"FLUORESCEIN SODIUM -AN ALTERNATIVE TO METHYLENE BLUE IN DETECTING SENTINEL AXILLARY LYMPH NODE IN CARCINOMA BREAST."

BY

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In partial fulfilment of the requirements for the degree of

M.S. GENERAL SURGERY

UNDER THE GUIDANCE OF

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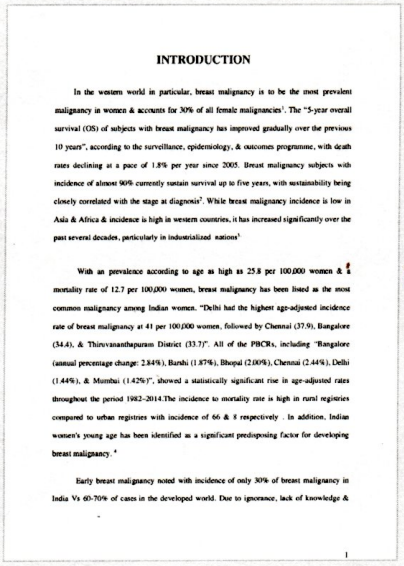

INTRODUCTION

In the western world in particular, breast malignancy is to be the most prevalent malignancy in women & accounts for 30% of all female malignancies[1]. The "5-year overall survival (OS) of subjects with breast malignancy has improved gradually over the previous 10 years", according to the surveillance, epidemiology, & outcomes programme, with death rates declining at a pace of 1.8% per year since 2005. Breast malignancy subjects with incidence of almost 90% currently sustain survival up to five years, with sustainability being closely correlated with the stage at diagnosis[2]. While breast malignancy incidence is low in Asia & Africa & incidence is high in western countries, it has increased significantly over the past several decades, particularly in industrialized nations[3]

With an prevalence according to age as high as 25.8 per 100,000 women & a mortality rate of 12.7 per 100,000 women, breast malignancy has been listed as the most common malignancy among Indian women. "Delhi had the highest age-adjusted incidence rate of breast malignancy at 41 per 100,000 women, followed by Chennai (37.9), Bangalore (34.4), & Thiruvananthapuram District (33.7)". All of the PBCRs, including "Bangalore (annual percentage change: 2.84%), Barshi (1.87%), Bhopal (2.00%), Chennai (2.44%), Delhi (1.44%), & Mumbai (1.42%)", showed a statistically significant rise in age-adjusted rates throughout the period 1982–2014.The incidence to mortality rate is high in rural registries compared to urban registries with incidence of 66 & 8 respectively . In addition, Indian women's young age has been identified as a significant predisposing factor for developing breast malignancy. [4]

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Signature of the candidate

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ABSTRACT

INTRODUCTION:

Fluorescein sodium (FS) is a fluorescent dye that is widely used in ophthalmic surgery and malignant brain tumour surgery. Ten percent FS, a widely available substance, is conventionally safe without any clearly associated adverse effects. Furthermore, FS was reported to be an effective tracer material for SLN mapping in colorectal tumours. However, the use of FS in SLNB of breast cancer is limited. Hence this study was conducted to determine the validity of fluorescein sodium in the identification of SLN in patients with carcinoma of the Breast.

MATERIAL AND METHODS:

A Prospective study was conducted among 30 patients with carcinoma breast aged >18 years with no clinically identifiable axillary lymph nodes at the Department of General Surgery, R.L. Jalappa Hospital and Research Centre, Sri Devraj Urs Medical College, Tamaka, Kolar. The study was conducted for a period of 1 year. Institutional Ethical clearance was obtained prior to the start of the study. Informed consent was obtained from all the patients recruited prior to the start of the study.

STATISTICAL ANALYSIS: Data was entered into a Microsoft Excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. The chi-square test was used as a test of significance for qualitative data. Continuous data were represented as mean and standard deviation. The validity of a test in the Screening of Disease was assessed through Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV) and Diagnostic accuracy.

RESULTS: Mean age was 53.13 ± 14.180 years. The majority of subjects were in the age group 51 to 60 years (26.7%). 20% were in stage T2N0M0, 30% were in T3N0M0 and 50% were in T4BN0M0. on HPE 86.7% had Infiltrating Ductal Ca, 10% had Lobular Ca and 3.3% had Squamous Cell Ca. Fluorescein Dye staining had a sensitivity of 84.21%, specificity was 30.99%, Positive Predictive Value was 39.51%, Negative Predictive Value was 78.57% and Diagnostic Accuracy was 49.54% in comparison with HPE. Methylene Blue staining had a sensitivity of 44.74%, specificity was 35.21%, Positive Predictive Value was 26.98%, Negative Predictive Value was 54.35% and Diagnostic Accuracy was 38.53% in comparison with HPE.

CONCLUSION: From the study findings it can be concluded that the detection of SLNs using fluorescein was high compared to methylene blue, both methods were feasible and safe in patients with breast carcinoma. Fluorescein dye had better validity in the diagnosis of Sentinel lymph node compared to Methylene blue in the Carcinoma of the breast.

KEYWORDS: Fluorescein dye, Methylene blue, Sentinel lymph node

CONTENTS

LIST OF TABLES

LIST OF FIGURES

46	Figure 46: Post Operative Pictures	**141**

LIST OF ABBREVIATIONS USED

ALMANAC:	Axillary Lymphatic Mapping Against Nodal Axillary Clearance.
ALNB:	Axillary Lymph Node Biopsy.
ALND:	Axillary Lymph Node Dissection.
ASHA:	Accredited Social Health Activist.
BCS:	Breast Conserving Surgery.
BSE:	Breast Self-Examination.
CME:	Cyclophosphamide Methotrexate Flurouracil.
CNB:	Core Needle Biopsy.
CUSUM:	Cummulative Sum.
EBC:	Early Breast Cancer.
ER:	Estrogen Receptor.
FNAC:	Fine Needle Aspiration Cytology.
FN:	False Negative.
FNR:	False Negative Rate.
FS	fluorescein sodium
G6PD:	Glucose -6 Phosphate Dehydrogenase.
HPE:	Histopathological Examination.
H & E:	Haematoxyllin And Eosin.
IHC:	Immuno Histo Chemistry.
IR:	Identification Rate.
LABC:	Locally Advanced Breast Cancer.
LM:	Lymphatic Mapping.
LR:	Local Recurrence.
PHC:	Primary Health Centre.

MAO:	Mono Amino Oxidase.
MBD:	Methylene Blue Dye.
MRM:	Modified Radical Mastectomy.
NACT:	Neo Adjuvant Chemotherapy.
NACTRT:	Neo Adjuvant Chemotherapy Radiotherapy.
NPV:	Negative Predictive Value.
OS:	Overall Survival.
PBCR:	Population Based Cancer Registry.
PPV:	Positive Predictive Value.
SE:	Sensitivity.
SLN:	Sentinel Lymph Node.
SLNB:	Sentinel Lymph Node Biopsy.
SP:	Specificity.
SPRT:	Sequential Probability Ratio Test.
SSRI:	Serotonin Re Uptake Inhibitor.
TN:	Total Negative.
TP:	Total Positive.

INTRODUCTION

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In the western world in particular, breast cancer is to be the most prevalent cancer in women and accounts for 30% of all female malignancies[1]. The "5-year overall survival (OS) of patients with breast cancer has improved gradually over the previous 10 years", according to the surveillance, epidemiology, and outcomes program, with death rates declining at a pace of 1.8% per year since 2005. Breast cancer patients with an incidence of almost 90% currently sustain survival for up to five years, with sustainability being closely correlated with the stage at diagnosis[2]. While breast cancer incidence is low in Asia and Africa and incidence is high in western countries, it has increased significantly over the past several decades, particularly in industrialized nations[3].

With an age-adjusted prevalence as high as 25.8 per 100,000 women and a mortality rate of 12.7 per 100,000 women, breast cancer has been listed as the most common carcinoma among Indian women. "Delhi had the highest age-adjusted incidence rate of breast cancer at 41 per 100,000 women, followed by Chennai (37.9), Bangalore (34.4), and Thiruvananthapuram District (33.7)". All of the PBCRs, including Bangalore (annual percentage change: 2.84%), Barshi (1.87%), Bhopal (2.00%), Chennai (2.44%), Delhi (1.44%), and Mumbai (1.42%), showed a statistically significant rise in age-adjusted rates throughout the period 1982–2014. The incidence to mortality rate is high in rural registries compared to urban registries with an incidence of 66 and 8 respectively. In addition, Indian women's young age has been identified as a significant risk factor for developing breast cancer. [4]

Early breast cancer constitutes only 30% of breast cancer in India Vs 60-70% of cases in the developed world. Due to ignorance, lack of knowledge and access to healthcare services, and social-cultural attitudes, the majority of the women (70%) present at an advanced stage. "Mammography, examination of the breast clinically, and examination of the breast by self",

are the three methods of breast cancer screening that are most frequently used worldwide. In developing countries like ours where mammography and breast clinical examination are expensive, breast self-examination is the most feasible technique for early breast cancer detection. In our country the involvement of ASHA is helping in raising awareness for diagnosing breastcarcinoma as early as possible before progresses, however, still many cases go unreported[5]. One of the most crucial breast cancer prognostic factors is the condition of the axillary lymph nodes. Regular axillary dissection is not beneficial for 70% to 80% of node-negative early breast cancer patients .[6]

One of the most significant prognostic indicators in breast cancer has been demonstrated to be the "sentinel lymph node (SLN)" status, a reliable predictor of axillary lymph node status.[7] With clinically negative lymph nodes, "sentinel lymph node biopsy (SLNB)" is a reliable and safe method that is widely used as a most acceptable procedure in breast cancer surgery. [8]

Diverse tracer materials, like "blue dye, radioisotopes, carbon nanoparticles, or a composite of these", have been used recently in performing SLNB of breast cancer at about this period[9, 10]. Yet there is no agreement on the most appropriate and effective tracer materials for SLNB[11]. Despite being much more economical and safe than other radioisotopes, the blue dye does have a lower detection accuracy [9]. The hazard of radiation contact and insufficient and incomplete lymph vessel mapping renders the usage of radioisotopes in SLNB more challenging.[12, 13] The exorbitant cost of carbon nanoparticles hinders their application and usage. Therefore, there is still a need for the development of novel tracer materials for SLNB.

A fluorescent dye named fluorescein sodium (FS) is extensively employed in ocular surgery and surgery for malignant brain tumours[14, 15]. A substance that is widely available which is 10% FS is universally regarded as secure and safe for usage with no known negative

or adverse effects. Moreover, it was discovered that FS is a useful and promising product for tracing and SLN mapping in colorectal cancers[16]. However, there is a limited application of FS in SLNB of breast cancer.

Hence this research was done to ascertain whether Fluorescein sodium is better than methylene blue dye in terms of the rate of characterisation of SLN in patients with breast carcinoma.

