[10] Other studies established that Lugol's iodine could possibly have a high potential for oral PMDs and oral carcinoma screening.

In this study, Methylene blue showed the best diagnostic efficacy as sensitivity was 89.4% and specificity was 66.6%. Nagaraju K et al. stated that the 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. Similarly, Riaz A et al4 reported that the sensitivity of methylene blue in determining dysplastic and carcinomatous changes was determined as 91.4%, but the specificity was determined as 66.6%. Other studies showed 72–100% sensitivity.[15,16]

CONCLUSION

It was concluded that methylene blue and lugols iodine are excellent noninvasive diagnostic tools for the early diagnosis of premalignant and malignant lesions of the oral cavity with high sensitivity and specificity. These non-invasive diagnostic tools should be applied to the screening of these patients for early diagnosis. Early identification of these lesions is important for prevention and management of malignancy.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
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2	Talha Asad Khan	Study design, Questionnaire design, Literature search.	
3	Khushbu Lohana	Data analysis, Suggestions, Data interpretation.	
4	Hafiz Mehmood	Patients selection, experiments and patients follow-up data acquisition.	