ORIGINAL ARTICLE

Sentinel Lymph Node Biopsy Using Methylene Blue Dye and Intraoperative Palpation Method in Node-negative Early Breast Cancer

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Abstract

Background: Early breast cancer with clinical or radiological node-negative axilla is treated with breast conservation surgery where lumpectomy with axillary dissection is done. Sentinel lymph node biopsy (SLNB) is an acceptable alternative to axillary clearance and has relatively lesser morbidity. SLNB methods include radioisotope (RI)-guided, blue dye-guided, or a combination of both. However, access to RI can be limited in certain geographic locations. **Objective:** This study aimed to determine the effectivity of methylene blue (MB) dye-guided SLNB as an alternative to RI. **Methods:** In this investigation, 43 clinically node-negative early breast cancer patients were prospectively enrolled. SLNB was performed using MB dye (1%) administered to the peritumoral or periareolar region. The histopathology reports of the harvested nodes were studied, and the results were computed using SPSS and 2×2 contingency table. **Results:** The sensitivity, specificity, false-negative rate (FNR), and accuracy of MB-guided SLNB in our study were 92.8%, 100%, 7.14%, and 97.7%, respectively. Conclusion: The use of MB dye along with intraoperative palpation after meticulous lymph node dissection in each level is more effective and has lower FNR than RI.

Keywords: SLNB, Methylene blue dye, Sensitivity, Specificity, Breast carcinoma

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Introduction

In breast cancer surgery, investigation of the status of axillary lymph node (ALN) is a pivotal prognostic factor that determines the next course of treatment for the patients. Axillary lymph node dissection (ALND) has been considered as a gold standard for post- operative pathological staging in invasive breast carcinoma. However, this process involves substantial morbidity such as paresthesia and arm numbness, lymphedema, and limited shoulder movement, which adversely affects the patient's quality of life (1, 2).

Hence, there is a paradigm shift in the surgical management of ALNs that evolved from complete axillary lymphadenectomy to a more conservative approach of axillary dissection. Eventually, the introduction of the sentinel lymph node biopsy (SLNB) in carcinoma breast surgery has revolutionized the pathological assessment of ALNs in early breast cancer patients. This method has minimized the occurrence of debilitating adverse events in post-operative patients caused by complete ALND (3). Hence, SLNB has been practiced widely as the standard procedure for axillary staging in early breast cancer patients (4–6). There are various tracer techniques employed to perform SLNB, and each has its own limitations and advantages. Some surgeons prefer to use radioisotopes (RI) or vital dyes alone, whereas others prefer dual- tracer-guided SLNB. However, the major limitations of radioactive tracer techniques are their high cost, technical complexity, and logistics (7). Furthermore, this technique requires a highly specialized and state- of-the-art department of nuclear medicine capable of handling RI sat the point-of-care unit, but most of the public tertiary care centers in developing nations lack this facility. Furthermore, the chance of radiation exposure to the patient and treating surgeon is another significant challenge in the use of radioactive colloid. Hence, the use of diagnostic dyes for SLNB is a viable alternative technique to RI-based methods.

Despite the availability of different dyes such as patent blue (PB), isosulfan blue, and indocyanine green, methylene blue (MB) is widely used due to its cost-effectiveness and easy availability. Besides, the use of the MB technique alone is practiced more in SLNB these days due to its technical ease of use and lesser chances of anaphylaxis and/or tissue necrosis compared with other dyes (8–10). Despite the wide acceptability of MB in SLND, some surgeons reported quick diffusibility of the dye into the peripheral tissues, which stains a large portion of the breast, thereby affecting the surgical procedure to some extent (11– 13). Similarly, few authors reported varying sensitivity, specificity, sentinel lymph node (SLN) identification rate, and false-negative rate (FNR) of MB dye in SLNB (14–17). It is therefore imperative to validate this economic and cost-effective SLNB technique to increase usage in public healthcare settings in developing nations like India. Hence, the objective of this study was to assess the accuracy of this tracer technique (using MB alone) in clinically and radiologically node-negative breast cancer patients.

