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Original Research Article

Exploring methylene blue as an effective alternative to radiotracer for sentinel lymph node biopsy in early breast cancer in resource-limited settings

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ABSTRACT

Background: Sentinel lymph node biopsy is the recommended approach in the evaluation of axilla during breast cancer surgery. This study evaluated the results of patients who underwent methylene blue sentinel lymph node biopsy.
Methods: The study included 35 female patients with T1, T2 and T3 tumours. 5 ml of 1% methylene blue was injected into the periareolar region. The axillary sentinel lymph node was found and removed, and axillary dissection was performed. The sentinel lymph node and axillary dissection specimen were histopathologically examined, and the results were compared.
Results: The sentinel lymph node was found in 33 (90%) patients. There were no complications associated with the use of methylene blue dye. Methylene blue-stained sentinel nodes were histologically negative for malignancy in 42% and positive in 58% of patients. Corresponding to sentinel nodes, the final axillary staging was negative in 60% and positive in 40% of patients. On diagnostic accuracy parameters, the sensitivity of the methylene blue method in early breast cancer (cT1-3N0M0) patients was 90.47%, and the specificity was 100%. The PPV of the technique under study was found to be 100% and NPV was 85.71%. The accuracy rate was 93%, and the false negative rate was identified as 9.5%.
Conclusions: Sentinel lymph node biopsy by methylene blue is a method that can be applied with high accuracy. Methylene blue can be considered as an alternative to radio colloid dye in sentinel lymph node biopsy in a limited resource setting.

Keywords: Methylene blue, Sentinel lymph node biopsy, Early breast cancer, Radiotracer alternative, Resource-limited settings, Breast cancer surgery

INTRODUCTION

Breast carcinoma is the most common malignancy among females in India and all over the globe.[1,2] In the year 2020, 178,361 new cases and 90,408 fatalities were attributed to breast carcinoma in India.[2] By 2025, breast cancer is estimated to reach 15% of the country's total incidence of cancer.[3,4]

The presence of axillary lymph nodal metastasis is essential for the assessment of breast carcinoma as well as the determination of staging.[5] Axillary lymph nodal staging remains the standard of care for all breast carcinomas, providing information important for individualized adjuvant and treatment. Due to the limited sensitivity of imaging techniques in axillary lymph node evaluation, the surgical evaluation of the axilla seems necessary. However, axillary lymph node dissection (ALND) may cause complications such as lymphedema, seroma, paraesthesia and numbness of the arm, shoulder dysfunction, and other short-term and long-term

complications limiting functionality and reducing quality of life.[6]

Currently, sentinel lymph node biopsy (SLNB) has taken over ALND as the standard of care for axillary lymph node staging in clinically and radiologically node-negative breast carcinoma.[7] As SLNB leads to less extensive dissection, it can decrease surgical morbidity in terms of shoulder dysfunction and lymphedema, without affecting diagnostic accuracy and prognostic information.[8]

Recently, various SLNB techniques have been described with good efficacy and safety data, including methylene blue dye, near-infrared (NIR) fluorescence imaging, 99 m-Technetium (99-Tc), and dual tracing modality.[9,10] Mapping sentinel lymph node locations with methylene blue dye alone results in an acceptable identification rate but an excessive false negative rate, according to the American Society of Breast Surgeons' recommendations.[11] NIR fluorescence imaging using indocyanine green (ICG) has been proven to have valid diagnostic performance for sentinel lymph node detection and showed a trend toward better axilla staging.[12] Dual tracing modality using ICG plus methylene blue has been demonstrated to improve the mapping performance and exhibit great potential as an alternative to traditional standard mapping methods.[13] Although mapping tracers are constantly evolving, their application in the real world is still unclear, especially in resource-limited settings.

In India, breast cancer surgeries are performed at small clinics to tertiary care hospitals, where advanced devices such as radioisotope tracers and near-infrared fluorescence imaging systems are difficult to acquire due to expensive maintenance and cost.[14] The SLNB is still not widely used in smaller centres across India due to reasons such as, e.g. huge population and geographical distribution, limited availability and cost to provide radioisotopes and gamma probes. The limitation to providing these sophisticated technologies for SLNB has been our major setback. The 1% methylene blue dye is a good option for this limitation due to its easy availability and safety. Although it is significant to validate the use of the same to overcome the limitation of performing SLNB, therefore we planned a study to identify the false negative rate and safety of using 1% methylene blue dye alone.

