Review Article

Methylene Blue Vital Staining in Diagnosis of Oral Lesions: A Narrative Review

Dr. T. Maheswaran[1], Dr. M. Radhika[2], Dr. S. Jeelani[3], Dr. I. Janani[4], Dr. S. Kokila[5], Dr. M. Saranya[6*]

[1]Professor, Department of Oral Pathology and Microbiology, Adhiparasakthi Dental College and Hospital, Tamil Nadu, India
[2]Assistant Professor, Department of Periodontics, JKKN Dental College and Hospital, Tamil Nadu, India
[3]Professor and Head, Department of Oral Medicine & Radiology, Dhanalakshmi Srinivasan Dental College, Tamil Nadu, India
[4]Associate Professor, Department of Oral Pathology and Microbiology, Adhiparasakthi Dental College and Hospital, Tamil Nadu, India
[5]Associate Professor, Department of Oral Pathology and Microbiology, Adhiparasakthi Dental College and Hospital, Tamil Nadu, India
[6]Associate Professor, Department of Oral Pathology and Microbiology, Vivekanandha Dental College for Women, Tamil Nadu, India

*Corresponding Author
Dr. M. Saranya
Associate Professor, Department of Oral Pathology and Microbiology, Vivekanandha Dental College for Women, Tamil Nadu, India

Abstract: Methylene blue vital staining is an emerging diagnostic adjunct for detecting potentially malignant oral disorders and malignancies. This chromophoric dye exhibits a selective affinity for dysplastic and neoplastic tissues through acidophilic binding to nucleic acids. Recent evidence has demonstrated promising sensitivity and specificity profiles, positioning methylene blue as a cost-effective and minimally toxic alternative to toluidine blue for community-based screening programs. However, its role remains complementary to histopathological examination, the definitive diagnostic standard.
Keywords: Methylene Blue, Vital Staining, Photodynamic Therapy, Potentially Malignant Disorders.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes a significant global health burden, ranking among the most prevalent malignancies worldwide, with particularly elevated incidence in South Asian populations, where tobacco consumption predominates [1]. Despite therapeutic advances, five-year survival rates remain suboptimal at approximately 50-60 percent, primarily attributable to delayed diagnosis at advanced disease stages [2]. Early detection through systematic screening of high-risk populations is the most promising strategy for improving clinical outcomes [3]. Vital staining techniques have emerged as valuable diagnostic adjuncts to conventional clinical examinations, facilitating the identification of suspicious lesions that require histopathological assessment [4]. This review provides a concise summary of the properties and applications of methylene blue in dentistry.

Chemical Properties and Staining Mechanism

Methylene blue, a thiazine dye with the molecular formula $C_{16}H_{18}ClN_3S$, functions as an acidophilic chromophore that preferentially binds to nucleic acids within epithelial tissues [2]. The staining mechanism parallels that of toluidine blue, exploiting the elevated DNA and RNA concentrations characteristic of dysplastic and malignant cells [3]. This physicochemical property enables differential uptake between normal mucosa and pathological lesions, manifesting as deep royal-blue coloration in areas harboring cellular atypia [1]. Critically, methylene blue demonstrates markedly reduced toxicity compared to toluidine blue, lacking the fibroblast cytotoxicity and oral ingestion hazards

associated with its predecessor [5]. This dye has established clinical utility in Barrett's esophagus surveillance and bladder cancer detection, providing a precedent for its application in oral mucosal screening [3].

Diagnostic Characteristics

Multiple prospective clinical investigations have systematically evaluated the accuracy of methylene blue staining for oral lesion detection. A comprehensive meta-analysis synthesizing seven studies encompassing 493 patients reported a pooled sensitivity of 90.3 percent and specificity of 68 percent, with a diagnostic odds ratio of 20.017 [6]. The sensitivities of the individual studies ranged from 89 to 95 percent for the combined potentially malignant disorders and malignancies, demonstrating a consistent discriminatory capacity across diverse patient populations [1-5]. Notably, the sensitivity approaches 100 percent specifically for invasive carcinomas, with marginally reduced performance for mild dysplastic lesions [3]. Positive predictive values consistently exceed 90 percent, whereas negative predictive values demonstrate greater variability, ranging from 73 to 87 percent [1-4]. False-negative results typically occur with homogeneous leukoplakias exhibiting minimal dysplastic changes, where pale blue staining may be misinterpreted as a negative result [1]. Conversely, false-positive staining may result from inflammatory processes or traumatized mucosa retaining the dye non-specifically [4].

Clinical Application

The standardized methylene blue staining protocol comprises the sequential application of preparatory and diagnostic rinse solutions [1]. Patients initially rinse with one percent lactic acid solution for 20 s to remove debris and establish consistent mucosal conditions [4]. Following gentle desiccation of the target areas, a one percent methylene blue solution was applied for 20 s, followed by a lactic acid post-rinse to eliminate mechanically retained stains [3]. Lesions exhibiting dark blue, deep focal staining are designated as positive, warranting an incisional biopsy for a definitive histopathological diagnosis [1]. Light or equivocal staining patterns require clinical correlation and longitudinal follow-up. The staining intensity correlates with nuclear density, enabling a rough approximation of dysplastic severity, although this remains subjective [4]. However, methylene blue serves as a screening adjunct rather than a diagnostic replacement, as histopathological examination remains the gold standard [6].

