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Original Research Article

Outcomes of Sentinel Lymph Node Biopsy in Early Breast Cancer using Methylene Blue and Fluorescein Dye in a Tertiary Care Centre of North East India

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Abstract:

Objectives: The currently recommended technique of sentinel lymph node biopsy (SLNB) in breast cancer is a combination of blue dye (commonly used isosulfan blue or Patent blue violet) and radiotracer-guided technique (Tc99m-labeled sulfur colloid). Neither of the blue dyes are currently marketed in India, are expensive to import, and not easily available. Nuclear medicine facilities are not available at most centers. This study involves using a combination of methylene blue dye and fluorescein dye to detect sentinel lymph nodes.

Materials and Methods: This is a prospective, observational study. Patients with first time detected T1 or T2, N0 and M0 breast cancer were eligible for this study and taken up for SLNB using dual dye technique (methylene blue dye and sodium fluorescein dye). N0 status was confirmed both clinically and radiologically. Patients with a previous history of malignancy and those who received neoadjuvant chemotherapy were excluded from this study. Statistical analysis was done with Statistical Package for the Social Sciences (SPSS) version 20.0.

Results: In this study we present outcomes of 50 patients who underwent sentinel lymph node (SLN) biopsy using a combination of methylene blue and fluorescein dye at our institute. Mean age was 51.8 years and range was 32-70 years. Sentinel node was successfully identified in 47 cases, thereby a 94% of identification rate was observed with this dual method technique and the median sentinel nodes were 2. Among a total of 98 detected sentinel nodes, 47(47.9%) had both blue dye and fluorescein dye uptake.

Conclusion: Fluorescein dye is a very cheap alternative to radioactive tracer technique; hence it can be used as an alternative especially in resource constrained countries like ours.

Keywords: Sentinel Lymph Node Biopsy, Dual Dye Technique, Methylene Blue Dye, Fluorescein Dye, Breast Cancer.

Introduction

The presence and extent of axillary lymph node involvement in breast cancer remains the strongest predictor of recurrence and survival. The presence of local metastases in the axillary nodes has been shown to reduce the 5-year survival rate of patients by approximately 28-40% [1,2].

Historically, axillary lymph node dissection (ALND) has been the standard of care for treating the axilla. However, lymph node metastases are found only in 40% of patients undergoing axillary lymph node dissection (ALND) [3, 4]. ALND is associated with complications such as lymphedema and stiff shoulders. In recent years, sentinel lymph node (SLN) biopsy (SNB) has been studied and used as an alternative to ALND in patients with negative SLN sampling. SNB is based on the hypothesis originally proposed by Cabanas [5]. The currently recommended technique of SLNB in breast cancer is a combination of blue dye (commonly used isosulfan blue or patent blue violet) and radiotracer-guided technique (Tc99m-labeled sulfur colloid).

Neither of the blue dyes are currently marketed in India, are expensive to import, and not easily available. Nuclear medicine facilities are not available in most centres of India and the portable

gamma probe used for intraoperative SLNB is expensive. There was aneed to identify cost-effective and accurate methods of SLNB in the Indian setting. Although another blue dye, methylene blue has been used previously by surgeons with similar identification rates [6-8]. This study involves using a combination of methylene blue dye and fluorescein dye to detect sentinel lymph nodes. Fluorescein sodium is a potential new cheap tracer for breast sentinel lymph node biopsy [9].

Materials and Methods

This is a prospective, observational study. Patients with first time detected T1 or T2, N0 and M0 breast cancer were eligible for this study and taken up for SLNB using dual dye technique (methylene blue dye and sodium fluorescein dye). N0 status was confirmed both clinically and radiologically. Patients with a previous history of malignancy and those who received NACT were excluded from this study.

Study Procedure

Females with early breast cancer ($cT_{1-2}N_0$) were taken up for this study. Clinically N_0 nodes were confirmed by USG Axilla. In case of suspicious nodes in USG, FNAC of nodes was done and only patients with negative nodes were taken for study.

During SLNB procedure, 1ml of methylene blue dye and 0.125 ml of sodium fluorescein dye(Figure 1) was mixed and diluted to 10ml with normal saline.

This mixture was injected in sub areolar and periareolar area in subdermal plane. Breast was massaged towards the axilla for 10 minutes and then axillary incision was made to detect sentinel node. Sentinel lymph node was detected by blue Colored nodes to naked eye and fluorescent nodes by blue light (Figure2).

