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Original Article

Assessment of Sentinel Nodes with Methylene Blue Dye in Carcinoma Breast is Feasible? A Pilot Study

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Abstract

Introduction: Management of axilla is an integral part of treatment of carcinoma breast. Axillary lymph node dissection has a well-established role in regional disease control and it provides information about the histopathological status which has significant prognostic and therapeutic implications.

Materials and Methods: Thirty five patients with breast cancers with stages T1-T3, N0, and one patient with T3 N1 M0 disease who had become node negative post chemo therapy were included in the study. 5 patients with breast cancer clinically node negative axilla were excluded from the study after they have found to have axillary nodes after ultrasound examination. Totally 36 patients were evaluated.

Results: This study demonstrates that sentinel node localization is possible with methylene blue dye alone with 88.88% localisation rate. Though limited by small sample size this study has shown a low false negative rate of 6.25%. which denotes that SLN biopsy using methylene blue dye alone is a highly reliable and predictable technique to stage the axilla in breast cancer patients.

Conclusion: This technique may help to avoid complete axillary lymph node dissection in sentinel node negative patients thereby minimising the morbidity of axillary lymph node dissection.

Key words: Axillary dissection, Breast cancer, Methylene blue dye, Sentinel node biopsy

INTRODUCTION

Management of axilla is an integral part of the treatment of carcinoma breast. Axillary lymph node dissection has a well-established role in regional disease control and it provides information about the histopathological status which has significant prognostic and therapeutic implications.[1] However, only around 30% of the of clinically node negative patients prove to be histopathologically node positive which means that 70% of clinically node negative patients undergo axillary dissection and are exposed to its morbidities such as neuropathies, seromas, and upper extremity lymphedema. This can be avoided with a sentinel lymph node biopsy (SLNB).[2] Published data till date use vital blue dye and/or^{99m} Technetium labeled colloid with gamma probe for the identification of SLNs. A combination of the two techniques has been found to be the best and is recommended for optimal outcome. Blue dye-guided SLN identification may be the only available option in countries with low resources due to the prohibitive price of gamma probes. This pilot study was done to analyze methylene blue dye uptake after peritumoral injection and to compare tumor positivity in nodes stained and unstained with blue dye in modified radical mastectomy (MRM) specimens.

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MATERIALS AND METHODS

Patients of breast cancer with clinically negative axilla or patients who had pre-operative treatment (chemotherapy and/or radiotherapy [RT]) and became clinically negative axillae, irrespective of initial axillary nodal status were included in the study, after obtaining informed consent.

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35 patients with breast cancers with stages T1-T3, N0, and one patient with T3 N1 M0 disease who had become node negative post chemotherapy were included in the study.[2] Patients with breast cancer clinically node negative axilla were excluded from the study after they have found to have axillary nodes after ultrasound examination. In total, 36 patients were evaluated. Inclusion and exclusion criteria are given below:

Inclusion Criteria

a. Patients with carcinoma breast with clinically negative axillary nodes
b. Patients who had pre-operative treatment (chemotherapy and/or RT) and now have clinically negative axillae, irrespective of initial axillary nodal status
c. Patients above 18 years ago with the ability to give consent.

Exclusion Criteria

a. Clinically palpable axillary nodes
b. Prior upper limb lymphedema
c. Prior breast/axillary surgery
d. History of blue dye allergy
e. Patients taking serotonergic drugs such as paroxetine and fluoxetine.

Comprehensive history was taken, and thorough clinical examination was done. Ultrasound examination of the axilla was done with real-time scanner with probe head of 7.5 MHz frequency transducer. Axillary lymph nodes were reported at the time of examination as abnormal on the basis of size criteria and morphology (short-axis diameter >10 mm, cortical thickening, and lobulation or loss of the normal hyperechoic hilum). The patient with abnormal axillary lymph nodes with the above-mentioned features on ultrasonogram was excluded from the study, and thus 5 patients were excluded from the study.