METHODS

This prospective observational study included 35 patients in the Department of Surgical Oncology, Apollo CBCC Cancer Care, Ahmedabad, from September 2017 to April 2019 after the approval of the hospital's ethical committee. All patients with true cut biopsy-proven carcinoma breast and having T1, T2 or T3 tumours and clinically node negative axilla were included in this study. Patients with metastatic, inoperable disease, history of previous breast surgery, clinically palpable nodes, and larger tumour size (T4) were excluded from the study.

All surgeries were done by the same breast cancer surgeon at the hospital. Non-probability consecutive sampling was done. At the operation table, prophylactic antibiotics were given at the time of induction to all the patients, and after draping, 3-5 ml sterilised 1% methylene blue dye was infiltrated with a 10 cc syringe in the periareolar area of the diseased breast (Figure 1). Gentle massage of the breast was done for 1-2 minutes, and then after 10 minutes, dissection was done in the axilla for localization of the sentinel lymph node by giving an incision in the axilla that incorporates the incision of modified radical mastectomy.

Figure 1: Peri areolar injection of methylene blue dye.

The blue-coloured lymphatic channels were followed, which led to the blue-stained node being defined as a sentinel lymph node (SLN) (Figure 2), which was carefully dissected out, and any other stained node if found in the vicinity, was also removed (Figure 3). According to staging, the modified radical mastectomy with axillary clearance or breast conservative surgery with axillary clearance was performed, and the SLN and mastectomy with axillary clearance specimen will be sent to the histopathology laboratory fixed in formalin in two separate bottles marked "A" and "B" respectively, and the report of the histopathology was compared. The outcome variables, such as e.g. sensitivity specificity, positive predictive value (PPV), negative predictive value (NPV), identification rate, and side effects, were evaluated.

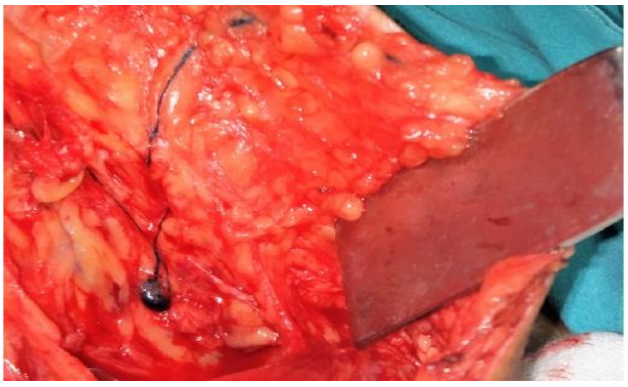

Figure 2: Level I sentinel node with lymphatic channels.

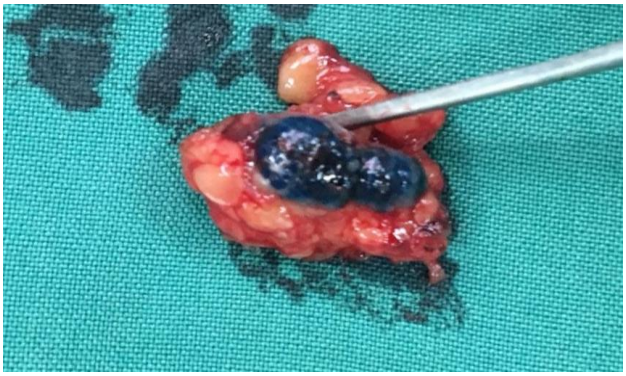

Figure 3: Isolated blue colour-stained sentinel lymph nodes.

Statistical analysis

Data was analysed by utilizing statistical package for the social sciences (SPSS) version 17. Mean and standard deviation were calculated for age tumour size. Frequency and percentage were calculated for gender, breast involvement and quadrant of breast involved, and accuracy of methylene blue dye. A 2×2 table was constructed to calculate the sensitivity, specificity, PPV, NPV and diagnostic accuracy of methylene blue dye to predict axillary status, taking histopathology as the gold standard. Effect modifier was controlled through stratification of age, gender, size of tumour, breast involvement and quadrant of breast involvement to see the effect of these on outcome variables.