AIMS AND OBJECTIVES

AIM AND OBJECTIVES

AIM:

To ascertain the validity of fluorescein sodium in the identification of SLN in patients with Breast carcinoma.

OBJECTIVES OF THE STUDY

1. To analyze and ascertain the identification of SLN using methylene blue in breast carcinoma patients.
2. To analyze the identification of SLN using fluorescein sodium in breast carcinoma patients.
3. To compare the identification of SLN using methylene blue and fluorescein sodium in breast carcinoma patients.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

During the 19th century, radical mastectomy was widely used as a clinical practice by Halsted. He published his paper on classical radical mastectomy done on 50 patients. In his research, he mentioned a very low local recurrence rate of ~6%. The operation removed the tumor containing the breast, underlying pectoralis muscle, and ipsilateral axillary lymph node and he also divided the pectoralis minor muscle[17]. It was associated with considerable morbidity during the postoperative period comprising sensory loss over the ipsilateral limb, restricted mobility of the ipsilateral shoulder, extensive lymphedema, disfigurement, and agony [18].

At the end of the 19th century, Dr Meyer from New York developed his own procedure which was essentially similar to Halsted's work which was published prior. The exception was that he excised skin widely with an adequate margin and closed the defect with a graft, excised pectoralisminor along with the pectoralis major and also performed ALND first followed by mastectomy[17].

R S Handley in 1969 adopted the method of conservative radical mastectomy (Patey mastectomy) where he performed the surgery on 143 patients. He preserved the pectoralis major muscle in contrast to the Halsted operation technique. The removal of the breast began from the midline and the breast together with the pectoral fascia and a few muscle fibers were peeled off the pectoralis major by sharp dissection. The pectoralis minor was excised to gain access to axilla[18]. Preservation of the axillary vein and nerves to the serratus anterior and latissimus dorsi was a crucial step in this procedure. The wound was drained using a suction tube[19].

Breast-conserving surgery is the primary surgical procedure for small to medium-sized breast malignancies and was pioneered in the 1960s [20]. Lumpectomy, which would be typically

followed by radiation therapy, is defined by the international consensus conference on BCS (Milan, 2005) as the total excision of the breast lump with a concentric margin of surrounding healthy tissue in small to medium-sized breast[21]. The conventional treatment for T1 and small T2 breast cancers now involves BCS followed by radiation therapy with tumor-free margins, although this approach carries a higher risk of local recurrence (LR), even though local recurrence is rare (1% per year), with survival rates comparable to radical operations[22].

Haagensen studied the route of metastases through the axillary lymph node filter and stated that the nodes of the central group are not only most often involved, but also most often exclusively involved[23]. In 1990, Morton et al introduced the lymphatic mapping (LM) of the first tumor-draining lymph node, the sentinel lymph node (SLN), at the annual meeting of the Society of Surgical Oncology. Morton states that the mapping and biopsy of the SLN are due to the afferent lymphatic channel from a primary tumor draining first to one or more SLNs in the regional lymphatic basin[24]. On analysis of standard hematoxylin-eosin-stained slides (12%), exclusively in immunohistochemically stained preparations (9%), and in 194 of 237 lymphatic basins, he correctly identified the sentinel node(s) and discovered metastases in 21%. 18% of 259 sentinel nodes had metastases, while just two of 3079 nodes from 194 lymphadenectomy specimens that had a sentinel node that could be identified did, resulting in a false-negative rate of less than 1%[25].

Giuliano and colleagues successfully adapted SNB for breast cancer and began a pilot study in 1991. This study was reported in 1994 after 174 lymphatic mapping procedures were performed using a vital dye injected at the primary breast cancer site. SNs were found in 114 (65.5%) of 174 operations, and axillary nodal status was correctly predicted in 109 of 114 (95.6%) cases. There was a noticeable learning curve, and in the primary process of the

investigation we observed the occurrence of false-negative sentinel nodes more often but sentinel nodes discovered in the most recent 87 treatments had a 100% accuracy rate. The sentinel node was the sole tumor-involved lymph node found in 16 of 42 (38.0%) clinically negative/pathologically positive axilla. In the 54 most recent surgeries, the anatomic position of the sentinel node was investigated; 10 patients showed only level II nodal metastases that could have been overlooked and neglected by sampling or low (level I) axillary dissection.[26]

Cabanas discovered a sentinel node in the penis lymphatic drainage system. The precise placement of such sentinel nodes was elucidated by lymphangiographic studies. It was noted that there was direct drainage from the penis to the lymph nodes connected to the superficial epigastric vein. The sentinel lymph node was located by placing the finger under the top flap in the direction of the pubic tubercle after creating an incision parallel to the inguinal ligament[27].

In order to visualize breast lymphatics, Kett et al. injected areolar blue dye as a contrast media [28]. They noticed passage to a solitary lymph node known as the "Sorgius node," followed by emptying through innumerable lymphatic arteries and lymph nodes to the collecting system encircling the axillary vein. 1980, we had seen the discovery of "primary draining nodes" by Christensen et al. using breast lymphoscintigraphy[29].

A prospective study with aim of determining of survival benefits of ALND and loco-regional control in breast-conserving surgery with whole breast radiotherapy and systemic therapy in patients with SLN metastases, and is named Z11 trail. They enrolled 891 women with clinically T1- or T2-invasive breast cancer without clinical lymphadenopathy and one or two SLNs containing metastases, which were identified by touch preparation, frozen section, or H & E staining. Tangential whole-breast radiation and breast-conserving surgery were employed to treat all patients. The axilla received no third-field irradiation. Patients who were diagnosed to

have SLN metastases on SLNB were randomized to undergo ALND or no further axillary care. The average patient follow-up period had been 6.3 years. Five-year overall survival and disease-free survival were 91.8% and 82.2% in the group of patients chosen at random to ALND, respectively, while they were 92.5% and 83.9% in the group who has undergone only SLNB[30].

Sentinel node biopsy is currently being done in the management of regional basins in earlybreast cancer. Limiting surgery and preserving the quality of life have emerged as crucial ideas in the treatment of breast cancer which showed a steady increase in cure rates over the past few decades. A successful and affordable treatment for breast cancer is SLNB[31].

In a 2007 study by S.P. Somashekhar S. et al.[32], SLN was detected in all 100 patients of the validation trial by using a combination of methylene blue dye and radioactive technetium 99m Sulphur colloid. There were a total of 27 instances with positive axillary nodes, of which 69% had SLN as the sole positive node for metastases. The overall sensitivity, specificity, positive predictive value, and negative predictive value of SLNB were, respectively, 96.2%, 100%, 100%, and 98.6%, with a false negative rate of 3.7%. It was determined that SLNB is successful in early breast cancer patients from the Indian population, in the subsequent 35 patients who underwent SLNB and then underwent complete axillary clearance.[32].

In a study done by **Gaurav Goel et al**.,[33] in December 2016 he did a SLNB using a combination of isosulphan blue dye and Tc 99 labelled Sulphur colloid 105 patients underwent sentinel lymph node biopsy followed by frozen section analysis. The sensitivity, specificity, PPV, and NPV of intra-operative sentinel lymph node biopsy analysis were 56.52 % (13/23), 97.56 % (80/82), 86.67 % (13/15), and 88.89 % (80/90) respectively. Overall diagnostic accuracy was 88.57 % [33].

In a study done by **Vipul V. Nandu et al.,**[34] in June 2017 in 35 patients for early breast cancer using methylene blue dye only, they found the sensitivity of SLNB to be 90.48% and specificity to be Negative predictive value is 85.71%, the positive predictive value is 90.48%, and overall SLNB accuracy is 88.57%.[34].

In a study done by **Jeffery ME et al.,**[35] in 2009 it was found that methylene blue dye is an accurate and cost-effective single agent for sentinel lymph node mapping in breast cancer. It was also noted that the Sentinel node identification rate is a more reasonable target for the assessment of the learning curves of surgeons than in false negative rate. Application of a tubular CUSUM (cumulative sum) chart, with SPRT (sequential probability ratio test) limit values based on a target identification rate of 85% and a reasonable choice of other parameters, demonstrates that experienced breast surgeons have completed the SLNB learning curve after 8 consecutive successful attempts using methylene blue[35].

In a study done by **Nighat Bakhtiar et al.,**[36] from January 2013 to September 2015 in 85 patients with early breast cancer they concluded methylene blue dye is a reliable and safe diagnostic modality for the detection of SLN because of its high accuracy, SLN was identified in 61 patients[36].

THE BREAST/MAMMARY GLAND:

It is one of the most significant structures in the pectoral region which is present in both sexes but rudimentary or primitive in males. After puberty, it is gradually matured and fully grown in females. It is a modified sweat gland and auxiliary reproductive organ of the female that produces milk after reproduction to nourish the fetus. Between the second and sixth ribs, as well as between the sternal border and the midaxillary line, the mature female breast can be found. The breast is made up of skin, subcutaneous tissue, and breast tissue, the latter of which contains both stromal and epithelial components. A high proportion of carcinomas arise in the upper outer quadrant of the breast[37]. The route of metastasis is through hematogenous and lymphatic spread however the dissemination of tumor cells via lymphatic drainage of the tumor is the most common metastatic route.

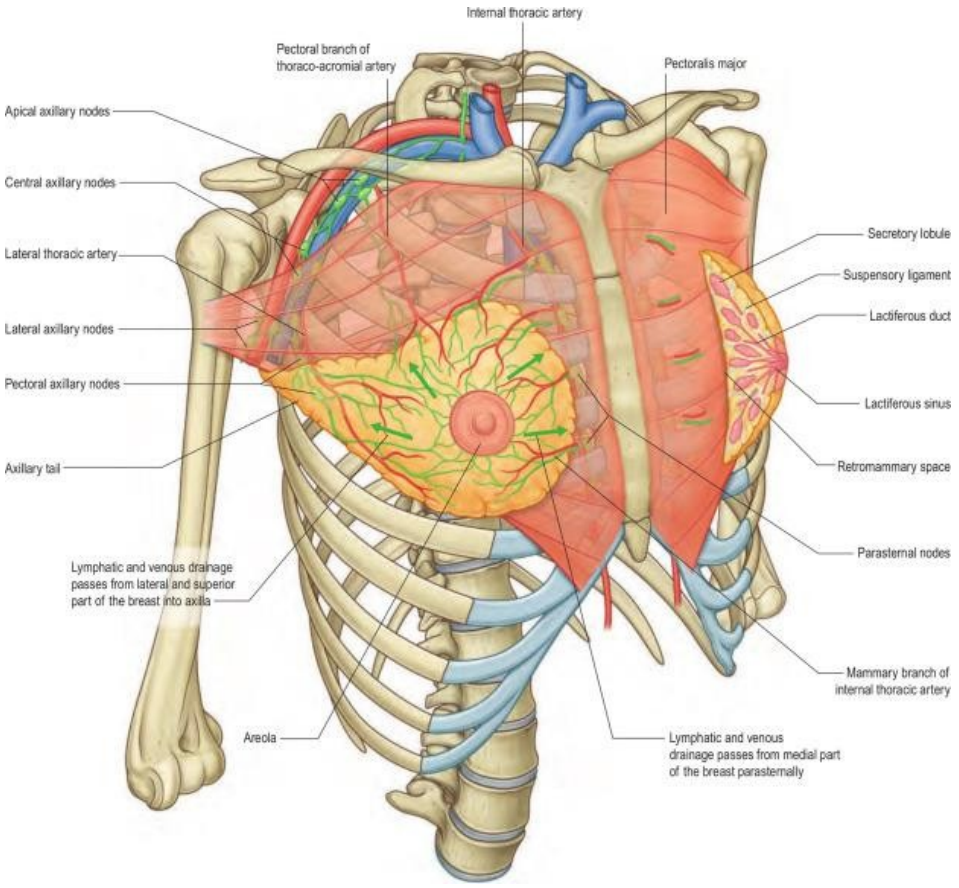


Figure 1: Anatomy of Breast, Pic courtesy: Grays Textbook of Anatomy.