Materials and methods

Patients and surgical procedure

This study was approved by the institutional ethics committee of Acharya Harihar Regional Cancer Centre, Cuttack, Odisha, India(letter no. 102-IEC-AHRCC).From January 2018 to February 2020, patients with early invasive female breast cancer (stageT1–T3,N0, M0) with clinically and radiologically node-negative axilla were included prospectively in this study (n=43).Of 51 identified patients, 3patients with progressive disease with skin involvement and 5 patients in whom SNLs could not be identified were excluded (Figure 1).The details of the 5 cases where SNLs could not be identified are given in Table 1. The small sample size precluded any statistical conclusion.

Patients with previous breast cancer surgery or axillary dissection, neoadjuvant therapy, distant metastasis, or any palpable or radiologically detected axillary nodes were excluded from the study. Patients with T4 or larger tumors, with inflammatory breast cancer, who were pregnant females, or with male breast cancer were also excluded from this study. Similarly, patients with any scar of previous surgery within the natural pathway of lymphatic drainage that blocks the lymphatics going toward axilla were not included. Patients who received radiation therapy to the chest wall for any other reason, and patients unwilling to participate in the study were also excluded. In this research, five experienced surgical oncologists participated, and the surgeries were performed after obtaining informed consent from the patients. The surgeries were performed as per standard protocol (18– 20), with minor modifications. Briefly, routine pre- operative assessment of surgical fitness, chest radiography, and ultrasonography was performed for all the enrolled patients. Before approximately 15 to 20 minutes of the surgery or immediately after the induction of anesthesia, 1% MB dye (3–5 mL based on breast volume) was injected in two equally divided doses. Half of the dose was administered in the peritumoral region close to the axilla, while the other half was administered to the periareolar site. Post-injection, for 2 to 5 minutes, gentle message was performed laterally to the tumor to prevent the migration of malignant cells to regional lymph nodes and to facilitate the movement of the MB dye. Mastectomy or lumpectomy was performed with standard principles, and then, the axilla was exposed.

Table 1. Characteristics of the 5 patients whose SLN were not identified intraoperatively

Patient no.	Age (years)	Clinical findings	Pre-operative biopsy	Stage/T-size	Intraoperative findings	Post-operative HPE	Comment
1	75	Lower outer quadrant breast lump (diameter, 3 cm),senile breast	Well-differentiated IDC	cT2,N0,M0	Lymph nodes could not be identified	Two lymph nodes were involved with the tumor	Senile breast, atrophied or blocked lymphatics due to tumor
2	42	Lower inner quadrant tumor (4 cm)	Well-differentiated IDC	cT2, N0	Excessive fatty breast and axilla were noted; no stain or unstained lymph nodes identified	No lymph nodes were involved; the total number of lymph nodes was6	Fatty breast, medial quadrant tumor location; developmental abnormality with tiny nodes or fewer overall nodes
3	65	Lower outer quadrant tumor of 5–6cmin size	Moderately differentiated IDC	cT3cN0	No stained lymph nodes identified; multiple round nodes seen	Four lymph nodes were positive	Blocked lymphatics and node involved
4	40	Upper outer quadrant tumor (size, 3 cm)	Well-differentiated IDC	cT2 N0	No stained lymph nodes seen	Lymph nodes were not involved	Dye did not reach lymph nodes; inadequate dye volume; inadequate massage
5	65	Central tumor (size, 2.5 cm); thin-built patient; senile breast	Well-differentiated IDC	cT2 N0	No stained lymph nodes seen	Lymph nodes were not involved	Dye did not reach lymph nodes; senile lymphatics

HPE, histopathological examination; IDC, invasive ductal carcinoma.

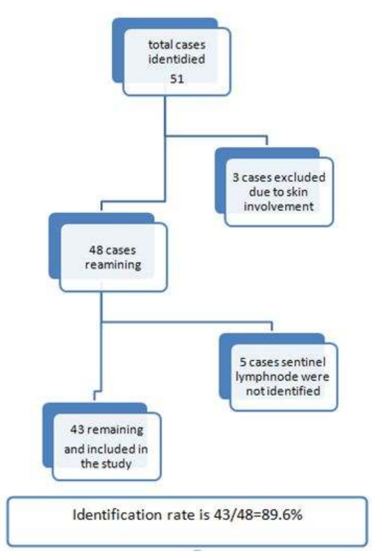

Figure 1. Patients included and identification rate.

