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Original Article

Efficacy Of Methylene Blue in Detecting Histopathologically Malignant Disease in Axillary Lymph Nodes in Breast Cancer

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ABSTRACT

Background: Breast cancer is currently the most common malignancy diagnosed in women worldwide, and axillary lymph node status remains a significant determinant of prognosis and adjuvant therapy. SLNB has become widely adopted over ALND in clinically node-negative patients due to its lower morbidity. Dual tracer techniques using radioisotopes and blue dyes have high detection rates; however, their applicability is limited in many low-resource settings because of high cost, limited availability, and regulatory constraints. Methylene blue is an inexpensive, safe, and widely available agent that provides a practical alternative for SLN mapping. **Objectives:** To assess the efficacy of methylene blue dye in detecting histopathologically malignant sentinel lymph nodes in patients with breast cancer. **Methods:** This was a prospective interventional study conducted for two years from Karnataka Medical College and Research Institute, Hubballi. The study enrolled 88 operable cases of breast cancer into an intervention group (injection of methylene blue peritumorally) (42) and control (46). Lymph nodes that were stained blue would be removed and subjected to histopathology. Sensitivity, specificity, predictive values, diagnostic accuracy, and false-negative rate are calculated. Statical significance is evaluated with a Chi-square test and Fisher's exact test. **Results:** Methylene blue had a 90.47% SLN detection rate. Metastatic involvement was significantly higher in stained nodes, with a p-value of 0.034. For sensitivity, the value was 87.87%; specificity, 22.2%; PPV, 80.5%; NPV, 33.3%; diagnostic accuracy, 73.8%; and the false-negative rate was 12.12%. There were no adverse reactions reported. **Conclusion:** Methylene blue is an effective and safe dye for SLN localization in breast cancer surgery. While specificity and negative predictive value remain modest, high detection rate and sensitivity make the technique a useful diagnostic adjunct, especially in settings without access to radioisotopes or sophisticated imaging. Given its low cost, ease of administration, and low profile of complications, methylene blue is a practical choice for SLNB, particularly in resource-poor settings. Future research into the combination of methylene blue with other techniques, such as ICG or portable ultrasound, may help mitigate some of the diagnostic deficiencies associated with methylene blue.

***Keywords**: Breast Cancer, Sentinel Lymph Node Biopsy, Methylene Blue, Axillary Lymph Node, Histopathology, Sensitivity, Diagnostic Accuracy, Resource-Limited Settings.*

INTRODUCTION:

Assessment of axillary lymph node metastasis in breast cancer is of paramount importance for oncologic decision-making, since it significantly influences prognosis and further guides postoperative therapeutic planning [1]. Breast cancer remains the most common cancer diagnosed in women worldwide, and nodal involvement continues to be one of the strongest predictors of disease progression and survival outcomes [2]. Traditionally, axillary lymph node dissection was considered the standard approach to staging, but it carries significant postoperative morbidity, such as lymphedema, chronic pain, sensory loss, and limitation of shoulder function [3]. The introduction of SLNB revolutionized axillary staging by providing for the selective assessment of lymph nodes and decreasing the necessity for complete dissection in patients with no nodal disease [4]. The SLN, being the first node to receive lymphatic drainage from the primary tumor, represents the most likely initial site of metastasis [5]. Therefore, accurate SLN identification is an absolute necessity for the precise assessment of axillary status.

Various agents have been tried for SLN mapping, including blue dyes-methylene blue, isosulfan blue-and radiocolloids like technetium-99m [6]. While radioisotope techniques are associated with very high detection rates, financial constraints, regulatory issues, and logistical difficulties in many settings lead to its restricted use in many places [7]. Isosulfan blue, another popularly used dye, is expensive and also causes rare severe hypersensitivity reactions [8]. Methylene blue, however, is inexpensive, easily available, and safe, with success rates comparable to more sophisticated modalities of identification [9]. A synthetic dye for several decades in many medical applications was first popularized in the late 1990s for SLNB [10]. Further studies have reported fairly consistent detection rates of 85% to 95% [11], with one meta-analysis describing a pooled identification rate of 91.2%, equivalent to radioisotope technique [7]. It also has an excellent safety profile, with only mild, transient discoloration and, rarely, allergic reactions [9]. Its low cost and easy availability make it especially useful in resource-constrained healthcare facilities without nuclear medicine facilities [6].

