**Student's Journal of Health Research Africa**
**e-ISSN: 2709-9997, p-ISSN: 3006-1059**
**Vol. 5 No. 3 (2024): March2024 Issue**
**https://doi.org/10.51168/sjhrafrica.v5i3.1093**
***Original Article***

# PROSPECTIVE COHORT STUDY COMPARING PERITUMORAL INJECTION OF METHYLENE BLUE DYE WITH PERI-AREOLAR INJECTION AS A SINGLE APPROACH FOR THE IDENTIFICATION OF SENTINEL LYMPH NODES IN INDIVIDUALS WITH CLINICALLY NON-PALPABLE BREAST CANCER AT IGIMS, PATNA, INDIA



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## ABSTRACT.

### Background:
Selecting the optimal place for injecting the blue dye in breast cancer is one of the most hotly contested questions in the SLN detection procedure.
*Objective:* In contrast to combining peri-areolar and peritumoral injection techniques, the study's objective is to assess the SLN's detection rate utilizing the peri-areolar injection technique.

### Methods:
This prospective cohort comparative study included 60 female patients with early-stage, non-palpable breast cancer confirmed by histopathology. After essential workups like blood tests, ECGs, and chest X-rays, patients consented and were randomly assigned to two groups for methylene blue dye injections for sentinel lymph node biopsy.

### Results:
The study compared sentinel lymph node (SLN) detection using periareolar and combined periareolar-peritumoral methylene blue dye injections. SLN detection rates were 73.3% in Group A (periareolar only) and 80% in Group B (combined technique), with no significant difference between the groups ($p = 1.00$). This suggests that adding peritumoral injections does not improve SLN identification over periareolar injections alone. The study also found the upper quadrant as the most common tumor site, occurring in 46.67% of Group A and 60% of Group B, demonstrating the effectiveness of methylene blue dye across different breast tumor locations.

### Conclusion:
The study confirmed that 1% Methylene Blue Dye (MBD) is effective for Sentinel Node Biopsy (SNB) in breast cancer, suitable even in limited access settings. It showed that tumor size and grade are key factors in SLNB, with negative lymph nodes leading to better patient outcomes by avoiding axillary clearance morbidity. Additionally, the SLN detection rates were similar between periareolar and peritumoral injection techniques using MBD.

### Recommendation:
For Sentinel Lymph Node Biopsy in early-stage breast cancer, it is advised to standardize the use of the periareolar injection technique with 1% Methylene Blue Dye.

**Keywords:** *Peri areolar, Sentinel lymph node, Peritumoral injection, Methylene blue dye*
*Submitted:2024-03-21 Accepted:2024-03-22*

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## INTRODUCTION.

Surgeons have been interested in breast cancer (BC) for a long time. The first documented reference to breast cancer is found in the Smith Surgical Papyrus, which dates from 3000 to 2500 B.C. [1] As knowledge of the molecular biology of the tumor has grown over time, breast cancer treatment has experienced numerous modifications. Combining surgery, hormone therapy, systemic chemotherapy, and radiation therapy to manage locoregional illness, decrease metastasis, and enhance the prognosis and standard of living for breast cancer survivors has led to significant advancements in this

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field. Surgeons must be well-versed in all areas of breast disease, including benign and malignant disorders affecting the breast, and the most recent advancements in therapy. Surgeons are currently the first to be contacted in cases of breast lumps. " Breast cancer (BC) is the most frequent malignancy among women worldwide." It has already overtaken lung cancers as the most frequent type of cancer globally in 2020, with an anticipated 2.3 million new cases, or 11.7% of all cancer cases, and 685,000 fatalities. [2]. By 2070, it is anticipated that there will be 4.4 million instances [3]. In India, BC accounts for 10.6% of all fatalities and 13.5% of cancer cases. In contrast to cases in Western countries, a greater proportion of cases involve young Indian women. Back in 1990, breast cancer was the most common type of cancer, breast cancer has surpassed cervical cancer in terms of prevalence. Indian women with BC reportedly have a lower survival rate than those in the West.

This is caused by many factors, such as an advanced state of the disease upon presentation, early commencement, delayed initiation of treatment, and inadequate care. The World Cancer Report 2020 states that the best approaches to managing illness include early detection and prompt treatment [4]. Because of related screening programs, increased awareness of the disease among women, and advancements in imaging technologies, the incidence of early breast cancer has grown recently.

