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Assessment Of Sentinel Lymph Node Using Methylene Blue Dye In Carcinoma Breast.

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ABSTRACT

Management of the 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. However, only around 30 percent of the clinically node-negative patients prove to be histopathological node positive which means that 70 percent of clinically node-negative patients undergo axillary dissection and are exposed to its morbidities like neuropathies, seromas, and upper extremity lymphedema. This can be avoided with a sentinel lymph node (SLN) biopsy. This study aimed to assess the feasibility of localization of the sentinel node with the blue dye alone and compare the nodal tumor positivity in relation to blue dye is positivity. 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-chemotherapy were included in the study.5 patients with breast cancer clinically node-negative axilla were excluded from the study after they were found to have axillary nodes after the ultrasound examination. Totally 36 patients were evaluated. This study demonstrates that sentinel node localization is possible with methylene blue dye alone with an 88.88% localization rate. Though limited by a 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. 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.

Keywords: Breast Cancer, Sentinel Node Biopsy, Methylene Blue Dye, Axillary Dissection

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INTRODUCTION

Sentinel lymph node biopsy (SLNB), a minimally invasive procedure, has been shown to accurately stage the axilla and promote lesser morbidity compared to axillary lymph node dissection (ALND). The mapping method has been among the most important factors affecting the identification rate and false negative rate of SLNB in breast cancer [1]. A combination of lymphoscintigraphy, blue dye, and intraoperative gamma probe for SLNB has currently been considered the gold standard for detecting sentinel nodes (SNs) in early-stage breast cancer [2]. In India however, SLNB for breast cancer has yet to become popular considering several factors, including patient characteristics (most already had the advanced-stage disease), unclear recommendations, and limited facilities for radioisotope use due to high costs and unavailability in most cancer centers [3]. The aforementioned factors are compounded by the fact that Indonesia consists of many islands, making access to cancer centers even more difficult. All such factors contributed to the difficulty in the application of SLNB. Hence, studies that attempt to address the aforementioned limitations in performing SLNB are imperative [4]. New knowledge on the proposed landmark involving intercostobrachial and medial pectoral nodes as the first group of axillary lymph nodes that receive lymphatic flow from the primary tumor has allowed the utilization of a blue dye as a single tracer for detecting SNs [5]. Lymphatic drainage from the primary tumor has been suggested to be consecutively spread in a stepwise fashion to the intercostobrachial and medial pectoral nodes [6]. Several blue dyes, such as patent blue, isosulfan blue, and methylene blue, have been commonly used in SLNB to map the axillary lymph nodes. Considering its better accessibility and considerably lower allergic risk compared to other blue dyes, some cancer centers have preferred to use methylene blue [7,8]. A combination of the two techniques is the best and is recommended for optimal outcomes [9]. Blue dye-guided SLN identification may be the only available option in countries with low resources due to the prohibitive price of gamma probes [10].