The axilla was accessed by a separate axillary incision, and the lateral and anterior skin flaps were raised, up to the angular vein, as in standard methodology in the case of breast- conserving surgery planning. When modified radical mastectomy was planned, the axilla was accessed through the same incision after raising the skin flaps laterally and anteriorly. Starting from the axillary tail of Spence, the blue lymphatics were searched and traced toward the nearest node. Then, the lymph nodes in the axillary tail; pectoral, central, lateral, and posterior groups; and interpectoral and apical nodes were searched meticulously in situ for blue-stained ones. Simultaneously, the axilla was palpated during surgery for the presence of any suspicious nodes (nodes >1cm or with rounded shape) harboring metastatic disease. The nodes that stained blue completely or partially (Figure 2 VI, VII) and suspicious nodes were considered as SLN and were harvested.

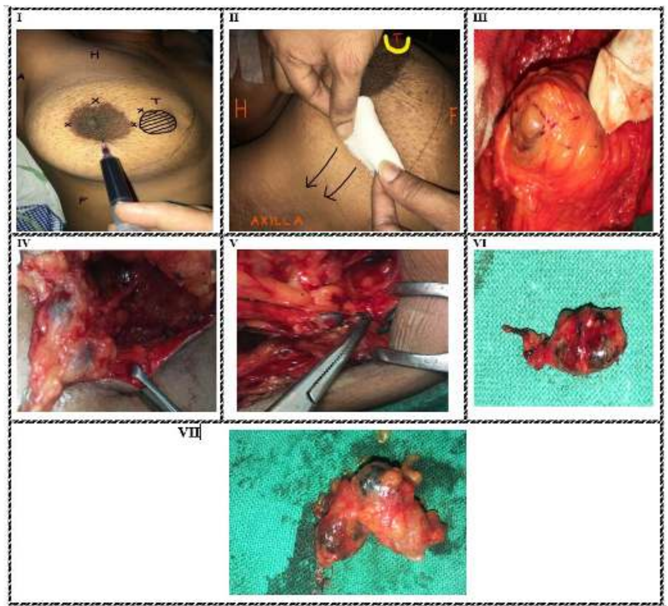

Figure 2. Site of methylene blue injection and harvested Sentinel lymph nodes. (I) H, head end of the patient; F, foot end of the patient; A, axilla; T, tumor;X, sites of peritumoral and periareolar injection of blue dye.(II)H, head-end side; F, foot-end side; black arrow, direction of massage toward the axilla, the yellow line indicated the border of the tumor.(III)Blue-stained lymphatics traced toward the lymph node.(IV)Two nodes stained faintly blue in situ both nodes adjacent to each other. (V)Blue-stained lymph node insitu. (VI)A single blue-stained lymph node dissected along with lymphatics.(VII)The conglomerate of the blue-stained lymph node.

Furthermore, level I and II nodal dissection were done, followed by level III lymphadenectomy, and sent for histopathology in separately labeled containers. Hematoxylin and eosin- stained slides of SLNs and all ALNs were carefully studied and compared for the presence or absence of metastasis.

The surgical plan was made on clinical grounds of operability, that is, disease stage and immunohistochemistry (IHC) were not considered in the pre-operative decision making and, thus, were not considered in our study. Our study aimed to validate the technical methodology for surgeons based on clinical grounds; thus, IHC was not included. To determine the effects of IHC on SLN technique, various factors would be compared with IHC, which requires multivariate analysis with a large number of sample size and will provide a completely different study direction.

Statistical analysis

Descriptive statistics, including the baseline characteristics and clinical and pathological findings, were analyzed using SPSS Statistical Package for Windows version 21.0 (IBM Corp., Armonk, NY, USA). The SNL detection rate, sensitivity, specificity, and FNRs were calculated using the 2×2 contingency table.

Results

Between January 2018 and February 2020,51 cases were identified, of which 3cases were excluded from the study, as discussed in the previous section. From the remaining 48 subjects, SNLs could not be identified intraoperatively in 5 cases; hence, the SNL identification rate in our study was 89.6% (Figure 1). The mean age of the patients in the studied cohort was 49.58 years, with minimum and maximum age of 32 and 69 years, respectively.