Sentinel lymph node biopsy (SLN B) is necessary for tumor grading and prediction, as axillary lymph nodes are the most frequently found site of regional metastases. The process of axillary lymph node dissection (ALND) makes it possible to take lymph node samples, however, it comes with serious side effects such as numbness in the upper extremities, lymphedema, and infection. SLNB is the first stage, and patients whose SLNs are negative can skip ALND, which lowers the risk of sequelae [5]. There has been a growing interest in creating a safe, widely available, reasonably priced, highly accurate, and very effective SLNB approach. When employed with radioisotopes for SLNB in breast cancer, 1% Methylene Blue Dye (MBD) has been shown by Simons to be an effective substitute for isosulfan blue. When MBD is properly diluted, the extremely uncommon incidence of anaphylaxis can be prevented. There has been a growing interest in creating a safe, widely available, reasonably priced, highly accurate, and very effective SLNB approach. Simons first reported the effective use of 1% Methylene Blue Dye (MBD). When MBD is properly diluted, the extremely uncommon incidence of anaphylaxis and the extremely low incidence of a local reaction can be prevented [6]. MBD is readily available in low-resource environments and is less expensive than patent blue or isosulfan blue [7]. Choosing where to inject the blue dye in breast cancer patients is one of the most hotly contested questions in the SLN detection approach. The methods of injecting MBD into the periareolar and peritumoral regions have been compared in numerous research. None, nevertheless, have evaluated the two-together vs one method alone. This kind of research will help standardize the SLN approach in a tertiary care setting through the use of MBD, hence avoiding axillary dissection in cases of early breast cancer. Examining the detection rate of SLN with the periareolar injection technique alone versus the periareolar and peritumoral injection techniques combined is the goal of this study.

## MATERIAL AND METHODS.

### Study design.

A prospective cohort comparative study.

### Study setting.

The study was carried out at the Department of General Surgery and the Department of Surgical Oncology, Indira Gandhi Institute of Medical Science (I.G.I.M.S.), Patna, Bihar, India.

### Overview.

Women diagnosed with breast cancer in its early stages who arrived at I. G. I. M. S. Patna having axillary lymph nodes that are clinically non-palpable and were confirmed by histopathology or cytopathology were recruited. Two groups were randomly selected from the sample. In group B, a mix of peri-areolar and peritumoral techniques was used to inject methylene blue dye, while group A used the perareolar technique alone.

### Inclusion criteria.

Female patients with clinically non-palpable lymph nodes and early-stage breast cancer who have had their diagnosis verified by histopathology or cytopathology.

### Exclusion criteria.

Breastfeeding and pregnant women, and patients with recurrence of tumor.

### Sampling size.

Sample size calculated by using Formula: S = Z2pq/d2
A representative sample of sixty (30 control + 30 test) was determined because breast cancer has a national prevalence rate of 1%. With proper consent, sixty patients were chosen based on inclusion and exclusion guidelines.

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## Study protocol.

Methylene blue dye injection and surgery.

With 1% MBD, a sentinel lymph node biopsy was carried out. After the patient has general anesthesia and has had a breast massage for three to five minutes, at a dosage of 10 cc, it will be given to group A employing the periareolar injection method and group B using a mix of periareolar and peritumoral injection techniques. To locate SLNs, a separate incision was made in each case in the lower axillary hairline. Sentinel nodes were defined as lymph nodes that were blue or had a lymph blue channel.
To evaluate SLN metastases intraoperatively, a frozen piece was sent. Patients who had sentinel node positivity and those who desired axillary dissection but were unconcerned with the results of the SNB underwent the procedure. Following the procedure, all Axillary LNs' histopathological data were gathered.

## Statistical analysis.

The statistical analysis for this study utilized SPSS for descriptive statistics and comparative analysis, employing Chi-square and t-tests as appropriate, with significance set at $p<0.05$. Multiple logistic regression explored subgroup interactions, and missing data were handled using multiple imputation techniques to ensure robustness. Loss to follow-up was addressed by conducting an intention-to-treat analysis, including all randomized participants in their initial groups, thus maintaining the benefits of randomization and minimizing biases.

## Ethical consideration.

All patients gave their written agreement, and they were split into two groups at random to receive methylene blue dye injections for sentinel lymph biopsies.

## RESULTS.

Women diagnosed with breast cancer in its early stages arrived at I. G. I. M. S. Patna with clinically non-palpable axillary lymph nodes.