RESULTS

The total number of patients was 33 from the final diagnostic accuracy analysis, as two patients in the study did not have blue stained nodes after injection of methylene blue dye. Therefore, these 2 cases were excluded from the final diagnostic accuracy analysis. The AJCC cancer staging manual (8th edition) was used for clinical and pathological staging of the tumour. The mean age of the study population was 54±10.63 years. The most frequently involved quadrant of breast in my study was upper outer quadrant (UOQ) – 42.85% (n=15) followed in decreasing frequency by upper inner quadrant (UIQ) and central quadrant – 14.28% (n=5), lower inner quadrant (LIQ) – 11.42% (n=4 and least frequent was lower outer quadrant (LOQ) – 8.57% (n=3). The 27 out of 35 patients underwent modified radical mastectomy (MRM). The clinical staging distribution was 14.28%, 74.3% and 11.42% in T1, T2 and T3 accordingly (Table 1).

The accuracy (identification rate) of the method under study was 93%, as 33 out of 35 patients had blue-stained lymph nodes. The blue-stained sentinel nodes were positive for metastasis only in 8 out of 33 patients, and the rest of the axillary nodes were negative for metastasis. These cases were not termed as false positives as existing evidence suggests only sentinel nodes may be positive if less than three nodes were positive. The most frequent number of targeted sentinel nodes removed was 2 45.45% (n=58), followed by four nodes – 18.18% (n=6), followed by three nodes – 14.14% (n=4), followed by five nodes – 3.03% (n=1) each. All positive sentinel nodes were in level I. The rest of the axillary nodes were noted in levels I, II, and III. Isolated level I node positivity was noted in 8 patients. Level I II were noted in 4 patients, and only one patient had positive nodes in levels I, II, and III (Table 2). The average number of the sentinel nodes was 2.31, and the rest of the axillary nodes were 14.8. The sensitivity and specificity of this method in predicting the axillary node involvement were 90.47% and 100%, respectively. The positive and negative predictive values (PPV and NPV) were 100% and 85.71% respectively (Table 3).

Table 1: General characteristics.

Characteristics	Value (%)
Total patients	n=35
Age (years)	54±10.63
Histopathology	
IDC	97 (n=34)
ILC	3 (n=1)
Quadrants involved	
Central	14.28 (n=5)
Lower inner	11.42 (n=4)
Lower outer	8.57 (n=3)
Upper inner	14.28 (n=5)
Upper outer	42.85 (n=15)
T stage	
T1	14.28 (n=5)
T2	74.30 (n=26)
T3	11.42 (n=4)
Surgery	
BCS	22.85 (n=8)
MRM	77.15 (n=27)
Number of sentinel nodes	
1	18.18 (n=6)
2	45.45 (n=15)
3	15.15 (n=5)
4	18.18 (n=6)
5	3.03 (n=1)

Table 2: Sentinel node characteristics.

Parameters	Sentinel nodes	Other axillary nodes
Average number	2.31	14.8
Average size (mm)	5×5	5×5
Site of nodal metastasis		
Level I	19	8
Level I, level II	0	4
Level I, level II, level III	0	1
Histopathology (%)		
Negative for metastasis	42.42 (n=14)	60.60 (n=20)
Positive for metastasis	57.88 (n=19)	39.40 (n=13)

Table 3: Efficacy of sentinel lymph node biopsy.

Diagnostic accuracy	Axilla metastasis	
	No.	%
Sensitivity	19/21	90.47
Specificity	12/12	100
PPV	19/19	100
NPV	12/14	85.71
Accuracy	31/33	93
False negative rate	NA	9.5

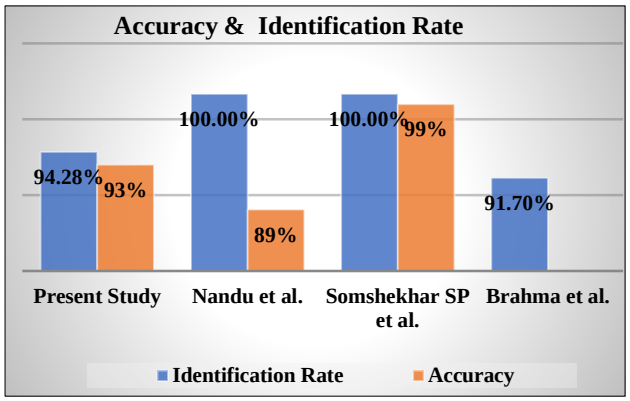

Figure 4: Comparison of accuracy and identification rate with other studies.