INNERVATION:

The second through sixth intercostal nerves' anterior and lateral cutaneous branches innervate the breast. The fourth intercostal nerve innervates the nipple.

BLOOD SUPPLY OF THE BREAST:

The breast's vascular system

1. **Thoracic Artery**: A branching of the subclavian artery, the internal thoracic (or internal mammary) artery runs parallel to the sternum's lateral margin, located just behind the internal intercostal muscles.
2. **Branches of the Axillary Artery:** Four branches may supply the breast.

   They are, in order of appearance:

   a) The superior thoracic artery

   b) The pectoral branches of the thoracoacromial artery

   c) The lateral thoracic arteries

   d) Unnamed mammary branches.
3. **Intercostal Arteries**: The third, fourth, and fifth intercostal arteries may also branch out into the lateral portion of the breast.

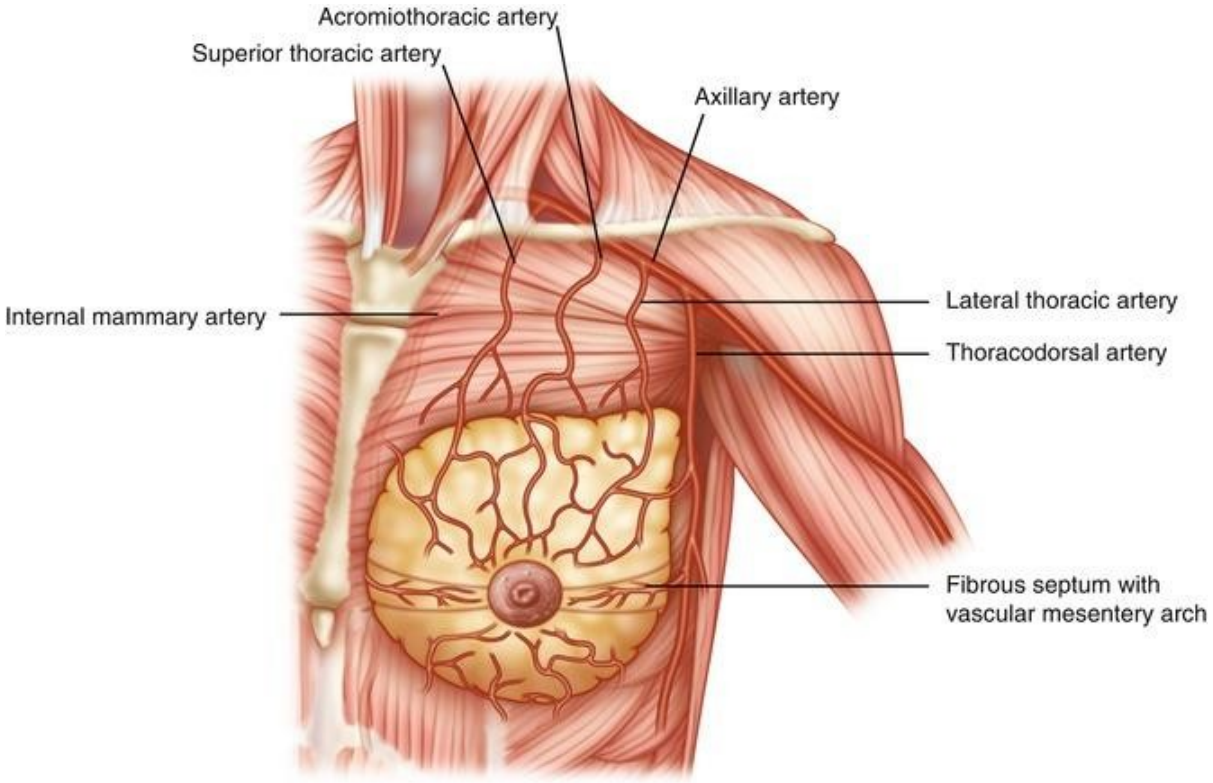


Figure 2: Blood supply of Breast, pic courtesy: Grays Textbook of Anatomy.

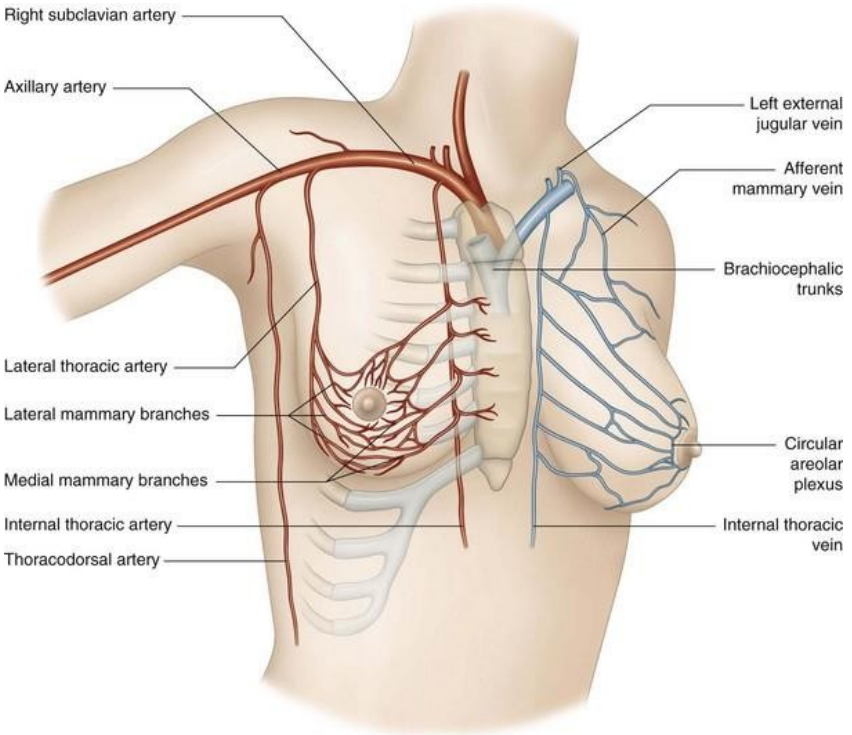


Figure 3: Anterior view of the blood supply

AXILLA:

1. The axilla is made up of the brachial plexus, axillary artery, lymph nodes, vein, fat, auxiliary breast tissue, skin, and subcutaneous glands.
2. The anatomic boundaries are as follows:
   a) Superiorly : the clavicle, scapula and first rib
   b) Posteriorly: subscapularis, teres major and latissimus dorsi muscle
   c) Anteriorly: pectoralis major and minor muscles.
   d) Medially: serratus anterior and first four ribs
   e) Lateral: coraco brachialis and short head of the biceps muscle

3. The area of lymph nodes is divided as follows:
   a) Level I: Lymph nodes lateral and inferior to the pectoralis minor muscle.
   b) Level II: Lymph nodes beneath the pectoralis minor muscle
   c) Level III: Lymph nodes deep and medial to the medial border of the pectoralis minor muscle.

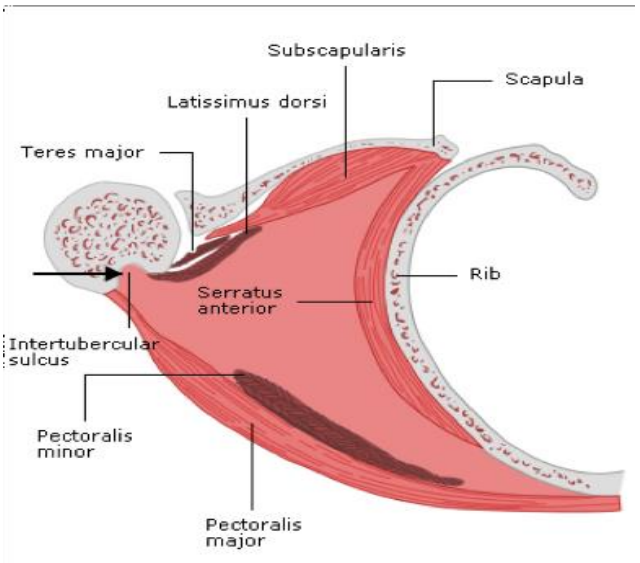


Figure 4: Anatomy of Axilla[38].

LYMPHATIC DRAINAGE:

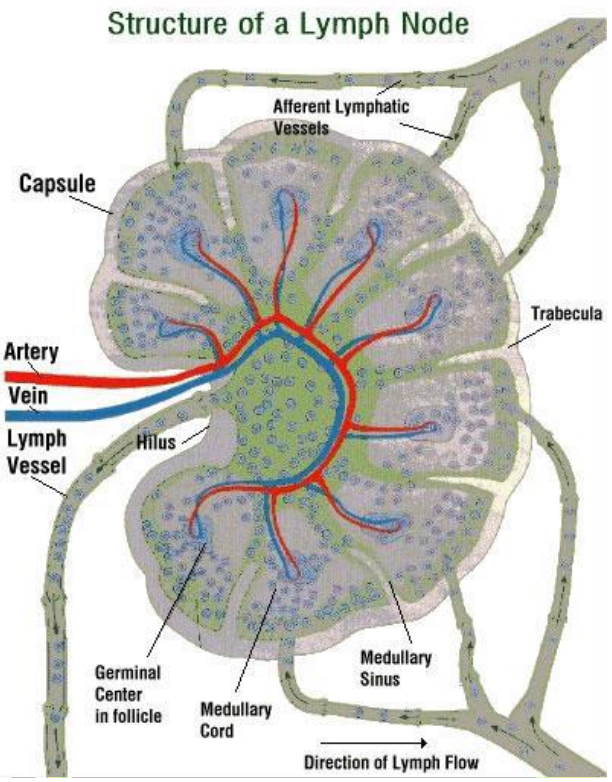


Figure 5: Structure of Lymph node

A typical lymph node is divided into compartments called lymph nodules and is encased in a connective tissue capsule. The lymph nodules are lymphocyte and macrophage-rich lumps that are divided by lymph sinuses. The afferent lymphatics, which bring lymph into the node and enter the node on the convex side, enter the node at various points along its periphery. The lymph travels through the lymph sinuses and then enters an efferent lymphatic channel at the hilum, which is an indentation in the lymph node's surface.