Technique

In all selected patients, MRM was done with an axilla first approach. After induction of anesthesia, peritumoral injection of 1% methylene blue dye (4 ml) at the 3, 6, 9, 12 o'clock positions was done. SLNs were looked for after raising the superior flap and opening the clavipectoral fascia, within 15 min from the time of injection. The stained nodes were removed initially and sent for histopathological examination (HPE). MRM was completed along with axillary lymph node dissection in all cases. The excised breast with the axillary tissue was sent for HPE to correlate with the findings of the SLNB (Figure 1).

Pathological Examination

Post-operative specimen of the primary tumor was examined under hematoxylin and eosin stain after preparing paraffin sections. Tumor grade, margin, tumor thickness, vascular invasion, lymphatic invasion, and pathological T stage were noted. Number of nodes harvested at each level and nodes positive for blue dye were separately noted. Lymph nodes were bisectioned along the long axis, and each half was separately examined after standard eosin and hematoxylin fixing and staining.

RESULTS

In this study, 36 patients were evaluated, with mean age of 51 years, and age range was 26-70 years. Breast cancer was the most common in the age group of 41-50 (4/36 [38.8%]) cases, followed by 51-60 years (13/36 [36.1%]) cases. In our series, left-sided lesions 19/36 (52.7%) were predominant over right sided lesions 17/36 (47.3%). The most commonly involved site was upper outer quadrant (20/36) followed by upper inner quadrant (8/36), lower outer quadrant (4/36), and central quadrant (4/36). T stage distribution includes T1 - 2/36 (5.56%), T2 – 28/36 (77.78%), and T3 - 6/36 (16.67%) (Table 1).

Sentinel node was successfully identified in 32/36 (88.89%). Among the 32 cases, there was skip metastases to Level II node in one patient. SLN was not identified in 4 cases 4/36 (11.11%). There was only one patient with node-negative axilla post-neoadjuvant chemotherapy. In that patient, also sentinel node was identified. When the histopathological status of axillary lymph nodes was compared to SLNs histopathology, it was seen that when sentinel node HPE was positive (16/32) cases, the rest of the axilla was positive in 3 cases and negative in 13 cases and when the sentinel node HPE was negative (16/32) cases,

Table 1: General characteristics

General patient characteristics	Number of patients (%)
Age distribution: 26-70 (mean 43 years)	
<50 years	18 (50)
50 years and above	18 (50)
Side	
Left	19 (52.8)
Right	17 (47.2)
Size	
T1	2 (5.56)
T2	28 (77.78)
T3	6 (16.67)
Site	
UOQ	20 (55.55)
UIO	8 (22.22)
Central	4 (11.11)
LOQ	4 (11.11)
Grade	
1	8 (22.22)
2	19 (52.8)
3	9 (25)

UOQ: Upper outer quadrant , UIO: Upper inner quadrant, LOQ: lower outer quadrant

the rest of the axilla was also negative in 15 cases except one case. The sensitivity, specificity, positive predictive value, and negative predictive values were 66.67%, 51.5%, 12.5%, and 93.75%, respectively (Table 2).

In this study, we have dissected 867 axillary lymph nodes and total no of blue nodes harvested 80 and non-blue nodes 787. Average sentinel node harvest was 2.22. When analyzing the factors affecting the nodal positivity, we found that <50 years of age, 56.25%, left sided 43.75% upper outer quadrant 37.5%. Grade 2, 62.5%, was associated with sentinel node histopathology positivity, but none of these factors except the grade were statistically significant. Lower the grade higher was the sentinel node identification rate (Table 3).

Table 2: Sentinel node histopathology positivity versus rest of axillary lymph node histo pathology positivity

Histopathology of the SLN	Histopathology of the rest of the axillary node			*P* value
	Positive	Negative	Total	
Positive	2	14	16	0.5442
Negative	1	15	16	
Total	3	29	32	

SLN: Sentinel lymph node

Table 3: Factors affecting sentinel node identification

General patient characteristics	Sentinel node-identified	Sentinel node not identified	Total	*P* value
Age				
<50	17	1	18	0.60
>50	15	3	18	
Side				
Right	16	3	19	0.70
Left	16	1	17	
Site				
UOQ	19	1	20	0.30
UIO	7	1	8	
Central	3	1	4	
LOQ	3	1	4	
Size				
T1	1	1	2	0.20
T2	25	3	28	
T3	6	0	6	
Grade				
1	8	0	8	0.08
2	17	2	19	
3	7	2	9	