The clinicopathological investigation revealed6 (14%), 20(46.5%), 12(28%), and 5(11.5%) patients with stage I, IIA, IIB, and IIIA, respectively. In our study, SNLs were found at level I of the axilla in 40 cases (93%), at level II of the axilla in 2 cases (4.7%), and at levels I and II of the axillary region in 1 case (2.3%). In this investigation, the mean number of SNLs and ALNs harvested per subject was 2.88 and 14.23, respectively. Furthermore, in the interventional study, a total of 614 lymph nodes were harvested, of which 124 were SNLs and 490 were non-SNLs. In addition, 24 (19.3%) of 124 SNLs were positive, 13(2.6%) of 490 non-SNLs were positive, and 4(0.8%) skipped metastatic ALNs (in one patient). The rest of the clinicopathological findings are presented in Table 2.

In this MB-guided SLNB technique, 13 cases were true positive (a), 29 cases were true negative (d), and 1 case was false negative (c). Hence, the sensitivity, specificity, FNR, and accuracy of our studied technique were92.8%, 100%, 7.14%, and 97.7%, respectively (Table 3).

Discussion

In the recent past, there is a paradigm shift in the surgical management of early breast carcinoma. Conservative surgery with SLNB has become a regular practice by surgical oncologists for axillary staging (21,22). Giuliano et al. (18), in 1994, first introduced this technique for axillary lymphatic mapping and lymphadenectomy in breast carcinoma, which has a sensitivity and specificity of 88.0%, and 100%, respectively. Since then, this axillary staging procedure, which has less morbidity, has been practiced and evaluated by different groups of surgeons.

As we discussed in the previous section, diverse tracer techniques, such as radioactive isotopes, vital dyes, and dual-tracer technique, are currently widely used in SLNB. Although dual-tracer techniques have been the gold standard for localizing the SLNs (23,24), the unavailability of nuclear medicine department in many public tertiary care centers and expensive vital dyes have prompted wide use of MB (8–10). However, the SNL identification rate, sensitivity, specificity, FNR, and accuracy of the tracer technique (using only MB) are of paramount concern.

In a recent study, Özdemir et al. (19) reported a sensitivity of 85%, specificity of 100%, identification rate of 94%, FNR of 15%, and accuracy of 93%of the MB technique. Similarly, Nandu and Chaudhari (20), in 2017, reported the sensitivity, specificity, FNR, and accuracy of MB technique in SLNB as 90.48%, 85.71%,9.52%, and 88.57%, respectively.

Our present study revealed the sensitivity, specificity, FNR, and accuracy of MB-guided SLNB as 92.8%, 100%, 7.14%, and 97.7%, respectively.

The sensitivity found in our study is higher than the sensitivity reported by Özdemir et al. (19) and Nandu and Chaudhari (20). Furthermore, our specificity is equal to the specificity achieved by Özdemir et al. (19).

In a recent randomized controlled trial, the dual-tracer technique was employed, where all patients received radionuclide tracer in the peritumoral region and were randomized to MB and PB groups.

In this study, the results (SLN identification rate and FNR) in the MB dual-tracer group (97.4% and 1.49%, respectively)

Table 2. Baseline characteristics, clinicopathological, and lymphadenectomy findings (n=43)

Baseline characteristics and clinicopathological details	
Age (years)	49.58±9.71 [32–69]
Clinical size of tumor	
0–2cm, T1 N0	8 (18)
2–5cm, T2 N0	25(58)
>5cm, T3 N0	10(23)
Stage I	6(14)
StageIIA	20(46.5)
StageIIB	12(28)
StageIIIA	5 (11.5)
IDC	41 (95.35)
Lobular carcinoma	1(2.3)
Mixed IDC+squamous carcinoma	1(2.3)
GradeI	6(14)
GradeII	26(61)
GradeIII	11(25)
LVSI +ve	15 (35)
PNI +ve	4(9)
Both LVSI and PNI +ve	3(7)
Upstaging N0→N1	11(25.5)
Upstaging N0→N2	3(7)
Total upstaging N0→N+	14(32.5)
BSC	11 (26)
MRM	32 (74)
Lymphadenectomy details	
Mean number of nodes harvested per patient	
Sentinel lymph nodes dissected in a case	2.88±1.23[1–5]
Lymph nodes harvested in a case	14.23±3.89[6–24]
Total number of lymph nodes harvested	614
Sentinel nodes (n=124)	24 (19.3)
Non-sentinel nodes (n=490)	13 (2.6)
Skip metastasis (out of 490)	4 (0.8)

Values are presented as mean±standard deviation [range] or n (%). IDC, invasive ductal carcinoma; LVSI, lymphovascular space invasion; PNI, perineural invasion; BCS, breast-conserving surgery; MRM, modified radical mastectomy.