Draining interstitial fluid from tiny lymphatic capillaries with a single layer of endothelial cells is the lymphatic system's main job. Lymph flow is produced by the contraction of smooth muscle and an osmotic pressure gradient. Pre-collectors with valves that help route the lymph that then drains into afferent lymphatic arteries on the cortex of the lymph nodes are

where lymphatic capillaries empty into. Lymph nodes are found all across the lymphatic system and serve as filters. Efferent lymphatic arteries that drain into major collecting vessels, which in turn eventually reach the thoracic duct or the right lymph duct, transmit the lymph out of the node from the hilum before it enters the venous circulation via a jugular anastomosis[39].

Except for the nipple and areola, the skin above the breast is drained by the superficial lymphatics. The lymphatics go radially to the lymph nodes nearby (axillary; internal mammary; supraclavicular and cephalic). The parenchyma of the breast is where the deep lymphatics exit. They also drain the areola and nipple. There can be variations in the lymphatic drainage of the breast. The walls of the lactiferous duct, sub areolar area, and interlobular connective tissue all contain connecting lymphatic plexus. On the adjacent deep fascia, there is a plexus of tiny arteries as well, although it has little impact on either normal lymphatic drainage or the early dissemination of cancer. When other routes are blocked, it provides an alternative for drainage.

The nodes are described in reference to the pectoralis minor from a surgical perspective. "The nodes behind the pectoralis minor muscle are the intermediate group (level II), the nodes below the muscle are the low nodes (level I), and the nodes between the pectoralis minor and collarbone are the higher or apical nodes (level III)".

The lymph nodes from the breast drain into the following lymph nodes:

1. Axillary lymph nodes:

    Anterior or pectoral group

    Posterior

    Lateral

    Central

    Apical

2. The internal mammary or parasternal nodes: lie along the thoracic vessels.

Among the axillary nodes, the lymphatics end mostly in the anterior group and partly in the posterior and apical groups. Lymph from the anterior and posterior group passes to the central and lateral groups and through them to the apical group, finally reaches to supraclavicular nodes. 75% of the lymph nodes from the breast drains into axillary nodes, 20% into the internal mammary nodes and 5% into the posterior intercostal nodes.

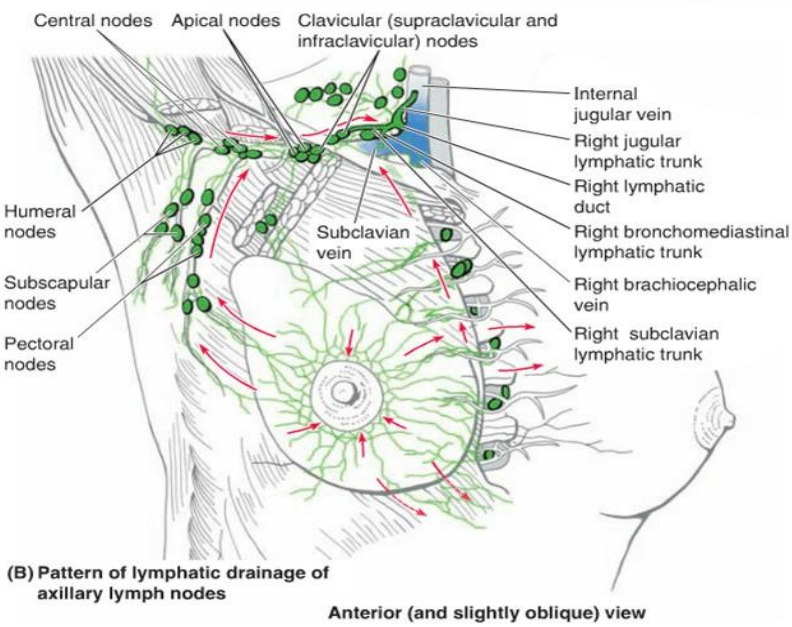


Figure 6: Anterior view of the lymph drainage of the breast

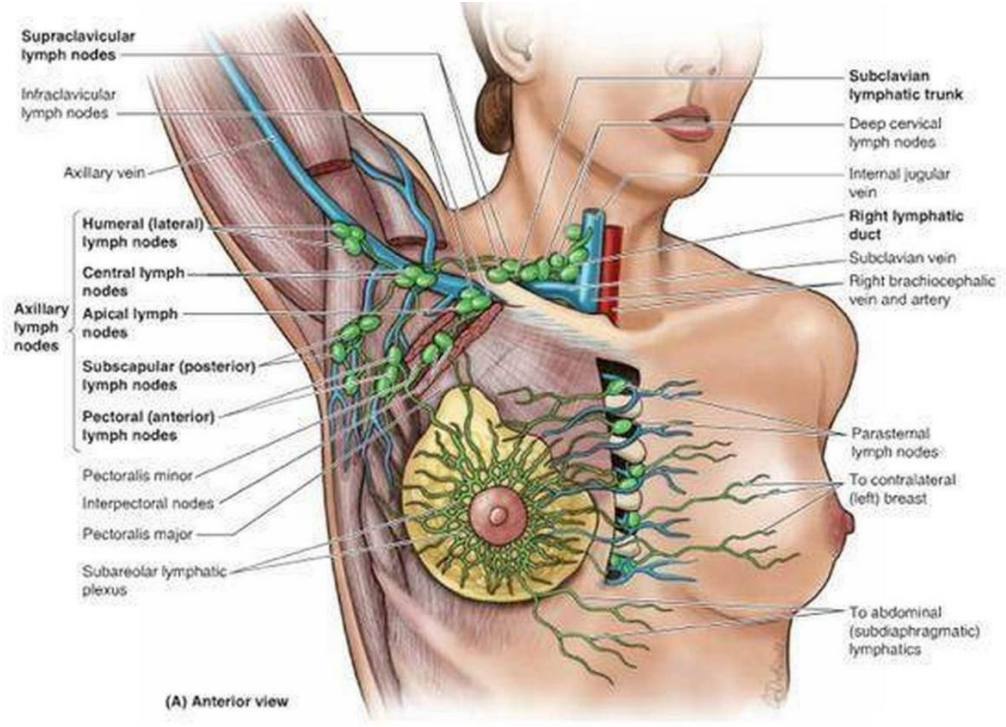


Figure 7: Lymphatic drainage of the Breast[40].

SUBAREOLAR PLEXUS OF SAPPEY:

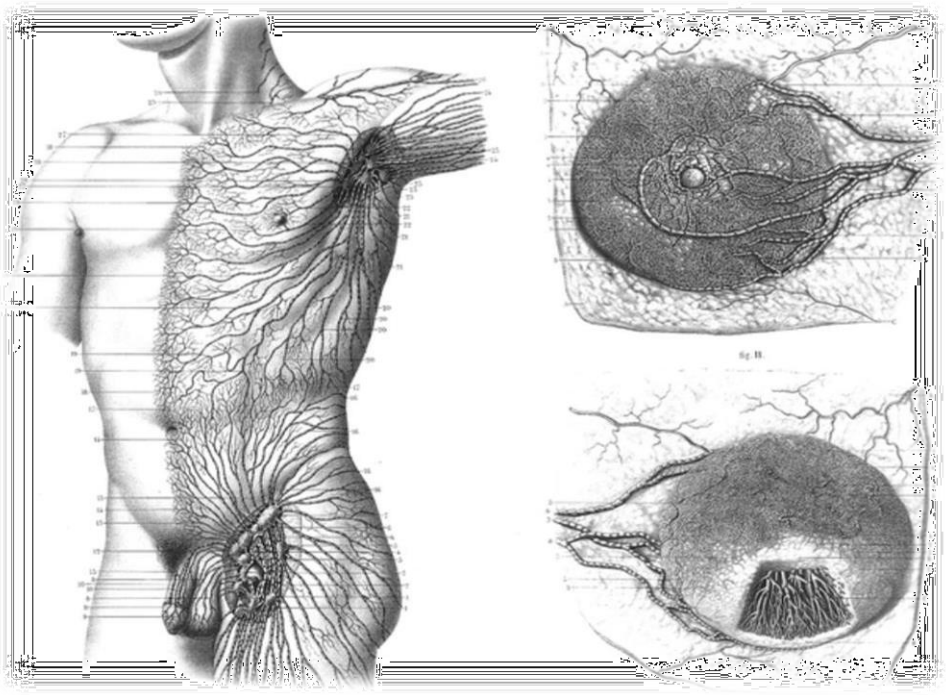


Figure 8: Sappey drawing of the superficial lymphatic of the upper torso (left) and female breast(right)[41].

A plexus of lymph vessels is present deep to the areola. This is the sub areolar plexus of sappey. Sub areolar plexus and most of the lymph from the breast drain into the anterioror pectoral group of lymph nodes. At the end of the eighteenth century, Sappey attempted to decipher the complicated lymphatic system of the breast by injecting mercury. Later, surgeons and nuclear medicine specialists used vital dyes and radioactive isotopes to visualize the lymphatic system[40].

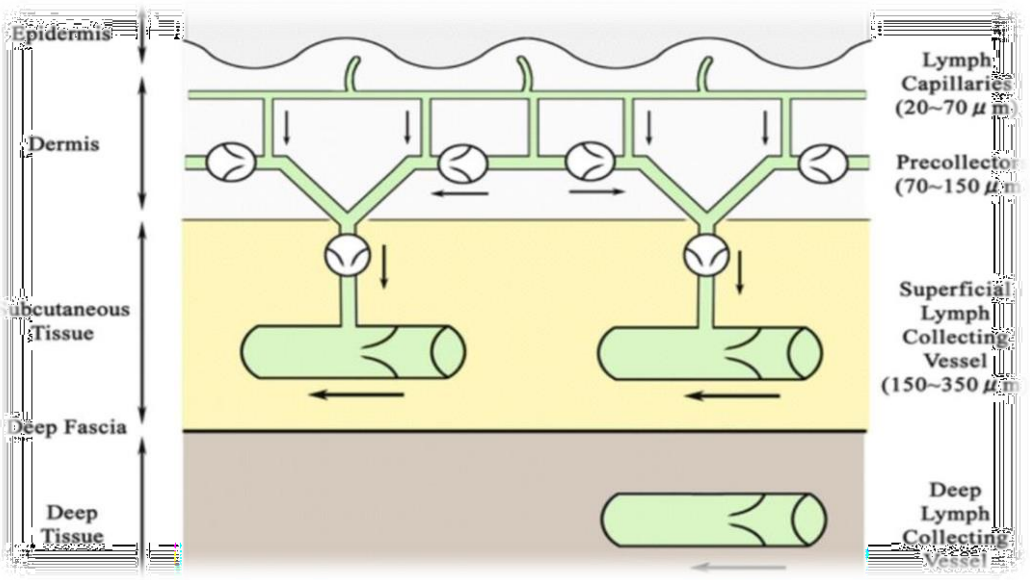


Figure 9: Relationship between lymph capillaries, pre collectors and lymph collecting vessels[41].