UOQ: Upper outer quadrant , UIO: Upper inner quadrant, LOQ: lower outer quadrant

DISCUSSION

The status of axillary lymph node remains the most important predictor of survival in women with invasive breast cancer, and this is used for making treatment decision.[1] Various methods of predicting axillary lymph node status have been described including clinical assessment, radiological, and operative procedures. Axillary lymph node dissection was earlier considered to be the gold standard for predicting the axillary lymph node status. Axillary lymph node dissection may be associated with significant morbidity such as post-operative pain in arm, chronic lymphedema of involved arm, neuropathy of arm, seroma formation, restricted shoulder mobility, and other complications.[3] SLNB has emerged as an effective diagnostic tool in staging axillary disease. The major advantage of SLNB is the lower complication rate compared with axillary lymph node dissection.[2]

This study was conducted to assess the feasibility of SLN localization using methylene blue dye alone. 36 patients were included whose axilla was clinically negative for lymphadenopathy. 35 patients were subjected to primary surgery and one patient was treated with neoadjuvant chemotherapy and subsequently became node negative. Although number of patients included was small ($N = 36$), it was comparable to studies done by Krag *et al.* ($N = 22$),[4] Borgstein *et al.* ($N = 33$),[5] Pijpers *et al.* ($N = 34$),[6] Ikeda *et al.* ($N = 29$),[7] Motta *et al.* ($N = 54$),[8] and Bassi *et al.* ($N = 40$).[9] 36 patients were evaluated with a median age of 51, and the study group was similar to what is reported in literature.

Sentinel node identification was higher in the age group of <50 years. Patient age was inversely correlated with the ability to identify the SLN. This finding has been reported previously and may be related to the inability of the blue dye to be taken up by the lymphatic system when injected into the fat-replaced postmenopausal breast. Özdemir *et al.*[10] studied 32 patients with a median age of 50. Mukherjee *et al.*[11] evaluated 27 patients with a median age of 43.

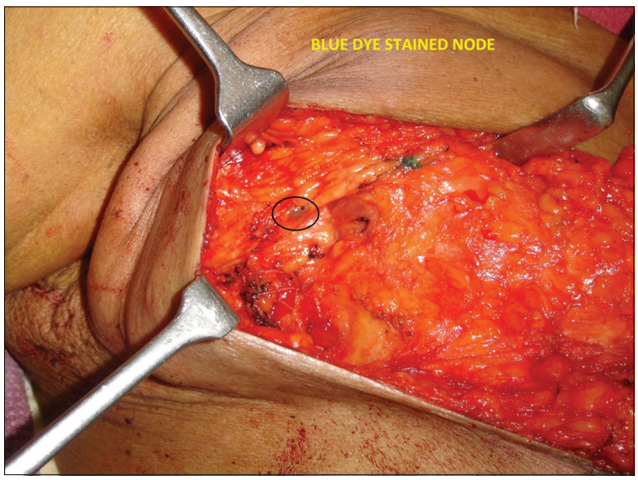

Figure 1: The methylene blue dye stained sentinel node

In this study, both right and left sides were more or less equally affected with slight predominance of left-sided lesions (19/36). Upper outer quadrant was involved in 20/36 (55.6%) of cases followed by upper inner quadrant 8/36 (22.2%), central quadrant 4/36 (11%), and lower outer quadrant (4/36). Sentinel node was identified readily in the upper outer quadrant tumors 95% followed by upper inner, central and lower outer quadrant locations with similar identification rate of 75%.

Right side (72%) and upper outer quadrant (75%) were the most common side and site of tumor location in a study by Ozdemir *et al*. 2013. In the study by Mukherjee *et al*., upper outer quadrant 44% was the most common site of tumor. Clinical tumor status include T1 2/36 (5.6%), T2 28/36 (77.8%) and T3 6/36 (16.7%) and Grade I - 8/36 (22.2%), Grade II - 19/36 (52.8%), Grade III - 9/36 (25%) with highest sentinel node identification in T3 and Grade I lesions about 100%. In this study, clinical characteristics did not affect sentinel node identification except tumor grade and it is similar to the results observed by Nano *et al*.[12] who studied clinical and histological factors associated with sentinel node identification.