Table 3. Sensitivity, specificity, accuracy, and false-negative rate in MB-guided SLNB

	Positive axilla	Negative axilla	Total
Sentinel node positive	13(a)	0(b)	13
Sentinel nodes negative	1(c)	29(d)	30
total	14	29	43
Parameter	**Formula**	**Results**	
Sensitivity (%)	a/a+c×100	92.8	
Specificity (%)	d/d+b×100	100	
Positive predictive value (%)	a/a+b×100	100	
Negative predictive value (%)	d/d+c×100	96.6	
Accuracy (%)	a+d/a+b+c+d×100	97.7	
False-negative rate (%)	c/a+cX100	7.14	

were significantly better than those obtained in the PB dual- tracer group (96.6% and 3.39, respectively) (p>0.05). Hence, considering the cost-effectiveness and easy availability, wider use of MB is recommended for low- resource facilities (25). The total cost for MBin the whole study for all 43 patients was 210INR (3 USD). A 5-mL syringe was used in each patient, which costs around 5 INR each, for a total of 215INR. The total cost of the whole study for 43 patients is approximately 425 INR (6 USD). The cost per patient in this study was approximately 10 INR (0.14 USD). There was no significant increase in the duration of surgery or anesthesia; hence, no extra cost was added to these parts. Additionally, the dual-tracer technique seems beneficial for less experienced surgeons and in cases where the chances of FNR and misidentification of SLNs are high, such as in patients in neoadjuvant therapy, those who had prior breast or axillary surgery, and those with obesity. Hence, the blue dye tracer technique seems ideal for patients with node-negative early breast cancer.

In one landmark study, Krag et al. (5), in 1993, reported 82% of SLN identification rate using only RIand γ-probe; meanwhile, in our study, we report a superior identification rate of 89.6% using only MB. Furthermore, Li et al. (26), in a prospective study, used 99mTc-rituximabradiotracer alone to perform SLNB. They found the sensitivity, specificity, and accuracy of the method as 97.4%, 100%, and 98%, respectively, which were similar to the results of our present study (using MB only).

The FNR observed in our study (7.14%) was lower than those of the two aforesaid studies. Additionally, the FNR of our study is almost equal to the average FNR of 69 trials (7.3%) reported by Kim et al. (23).

As we discussed in the previous section, the use of RI has a diverse logical challenge, starting from the infrastructure to the handling of the RI, staff training, isotope disposal, and legislative concerns. The schedule of the surgeries is restricted due to the short half-life (6 hours) of the RI, and the injection has to be administered by the nuclear medicine experts. Furthermore, radiation exposure is possible. Hence, the use of a single dye tracer, most preferably MB, is commercially more viable than other dyes and RIs.

Conclusion

Institutions should have a validated SLNB technique when using a single-agent dye and such technique should have a low FNR that is within acceptable range. It may take training and revision of technique to achieve a low FNR. Our technique uses MB dye along with intraoperative palpation method after meticulous dissection of lymph nodes in each level. Our study provides a detailed methodology and different modifications to the standard technique of using a single-agent MB with acceptable time and easy transfer of knowledge among residents and other surgeons. Such validation studies will help trainee surgeons and surgical residents to start similar validation methods in various surgeries other than breast surgery.