Yet, despite its advantages, methylene blue does not come without its shortcomings. False-negative findings, or undetected metastatic nodes, do occur-in incidences between 5% and 10% [3]. These may result from various technical errors, anatomical abnormalities in lymphatic drainage, or even the learning curve for SLNB [5]. However, it seems that with standardized techniques and sufficient surgical experience, some of these risks can indeed be lowered [1]. Another disadvantage is that methylene blue staining is transient; timing between dye administration and node removal should be optimal [11].

AIMS & OBJECTIVES: To determine the efficacy of methylene blue dye in detecting histopathologically malignant disease in axillary lymph nodes in breast cancer.

MATERIALS & METHODS:

Source of the data: The present study is carried out in the Department of General Surgery, Karnataka Medical College and Research Institute, Hubballi. The patients of Breast Carcinoma admitted in Department of Surgery KMCRI Hubballi during study period.

Method of collection of data

Study Design: A Prospective Interventional study

Study period: April 2023 to April 2025

Study duration: 24 months

Place of study: Department of General Surgery KMCRI, Hubballi

Key Characteristics of the Study Design:

1. Prospective-Nature: The study's prospective design represents a significant strength, as emphasized in the presentation. Patients were enrolled consecutively and data was collected in real-time during their diagnostic and treatment journey, to minimize recall bias that often plagues retrospective studies. This approach allowed for precise documentation of methylene blue administration, surgical findings, and histopathological outcomes.

2. Interventional-Component: As an interventional study, the methylene blue dye is actively administered to participants rather than merely observing existing practices. The intervention protocol: a 2ml dose of 1% methylene blue diluted to 5ml with normal saline, injected in the peritumoral region before anesthesia induction. This active 35 intervention differentiates the study from observational research and provides direct evidence of the dye's performance characteristics.

Inclusion criteria

1. Operable cases of breast carcinoma admitted in Department of General Surgery KMCRI, Hubballi during the study period.

Exclusion criteria

1. Previous breast or axillary surgery (risk of altered lymphatic drainage).
2. Prior chemotherapy/radiotherapy
3. Allergy to contrast media or methylene blue.
4. Pregnancy.

Study methodology

Preoperative Preparation

- **Informed Consent:** Detailed explanation of the procedure, risks, and benefits was provided.
- **Pre-anesthesia Workup:** Routine tests (blood work, ECG) and imaging (mammography/USG) and pathological (FNAC/Tru-cut) were conducted. Methylene Blue Injection Protocol
- **Dye Preparation:**
  A. 2 mL of 1% methylene blue diluted with 3 mL normal saline (total: 5 mL). 36
  B. Concentration chosen based on Ahmed et al. [6], ensuring optimal staining with minimal side effects.
- **Injection Technique:**

A. Peritumoral injection (around tumor periphery) for effective lymphatic uptake.
B. Administered before anesthesia to allow 10-15 minutes for dye migration.
C. Gentle massage at the injection site to enhance dye spread.

Surgical Procedure

- Axillary Exploration:
  A. After mastectomy, the axilla was examined.
  B. Blue-stained lymph nodes (SLNs) were identified and excised.
- Histopathological Examination (HPE):
  A. SLNs were sent for HPE.
  B. Non-stained nodes (if any) were also analyzed for comparison.

Postoperative Monitoring

- Urine Discoloration: Expected and temporary (resolved in 24-48 hours).
- Complications: Monitored for allergic reactions, skin discoloration, or infection.