Figure 1: Fluorescein sodium dye (20%w/v)

Figure 2: Blue light

The detected nodes were sent for the frozen section. If nodes came to be positive or no nodes were detected then complete axillary Lymph Node Dissection (ALND) was done. If nodes came to be negative, then no ALND was done.

The primary aim of the study was to find the sentinel lymph node detection rate when both methylene blue and fluorescein dye was used.

We have also tried to find correlation between SLN detection rates with factors like site of primary, T stage and tumor grade.

Statistical analysis was done with Statistical Package for the Social Sciences (SPSS) version 20.0. The data was checked for outliers, typos, and missing values. Group comparison against various categorical variables was performed using the chi-square test of association. A p-value < 0.05 was taken as significant.

Results

In this study we present outcomes of 50 patients who underwent sentinel lymph node (SLN) biopsy using a combination of methylene blue and fluorescein dye at our institute.

Mean age was 51.8 years and range was 32-70 years. Table 1 shows clinicopathological data of the patients.

Table 1: Clinico-pathological data

Characteristics	**Results**
Age(Mean)	51.8 years(±10.852)
Age(Median)	50 years
Incidence	
≤40	19(38%)
>40	31(62%)
Side	
Right	27(54%)
Left	23(46%)
Pathological type	
Carcinoma in situ	6(12%)
Invasive ductal carcinoma	42(84%)
Invasive lobular carcinoma	2(4%)
Tumor Grade	
1	11(22%)
2	24(48%)
3	15(30%)
Molecular typing	
Luminal A	10(20%)
Luminal B	29(58%)
Her2-neu enriched	3(6%)
TNBC	7(14%)
T-stage	
T1	22(44%)
T2	28(56%)
Site	
Upper Outer Quadrant	28(56%)
Upper Inner Quadrant	10(20%)
Lower Outer Quadrant	7(14%)
Lower Inner quadrant	2(4%)
Central quadrant	3(6%)

TNBC: Triple negative breast cancer

Sentinel node was successfully identified in 47 cases (94%) and the median sentinel nodes were 2 [Table 2]. Among a total of 98 detected sentinel nodes, 47(47.9%) had both blue dye and fluorescein dye uptake [Table 3] [Figure 3& 4]. On statistical analysis, none of the factors, that is age, side, site, T stage or grade affected the sentinel node detection significantly. [Table 4]

