



Methylene Blue as a Diagnostic Aid in The Early Detection and Screening of Oral Cancers and Precancerous Lesion

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Abstract

Objective: To assess the reliability and sensitivity of in vivo methylene blue staining as a diagnostic tool for screening of oral malignant and premalignant lesions.

Methodology: The cross-sectional study was conducted at the oral and maxillofacial surgery department, Foundation University College of Dentistry & Hospital, Islamabad, from October 2023 to December 2024. The screening for oral cancers and precancerous lesions was conducted on 77 patients. Clinical data on patients' gender, age, followed by a systematic oral examination to clinically diagnose suspicious lesions on routine examination. Chair side Methylene blue dye uptake was compared with Biopsy report (Gold standard).

Results: Of the 77 patients, 64 patients were found to have oral cancer. The result indicated sensitivity of 93.8%, specificity of 53.8%, positive predictive value of 90.9%, negative predictive value of 63.6% and accuracy of 87%.

Conclusion: Methylene blue staining is a reliable diagnostic tool for oral cancer screening, particularly for high-risk individuals. Its utility is especially significant in settings where access to advanced diagnostic tools may be limited, as it provides a cost-effective, non-invasive, and relatively simple method to improve diagnostic accuracy and prioritize individuals for further evaluation or biopsy.

Keywords: Biopsy, Methylene blue, Neoplasm, Squamous Cell Carcinoma.



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Introduction

Squamous cell carcinoma is the most common malignant neoplasm of oral region in the world, with significant geographic variations in its occurrence.¹ These cancers have a poor survival rate, estimated between 30% and 80% over a span of five years.²

Oral suspected lesions can be missed on clinical examinations as they might not present significant clinical features, often resembling normal mucosa in colour. Early lesions may also mimic inflammatory and reactive conditions, complicating diagnosis. The lack of a definitive approach for the early detection of oral cancers contributes to delays in diagnosis and poor prognosis.²

Several variants of oral cancers can arise from the oral cavity disorders and certain precancerous lesions also known as Potential Malignant disorders (PMDS).³ Such lesions include epidermolysis bullosa, dyskeratosis congenita, discoid lupus erythematosus, erythroplakia, leukoplakia, and oral submucous fibrosis. Many of these lesions may be asymptomatic and can appear benign during clinical assessments.^{3,1} While biopsy and histological analysis of the oral cavity serve as the gold standard for identifying oral precancerous lesions, not all patients are willing or able to undergo this procedure, especially when the lesions appear normal. Furthermore, biopsy results can be influenced by several factors, including the depth and size of the biopsy, specimen quality, the processing techniques used, and the pathologist's expertise.⁴

Several chairside, non-invasive methods have emerged in the last few decades, such as use of fluorescent light (Vizilite), application of dyes such as Toluidine Blue (TB), Lugol's Iodine (LI), Acetic Acid (AA), and Methylene Blue (MB), with varying success at detecting oral precancerous lesions.^{5,6}

Methylene Blue (MB) has conventionally been used for histopathological and surgical procedures. It has a potential for screening of various tumors and has been used to detect neoplasm of the gastrointestinal tract, prostate, and bladder.⁸ It shares similarities with Toluidine Blue, particularly in its acidophilic properties and strong affinity for acids, including DNA, which is observable through its deep blue coloration during clinical use and notable sensitivity.^{7,8} Methylene blue is typically viewed as having a lower toxicity than toluidine blue, which is an imperative consideration in certain patients.⁹

Early detection of oral squamous cell carcinoma remains a challenge due to its subtle presentation and the limitations of invasive diagnostic methods like biopsy. While methylene blue has shown promise as a non-invasive, cost-effective screening tool. This study addresses that gap by evaluating methylene blue's sensitivity and reliability, offering valuable insights for improving early diagnosis, especially in resource-limited

settings. Methylene blue should be used as an adjunct to biopsy, not a replacement, due to its lower specificity. Therefore, this study was conducted to evaluate the sensitivity and diagnostic accuracy of methylene blue staining in the early diagnosis of malignant oral mucosal lesions.