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References

1. He L, Qu H, Wu Q, et al. Lymphedema in survivors of breast cancer.Oncol Lett. 2020; 19(3): 2085-96.
2. Manca G, Tardelli E, Rubello D, et al. Sentinel lymph node biopsy in breast cancer: a technical and clinical appraisal. Nucl Med Commun. 2016; 37(6): 570-6.
3. Yuan Q, Wu G, Xiao SY, et al. Identification and preservation of arm lymphatic system in axillary dissection for breast cancer to reduce arm lymphedema events: a
4. Mathelin C, Croce S, Brasse D, et al. Methylene blue dye, an accurate dye for sentinel lymph node identification in early breast cancer. Anticancer Res. 2009; 29: 4119-25.
5. Thevarajah S, Huston TL, Simmons RM. A comparison of the adverse reactions associated with isosulfan blue versus methylene blue dye in sentinel lymph node biopsy for breast cancer. Am J Surg. 2005; 189(2):236-9.
6. Eldrageely K, Vargas MP, Khalkhali I, et al. Sentinel lymph node mapping of breast cancer: a case-control study of methylene blue tracer compared to isosulfan blue. Am Surg. 2004; 70(1):872-5.
7. Blessing WD, Stolier AJ, Teng SC, et al. A comparison of methylene blue and lymphazurin in breast cancer sentinel node mapping. Am J Surg. 2002; 184(4):341-5.
8. Masannat Y, Shenoy H, Speirs V, et al. Properties and characteristics of the dyes injected to assist axillary sentinel node localization in breast surgery. Eur J Surg Oncol. 2006; 32(4):381-4.
9. Narui K, Ishikawa T, Kito A, et al. Observational study of blue dye-assisted four-node sampling for axillary staging in early breast cancer. Eur J Surg Oncol. 2010; 36:731-6.
10. Mathelin C, Croce S, Brasse D, et al. Methylene blue dye, an accurate dye for sentinel lymph node identification in early breast cancer. Anticancer Res. 2009; 29:4119-25.
11. Vohra LM, Memon AA, Khaliq T, et al. Sentinel lymph node biopsy in breast cancer by using isosulfan blue. Pak J Med Sci. 2009; 25:786-90.
12. Ollila DW, Brennan MB, Giuliano AE. The role of intraoperative lymphatic mapping and sentinel randomized clinical trial. Ann Surg Oncol. 2019; 26(11): 3446-54.
13. Lyman GH, Giuliano AE, Somerfield MR, et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol. 2005; 23:7703-20.
14. Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph node dissection in clinically node-negative patients with breast cancer: overall survival findings from the NSABP B-32 randomised phase 3 trial. Lancet Oncol. 2010; 11:927-33.
15. Mansel RE, Fallowfield L, Kissin M, et al. Randomized multi-center trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC trial. J Natl Cancer Inst. 2006; 98:599-609.
16. Guo J, Yang H, Wang S, et al. Comparison of sentinel lymph node biopsy guided by indocyanine green, blue dye, and their combination in breast cancer patients: a prospective cohort study. World J Surg Oncol. 2017; 15(1): 196.
17. Zaazou M, Mahran K, Merhem I. Sentinel lymph node biopsy using methylene blue dye in early breast cancer. Is it truly reliable? Egypt J Surg. 2010; 29:132-6. Lymphadenectomy in the management of patients with breast cancer. Adv Surg. 1999; 32:349-64.
18. Giuliano AE, Kirgan DM, Guenther JM, et al. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg. 1994; 220(3): 391-401.
19. Özdemir A, Mayir B, Demirbakan K, et al. Efficacy of methylene blue in sentinel lymph node biopsy for early breast cancer. J Breast Health. 2014; 10(2): 88-91.
20. Nandu VV,Chaudhari MS. Efficacy of sentinel lymph node biopsy in detecting axillary metastasis in breast cancer using methylene blue. Indian J Surg Oncol. 2017; 8(2): 109-12.
21. Zurrida S, Veronesi U. Milestones in breast cancer treatment. Breast J. 2015; 21:3-12.
22. Giuliano AE, Gangi A. Sentinel node biopsy and improved patient care. Breast J. 2015; 21:27-31.
23. Kim T, Giuliano AE, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in early-stage breast carcinoma: a meta-analysis. Cancer. 2006; 106:4-16.
24. Ahmed M, Purushotham AD, Donek M. Novel techniques for sentinel lymph nodebiopsy in breast cancer: a systematic review. Lancet Oncol. 2014; 15:351-62.
25. Seenu V, Suhani S, Srivastava A, et al. Optimization of sentinel lymph node identification techniques in the Indian setting: a randomized clinical trial. Indian J Cancer. 2019; 56(2): 114-8.
26. Li J, Chen X, Qi M, et al. Sentinel lymph node biopsy mapped with methylene blue dye alone in patients with breast cancer: A systematic review and meta-analysis. PLoS One 2018;13(9):2043