Table 2: Detection of SLNS(s) in patients with early breast cancer

Number of SLNs detected	N	%
1	12	25.5
2	22	46.8
3	10	21.2
4	3	6.4
Total	47	100%

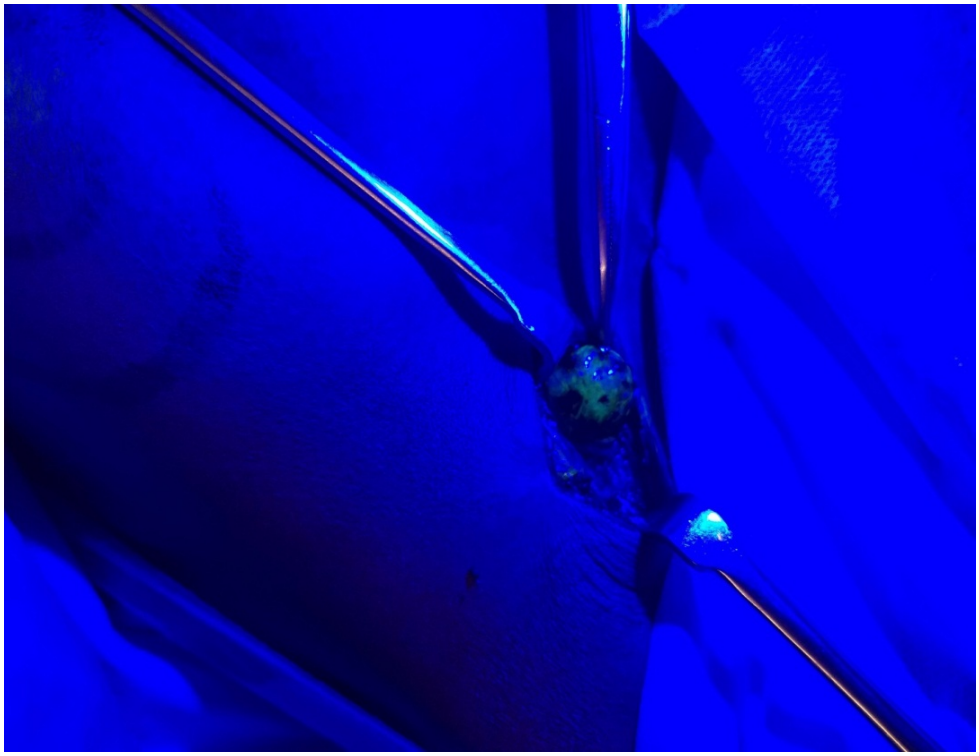

Figure 3: Fluorescent node under blue light

Figure 4: Different patterns of staining

Table 3: Staining in sentinel nodes

Staining	Number of nodes	%age
Blue and fluoroscent	47	47.9%
Blue only	33	33.6%
Fluoroscent only	18	18.3%
Total	98	100%

Table 4: Factors affecting sentinel node identification

Patient's characteristics		Number of patients	Sentinel node identified	%age	p-value
Age	≤40	19	18	94.7	0.326
	>40	31	29	93.5	
Side	Right	27	25	92.6	0.603
	Left	23	22	95.6	
Site	Upper Outer Quadrant	28	26	92.8	0.343
	Upper Inner Quadrant	10	9	90	
	Lower Outer Quadrant	7	7	100	
	Lower Inner quadrant	2	2	100	
	Central quadrant	3	3	100	
T-stage	T1	22	21	95.4	0.314
	T2	28	26	92.8	
Grade	G1	11	10	90.9	0.096
	G2	24	23	95.8	
	G3	15	14	93.3	

Out of 47 cases in which sentinel node was detected, 23 cases (48.9%) had positive nodes on frozen section (on final histopathological examination, there was no discrepancy with the frozen section report in both positive and negative nodes) and none of the factors (age, side, site, T stage or grade) significantly affected node positivity [Table 5].

Table 5: Factors affecting sentinel node positivity

Patient's characteristics		Number of patients with SLN identification	Node positivity (% age)	p-value
Age	≤40	18	9(50.0)	0.196
	>40	29	14(48.2)	
Side	Right	25	12(48)	0.795
	Left	22	11(50)	
Site	Upper Outer Quadrant	25	13(52)	0.256
	Upper Inner Quadrant	10	5(50)	
	Lower Outer Quadrant	7	3(42.8)	
	Lower Inner quadrant	2	1(50)	
	Central quadrant	3	1(33.3)	
T-stage	T1	21	11(52.3)	0.922
	T2	26	12(46.1)	
Grade	G1	10	6(60)	0.693
	G2	23	11(47.8)	
	G3	14	6(42.8)	

Discussion

Axillary lymph node status is the most important predictor of survival in women with invasive breast cancer and is used to guide treatment decisions. Various methods of predicting axillary lymph node status are described, including clinical evaluation, imaging, and surgical methods. Axillary lymph node dissection was previously considered the gold standard for predicting axillary lymph node status. However ALND can cause significant morbidity such as postoperative arm pain, chronic lymphedema of the affected arm and arm neuropathy, seroma formation, decreased shoulder mobility, and other complications. Sentinel lymph node biopsy has emerged as an effective diagnostic tool for staging axillary disease of early-stage breast cancer. A major advantage of sentinel lymph node biopsy is the lower incidence of complications as compared to axillary lymph node dissection. The present study was conducted to assess the feasibility of sentinel lymph node localization using combination of methylene blue dye and fluorescein dye.

Fifty patients whose axilla was clinically negative for lymphadenopathy were included. All patients underwent primary surgery and none were treated with neoadjuvant chemotherapy. Although the number of patients included was small (N = 50), it was comparable to the study by Krag et al [10] (N = 22), Borgstein et al [11] (N = 33), and Pijpiers et al [12] (N = 34), Ikeda et al. [13] (N = 29), Motta C et al. [14] (N = 54), Seenu and Bassi et al. [15] (N = 40). Fifty patients with a median age of 50 years were evaluated and the study group was similar to that reported in the literature. The majority of patients (62%) belonged to the elderly group over 40 years old. Identification of the sentinel lymph node was higher in the younger age group. However, it was not statistically significant.