Mechanism of metastasis to lymph node:

Kawada and Taketo outlined the process through which lymph nodes are altered by metastasis[42]. The term "macro-metastasis" was used to denote such metastasis when the metastatic node measured well over 2 mm on the histological section[43]. Nodes that ranged in size from 0.2 mm to 2 mm were categorized as "micro-metastatic" nodes. "Isolated tumor cells" are defined as tumor cells that are smaller than 0.2 mm in size[44]. Micro-metastasis and isolated tumor cells have little impact on disease-free survival and do not raise the likelihood of loco-regional recurrence. However, the likelihood of a local recurrence is increased by macro-metastasis in the lymph node. Therefore, it's crucial to find the lymph node that has a macro-metastasis. Angiogenesis is shown by Kahlert et al.[45] to be essential for the development of tumors in both humans and animals. Figure 10 illustrates the angiogenesis process in detail.

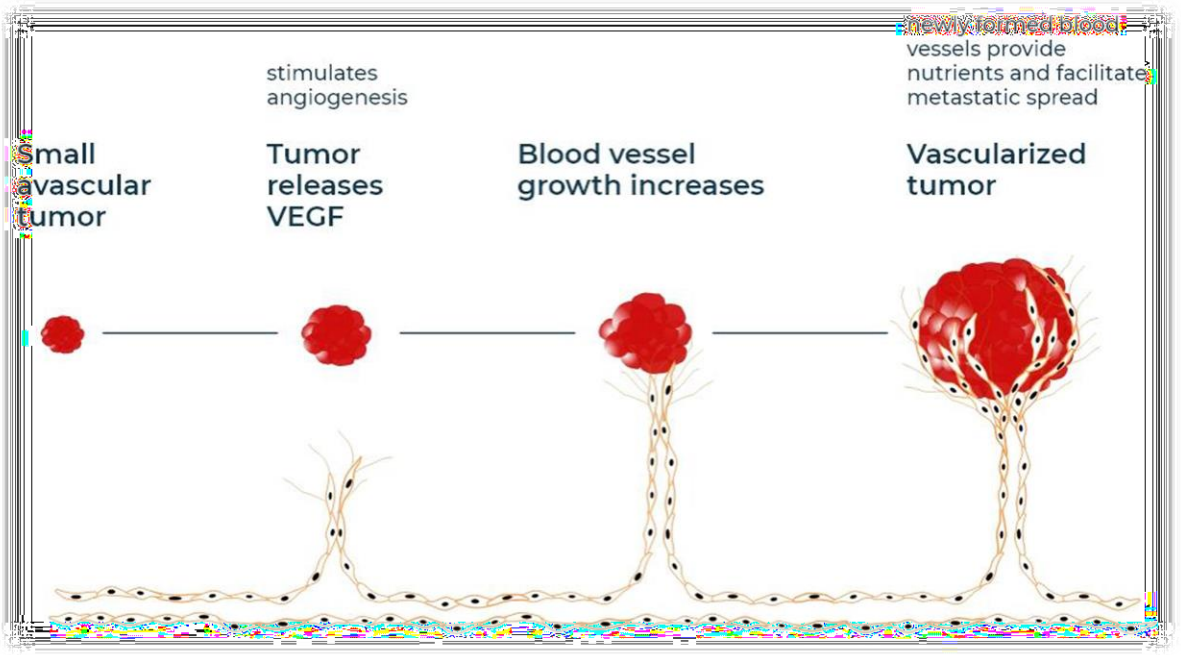


Figure 10: Process of angiogenesis

Vascular endothelial growth factors (VEGF family) are secreted by tumor cells once they acquire a diameter of 0.2-2 mm (VEGF-A, B, C and D)[46]. Neo-vascularization is correlated to the expansion and development of cancer cells inside a sentinel lymph node. The lymph node containing tumor cells larger than 1 mm in diameter has more blood flowing to it due to this neo-vasculature of blood vessels [47, 48]. Doppler ultrasonography flowmetry can identify this increased blood flow[49, 50].

Surgery-based axillary lymph node group classification

1. Axillary vein group
2. External mammary group
3. Scapular group
4. Central group
5. Sub clavicular group
6. Interpectoral or Rotters group

The link between the ALN and the pectoralis minor muscle is another way that surgeons describe them.

- Level I- Lymph nodes located lateral or below the lower border of the pectoralis minor muscle and include external mammary, scapular and axillary vein nodal groups.
- Level II- lymph nodes deep or posterios to the pectoralis minor and include central and some of the subclavicular lymph node group.
- Level III – Lymph nodes medial or superior to the upper border of the pectoralis minor and include the subclavicular lymph node group.

Tumor Classification

American Joint Committee on Cancer Staging Primary Tumors (T):

T_X: Primary tumour cannot be assessed.

T_O: No evidence of primary tumour.

T_{is}: carcinoma in situ (DCIS; LCIS; PAGET).

T_1: Tumour < / = 20 mm in greatest dimension.

T_1m_1: Tumour </= 1mm in greatest dimension.

T_1a: Tumour >1 mm but < / = 5mm in greatest dimension.

T_1b: Tumour >5 mm but < / = 10mm in greatest dimension.

T_1c: Tumour > 10mm but < / = 20 mm in greatest dimension.

T_2: Tumour > / = 20 mm but < / = 50mm in greatest dimension.

T_3: Tumour > 50 mm in greatest dimension.

T_4: Tumour of any size with direct extension to the chest wall / and or skin. (Ulceration or skin nodules)

T_4a: Extension to the chest wall not including only pectoralis muscle adherence/ invasion.

T_4b: Ulceration / ipsilateral satellite nodule/oedema of skin.

T_4c: a + b.

T_4d: Inflammatory carcinoma.

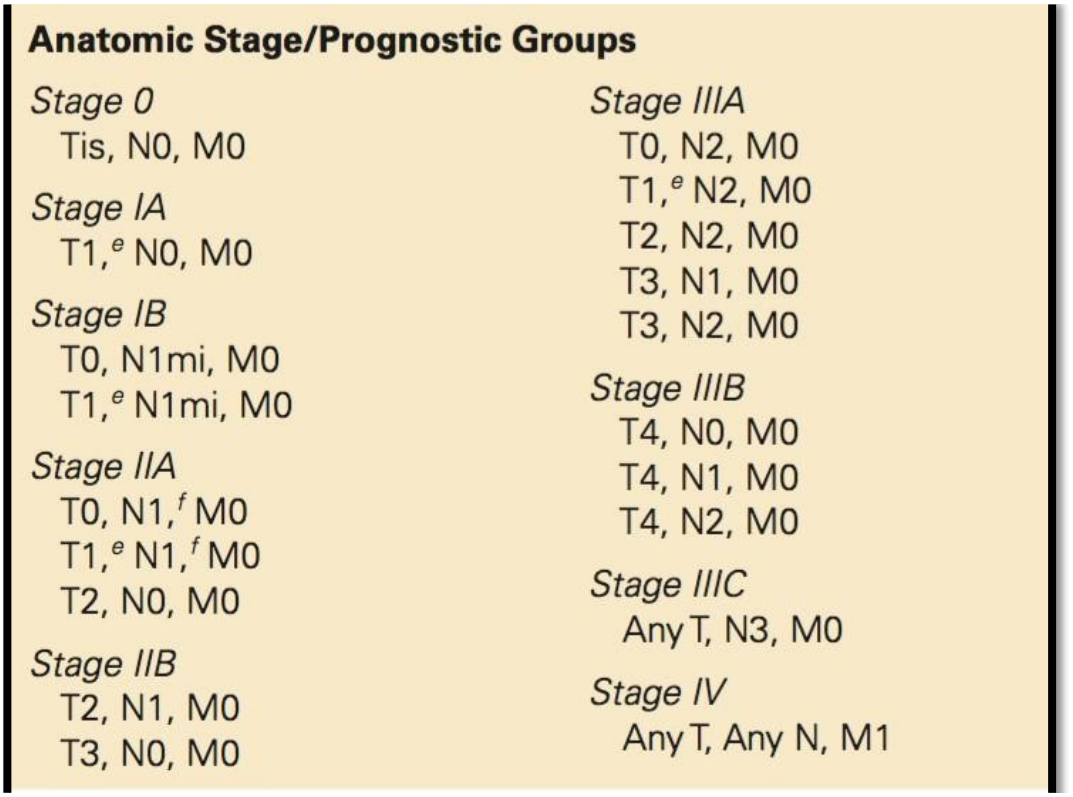
Regional Lymph Nodes (N):

- N_x: Regional lymph nodes cannot be assessed. (Previously removed).
- N_o: No regional lymph node metastasis.
- N_1: Metastasis to movable ipsilateral Level I and II axillary lymph nodes.
- N_2: Metastasis in ipsilateral level I and II axillary lymph nodes that are clinically fixed or matted or in clinically detected ipsilateral internal mammary nodes in absence of clinically evident lymph node metastases.
- N_3: Metastases in ipsilateral infraclavicular (level III) axillary lymph nodes with or without level I and level II axillary lymph node involvement; or in clinically detected ipsilateral internal mammary lymph nodes with clinically evident level I and level II axillary lymph node metastases or metastases in ipsilateral supraclavicular lymph node (s)with/without axillary/ internal mammary lymph node involvement.

Distant Metastasis (M):

- M0: no clinical/radiological evidence of distant metastases.
- cM0(it): No clinical/ radiographic evidence of distant metastases but deposits of molecularly / microscopically detected tumour in circulating blood; bone marrow / other non-regional nodal tissue that are no greater than 0.2 mm in a patient without symptoms/ signs of metastases.
- M_1: Distant detectable metastases as determined by classic clinical/ radiographic means/ and or histologically proven > 0.2 mm.

Staging of Carcinoma Breast:



Anatomic Stage/Prognostic Groups

Stage 0
Tis, N0, M0

Stage IA
T1,[e] N0, M0

Stage IB
T0, N1mi, M0
T1,[e] N1mi, M0

Stage IIA
T0, N1,[f] M0
T1,[e] N1,[f] M0
T2, N0, M0

Stage IIB
T2, N1, M0
T3, N0, M0

Stage IIIA
T0, N2, M0
T1,[e] N2, M0
T2, N2, M0
T3, N1, M0
T3, N2, M0

Stage IIIB
T4, N0, M0
T4, N1, M0
T4, N2, M0

Stage IIIC
Any T, N3, M0

Stage IV
Any T, Any N, M1

Figure 11: Staging of Carcinoma Breast[51].