Methodology

This cross-sectional study was conducted from October 2023 to April 2024. The research protocol was approved (FF/FUCD/632/ERC/51) by the Ethical Review Committee, Foundation University College of Dentistry & Hospital, Islamabad. Detailed history and clinical examination of patients presenting to the OMFS department was carried out, and patients with suspected oral lesions were selected, and informed consent was taken for this.

Our study, comprised patients of both genders, aged between 20 and 70 years. Included participants were those presenting with non-healing ulcers or patches persisting for more than two weeks, or cases unresponsive to local or systemic treatments. Additionally, only patients without prior biopsy of the lesion site were considered. Patients with recurrent lesions or a history of previous radiation therapy to the affected area were excluded from the study.

Methylene blue was applied as dye rinse, a compound of 1% malachite, diamethyl-sulfoxide, glycerol and 1% methylene blue solution (solution A). Another solution (solution B) was prepared with purified water, raspberry flavor, and 1% lactic acid; it was used as a pre- and post-rinse solution solution.^{5,7}

Participants were asked to rinse their mouth with solution B for the duration of 20 seconds in order to obtain a consistent oral environment by getting rid of hypersalivation and food debris, if any. In addition, with the help of a triple syringe and a gauze, the mucosa of the intended area was vigilantly dried. Once the area was dried, solution A was applied. After 20 sec wait, patients were instructed to rinse again for 20 sec with solution B. The stain intensity was noted after rinse with solution B by one observer. Figure I, II, III, IV and V. The indicators of positive reaction include local, stippled and concentrated blue stains while wide and faint blue stains indicate a negative reaction. To prevent ambiguous results, cotton rolls were utilized to apply solution B to clear the stained surface. If the stain retained, positive result was recorded and vice versa. Pre and post-pictures of lesions were taken before proceeding with the biopsy. The area with highest uptake of the dye in the suspected lesions were then biopsied under local anesthesia and sutures were applied after biopsy. All biopsy specimens were preserved in neutral buffered 10% formalin and sent for standard histopathological evaluation to the pathology laboratory.

Histopathology reports of lesion were classified as

(1) Benign (2) Precancerous and (3) Cancerous lesion. Benign lesions were considered as histopathology negative, cancerous and precancerous lesions as histopathology positive. The histopathology results of the lesions were classified as: hyperkeratosis (Benign lesions), verrucous hyperplasia and dysplasia (precancerous lesions), verrucous carcinoma and squamous cell carcinoma (Oral cancer). The data was analysed with SPSS 22. The results of the histopathology (gold standard) were cross tabulated against the methylene blue staining result to construct a 2 x 2 table for calculation of sensitivity and specificity, positive predictive value, negative predictive value and accuracy.

Results

A total of 77 patients were included in this study, with a mean age of 61 ± 9.7 years. Among the participants 69 (89.6%) were females. Of the total cases, 64 (83.1%) were diagnosed with oral cancer, while 13 (16.8%) were diagnosed with benign lesions. However, no pre-

cancerous lesion was reported. The buccal mucosa was the most commonly affected by lesions (36.4%), followed by the alveolar mucosa (35.1%), whereas the palate was observed to be the least involved among patients (Table 1).

Among the 77 cases, 64 patients had biopsy proven malignant cases. Out of all who were biopsy proven malignant ($n=64$) patients, 60(93.8%) were observed with dark blue stained lesion on methylene blue application (True positive). False positive was observed in 6 patients (histopathologically benign but stained dark blue), whereas false negative were seen in 4 patients (histopathologically malignant but with faint methylene blue staining). Faint or no staining (True negative) were observed in 7 patients (53.8%) out of 13 cases. Moreover, no precancerous lesion was noted. (Table II)

The specificity and sensitivity of the methylene blue staining method were found to be 53.8% and 93.8%, respectively, with an overall accuracy of 87% as shown in table 3.



A **B** **C**

Figure. I. Clinical photograph showing potentially traumatic lesion at left lateral border of the tongue.

A) After application of solution B for 20 sec.

B) After application of solution A for 20 sec.

C) A localised deep blue uptake of methylene blue dye after use of solution B.

The histopathology result revealed, moderately differentiated squamous cell carcinoma.