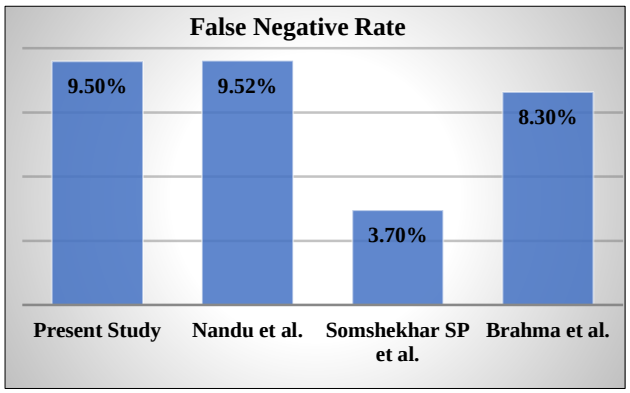

Figure 5: Comparison of false negative rate with other studies.

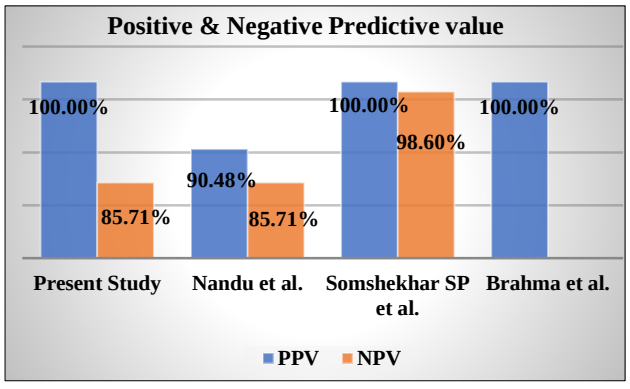

Figure 6: Comparison of PPV and NPV with other studies.

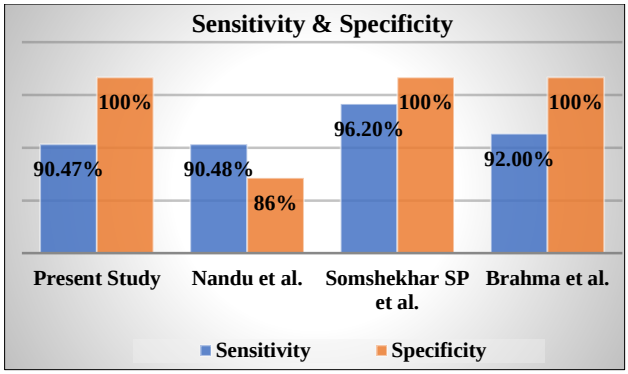

Figure 7: Comparison of sensitivity and specificity with other studies.

DISCUSSION

In many developing countries, including India, breast cancer patients often present at a more advanced stage, leading to higher mortality rates compared to developed nations.[1,2] Following the practice-changing results of sentinel lymph node biopsy (SLNB) trials, several centres in India have begun adopting SLNB.[3,4] However, its use remains limited primarily to tertiary care centres. The standard technique for lymphatic mapping, which involves a combination of radioisotopes and blue dye, is not widely accessible due to the limited availability of radiotracers, blue dye, and nuclear medicine facilities. This is particularly problematic for cancer treatment centres in non-metropolitan areas, where access to radiotracers is restricted. To address this, methylene blue alone was employed in our study to identify sentinel lymph nodes (SLNs) in early-stage breast cancer patients.