Either isosulfane blue or methylene blue can be used as a dye in SLNB. Methylene blue is cheaper, more easily obtainable, and is a dye with fewer complications as compared to isosulfane blue. Hypersensitivity reactions which may also be fatal are reported at a rate of 0.6-2.5% following isosulfane blue injection. Skin necrosis, if injected intradermally, fat necrosis, and fibrosis over the injection site are among complications of methylene blue. However, in this study, no such complications related to methylene blue were encountered. In studies conducted in our country isosulfane blue was often preferred.[13] In the literature, there are many studies showing that methylene blue can be used safely and with high success as an alternative to isosulfane blue. Simmons *et al*.[14] have identified the SLN in 104 of 112 patients by using methylene blue and reported that SLN represented axillary status in 96.9% of patients. Blessing *et al*.[15] compared isosulfane blue and methylene blue and found the accuracy rate as 88.5% with isosulfane blue and as 92.7% with methylene blue.

In this study, also sentinel node identification with blue dye alone was 88.88%. In comparision, other studies which have reported sentinel node identification with methylene blue dye alone, ranging from 65% to 94% (Blessing *et al*. Simmons *et al*., Nour, 2006),[16] slightly improved rates with combination of both radioactive colloid and blue dye (94-100%).

In this study, we dissected 867 axillary lymph nodes from 36 patients and subjected for HPE for evidence of metastasis. We could identify 80 blue stained SLN during the procedure with average of 2.2 sentinel node This finding is in conjunction with identification rates of several authors such as Giuliano *et al*.[17] (1.8), Motomura *et al*.[18] (1.8), Cserni[19] (1.3), Cox *et al*.[20] (1.92), Hill *et al*.[21] (2.1), Ikeda *et al*.[7] (1.95), and Albertini *et al*.[22] (2) Increasing the mean number of SNs removed may improve accuracy.

In this study of 36 cases, the SLN detection rate was over 88.8%, and the negative predictive value was 93.75%. The rate of false-negative result best defines the accuracy of SLNB. In this study, false-negative result was seen in one patient (6.25%). This is comparable with those of other published studies by Blessing *et al.,* Simmons *et al.,* Nour, 2006.[14-16]

Only one patient was post neoadjuvant chemotherapy in this study. She initially had T3 N1M0 disease and became node negative after three cycles of neoadjuvant chemotherapy with 5 flurouracil, adriamycin and cyclophosphamide (after chemotherapy became node negative). In that patient, we could identify the sentinel node and could accurately predict the axillary status, as both sentinel nodes and rest of the axillary nodes were positive for malignancy.

Our results indicate that SLNB can reliably predict the axilla status such that when sentinel node is negative for metastases, axillary dissection can be safely omitted.

A recent survey on SLNB distributed by American Society of Breast Diseases Rapid Response Panel demonstrates that SLNB is considered to be the standard of care by 85% of the members who responded. It has been suggested that surgeons should demonstrate an SLN identification rate of more than or equal to 90% and a false negative rate of less than 5% before they offer SLNB without completion axillary dissection.[23] However, before SLNB becomes the undisputed standard of care, randomized trials will have to show no difference in axillary recurrence, and overall survival between SLNB alone and SLNB followed by axillary dissection in patients with negative sentinel node(s). Blue dye along with Tc99m mapping theoretically increases the accuracy of test but from various validation studies it is clear that blue dye technique alone can be used when Tc99m mapping facility is not available. Our study demonstrates that sentinel node localization is possible with methylene blue dye alone. Although limited by a small sample size, this study has shown a low false negative rate of 6.25% which denotes that SLNB using methylene blue dye alone is a highly reliable and predictable technique to stage the axilla in breast cancer patients. This technique may help to avoid complete axillary lymph node dissection in sentinel node negative patients thereby minimizing the morbidity of axillary lymph node dissection.

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