A **B** **C**

Figure. II. Clinical photograph showing potentially pre-malignant oral lesion at left Buccal mucosa.

A) After application of solution B for 20 sec.

B) After application of solution A for 20 sec.

C) A localised faint blue uptake of methylene blue dye after application of solution B.

The histopathology results revealed, keratosis with no dysplasia.



A **B** **C**

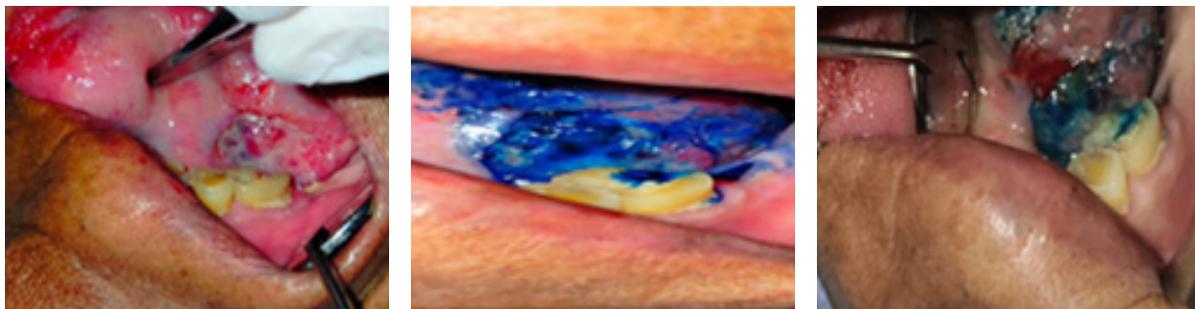
Figure. III. Clinical photograph showing potentially malignant oral lesion at left side of posterior hard palate.

A) After application of solution B for 20 sec.

B) After application of solution A for 20 sec.

C) No uptake of methylene blue dye after use solution B.

The histopathology results revealed, acute on chronic nonspecific inflammation.



A **B** **C**

Figure. IV. Clinical photograph showing potentially malignant oral lesion at lateral border of right side of tongue

A) After application of solution B for 20 sec.

B) After application of solution A for 20 sec.

C) A localised deep blue uptake of methylene blue dye after use solution B.

The histopathology results revealed, moderately differentiated squamous cell carcinoma.



A **B** **C**

Figure. V. Clinical photograph showing potentially pre-malignant oral lesion at right buccal mucosa

A) After application of solution B for 20 sec.

B) After application of solution A for 20 sec.

C) Faint blue uptake of methylene blue dye after use solution B.

The histopathology results revealed, well differentiated squamous cell carcinoma.

Discussion

Early diagnosis and prompt treatment are pivotal for improving patient survival. Timely diagnosis can de-

crease the morbidity and its associated treatment outcomes, such as significant loss of function, aesthetic disfigurement, psychological impact and poor quality of life.¹⁰

Table 1. Baseline characteristics of subjects (N=77)

	Variable	n (%)
Gender	Male	8 (10.3)
	Female	69 (89.6)
Site of biopsy	Buccal mucosa	28(36.4)
	Tongue	13(16.9)
	Alveolar mucosa	27(35)
	Palate	2(2.6)
	Lip	7(9.1)

Table 2. Efficacy of methylene blue dye application in histopathology examination

Methylene blue dye application	Histopathology examination		Total
	Positive n (%)	Negative n (%)	
Deep blue stains	60 (93.8%)	6(46.15%)	66
Faint blue stains	4 (6.2%)	7(53.8%)	11
Total	64	13	77

Table 3. Sensitivity, Specificity & predictive values

Sensitivity	True positive____ True positive +False negative	93.75%
Specificity	True negative____ True negative + False negative	53.8%
Positive predictive value	True positive____ True positive + False positive	90.9%
Negative predictive value	True positive____ True negative + False negative	63.6%
Accuracy	True positive + True negative True positiv e+ False positive + False negative + True negative	87%

Ya-Wei Chen et al reported that the patients' ages varied between 31 and 82 years (41 ± 15 years), with the ratio of male to female being 51:7.¹¹ In our study, maximum number of patients reported were females n=69 (89.6%) and minimum number of males n=8 (10.3%). As, our institute is designated for entitled females and families of veterans. In our study most patients that underwent biopsy were found with malignant lesions n=64(83.1%). It indicates that most patients are seeking dental intervention for their lesions, late.