Screening program:

EBC constitutes only 30 % of breast cancer in India vs 60-70 % of the cases inthe developed world. Most of the women (70 %) present in the advanced stage; this is brought on by ignorance, a lack of access to healthcare resources, and societal and cultural norms. Mammography, clinical breast examination, and breast self-examination are the three most often utilized methods for breast cancer screening on a global scale. In developing countries like ours where mammography and clinical breast examination can be expensive, BSE is the most reasonable and feasible for early breast cancer detection. In India, the involvement of ASHA has increased compliance towards screening for breast cancer in asymptomatic women in rural communities. The training of women through the trained ASHA is similar to a nuclear

chain reaction that produces employees who are taught from house to home without any additional financial strain. Because of various programs organized at the PHC level, ASHA and various breast clinics in the urban setup, there is an increased awareness of breast carcinoma screening in Indian women.

Triple assessment test:

It is a combination of three tests that is palpation, radiological imaging, and percutaneous biopsy. Thoroughly performed pre-surgery diagnostics are essential for all disease management decisions. In our study of patients with carcinoma of the mammary gland, axillary lymph node status was of paramount importance as a prognostic factor and predictor of survival and for our inclusion criteria. In our study the diagnostic modality used was BSE; breast and axillary ultrasound and tumor cytology. The sensitivity of this radiological diagnosis of breast cancer grows as you become older as the breasts get less dense. 5% of breast cancer is missed on screening mammography.

In a research conducted by V. Dialini et al. [38], the author found that while clinical palpation alone has a low sensitivity of 31.6%, it can boost sensitivity by a statistically significant amount (P 0.001) when paired with axillary ultrasound. Using combined diagnostic modalities, specificity remained high at 91.6% and NPV rose from 63.4% to 73%[38].

A useful tool for nodal staging is ultrasound. According to Vaidya et al., the combination of clinical palpation and axillary ultrasonography increased the sensitivity of detecting axillary lymph nodes from 70% to 82%. However, the technical expertise and experience of the operator have a significant impact on accuracy.[52]

The examination of breast illness, whether it is asymptomatic and only suspected after screening mammography or manifests symptomatically in the patient, is increasingly using core needle biopsy (CNB). The procedure's outcome typically yields a conclusive diagnosis or

at the very least information that may be utilized to plan the patient's future care. Estrogen and progesterone receptors and HER2 can be reported[53]. Although FNAC is the least intrusive method for getting a cellular diagnosis, false negatives can occasionally occur, primarily due to sampling error, and invasive cancer cannot be differentiated from in situ illness.

Biomarkers:

ER, PR, HER2, and Ki-67 protein gene products are commonly analyzed by immunohistochemistry (IHC) analysis in carcinoma of the mammary gland and contribute as substantial prognostic but also predictive indicators that aid in predicting therapy sensitivity. Estrogen receptor (ER) exists in two main forms, ERα and ERβ. The current IHC measurements detect only ERα. Similarly, progesterone (PR) exists in two forms, dubbed PRA and PRB. Currently used IHC assays detect both these forms of PR[54]. The main clinical application of steroid hormone receptors i.e., ERα and PR is in selectingpatients with invasive breast cancer for treatment with endocrine therapy. As predictive markers for endocrine therapy, ER and PR are used in the neoadjuvant, adjuvant and advanced disease settings[54].

The HER2 gene, which encodes the growth factor receptor HER2, is amplified and over-expressed in 25 to 30 per cent of breast cancers, increasing the aggressiveness of the tumor[55]. Ki-67, which is expressed in the cell nucleus during the S, G1, G2, and M phases of the cell cycle, is connected to cellular proliferation. An immunohistochemistry method is used to find Ki67. Throughout the cell cycle, ki-67 expression varies and peaks during mitosis. Chromosome 10q25-ter contains the MKI67 gene, which codes for the protein Ki-67. It was discovered that estrogen receptor-negative cells in normal breast tissue have low levels of Ki-67 expression. The proliferation of cancerous cells may be evaluated using the monoclonal antibody Ki-67 immunostaining[56].

Axillary Surgery:

A thorough axillary dissection, sentinel lymph node biopsy, sampling, removal of nodes beyond and lateral to the pectoralis minor, and other procedures are among the alternatives that can be utilized to treat the axilla (level III). ALND has been the standard approach for patients with axillary metastases. Axillary nodes are the most typical first location of tumor dissemination in breast cancer. Numerous aspects of the underlying tumor, such as tumor size and clinical stage, are correlated with the prevalence of axillary involvement at the time of diagnosis.[57].

Since the development of the modified radical mastectomy, axillary lymph node dissection (ALND) levels I, II, and III have been the accepted axillary nodal staging techniques in breast cancer. To prevent misclassification, it is advised that the axillary lymph node yield with the proper surgical technique be at least 10 lymph nodes. According to a big Danish study, erroneous nodal staging with considerably greater recurrence rates and worse survival outcomes occurred if fewer than 10 negative lymph nodes were recovered.[58]

Sentinel Lymph Node Biopsy:

Sentinel Node

The first lymph node to drain the breast region that has a tumor is known as the sentinel lymph node[19]. A minimally invasive procedure known as sentinel node biopsy is used to identify individuals with occult lymph node metastases who may benefit from further local or systemic treatment. Sentinel node biopsy is founded on two fundamental ideas: that a first lymph node serves as an efficient filter for tumor cells and that there exists an ordered and predictable pattern of lymphatic outflow to a regional lymph node basin. In the randomized Axillary Lymphatic Mapping Against Nodal Axillary Clearance trial, the absolute incidence of

lymphedema in the sentinel node biopsy group was 5% at 12 months, with an RR of 0.37 (95% CI = 0.23 to 0.60) compared with the axillary clearancegroup in an intention-to-treat analysis[51].

Sentinel node biopsy is an option for the majority of individuals with stage I and stage II cancer. Pregnancy, nursing, and locally advanced breast cancer (LABC) are among the conditions that make the operation contraindicated. Because lymph nodes with a significant tumor load may not absorb the mapping agent, care should be taken to remove any palpably aberrant nodes intraoperatively. Without pathologic proof, direct dissection should be avoided since physical examinations have quite a false-positive probability of between 40% and 60%. Lymphatic mapping was formerly believed to be contraindicated in multi-centric malignancies and T3 primary tumors, however, investigations have demonstrated that SLN biopsy is reliable in these situations.

The selection and dose of the blue dye and radioactive tracer, the particle size and type of the radioactive tracer's carrier protein, where in the breast to inject them and how deeply, as well as a number of technical issues, all have an effect on the identification rate (IR) and false negative rates (FNR) when performing SLNB. In most circumstances, dual mapping using blue dye and radiolabeled colloid delivers the greatest sensitivity for the detection of nodal metastases[59]. Patent Blue V, isosulphan blue, and methylene blue are the most extensively used blue dyes. Blue dye is not recommended for pregnant women since that can cause allergy in 1% of procedures[60]

Technetium -99, a radioactive tracer with a half-life of six hours, is the mainstream technology. The radioactive tracer's drainage further into the lymphatic system after injection and where it travels in the lymphatics are both impacted by the particle size of the carrier protein. On the day of surgery or the day before, the radioactive tracer is injected into the breast. When administering an injection the day before surgery, a greater dosage is necessary. When

the patient is anaesthetized and carefully watched, the blue dye is administered in the operating room. A portable gamma probe is used by the surgeon during surgery to check for radiation in the axillary area[61].

The pathology department receives the SLNs directly to do a frozen section histopathological study. If there are metastases in the SLNs, the surgeon can continue with ALND during the same procedure thanks to intraoperative analysis. The most used technique is frozen section analysis, which has an average sensitivity of about 75%. SLNs are bisected along the longitudinal axis, and 2-mm-thick multiple sections are taken and stained with hematoxylin and eosin (H & E). On average, 12–14 sections are made depending on the size of the lymph node. Larger nodes are sectioned at three different levels. Two oncopathologists examine the sections independently. The entire procedure takes 15–20 min and prolongs surgery. The remaining frozen tissues are fixed in formalin, embedded in paraffin, and sectioned. The SLNs are later diagnosed usingpermanent sections and H & E staining. Other dissected non-SLNs are also examinedusing the same methods.

Lymphedema:

Following breast cancer therapy, lymphedema can be an irreversible and enduring illness with severe detrimental and negative effects on quality of life. The buildup of fluid in the interstitial tissues as a result of the lymphatic system's inadequate and impaired ability to transfer lymph fluid culminates in lymphedema, a persistent long-term swelling. Due to improvements and advances in medical care and surgical techniques like sentinel lymph node biopsy (SLNB), incidence rates for lymphedema in patients treated for breast cancer have significantly decreased, with a 5-8% incidence in patients who undergo SLNB and a 14-16% incidence in patients who undergo axillary lymph node dissection (ALND), including only levels I and II, patients[62].

The total incidence of lymphedema at the operative location was 41.1%, or 95 individuals, in research by Pramod et al.[63] from January 2004 to December 2007 in 231 patients who had axillary lymph node dissection at Amrita hospital in India. He concluded that the impact of lymphedema on patients, physical and psychological help can be enormous, long-lasting, and often permanent sequela to their quality of life[63].

Post-operative radiotherapy increases the risk of and aggravates lymphedema, although some study does not agree with it. **Edward et al**., studied the incidence of lymphedema after breast cancer treatment by volumetric method and subjective assessment of swelling and found no significant relationship between axillary irradiation and lymphedema[64.]

In the ALMANAC trial conducted from 1999 to 2003, in various institutions 1031 patients were randomly assigned to undergo SLNB (515 patients) or standard axillary surgery (516 patients). According to the institution's policy, patients with SLN metastases should undergo either axillary clearing or should receive axillary radiation. The relative risk of lymphedema and sensory loss for the SLNB group compared to the axillary surgery group were 0.37 and 0.37 respectively. Overall patients recorded quality of life and arm function scoring were statistically significantly better in the SLNB group throughout. SLNB should be the therapy of choice for patients with early-stage breast cancer and clinically negative nodes, according to the trial's findings, as it is associated with a higher quality of life than traditional axillary treatment[65].

Radiotherapy:

For patients with invasive breast cancer treated with breast-conserving surgery adjuvant radiation to the breast reduces the probability of a breast recurrence and improves outcome. For T1N0 or T2N0 patients, mastectomy and SLN dissection provide effective control. Patients with stage III breast cancer have high rates of loco-regional recurrence after treatment with MRM and adjuvant or neoadjuvant chemotherapy. Various studies have shown that post-mastectomy radiation significantly improves the outcome of the patient. Radiotherapy to the chest wall after mastectomy is indicated in patients with very huge tumors; and those with a large number of positive nodes or extensive lympho-vascular invasion.