MATERIALS AND METHODS

This prospective study was carried out in the Department of Surgical Oncology in collaboration with the Department of Pathology, Government Medical College, Villupuram, Tamil Nadu, India in the year February 2018 to October 2018. Patients of breast cancer with clinically negative axilla or Patients who had preoperative treatment (chemotherapy and /or RT) and became clinically negative axillae, irrespective of initial axillary nodal status were included in the study, after obtaining informed consent. 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-chemotherapy were included in the study.5 patients with b r e a s t cancer clinically node-negative axilla were excluded from the study after they were found to have axillary nodes after ultrasound examination. Totally 36 patients were evaluated. Inclusion Criteria: Patients with carcinoma breast with clinically negative axillary nodes. Patients who had preoperative treatment (chemotherapy and /or RT) now have clinically negative axillae, irrespective of initial axillary nodal status. Patients above 18 yrs age with the ability to give consent. Exclusion criteria: Clinically palpable axillary nodes, Prior upper limb lymphoedema, Prior breast / axillary surgery, History of blue dye allergy, Patients taking serotonergic drugs like paroxetine, fluoxetine, etc. Comprehensive history was taken and a thorough clinical examination was done. Symptoms were elaborated on in detail. Side, T stage of the tumor and type of tumor were noted. Axilla was examined for palpable lymph nodes. Only patients who were clinically negative for lymph nodes at the initial presentation or became clinically nodes after neoadjuvant chemotherapy were taken in the study. Ultrasound Examination*:* Ultrasound examination of the axilla was done with a real-time scanner with a probe head of a 7.5 MHz frequency transducer. Axillary lymph nodes were reported at the time of examination as abnormal based on size criteria and morphology (short-axis diameter > 10 mm, cortical thickening, and lobulation or loss of the normal hyperechoic hilum). If any patient was found to have axillary lymph nodes with the above-mentioned features on ultrasonogram they were excluded from the study. Thus 5 patients were excluded. In all selected patients Modified Radical Mastectomy (MRM) was done with an axilla first approach. After induction of anesthesia, peritumoral injection of 1% Methylene blue dye (4ml) at the 3, 6, 9, and 12 o'clock positions was done. Sentinel lymph nodes were looked for after raising the superior flap and opening the clavipectoral fascia, within 15 minutes from the time of injection. The stained nodes were removed initially and sent for histopathological examination. Modified radical mastectomy was completed along with axillary lymph node dissection in all cases. The excised breast with the axillary tissue was sent for histopathological examination to correlate with the findings of the sentinel lymph node biopsy. The postoperative 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. The number of nodes harvested at each level and nodes positive for blue dye were separately noted. Lymph nodes were bisectional along the long axis and each lymph node half was examined after fixing and staining. For both blue dye and non- blue dye nodes, the following features were analyzed separately. The number of nodes harvested and level of nodes several nodes positive for tumor deposits and presenting with features like extracapsular spread and lymphovascular invasion.

Statistical Analysis

Package for the Social Sciences (SPSS) (version 17.0 software, USA). Quantitative data are described as mean and standard deviations. Data were also presented graphically with bar diagrams and pie charts. Data were explored for any outliers, typing errors, and missing values. Comparison of groups was carried out for various categorical variables using the Chi-square test of association and Univariate logistic regression analysis to find out any statistical association between categorical variables. The mean values of age and other quantitative variables were analysed using one-way ANOVA A p-value (two-tailed) < 0.05 was taken as significant.

RESULTS

In this study, 36 patients were evaluated, with a mean age of 51 years, and the age range was 26-70 years. Incidence was most common in the age group of 41-50. {14/36(38.8%)} cases, followed by51-60 years{13/36(36.1%)}cases. In our series sided lesions19/36{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) respectively. T Stage distribution includes 1- 2/36(5.56%) , T 2 – 28/36 (77.78%) , T3 –6/36(16.67 %)

TABLE 1: GENERAL CHARACTERISTICS

General Patient characteristics:		No. of patients	Percentage
Age distribution 26-70(mean 43 years)	Less than 50 years	18	50
	50 years & 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
	UIQ	8	22.22
	CENTRAL	4	11.11
	LOQ	4	11.11
Grade	1	8	22.22
	2	19	52.8
	3	9	25

TABLE 2: node identification rate

Sentinel node	Frequency	Percentage
Identified	32	88.89
Not identified	4	11.11
Total	36	100

Sentinel node was successfully identified in 32 cases 32/36(88.89%). Among the 32 cases, there were skip metastases to level II nodes 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 sentinel node was identified.

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Table 3: Histopathology Of The Sentinel Lymph Node

Histopathology of the sentinel lymph node	Frequency	Percentage
Positive	16	50
Negative	16	50
Total	32	100

Sentinel node histopathology positivity VS rest of axillary lymph node histo pathology positivity.

Table 4: Comparison Of Histopathology Sentinel Node & Rest Of Axilla

Histopathology of the sentinel lymph node	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	

When the histopathological status of axillary lymph nodes was compared to Sentinel lymph nodes 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 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. In the present study, we have dissected 867 axillary lymph nodes and the total no of blue nodes harvested was 80 and non-blue nodes 787. The average sentinel node harvest was 2.22.

