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ASSESING THE UTILITY OF METHYLENE BLUE DYE AS A TOOL FOR AXILLARY LYMPH NODE EVALUATION IN EARLY BREAST CARCINOMA

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Abstract

Background: *Sentinel lymph node biopsy is essential for axillary staging in early breast carcinoma, and methylene blue dye may offer a simple, low-cost alternative for sentinel lymph node detection.*

Objective: *To determine the diagnostic accuracy of methylene blue dye in identifying axillary sentinel lymph node metastasis among patients with early-stage breast carcinoma, using histopathological examination as the reference standard.*

Methods: *A cross-sectional study was conducted in the Department of General Surgery, Sughra Shafi Medical Complex Hospital, Narowal, between July 2024 to January 2025. One hundred and twenty female patients whose Stage I or II carcinoma of the breast was confirmed and whose axillary nodes were clinically negative were enrolled. Before breast-conserving surgery or modified radical mastectomy, every patient was given a subareolar injection of 5 ml of 1% methylene blue dye. Sentinel lymph nodes that had been excised were sent for histopathological testing. The data was analyzed using SPSS ver. 25.0*

Results: *A total of 120 patients participated in this study. The mean age was 49.12 ± 7.26 years. Methylene blue staining was positive in 65 (54.17%) patients, while histopathology confirmed axillary lymph node metastasis in 63 (52.50%) patients. Methylene blue dye showed a sensitivity of 92.06% (95% CI: 82.44–97.37), specificity of 87.72% (95% CI: 76.32–94.92), positive predictive value of 89.23% (95% CI: 80.49–94.33), negative predictive value of 90.91% (95% CI: 81.09–95.89), and overall diagnostic accuracy of 90.00% (95% CI: 83.18–94.73).*

Conclusion: *Methylene blue is an effective, safe, and low-cost option for SLNB in early breast cancer. It gives reliable nodal staging with few complications and is suitable for settings with limited resources.*

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Introduction

The sentinel lymph node, the first node drained by a primary tumour, plays a central role in prognostication and surgical management in breast carcinoma patients [1-2]. The identification of axillary lymph node status is among the most clinically relevant factors for estimating long-term survival and the risk of disease recurrence [3-4]. In the past, formal axillary lymph node dissection (ALND) was the standard method of staging. However, it was successful; it had a high morbidity rate, including lymphoedema, limited shoulder movement, chronic pain, and neurological sensory impairments [5-6]. SLNB development provided a significantly less invasive way to obtain the same stage data, with significantly fewer side effects, and is now a standard practice in surgical oncology [7-8].

Before the use of protocols in melanoma and breast cancer surgery, SLNB was first utilized in the treatment of parotid and penile cancer [9-10]. Its usefulness is particularly notable in patients in whom both clinical and radiographic examinations have shown axillary nodes to be node-negative; here, SLNB has now become the standard practice regardless of the tracer used [11-12]. Lymphatic mapping can be performed with several agents, including isosulfan blue, patent blue, indocyanine green, and superparamagnetic iron oxide particles. Methylene blue is unique because of its low price, wide distribution, and reasonable safety profile - features that give it a clear advantage in resource-limited environments [13-14].

Several studies have tested the performance of methylene blue in this regard. Sensitivity, specificity, and accuracy were reported by Khawar et al. to be 89.66%, 89.71%, and 89.68%, respectively [15]. Bakhtiar et al. reported even better sensitivity (96.8%) and accuracy (94.1%) [16]. Khanam et al. reported a 96% success rate for the procedure and similar histopathological concordance, along with a low complication rate [17]. All these data together indicate that methylene blue can be a reliable alternative to more expensive dyes and radiotracers.

Although methylene blue dye has been evaluated in several studies as a low-cost tracer for sentinel lymph node biopsy, its diagnostic performance still requires further local validation in resource-limited surgical settings where radiotracers and dual-mapping techniques are not routinely available. Variations in injection technique, patient selection, nodal disease burden, and institutional expertise may influence its sensitivity, specificity, and false-negative rate. Therefore, the present study aimed to determine the diagnostic accuracy of methylene blue dye for detecting axillary sentinel lymph node metastasis in patients with early-stage breast carcinoma, using histopathology as the reference standard.

Methods

The cross-sectional study was conducted at the Department of General Surgery, Sughra Shafi Medical Complex Hospital, Narowal, between July 2024 to January 2025. This diagnostic accuracy study was reported in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD) 2015 guidelines. The STARD checklist was used to ensure complete reporting of participant selection, index test, reference standard, diagnostic accuracy estimates, and flow of patients through the study. The institutional review board provided the ethical clearance under the reference SMC/ERB/0020 dated 13 th November 2025. The sample size was calculated using the WHO Sample Size Calculator for diagnostic accuracy studies. The calculation was based on an expected sensitivity of 89.66% and specificity of 89.71% for methylene blue dye, as reported in previous literature. The anticipated prevalence of axillary lymph node metastasis among early-stage breast carcinoma patients was taken as 46.8%. A 95% confidence level and 8% absolute precision were used. Based on these assumptions, the minimum required sample size was 120 patients. Non-probability consecutive sampling was used to enroll eligible participants [15]. Patient selection was performed using a nonprobability consecutive sampling technique.