Hormone Therapy:

Tamoxifen is widely used as a hormonal treatment for carcinoma breasts. Tamoxifen appears to have positive benefits in lowering the risk of tumors in the contralateral breast (IBIS-I and BSABP-P1 studies). At least five years of endocrine therapy, either 20 mg of tamoxifen per day if the patient is premenopausal or the AIS (anastrozole, letrozole, and exemestane) if the patient is postmenopausal, will be beneficial for hormone receptor-positive women.

Chemotherapy:

Chemotherapy using a first-generation regimen such as 6 monthly cycles of CMF achieves a 25% reduction or relapse over a period of 10-15 yrs. Neoadjuvant chemotherapy is being used in many centers for large but operable tumors that would traditionally require a mastectomy. Reduced tumor volume will make breast-conserving surgery possible. Herceptin will be administered to patients who test positive for Her2neu as part of their therapy, which will aid in achieving a high response rate. In triple-negative patients, neoadjuvant

chemotherapy is also employed (ER, PR, Her2neu).

Lymphatic mapping techniques for sentinel node biopsy

Due to the fast expansion of sentinel node biopsy and lymphatic mapping, surgical organizations have produced a number of modifications, and many technological elements are developing, including :

- Choice of mapping label
- Radioisotope quantity and processing
- Injection site
- Timing of injection
- The use of preoperative lymphoscintigraphy
- Technique
- Radioisotope
- Blue dye
- Both radioisotope and blue dye
-

In 1993, Krag and colleagues, utilising technetium-99 m Sulphur colloid and a portable gamma probe, published the first description of the use of radioisotope alone for breast cancer[66]. With a false negative rate of 11%, the sentinel node identification rate was 98%. The most common isotope for lymphatic mapping in the United States is technetium-99 m Sulphur colloid. Technetium-99 m colloidal albumin is employed in Europe. The dosage is between - 0.1 and 4 mCi.

Blue dye:

Numerous studies on melanoma included the dye isosulphan blue (also known as Lymphazurin I%). Giuliano and colleagues reported using isosulphan blue dye as the only agent in SLNB for breast carcinoma[67]. The possibility of potentially fatal allergy and anaphylactic responses is a drawback. The rate of allergic reactions is reported to be between 1% and 3%. They consist of anaphylaxis, urticaria, rash, pruritus, and hypotension. Isosulphan blue dye is generally utilized because it produces good lymphatic mapping findings in patients with breast carcinoma.

Lymphatic mapping for breast cancer has also proved successful using methylene blue dye. Methylene blue was employed by Simmons et al. to identify lymphatic mapping in breast cancer[68]. Blessing et al. evaluated the recognition rates of methylene blue and isosulphan blue in 2002 and discovered comparable results[69]. Some writers prefer methylene blue since it is less expensive and has fewer drawbacks. Subcutaneous injection of methylene blue is necessary. Severe skin responses, including necrosis and dermolysis, have been caused by accidental injections into the dermis.

Combination of blue dye and radioisotope

The sentinel node detection rate is increased by combining blue dye with a radioisotope. The effective application of prospective lymphatic mapping using blue dye and radioisotopes was initially described by Albertini et al[70]. The outcomes show that the combination increases the rate of SLN recognition, and dual agent lymphatic mapping has gained widespread acceptance[71]. Given the potentially fatal allergic responses caused by the isosulphan blue dye, several centers have chosen to rely only on radioisotope mapping.

The injection site for mapping agents

1. Peritumoural injection.
2. Sub areolar and dermal injection.

1. Peritumoural injection

The intramammary lymphatic channel that metastases have travelled through is mimicked by peritumoural injection. The peritumoural injection was employed in the early data about sentinel node biopsies. This procedure has proven to be challenging and time-consuming for patients with non-palpable tumors since it calls for the employment of additional imaging modalities to direct the peri-tumoral injection of radioisotopes. Additionally, there is a larger risk of shine-through with peri-tumor injections, wherein leftover radioactivity from the injection site generates false-positive background activity picked up by the gamma probe in the axilla. These factors have led to the testing of other injection locations.

2. Sub areolar and dermal injection

The sub-areolar plexus of Sappey is where the majority of breast tissue lymphatics go before continuing on to the axillary basin. Sub areolar and dermal injection can be used to study non-palpable and multi-centric tumors without the shine-through effect. Blue dye injections into the sub areolar and dermis may significantly discolor the skin and may linger for months. Up to 10% of breast malignancies show non-axillary drainage of lymph with SLN located in the internal mammary or supraclavicular nodal basins. As a result, not all breast tumors may drain in the same manner as the surrounding skin and nipple regions[72].

Preoperative lymphoscintigraphy

A pre-operative lymphoscintigram is frequently given to patients undergoing radioisotope lymphatic mapping to help identify sentinel lymph nodes (SLNs). Anterior and lateral views, as well as precise patient placement, are included in the pre-operative lymphoscintigram to maximize transit time and drainage[73]. After 20 minutes of scanning, the patient is brought to the operating room for SLNB. Images are repeated until the SLN basin is identified. Pre-operative lymphoscintigram was examined by McMasters and colleagues[74] in the context of breast cancer. Of the 588 patients in the research, 348 had pre-operative lymphoscintigrams, whereas the remaining 240 did not. Of the 240 individuals who did not get preoperative scanning, the SLN was found in 221 of them. The false-negative rate in these individuals was 1.6%. The authors discovered no discernible difference between patients undergoing PL and those who underwent surgery without scanning in terms of the SLN detection rate, false-negative rate, or quantity of SLNs removed.

Borgstein and associates looked at the function of PL in people with breast cancer. Even when delayed pictures were produced, the scientists discovered that the intraoperative gamma probe was more sensitive than the PL in finding radioactive nodes in the axilla. Preoperative lymphoscintigraphy may not be able to increase the accuracy of SLNB, and some centers have given up on the practice in favor of using the intraoperative gamma probe to identify radioactive SLNs[75].

Timing of radioisotope injection

One or two days are required to complete the radioactive lymphatic mapping technique[76]. The morning of surgery is when the isotope injection is administered, and until the SLN is found, serial imaging is performed one to several hours afterwards. The adoption of a

2-day mapping process with radioisotope injections one day before to the surgery has been made necessary due to the impact of delays on patients and the operating room. According to the most recent research, the 2-day lymphatic mapping approach is a trustworthy and safe way to identify SLNs[77].

Technique

1. Blue dye

In the operating room, the patient is prepared and covered. The surgeon administers 3-5ml of blue dye subcutaneously or intravenously. An inferior axillary incision is used to access the axillary fascia. Lymphatic pathways leading to the blue-stained node are carefully examined. The nodes at the end of the blue lymphatic channel and all other nodes are eliminated. Because the dye transit time is quick and blue staining of distant, non-sentinel axillary LNs is not unusual, it is important to identify the blue node and the blue node closest to the tumor in the axilla. The most frequent technical error is failing to recognize the node at the end of the blue lymphatic channel as a sentinel node, regardless of whether the node itself looks blue[78].

2. Radioactive colloid

Colloids with technetium-99m labelling typically have particle sizes between 20 and 100 nm and injection volumes between 0.2 and 1.0 mL. To achieve a significant radio-colloid absorption in the lymph node and a high target signal intensity, the amount of radioactivity must be calculated in accordance with the interval between radio-colloid injection and the surgical procedure[79].

A probe with a sensitivity of 10 cps/kBq will be required, along with an activity of 150–250 megabecquerels (MBq), if surgery is scheduled for 24 hours following the injection. The quantity of radioactivity required is halved with a nuclide half-life of 6 hours (Technetium-99) and an injection period of 6 hours prior to SLNB. 10 to 50 MBq are enough for 1-day

treatments. Tracers can be administered intravenously, intratumorally, topically, or sub areolar into the peri tumoral tissue. Three hours after the tracer injection, the patient is lying on the gamma-camera bed for imaging. Using a twin-head gamma camera with low energy, high-resolution collimator, anterior and 45° anterior oblique static scintigraphy pictures are generated. Any alleged sentinel node's location can be examined. For the purpose of making a final choice for additional loco-regional and systemic therapy, the postoperative examination offers the SLN's final histopathologic state.

The SLN must be regarded as the first "hot spot" shown on imaging. Using the portable gamma probe and the skin mark as guidance, the surgeon will identify the lymph node with the greatest radioactivity during the procedure. Sentinel nodes are all nodes that have radioactive counts greater than 10 times the background count as determined by a portable gamma probe in the ante cubital fossa[80]. Any other similar lymph nodes should be removed as well. Any lymph node excised should be rechecked by the probe to confirm that it is radioactive before submitting it for histological investigation[81].

3. Intraoperative evaluation

At the beginning of the SLN era, intraoperative evaluation was simply performed to assess the nodal tissue because the tissue removed might not represent a legitimate lymph node[82]. Only when it has a direct impact on immediate treatment is an intraoperative assessment performed. Frozen sections and imprints have both shown to be quite effective at finding metastases within the operation[83].

4. Frozen section

Each SLN is divided into pieces at an interval of 0.2 cm. Each resulting 0.2 cm SLN tissue slice is processed to provide at least three distinct layers of tissue for frozen section

analysis, with the most superficial 25% of thickness being used. The usual Hematoxylin and Eosin (H & E) staining is next applied by hand to these frozen sections. The residual tissue from each 0.2 cm SLN tissue segment that results, corresponding to 75% of that tissue's thickness, is subsequently submitted for standard processing[84].

5. Imprint cytology

Depending on which institution is providing the assessment, the removed lymph nodes that we're submitted for intraoperative examination are analyzed using one of two procedures. The SLN is divided using the first technique along its long axis. The hilum and the marginal sinus are preferably included in the cross-sections of the maximal diameter, which are carefully obtained. By gently contacting the sliced surface of the SLN to a glass slide, two impressions are created for each half of the lymph node. Each pair's imprints are air-dried before being dyed with Diff-Quik. Hematoxylin and eosin are used to stain the second impression from each surface after it has been instantly fixed in 95% ethanol for three minutes (H & E). The second approach involves cutting the SLN into 4-mm slices, making impressions of each cut surface, letting them air dry, and then staining them with Diff-Quik[85].

6. Post-operative examination

The thoroughness and breadth of the SLN histopathologic work-up should depend on how important the findings are for making subsequent treatment decisions. The clinical ramifications of a false-negative staging due to undiagnosed micro-metastasis and the prognostic importance of micro-metastasis, as well as their predictive function for the involvement of non-SLNs, are still up for discussion. However, a thorough search for all micro-metastases is not warranted given the clinical consequences of SLNs' participation in micro-

metastases. Undoubtedly, during a thorough pathologic investigation, every macro-metastasis should be found. A single lymph node embedding and uniform distribution of the histologically investigated levels. To do this, 3 mm thick SLN slices may be produced for step sectioning and macroscopic inspection. Slices should be checked at regular, 500um intervals in cases of negative macroscopic results (maximum, six steps). With this method, there is a 100% potential detection rate for macro-metastasis[86].