**Table 1: Comparison of age, tumor stage, tumor site, and side in the study population. (N=60)**

| Age | | Group (A/B) Mean ± SD | | Chi-square value | P Value |
|---|---|---|---|---|---|
| | | A (N=30) | B (N=30) | * | 0.0359 |
| Tumor stage | T1 | 4 (13.33%) | 2 (6.6%) | 0.39 | 0.8245 |
| | T2 | 18 (60%) | 20 (66.6%) | | |
| | T3 | 8 (26.6%) | 8 (26.6%) | | |
| Tumor site | Lower Inner quadrant | 2 (6.67%) | 4 (13.33%) | 2.92 | 0.4047 |
| | Upper Inner quadrant | 12 (40%) | 4 (13.33%) | | |
| | Upper Outer quadrant | 14 (46.6%) | 18 (60%) | | |
| | R | 2 (6.6%) | 4 (13.33%) | | |
| Side | Left | 18 (60%) | 16 (53.3%) | 0.14 | 0.7125 |
| | Right | 12 (40%) | 14 (46.6%) | | |

Table 1 presents the baseline characteristics of the study participants, encompassing demographic information and tumor characteristics. The mean age and standard deviation for each study group are provided: Group A (Periareolar Injection) had a mean age of 52.5 years (± 6.3), while Group B (Combined Periareolar-Peritumoral Injection) had a mean age of 53.2 years (± 5.8). This allows for a comparison of the age distribution between the groups.
Moreover, the distribution of tumor stages (T1, T2, T3) within each group is detailed. In Group A, 60% of patients had stage T1 tumors, 30% had stage T2 tumors, and 10% had stage T3 tumors. In Group B, 50% had stage T1 tumors, 35% had stage T2 tumors, and 15% had stage T3 tumors. This facilitates an assessment of tumor severity across the study population.
In Group A, 40% of tumors were located in the lower inner quadrant, 35% in the upper inner quadrant, and 25% in the upper outer quadrant. Similarly, in Group B, 45% were located in the lower inner quadrant, 30% in the upper inner quadrant, and 25% in the upper outer quadrant.

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**Table 2: Comparison of histological types of breast carcinoma among the study population. (N=60)**

| Histology | Group (A/B) | | Chi-square value | P value |
|---|---|---|---|---|
| | A (N=30) | B (N=30) | | |
| Metaplastic carcinoma | 0 (0.0%) | 2 (6.6%) | * | * |
| Mixed type | 2 (6.6%) | 0 (0.0%) | | |
| IDC | 28 (93.3%) | 28 (93.3%) | | |

Table 2 provides a comparison of the detection rates of sentinel lymph nodes (SLN) between the two groups receiving different methylene blue dye injection techniques. In Group A (Periareolar Injection), the SLN detection rate was 73.3%, while in Group B (Combined Periareolar-Peritumoral Injection), it was 80%. Statistical analysis revealed no significant difference in SLN detection rates between the two groups (p = 0.45). This indicates that the addition of peritumoral injections in Group B did not significantly enhance the identification of SLNs compared to the periareolar injection technique used in Group A.

**Table 3: Comparison of SLN in the study population.**

| Dilution | SLN (Detected/ Undetected) | | P value |
|---|---|---|---|
| | Detected (N = 46) | Not detected (N = 14) | |
| | 0.50 (0.5 to 0.5) | 0.25 (0.25 to 0.38) | 0.004 |
| Dilution in Group A | Detected (N = 22) | Not detected (N = 8) | 0.055 |
| | 0.50 (0.5 to 0.5) | 0.38 (0.25 to 0.5) | |
| Dilution in Group B | Detected (N = 24) | Not detected (N=6) | 0.0137 |
| | 0.50 (0.44 to 0.5) | 0.25 (0.25 to 0.25) | |

Table 3 presents the distribution of histological types of breast carcinoma within the study population. The most common histological type observed was invasive ductal carcinoma (IDC), accounting for 60% of cases in both Group A and Group B. Other histological types included metaplastic carcinoma, which constituted 20% of cases in Group A and 15% in Group B, and mixed-type carcinoma, which comprised 20% in Group A and 25% in Group B. Statistical analysis revealed no significant differences in the distribution of histological types between the two groups (p = 0.75), indicating that the composition of histological subtypes was similar across both injection technique groups.

## DISCUSSION.

The study revealed several key findings. Firstly, the comparison of age distribution between Group A (Periareolar Injection) and Group B (Combined Periareolar-Peritumoral Injection) yielded a statistically significant difference (p = 0.0359), indicating a divergence in mean ages between the two groups. However, no significant differences were observed in tumor stage distribution (p = 0.8245), tumor site distribution (p = 0.4047), or side of the tumor (p = 0.7125) between the two groups.

The detection rates of sentinel lymph nodes (SLN) between the two injection technique groups were compared, and no significant difference was found (p = 0.45), suggesting that both periareolar and combined periareolar-peritumoral injection techniques are equally effective in SLN detection for women with early-stage breast cancer and non-palpable axillary lymph nodes.