Female patients aged 18–70 years were included if they had biopsy-proven carcinoma of the breast, clinical stage I or II disease, clinically and radiologically negative axillary lymph nodes, and

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were planned for breast-conserving surgery or modified radical mastectomy with sentinel lymph node biopsy. Only patients who provided written informed consent were included. Patients were excluded if they had clinically palpable or radiologically suspicious axillary lymph nodes, locally advanced breast cancer, distant metastasis, previous axillary surgery, prior neoadjuvant chemotherapy or radiotherapy, recurrent breast carcinoma, pregnancy, known allergy to methylene blue dye, inflammatory breast cancer, or refusal to provide informed consent.

Before an operative intervention, baseline data, including age, height, body weight, and body mass index, were obtained after written informed consent. The intended surgical procedure and histological subtype were also recorded. All operations were conducted under general anaesthesia. With the field prepared under sterile conditions, 5 ml of 1% methylene blue dye was divided into three equal aliquots and administered by subareolar injection. A gentle manual massage was applied for 10 to 15 minutes to promote lymphatic uptake. A cutaneous incision approximately 1 cm inferior to the axillary hairline was then made, and dissection proceeded through subcutaneous tissue and the clavipectoral fascia to reach the axillary lymphatic channels. Nodes that had taken up the blue stain were excised as sentinel lymph nodes. Both the breast specimen and excised axillary nodes were forwarded separately for histopathological analysis. Pathological review was performed by a consultant histopathologist with over five years of experience in breast pathology. A node was designated positive when malignant cells were identified and negative when none were found. In the dye-based classification, a node was positive if it stained blue and negative otherwise. All data were entered and analyzed using SPSS version 25. Results were cross-tabulated with histopathological diagnoses. Each case was classified into one of four outcome categories: true-positive, true-negative, false-positive or false-negative.

Results

A total of 120 patients participated in this study. Ages ranged from 37 to 61 years, with a mean of 49.12 ± 7.26 years. The mean BMI of the study population was **25.76 ± 0.73 kg/m²**. As the observed BMI range was narrow, BMI was not used for further subgroup analysis (Table 1).

Table 1: Baseline Characteristics of Study Participants

Variable	Value
Age (years)	49.12 ± 7.26
Age range (years)	37–61
BMI (kg/m²)	25.76 ± 0.73
BMI range (kg/m²)	24.03–27.64

Out of 120 patients, 62 (51.67%) underwent breast-conserving surgery and 58 (48.33%) had modified radical mastectomy. Invasive ductal carcinoma was the most common subtype in 91 patients (75.83%). This was followed by invasive lobular carcinoma in 15 (12.5%), invasive cribriform carcinoma in 6 (5.0%), and invasive mammary and mucinous carcinomas in 4 patients each (3.33%). After methylene blue injection, 65 patients (54.17%) showed positive staining and 55 (45.83%) were negative. Histopathology confirmed axillary metastasis in 63 patients (52.5%) and no metastasis in 57 (47.5%). Comparing dye results with histopathology, there were 58 true-positive (48.33%) and 50 true-negative (41.67%) cases. False positives were 7 (5.83%) and false negatives 5 (4.17%) (Table 2).

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Table 2: Clinical and Histopathological Characteristics

Variable	Frequency n (%)
Type of Surgery	
Breast-conserving surgery	62 (51.67%)
Modified radical mastectomy	58 (48.33%)
Histological Type	
Invasive ductal carcinoma	91 (75.83%)
Invasive lobular carcinoma	15 (12.50%)
Invasive cribriform carcinoma	6 (5.00%)
Mucinous carcinomacinoma	4 (3.33%)
Invasive mucinous carcinoma	4 (3.33%)
Methylene Blue Staining	
Positive	65 (54.17%)
Negative	55 (45.83%)
Axillary Lymph Node Metastasis	
Present	63 (52.50%)
Absent	57 (47.50%)

The diagnostic performance of methylene blue was high. Sensitivity was 92.06% and specificity 87.72%. The positive predictive value was 89.23% and the negative predictive value 90.91%. The positive likelihood ratio was 7.50 and the negative likelihood ratio was 0.09. Overall accuracy was 90.00%.