In this study, 35 cases met the inclusion and exclusion criteria, but two were excluded, resulting in 33 cases being analysed. The mean number of SLNs harvested in this study was 2.3, which compares favourably to the 1.8 in the study by Nandu et al and 2 in the studies by Somshekhar et al and Brahma et al.[15-17] The mean number of axillary lymph nodes harvested in the present study was 14.8, compared to 17.34 in the study by Nandu et al, 16 in the study by Somshekhar et al, and 14.8 in the study by Brahma et al.[15-17]

Our study demonstrated a sensitivity of 90.47%, similar to the 90.48% reported by Nandu et al, 96.2% by Somshekhar et al, and 92% by Brahma et al.[15-17] The specificity in this study was 100%, matching the findings of Somshekhar et al and Brahma et al, while Nandu et al reported a specificity of 85.71%.[15-17] The NPV in our study was 85.71%, the same as that reported by Nandu et al.[17] In contrast, the study by Somshekhar et al showed a higher NPV of 99%.[15] The PPV in our study was 100%, consistent with the findings of Somshekhar et al, while Nandu et al reported a PPV of 90.48%.[15-17]

The identification rate in this study was 94.28%, compared to 100% in the studies by Nandu et al and Somshekhar et

al, and 92% in the study by Brahma et al.[15-17] The accuracy rate in our study was 93%, higher than the 88.57% reported by Nandu et al, but lower than the 99% reported by Somshekhar et al.[15-17] The false-negative rate (FNR) in this study was 9.5%, similar to the 9.52% reported by Nandu et al and the 8% reported by Brahma et al.[17] In contrast, Somshekhar et al reported a lower FNR of 3.7%, likely due to their use of the dual method (radiocolloid + dye).[15]

In two cases, no blue-stained nodes were identified in the target area. Technical failure in these cases could have been due to altered or multiple lymphatic drainage pathways, as seen in multifocal disease, higher BMI or obesity, or improper injection technique. There were no significant complications associated with methylene blue dye, such as anaphylactic reactions, skin flap necrosis, or wound infections. All patients had green-coloured urine on the first postoperative day, which returned to normal within 24 hours, a known benign side effect of methylene blue absorption.

In the context of early breast cancer management, the paradigm has shifted toward more conservative treatments, with SLNB replacing axillary lymph node dissection (ALND) for axillary staging in early-stage disease. However, in India, most breast cancer cases present at more advanced stages, making ALND the more common practice. Increasing awareness of breast cancer screening and early detection may lead to more frequent use of SLNB, reducing the costs associated with treatment. Shorter hospital stays and fewer complications, such as lymphedema and motor function compromise, can result from the reduced need for axillary dissection.

Two decades ago, breast-conserving surgery (BCS) was proven to be as effective as mastectomy, and today, we stand on the brink of a similar shift where routine ALND may no longer be necessary for a large proportion of patients with early-stage breast cancer. Notably, 58% of metastatic foci in this study were confined to the SLNs, with nearly 30% restricted solely to these nodes. According to the ACOSOG Z0011 and AMAROS studies, patients with small tumour plans for BCS and whole breast radiation may avoid ALND even in the presence of positive SLNs.[18,19] However, most of our patients did not meet these criteria, as they had larger median tumour sizes, with 12% in stage III, and underwent mastectomy more often than BCS.

The lower NPV in our study, compared to previous studies, may be explained by the two false-negative cases, which had larger median tumour sizes (4 cm) and higher tumour grades (75% grade 3). Tumours larger than 3 cm and higher-grade tumours may have a higher risk of nodal metastasis and lymphatic system blockage, leading to false-negative results. Surgeons should exercise caution when performing SLNB with methylene blue alone in patients with grade 3 tumours and tumour sizes greater than 3 cm. In such cases, it is advisable to inspect non-blue suspicious lymph nodes to minimize false-negative rates.

Limitations

This study was conducted in a corporate hospital, where the number of early breast cancer cases was limited, resulting in a small sample size. The false-negative rate (FNR) in this study was 9.7%, which is slightly higher than the accepted range of 5-7% in landmark SLNB studies. Additionally, fine-needle aspiration cytology (FNAC) of axillary nodes was not performed to confirm the clinical node-negative status.

CONCLUSION

This study demonstrates that SLNB in breast carcinoma can be successfully performed using methylene blue dye alone in settings with limited access to standard radiotracers. Surgeons should consider several important factors: in patients with high-grade or larger tumours, identifying only blue-stained nodes may be insufficient. Non-blue suspicious lymph nodes should be carefully examined to reduce false-negative results. Additionally, a thorough understanding of the anatomical location of SLNs in the axilla is crucial to increasing identification rates when using methylene blue dye alone.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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