SAMPLE SIZE: Sample Size was calculated by using following equation.

$$n = \frac{Z2 * PQ}{d2}$$

n = sample size
Z = Standard normal variate for =0.05 (95%CI) = 1.89
P= 90.48% (sentinel lymph node positive for malignant cell)
Q = 9.52%
Absolute precision=d=10

By substituting the values in above formula, the minimum required sample size was 30. However, 88 patients undergoing Modified radical mastectomy (MRM) and Breast conserving surgery (BCS) with axillary dissection were enrolled in the study with 42 allotted to study group and another 46 allotted to control group.

Statistical analysis

Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test or Fischer's exact test (for 2x2 tables only) was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two quantitative variables.

RESULTS:

The table compares the demographic and clinicopathological characteristics of patients with methylene blue–positive nodes (Group 1) and methylene blue–negative nodes (Group 2) in order to assess its association with histopathologically confirmed malignancy. Age distribution reveals marked differences: Group 1 has a larger number of cases in the category of 40–45 years (28.57%) and 55–60 years (23.80%), indicating a predilection towards relatively young to middle-aged cases. On the other hand, Group 2 contains more age variability with relatively higher frequencies in the 45–50 years (26.08%), 50–55 years (26.08%), and 60–65 years (13.04%) age categories. This suggests that methylene blue uptake is more frequently seen among relatively younger patients.

Regarding tumour type, the most prevalent diagnosis for both groups is invasive ductal carcinoma, making up over 80% of cases. However, invasive lobular carcinoma is found to be more commonly seen in Group 1 at 19.04% compared to the 8.69% in Group 2, while DCIS is only present within Group 2. These findings suggest that positive methylene blue is more indicative of invasive histologies.

The lymph node status demonstrates a strong correlation with methylene blue staining. Histopathologically positive lymph nodes are 78.57% in Group 1 compared to 56.52% in Group 2, indicating the possible application of methylene blue in highlighting malignant nodal involvement. Also, within Group 1, there are more cases with 1–4 positive nodes, while in Group 2, patients with higher nodal burden (5–8 nodes) are present, though fewer in number. Methylene blue staining therefore correlates well with histopathological positivity, indicating its role as an intraoperative marker. The chi-square test reveals a statistically significant association between the staining of lymph nodes and the presence of metastatic cancer cells ($p < 0.05$), which underscores the potential of methylene blue to assist in accurate cancer detection.

Table 1: Comparison the demographic and clinicopathological characteristics of patients with methylene blue–positive nodes (Group 1) and methylene blue–negative nodes (Group 2)

Variables	Sub-variables	Group1 (Yes)	Group 2 (No)	Total
Age	30-35	2 (4.76%)	2 (4.34%)	4 (4.54%)
	35-40	2 (4.76%)	4 (8.69%)	6 (6.81%)
	40-45	12 (28.57%)	2 (4.76%)	14 (15.90%)
	45-50	9 (21.42%)	12 (26.08%)	21 (23.86%)
	50-55	4 (9.52%)	12 (26.08%)	16 (18.18%)
	55-60	10 (23.80%)	6 (13.04%)	16 (18.18%)
	60-65	0	6 (13.04%)	6 (6.81%)
	65-70	4 (9.52%)	2 (4.76%)	6 (6.81%)
Diagnosis	IDC	34 (80.95%)	38 (82.60%)	72(81.81%)
	ILC	8 (19.04%)	4 (8.69%)	12 (13.63%)
	DCIS	0	4 (8.69%)	4 (4.54%)
LN status in HPR	Positive	33 (78.57%)	26 (56.52%)	59 (67.04%)
	Negative	9 (21.42%)	20 (43.47%)	29 (32.95%)
			P =0.028	
No of positive nodes among stained	0	21 (50%)	20 (43.47%)	41 (46.59%)
	1	9 (21.42%)	0	9 (10.22%)
	2	2 (4.76%)	6 (13.04%)	8 (9.09%)
	3	5 (11.90%)	6 (13.04%)	11 (12.5%)
	4	5 (11.90%)	4 (8.69%)	9 (10.22%)
	5	0	4 (8.69%)	4 (4.54%)
	6	0	4 (8.69%)	4 (4.54%)
	8	0	2 (4.34%)	2 (2.27%)
			X2=21.09	P= 0.0036
Total		42 (100%)	46 (100%)	88 (100%)