Table 5: General Patient Characteristics On Identified & Non – -Identified Sentinel Node

General Patient Characteristics		Sentinel node Identified	Sentinel Node -Not Identified	Total	P value
Age	<5o	17	1	18	0.6
	>50	15	3	18	
Side	Right	16	3	19	
	Left	16	1	17	0.7
Site	UOQ	19	1	20	
	BIO	7	1	8	
	CEN	3	1	4	
	LOQ	3	1	4	0.3
Size	T1	1	1	2	
	T2	25	3	28	
	T3	6	0	6	0.2
Grade	1	8	0	8	
	2	17	2	19	
	3	7	2	9	0.0

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% were associated with sentinel node histopathology positivity but none of these factors except the Grade were statistically significant. The lower the Grade higher the sentinel node identification rate.

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DISCUSSION

The status of the axillary lymph node remains the most important predictor of survival in women with invasive breast cancer and this is used for making treatment decisions. Various methods of predicting axillary lymph node status have been described including clinical assessment and radiological and operative procedures [11]. 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 morbidities such as post-operative pain in the arm, chronic lymphedema of the involved arm, neuropathy of the arm, seroma formation, restricted shoulder mobility, and other complications. Sentinel Lymph node biopsy has emerged as an effective diagnostic tool in staging axillary disease [12]. The major advantage of Sentinel lymph node biopsy is the lower complication rate compared with Axillary lymph node dissection. The present study was conducted to assess the feasibility of sentinel lymph node 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 [13]. Although several patients included were small (N= 36) 36 patients were evaluated with a median age of 51 and the study group was similar to what is reported in the 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 [14]. In this study, both right and left sides were more or less equally affected with a slight predominance of left-sided lesions (19/36). The upper outer quadrant was involved in (20/36)55.6% of cases followed by the upper inner quadrant (8/36) at 22.2%, the central quadrant ((4/36) at 11%, and the lower outer quadrant. (4/36). Sentinel node was identified readily in the upper outer quadrant tumors at 95% followed by upper inner, central, and lower outer quadrant locations with a similar identification rate of 75%. Clinical tumor status include T1 (2/36) 5.6%, T2(28/36) 77.8% and T3(6/36)16.7% and gradeI-I- (8/36)-22.2%, grade II-(19/36)52.8% grade- III(9/36)25% with highest sentinel node identification in T3 & Grade I lesions about 100% [15]. Either isophane blue or methylene blue can be used as a dye in sentinel lymph node biopsy. Methylene blue is cheaper, more easily obtainable, and is a dye with fewer complications as compared to isophane blue. Hypersensitivity reactions which may also be fatal are reported at a rate of 0.6 to 2.5 % following isosulphane blue injection. Skin necrosis, if injected intradermally, fat necrosis, and fibrosis over the injection site are among the complications of methylene blue [16]. However, in the present study, no such complications related to methylene blue were encountered. In studies conducted in our country, isophane blue was often preferred. In the literature, many studies are showing that methylene blue can be used safely and with high success as an alternative to isophane blue [17]. In the present study, we dissected 867 axillary lymph nodes from 36 patients and subjected them to histopathological examination for evidence of metastasis [18]. In this study of 36 cases, the sentinel lymph node detection rate was over 88.8% and the negative predictive value was 93.75%. The rate of false-negative results best defines the accuracy of Sentinel lymph node biopsy. In this study, a false-negative result was seen in one patient. (6.25%). Only one patient was post-neoadjuvant chemotherapy in this study. She initially had T3 N1M0 disease and became node negative after 3 cycles of neo-adjuvant chemotherapy with 5 fluorouracil, adriamycin, and cyclophosphamide (y c T3N0M0) [19]. In that patient, we could identify the sentinel node and could accurately predict the axillary status, as both sentinel nodes and the rest of the axillary nodes were positive for malignancy. Our results indicate that SLNB can reliably predict the axilla status such that when t h e sentinel node is negative for metastases, axillary dissection can be safely omitted. A recent survey on SLNB distributed by the 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. SLNB becomes the undisputed standard of care, randomized trials will have to show no difference in axillary recurrence and overall survival (OS) 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 the test but from various validation studies, it is clear that the blue dye technique alone can be used when a Tc99m mapping facility is not available [20].

CONCLUSIONS

This study demonstrates that sentinel node localization is possible with methylene blue dye alone. Though limited by a 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. 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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