Comparative Analysis

Direct comparative investigations have demonstrated that methylene blue performance is comparable to that of the more extensively studied toluidine blue, achieving sensitivities of 90 percent versus toluidine blue's reported 72–100 percent range [5]. Methylene blue's superior safety profile and reduced cost position it advantageously for large-scale community screening initiatives [2]. Emerging evidence suggests that double-staining protocols combining methylene blue with Lugol's iodine may enhance diagnostic accuracy beyond single-dye approaches [6]. This dual-staining methodology exploits complementary mechanisms, with methylene blue targeting nucleic acids and iodine staining glycogen-depleted dysplastic epithelium. Preliminary investigations have reported 100 percent sensitivity and specificity with combined staining protocols, although validation in larger cohorts remains necessary [6]. The American Dental Association systematic review acknowledges vital staining as a triage tool for evaluating oral potentially malignant disorders, emphasizing its integration with comprehensive clinical assessment [7].

Therapeutic Applications: Photodynamic Therapy

Beyond its diagnostic utility, methylene blue has therapeutic potential as a photosensitizer for photodynamic therapy targeting oral premalignant lesions and carcinomas. Methylene blue-mediated photodynamic therapy significantly downregulates matrix metalloproteinase-9 expression in oral squamous cell carcinoma and leukoplakia cell lines, suggesting anti-invasive and anti-metastatic effects [8]. Clinical trials investigating five percent methylene blue photodynamic therapy for oral leukoplakia treatment are currently underway to evaluate its efficacy compared to conventional aminolevulinic acid-based protocols. The dual diagnostic and therapeutic functionality of methylene blue positions it as a versatile tool spanning the prevention-treatment continuum for oral potentially malignant disorders.

CONCLUSION

Methylene blue vital staining is a clinically validated, cost-effective screening adjunct for oral potentially malignant disorders and squamous cell carcinomas in high-risk populations. Its favorable toxicity profile relative to toluidine blue, coupled with its comparable diagnostic performance, supports its implementation in community-based screening programs. However, methylene blue should complement rather than replace histopathological examination, which remains essential for definitive diagnosis and treatment planning in dental clinical practice.

REFERENCES

1. Gupta, M., Shrivastava, K., Raghuvanshi, V., Ojha, S., Gupta, A., & Sasidhar, S. (2019). Application of *in vivo* stain of methylene blue as a diagnostic aid in the early detection and screening of oral cancerous and precancerous lesions. *Journal of oral and maxillofacial pathology: JOMFP*, *23*(2), 304. https://doi.org/10.4103/jomfp.JOMFP_242_17
2. Riaz, A., Shreedhar, B., Kamboj, M., & Natarajan, S. (2013). Methylene blue as an early diagnostic marker for oral precancer and cancer. *SpringerPlus*, *2*(1), 95. https://doi.org/10.1186/2193-1801-2-95
3. Lejoy, A., Arpita, R., Krishna, B., & Venkatesh, N. (2016). Methylene Blue as a Diagnostic Aid in the Early Detection of Potentially Malignant and Malignant Lesions of Oral Mucosa. *Ethiopian journal of health sciences*, *26*(3), 201–208. https://doi.org/10.4314/ejhs.v26i3.2
4. Chen, Y. W., Lin, J. S., Wu, C. H., Lui, M. T., Kao, S. Y., & Fong, Y. (2007). Application of in vivo stain of methylene blue as a diagnostic aid in the early detection and screening of oral squamous cell carcinoma and precancer lesions. *Journal of the Chinese Medical Association: JCMA*, *70*(11), 497–503. https://doi.org/10.1016/S1726-4901(08)70048-0
5. Chen, Y. W., Lin, J. S., Fong, J. H., Wang, I. K., Chou, S. J., Wu, C. H., Lui, M. T., Chang, C. S., & Kao, S. Y. (2007). Use of methylene blue as a diagnostic aid in early detection of oral cancer and precancerous lesions. *The British journal of oral & maxillofacial surgery*, *45*(7), 590–591. https://doi.org/10.1016/j.bjoms.2006.08.017
6. Hwang, S. H., Kim, S. W., Song, E. A., Lee, J., & Kim, D. H. (2021). Methylene Blue as a Diagnosis and Screening Tool for Oral Cancer and Precancer. *Otolaryngology--head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery*, *164*(2), 271–276. https://doi.org/10.1177/0194599820947686
7. Lingen, M. W., Tampi, M. P., Urquhart, O., Abt, E., Agrawal, N., Chaturvedi, A. K., Cohen, E., D'Souza, G., Gurenlian, J., Kalmar, J. R., Kerr, A. R., Lambert, P. M., Patton, L. L., Sollecito, T. P., Truelove, E., Banfield, L., & Carrasco-Labra, A. (2017). Adjuncts for the evaluation of potentially malignant disorders in the oral cavity: Diagnostic test accuracy systematic review and meta-analysis-a report of the American Dental Association. *Journal of the American Dental Association (1939)*, *148*(11), 797–813.e52. https://doi.org/10.1016/j.adaj.2017.08.045
8. Le, M. N., Wuertz, B. R., Biel, M. A., Thompson, R. L., & Ondrey, F. G. (2022). Effects of methylene blue photodynamic therapy on oral carcinoma and leukoplakia cells. *Laryngoscope investigative otolaryngology*, *7*(4), 982–987. https://doi.org/10.1002/lio2.772.