The development of cancer in the oral mucosa is well established, involving the initial presence of precancerous lesion that subsequently progresses into cancer. Oral mucosal dysplasia detection could halt the lesion progression and prevent malignant transformation. In 1964, Niebel and Chomet pioneered using dye materials, such as toluidine blue as a diagnostic tool to diagnose precancerous lesion or oral cancer.¹² The technique efficacy has been evaluated in numerous

studies, yielding sensitivities ranging from 72% to 100% and specificities between 45% and 67% in identifying suspicious malignancies.¹³

The specificity and sensitivity were 53.8% and 93.8% respectively. Whereas the overall accuracy was 87%, which falls within the range of sensitivities (52–100%) reported in previous research conducted by Epstein et al¹⁴ and Mashberg et al¹⁵ highlighting the consistent diagnostic performance while providing specific insights into the local population's context.

Similar results were reported by a-Wei et al¹⁶, which reported a specificity rate of 69% and thereby supporting the reliability of methylene blue in similar clinical contexts.

A study conducted by Soman C et al¹⁷ reported an overall sensitivity of 90.6%, with specificity of 57.1%. In this study negative predictive value was observed in 84.2% and positive predictive value 70.7%. In our study, neg-

ative predictive value was noted by 63.6% and positive predictive value was 90.9%.

A study conducted by Riaz et al⁸ reported 91.4% sensitivity, 66.6% specificity, 97.7% PPV, 33% NPV and diagnostic accuracy was 90% when methylene was used as diagnostic indicator. In present study accuracy was of 87%. Based on these findings, the study concluded that Methylene blue staining is a reliable diagnostic tool for oral cancer screening, particularly for high-risk individuals.

A study conducted by Gupta et al⁵ demonstrated that methylene blue staining revealed a specificity of 91% and sensitivity of 89%, indicating its high accuracy in detecting oral cancer lesions. Furthermore, the study reported a positive predictive value (PPV) of 97%, signifying that the majority of positive test results were true positives, and a negative predictive value (NPV) of 73%, reflecting its reasonable ability to correctly identify non-cancerous cases. Methylene blue staining can serve as a valuable diagnostic adjunct, particularly in large-scale, community-based oral cancer screening programs targeting high-risk populations.

In our study, there was a limitation in screening of clinical precancerous lesions during study time frame, as majority of them were ultimately diagnosed to be malignant or benign on histopathological results. As, our institute is designated for entitled females and families of veterans. In our study, most patients who underwent biopsy were found to have malignant lesions. It indicates that most patients are seeking dental intervention for their lesions, late. Hence the study has a limitation that pre-cancerous lesions are not in the given sample. However, it has a significant benefit for general practitioners and specialists to perform biopsy specifically from the site of maximum dye uptake by the lesion and not to overlook or miss out specific areas of the lesion, thus improving the quality of biopsy and histopathology results.

Conclusion

Methylene blue staining is a practical and affordable tool in large-scale, community-based oral cancer screening programmes, especially among high-risk populations. Its diagnostic value is particularly significant in resource-limited settings, where access to advanced imaging or histopathological assessment may be scarce. By providing a non-invasive, easy-to-implement method to improve lesion detection and prioritize individuals for further diagnostic tests or biopsy, methylene blue staining plays a valuable role in early detection and treatment of potentially malignant oral conditions.

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Authors' Contribution Statement

NA contributed to the conception, design, acquisition, interpretation of data, drafting of the manuscript, and final approval of the version to be published. MWI contributed to the design, acquisition, analysis, drafting of the manuscript, and critical review of the manuscript. NSM contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. NA contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. MH contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. RRG contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. All authors are accountable for their work and ensure the accuracy and integrity of the study.

Conflict of Interest

Authors declared no conflict on interest

Grant Support and Financial Disclosure

None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.