In this study, both the right and left sides were affected approximately equally, with 54% (27/50) of lesions on the right side, thus right side being slightly predominant. Upper outer quadrant was involved in 56% (28/50) of cases followed by upper inner quadrant in 20% (10/50). Sentinel node identification rate was 100% for lower outer quadrant, lower inner quadrant and central quadrant. It was 92.8% for upper outer quadrant and 90% for upper inner quadrant.

In a study by AltanOzdemir et al., the right side (72%) and upper outer quadrant (75%) were the most common side and site of tumor localization. [16]. In another study, by Arindham Mukherjee et al. [17] the upper outer quadrant was the most common tumor site at 44%. Clinical tumor status include T1 in 44% (22/50) and T2 in 56% (28/50), and grade 1 in 22% (11/50), grade 2 in 48% (24/50) & grade 3 in 30% (15/50) with highest sentinel node identification of 100% in T1 & Grade 2 lesions, 95.4% and 95.8% respectively.

In our study, none of the clinical characteristics affected sentinel node identification rate significantly, however better identification rates were seen in T1 tumors with grade 2 histology and all quadrants except upper outer quadrant and upper inner quadrant. Similar results were reported by Kollias et al. [18] who investigated clinical and histological factors associated with the identification of sentinel lymph nodes.

For sentinel lymph node biopsy, either isosulfane blue or methylene blue can be used as a dye. Methylene blue is a cheaper, more readily available dye than isosulfan blue. Possible fatal hypersensitivity reactions have been reported at a rate of 0.6-2.5% after injection of isosulfane blue. Skin necrosis, fat necrosis, and fibrosis at the injection site due to intradermal injection are one of the complications of methylene blue. However, no such complications associated with methylene blue occurred in this study.

There are many studies in the literature showing that methylene blue is safe to use and is a very successful alternative to isosulfane blue. Simmons et al. identified sentinel lymph nodes in 104 of 112 patients using methylene blue, and reported that sentinel lymph nodes showed axillary status in 96.9% of patients [19]. Segen et al. [20] comparing isosulfane blue and methylene blue, the accuracy rate was 88.5% for isosulfane blue and 92.7% for methylene blue. Das G et al. [21] presented data from our institute, with a 96% SLN detection rate using only methylene blue dye.

In our study, sentinel node identification with combination of blue and fluorescein dye was 94%. Compared to other studies reporting a sentinel lymph node identification rate of 65-94% with methylene blue dye alone [Simmons et al (19), Blessing et al [20] and Nour-Golshan et al [22]], our rate was similar or slightly higher with the combination of methylene blue and fluorescein dye. In the study by Chang YW et al. [23], the detection rate of SLN stained with fluorescein was 93.4%.

In the present study, we could identify a total of 98 sentinel nodes (with mean of 1.96). Among these, 33(33.6%) were stained blue color only, and 18(18.3%) with yellow color under blue light (fluorescein node) and 47 (47.9%) were stained with both. It can be seen that blue colour was seen in 80 out of 98 nodes (81.5%), fluorescence was seen in 65 out of 98 nodes (66.3%). In abstract of study by Srivastava A et al [24], blue nodes were identified in 64 out of 86 patients (74.4%) and while in 54 out of 86 patients fluorescent nodes could be identified (62.8%).

Mean number of sentinel nodes detected by different authors are, 2 by Albertini et al [25], 1.8 by Giuliano et al [26], 1.95 by Ikeda et al [13], 1.3 by Cserni et al [27], 2.1 by Linehan DC et al [28].We did axillary lymph node dissection in 26 (3 due to non-identification of sentinel node and 23 due to positive sentinel node) patients out of 50.

Blue dye with Tc99m mapping theoretically improves the accuracy of the test, but various studies have shown that blue dye can be used alone if the Tc99m mapping feature is not available. As Tc99m mapping technology is not available in developing countries, so finding alternatives is essential. In our study we have tried to solve this problem by using sodium fluorescein dye, which is another cheap dye (less than 5 rupees cost for one patient). However further studies are needed to establish its efficacy.

Conclusion

In our study sentinel lymph node detection rate with combination of methylene blue and fluorescein dye was found to be similar to that with

methylene blue dye alone (from previous studies). However it is a safe procedure and this dual modality technique may yield better results once the technique is standardized. Further studies are required to find the role of fluorescein dye in sentinel node biopsy. That said, fluorescein dye is a very cheap alternative to radioactive tracer technique, hence it can be used as an alternative especially in resource constrained countries like ours.

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