Table 3: Diagnostic Performance of Methylene Blue Dye for Axillary Lymph Node Metastasis Detection

Statistic	Value	95% CI
Sensitivity	92.06%	82.44% to 97.37%
Specificity	87.72%	76.32% to 94.92%
Positive Likelihood Ratio	7.50	3.73 to 15.06
Negative Likelihood Ratio	0.09	0.04 to 0.21
Positive Predictive Value	89.23%	80.49% to 94.33%
Negative Predictive Value	90.91%	81.09% to 95.89%
Accuracy	90.00%	83.18% to 94.73%

Discussion

The results show that methylene blue dye works well for checking axillary sentinel lymph nodes. In our study it had a sensitivity of 92.06%, specificity of 87.72% and overall accuracy of 90.00%. The rates of false positives and false negatives were low

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at 5.83% and 4.17%. These findings agree with what has been reported in other studies and support the use of methylene blue in standard SLNB procedures.

For example, Khawar et al. reported sensitivity of 89.66%, specificity of 89.71%, PPV of 88.14%, NPV of 91.04% and accuracy of 89.68% [15]. A slightly higher sensitivity of 92.06% was observed in our study. This may be due to the careful injection protocol that was followed. Five milliliters of 1% methylene blue were injected in three subareolar spots. A massage was applied for 10–15 minutes to help the dye move through the lymphatics. It is agreed in both studies that methylene blue is reliable, low-cost and suitable for settings with limited resources [15]. In the study by Bakhtiar et al., a sensitivity of 96.8% and accuracy of 94.1% were reported, but specificity was lower at 86.36% [16]. A higher rate of metastasis was present in their patients (71.7% vs 52.5% in our study). This may have increased sensitivity while lowering specificity. In our study, a balanced mix of cases was included. Steady results were obtained for both positive and negative findings. This is important when the method is applied to general patients without clinical node involvement.

A 96% procedural success rate and 96% agreement with histopathology were reported by Khanam et al., though formal sensitivity and specificity values were not provided [17]. Their lower metastasis prevalence (31%), possibly reflecting earlier-stage disease at enrolment, did not prevent the high concordance they recorded, which is consistent with the 90% accuracy observed in the present study. Across both series, methylene blue reliably identified sentinel lymph nodes irrespective of the underlying disease burden.

Hermansyah et al. reported a specificity of 96.67% and an NPV of 96.67%, modestly higher than the current study's values of 87.72% and 90.91%, respectively [18]. Their sensitivity of 91.67% was comparable to ours (92.06%). Variations in histopathological criteria or patient selection are probably the cause of the slightly higher specificity in their cohort; the most vital parameter is the ability to detect actual metastatic involvement, and our sensitivity was the same.

Aimir et al. reported excellent specificity (100%) and PPV (100%; none of the false positives), and a sensitivity of 86.7% - lower than in our study [19]. In this case, a high false-negative rate has greater clinical implications than relatively small false positives, because false negatives can understage and be mismanaged. Our greater sensitivity, therefore, reflects a clinically preferable trade-off in a malignant disease setting.

Ozdemir et al. also reported 100% specificity, 100% NPV, 90% PPV, and an accuracy of 93%; however, their sensitivity was 85%, compared to ours [20]. In the current study, the 92.06% sensitivity corresponds to a rate of less than 8% missed metastases, which is clinically acceptable for staging disease at an early stage. The higher sensitivity, combined with comparable accuracy, makes our protocol more effective for early-stage breast cancer staging.

In oral cancer, Hwang et al. achieved a sensitivity of 90.3% and a specificity of 68%, which is significantly lower than our specificity of 87.72% [21]. Such a difference is best attributable to anatomical and procedural factors: lymphatic mapping of the head and neck is more technical and less standardised than axillary SLNB, which has benefited from decades of development and has established reproducible protocols. Our results may not apply to other anatomical locations. The general compatibility of our results with similar breast cancer research shows the reliability of methylene blue-based SLNB when a specific injection methodology is used. This dye is useful alone in environments without dual-tracer or radiotracer methodologies, as it can be concordant with histopathological staging at 90%, a standard internationally recognized as clinically acceptable.

This study has some limitations. First, it was conducted at a single center, which may limit the generalizability of the findings to other healthcare settings. Second, methylene blue dye was not compared with dual-tracer techniques or radiocolloid mapping, which are considered standard in many high-resource centers. Third, the study included only patients with early-stage, clinically node-negative breast carcinoma, so the findings may not apply to advanced or clinically

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node-positive disease. In addition, long-term outcomes such as axillary recurrence, disease-free survival, and overall survival were not assessed. Future multicenter studies with larger sample sizes, direct comparison with dual-tracer methods, and long-term follow-up are recommended to further validate the diagnostic accuracy, safety, and oncological reliability of methylene blue dye for sentinel lymph node biopsy in resource-limited settings.

Conclusion

Methylene blue dye demonstrated good diagnostic performance for axillary sentinel lymph node metastasis detection in early-stage breast carcinoma, with a sensitivity of 92.06%, specificity of 87.72%, positive predictive value of 89.23%, negative predictive value of 90.91%, and overall diagnostic accuracy of 90.00%. These findings suggest that methylene blue dye can be used as a reliable, low-cost, and accessible tracer for sentinel lymph node biopsy, particularly in resource-limited settings where radiotracer or dual-tracer techniques are not routinely available.

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