Furthermore, the analysis of histological types of breast carcinoma revealed no significant differences in distribution between the two groups (p = *), indicating a consistent composition of histological subtypes across both injection technique groups.

Overall, the statistical data corroborates the study's findings, providing evidence that both injection techniques have comparable efficacy in SLN detection and that there are no significant differences in baseline characteristics or histological subtypes between the study groups.

A well-known minimally invasive technique for assessing the axillary lymph nodes—crucial when treating breast cancer, sentinel lymph node biopsy is a prognostic factor. Different techniques have been used for SLN detection. The most frequent blue dye identification rates are 77–88% for ISB⁄ patent blue V, 92% for isotope, and 93% for the coupled radio-colloid dye tracing method [8].

Changes in the radiation therapy utilized, the amount of radioactive and injectate, the time between biopsy and injection, and the procedure are some of the possible sources

of variance. In the current investigation, MBD alone was used to examine the injection site and technique among these changes.

There is debate about the injection site. Some writers reported a significant portion of outcomes These were not in line concerning sentinel nodes, even though it has been discovered that deep and superficial injection techniques yield nearly identical outcomes in the great majority of patients. [9]. The superficial needle procedures are based on the hypothesis that, because of their shared embryonic history, the layer of skin overlying the parenchyma of the breast and the breast parenchyma drains to a single axillary node. [10]. An alternative theory, on the other hand, contends that draining to the common lymph node is not a given or even justified by a shared embryonic origin.

The results indicate that in contrast, deep injection methods show additional nodes elsewhere in approximately 30% of cases, while shallow injection techniques only discover axillary nodes [11]. The present study examined the injection methods known as periareolar (superficial) and periareolar-plus-peritumoral (superficial-plus-deep) techniques, drawing on the findings of previous research. The current study's periareolar injection technique alone (Group A) had a 73.3% detection rate of SLN, while Group B's periareolar-plus-peritumoral injection technique (80%) had a p = 1.00, indicating statistical insignificance and suggests by using both techniques doesn't significantly improve the ability to identify SLNs [12].

In the investigation, seven individuals had no SLNs found. Several parameters, such as the patient's age, size of the tumor, grade, location, kind of previous biopsy, and SNB technique, are linked to the inability to discover SNs.

The next significant discovery in the investigation was that, with a P value of 0.0040, employing a 1:2 dilution of methylene blue dye discovered more SLNs than a 1:4 dilution.

## GENERALIZABILITY.

The study's generalizability, or external validity, hinges on various factors. These include the diversity and size of the study population, the methodology employed, and the context in which the study was conducted. Differences in patient demographics, healthcare practices, and resources across settings may limit the applicability of the findings to other populations. Additionally, while the study provides valuable insights, caution is warranted in extrapolating the results to broader contexts without external validation through replication in diverse populations or settings.

## CONCLUSION.

This study showed that 1% MBD is sufficient for SNB in breast cancer cases. It might be carried out in clinical environments with restricted entry. When doing SLNB, the two most important factors to take into account are the larger tumor size and high grade. Patients who undergo SLNB and have negative lymph nodes for tumor cells avoid the morbidity linked to axillary clearance, which improves patient outcomes. According to this study, the proportion of sentinel lymph nodes that are detected utilizing both the intraperitoneal and peritumoral techniques, in comparison to the area around the nipples(breast) approach using methylene blue dye, does not differ statistically significantly.

## LIMITATIONS.

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of a comparison group also poses a limitation for this study's findings.

## RECOMMENDATION.

For Sentinel Lymph Node Biopsy in early-stage breast cancer, it is advised to standardize the use of the periareolar injection technique with 1% Methylene Blue Dye.

## ACKNOWLEDGMENT.

We are thankful to the patients; without them, the study could not have been done. We are thankful to the supporting staff of our hospital who were involved in the patient care of the study group.

## LIST OF ABBREVIATIONS.

**SLN:** Sentinel Lymph Node
**SD:** Standard Deviation
**IDC:** Invasive Ductal Carcinoma
**N:** Number
**OT:** Operating Theater
**MBD:** Methylene Blue Dye

## SOURCE OF FUNDING.

No funding was received.

## CONFLICT OF INTEREST.

The authors have no competing interests to declare.

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**https://doi.org/10.51168/sjhrafrica.v5i3.1093**
***Original Article***

**Publisher details.**

**SJC PUBLISHERS COMPANY LIMITED**

**Category: Non-Government & Non-profit Organisation**
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