Table 2 shows Methylene blue demonstrates high sensitivity (87.87%), indicating it is effective in detecting malignant axillary lymph nodes, and its positive predictive value (80.5%) shows that most positive results truly represent malignancy. However, the low specificity (22.2%) and negative predictive value (33.3%) indicate a high false-positive rate and that negative results are unreliable for excluding disease. With an overall accuracy of 73.8%, methylene blue is useful as a screening tool but cannot replace histopathology, especially for ruling out malignancy

Table 2: Accuracy of Methylene Blue

Metric	Value
Sensitivity	87.87%
Specificity	22.2%
Positive predictive value	80.5%
Negative predictive value	33.3%
Accuracy	73.8%

DISCUSSION:

The present study demonstrated a sentinel lymph node (SLN) detection rate of 90.47% using methylene blue, aligning well with global literature. For instance, Goyal et al. (2016) [5] and Tew et al. (2005) [11] also reported detection rates above 89%, confirming the dye's consistency across varied clinical settings. A meta-analysis by Noguchi et al. (2015 [7] observed a pooled identification rate of 91.2%, supporting methylene blue's clinical utility as a standalone tracer, especially in resource-constrained environments The sensitivity observed in the study was 87.87%, indicating robust ability in identifying metastatic nodes. However, specificity was relatively low at 22.2%, suggesting a higher false-positive rate, possibly due to reactive or inflamed nodes being mistaken for malignancy. Compared to studies using dual tracers like Indocyanine Green (ICG) + Technetium-99m (99mTc), which reported sensitivity around 92.6% and specificity between 80–90%, the methylene blue-only method shows slightly lower diagnostic precision but remains acceptable in settings lacking nuclear medicine infrastructure. In the present study showed a statistically significant association between lymph node staining and malignancy (Chi-square p = 0.028; Fisher's Exact Test p = 0.034), confirming methylene blue's diagnostic relevance. However, the false-negative rate (FNR) was 12.12%, marginally above the ASCO-recommended threshold of 10%, and higher than in studies using dual-agent techniques, such as SPIO or 99mTc, which reported FNRs as low as 8.4%. Despite these limitations, the findings affirm methylene blue's utility where advanced options are unavailable. To further reduce FNR and improve specificity, future protocols may consider combining methylene blue with low-dose ICG or ultrasound 63 64 guidance. Structured training and stricter timing protocols could also minimize detection variability.

Comparison of SLN Detection Rates Across Studies

Study	Tracer used	Sample size	SLN detection rate	P-value
Present study	Methylene blue	42	90.47%	0.028
Ozmen et al [12]	Methylene blue + 99m Tc	200	93.0	>0.05
Vermersch et al [13]	ICG+99mTc	100	100	0.09

Comparative diagnostic Metrics

Study	Sensitivity	Specificity	PPV	NPV	Accuracy	P-Value
Present study	87.87	22.2	80.5	33.3	73.8	0.034
Vermersch et al. (2019)	92.6 (ICG), 85.2 (99mTc)	Not Specified	86.5	89.5	High	0.09
Zada et al. (2016)	~90	~85	89.5	87.5	~95	<0.001

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CONCLUSION:

This study illustrates that methylene blue is a safe, effective, and inexpensive tracer for sentinel lymph node mapping in breast cancer surgery. For the identification of metastatic axillary nodes, methylene blue is a reliable method with a high detection rate and good sensitivity, especially in institutions that do not have facilities to prepare radioisotopes. Although specificity and negative predictive value were lower, and the false-negative rate slightly exceeded ideal standards, methylene blue remained clinically useful with no significant complications. Given its accessibility and cost-effectiveness, methylene blue serves as a practical single-agent alternative. Larger multi-centre studies are recommended to further strengthen evidence and refine SLNB protocols.

Declaration:

Conflicts of interests: The authors declare no conflicts of interest.
Author contribution: All authors have contributed in the manuscript.
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