7. Immunohistochemistry (IHC)

The use of cytokeratin may facilitate the discovery of anomalies. It is controversial whether or not immunohistochemistry should be used to assess SLNs. If the objective is to appropriately stage the illness while excluding micro-metastasis, IHC should be carried out.

8. Molecular pathology

The tumor cell has been identified utilizing RT-PCR -based molecular techniques. Analysis using RT-PCR is a very sensitive technique. Finding a particular RNA sequence for a single tumor is challenging, though. Hence, using a panel of markers may be essential. High sensitivity carries the possibility of a false-positive result, though. IHC-detected metastases had no effect on overall survival, according to the findings of the American College of Surgeons Oncology (ACOSOG) study Z0010[87]. Thus, regular IHC or PCR is not advised in the guidelines released by ASCO 2014, NCCN 3.2014, and others for the appraisal of SLNs[88].

9. Documentation of Histopathological Findings

Standardized documenting of the histologic results is required by SLNB. Each SLN must be supplied individually and with a unique identification number for histopathologic analysis. The surgeon should note the area, the order of removal, and the detecting technique (including

target count). The 7th edition of the UICC TNM classification system uses the pathologic lymph node (pN) category to describe the histopathologic findings.

Methylene Blue Dye:

Molecular Formula: C16H18CIN3S Molecular Weight: 319.851 G/MOL.

Indication in Surgery:

1. Surgical staining of lymph node
2. Staining of the fistulous tract.

It is a synthetic base dye that, when injected into the lymphatic bed of a tumor during oncological surgery, stains negatively charged cell components including nucleic acids, staining lymph nodes draining from the tumor and assisting in the visual localization of cancer SLN.

Contrindication:

Patients with severe renal insufficiency and those who have acquired hypersensitive responses to it. Because it might induce severe hemolysis and is also contraindicated in those with Heinz body anemia and G6PD deficiency.

Drug Indication:

Given that it is an MAO inhibitor, it may interact negatively with both SSRIs and MAO inhibitors to produce severe serotonin poisoning. Dapsone interaction results in the formation of hydroxyl-amine, which then oxidizes to produce hemolysis.

Adverse Effects:

When taken at therapeutic quantities (2 mg/kg), the medicine is safe, but large dosages

can be harmful. Cardiovascular arrhythmias, coronary vasoconstriction, reduced cardiac output, renal blood flow, mesenteric blood flow, increased pulmonary vascular pressure, and self-limiting pulmonary skin and mucosal discoloration are all signs of toxicity. Due to its reactive tissue characteristics, it has been reported that female patients with breast carcinoma who underwent SLNB localizing with a peritumoural injection of methylene blue dye had fat necrosis followed by dry skin gangrene.

Additionally, hemolytic anemia, which is characterized by the production of Heinz bodies, maybe the cause, particularly in individuals with severe renal insufficiency and G6PD deficiency. Urine becomes blue in color, and it may also interfere with the light emission from pulse oximeters, giving erroneously low oxygen saturation values. Due to its ability to block monoamine oxidase, methylene blue may induce potentially lethal serotonin poisoning at levels more than 5 mg/kg. It also occasionally has the potential to cause severe anaphylactic shock.

Figure 12: Methylene blue dye vial.

Fluorescein sodium Dye:

Von Baeryer first synthesized the fluorescein dye (C20H12O5) in 1871. It can be prepared from phthalic acid anhydride and resorcinol in the presence of zinc chloride.

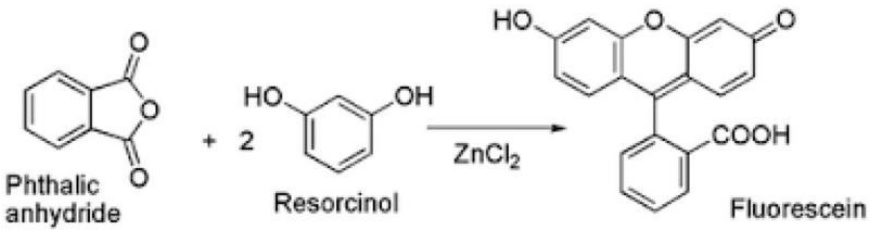


Figure 13: Synthesis of fluorescein dye.

The fluorescein reacts with the sodium salt to produce the alkaline solution of sodium fluorescein that is used in angiography (C20H10O5 Na2). Ehrlich utilized the colorant for the first time in ophthalmology and noticed it in aqueous humor. This prompted studies into the creation and movement of aqueous humor.

Properties of fluorescein[89]

Chemical properties:

Sodium fluorescein is a crystalline powder that dissolves in water and has a melting point of 315 ^{o}C and a molecular weight of 376.27. In an aqueous solution, the color ranges from dark red to yellow-green depending on the concentration. Utilizing ultraviolet light, the color may be seen at dilutions as low as 1:1000000. Around pH 7.5, fluorescence is at its best. Only dissociated fluorescence is seen. The emission is at 530 nm, whereas the ideal excitation point is at 485 nm. Blood showed a 465 nm absorption peak and a 525 nm emission peak[89].

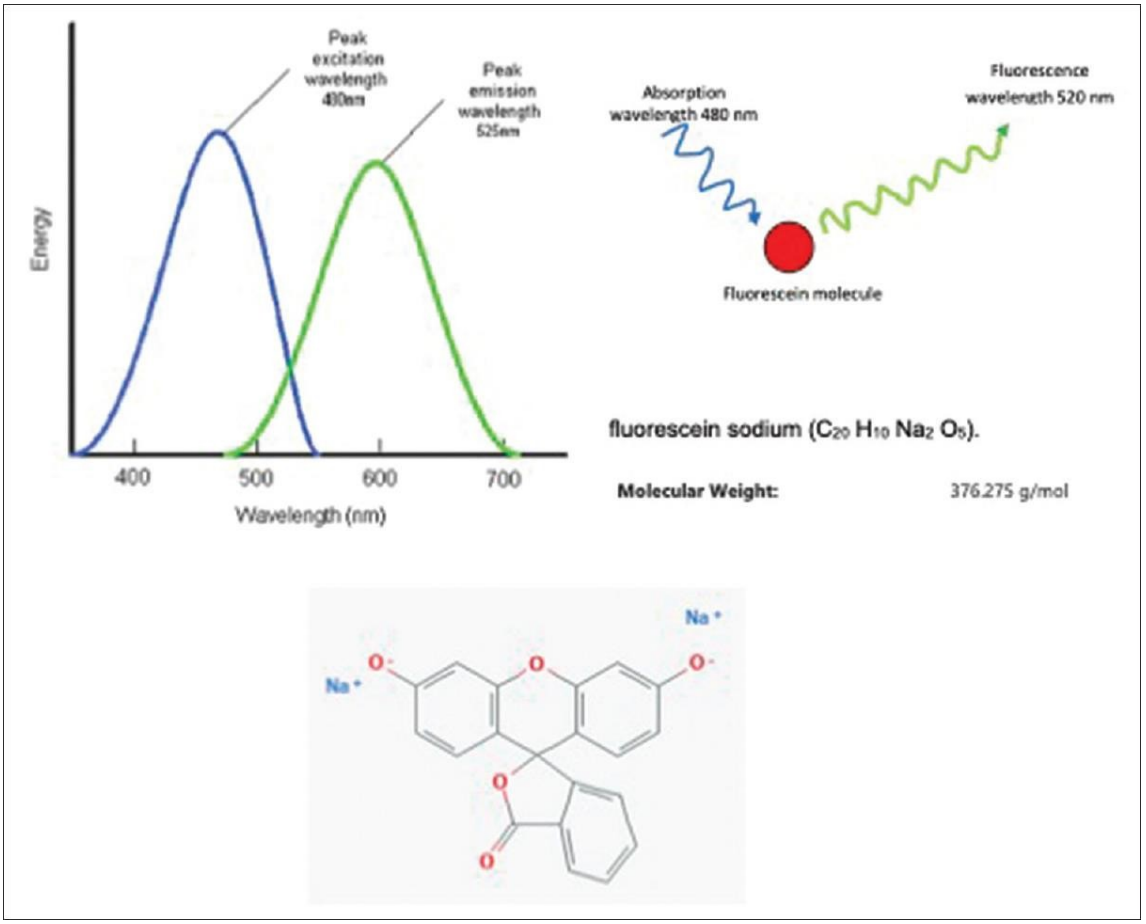


Figure 14: Fluorescence from Fluorescein excited by blue light of 480 nm wavelength (absorption maxima) emitting green fluorescence with wavelength 520 nm (emission maxima).

Figure 15: AK – Fluor 10 % Fluorescein Injection

Biochemical properties:

When administered intravenously, fluorescein is absorbed by the albumin component of plasma protein. The other 20% of the fluorescein is free and circulates in the retinal and choroid tissues, where it may be seen. The remaining 80% of the fluorescein is protein-bound, mostly to albumin and unavailable for fluorescence. A little function is played by globulin and fibrinogen. The highest binding impact is reached between pH 6 and pH 7. Fluorescein molecules do not diffuse inside cells; instead, they only accumulate on the blood corpuscle's surface.

Pharmacological properties:

The fluorescein dye is well tolerated with very few side effects. The dose is 15 mg/kg body weight, and used in FFA is usually 1000mg in 20% concentration. The dye should be used cautiously in pregnancy and renal failure, safe for newborn infants and young children. The skin may stain yellow for 4 to 6 hours and is excreted via urine.

Adverse effects

Complications and negative consequences can be divided into four categories: mild, moderate, severe, and fatal. 5 to 10% of people had a milder response, which includes pruritis, nausea, vomiting, and sneezing. If there is dye extravasation into the skin, it may hurt and be eased by cold compression, and occasionally it may result in skin sloughing. The patient must spend the night in a cardiac unit under observation if an accidental arterial injection is thought to have occurred. Urticaria, skin eruptions, thrombophlebitis, pyrexia, local tissue necrosis, temporary nerve palsy, and syncopal attack are considered moderate responses. It requires medical attention. Laryngeal edema, bronchospasm, anaphylaxis, shock, tonic seizures,

myocardial infarction, and cardiac arrest are examples of severe responses. All of them require urgent, serious medical care.

A fluorescent dye called fluorescein sodium (FS) is frequently utilized in ocular surgery and surgery on malignant brain tumours[9, 10]. The chemical 10% FS, which is readily accessible, is generally safe and has no obvious side effects. The use of FS as a tracer material for SLN mapping in colorectal tumors has also been described[11]. However, there is limited use of FS in SLNB of breast cancer. According to the literature, fluorescein can be utilized in SLN biopsy for breast cancer without running the risk of radiation exposure. In addition, good lymphatic route visualization during the surgery might serve as a guide to locating the sentinel node.

Important trials and current guidelines in the management of SLN-positive patients.

1. ACOSOG Z –0010

Sentinel node (SN) and bone marrow (BM) micro-metastases in women with clinical T1/T2 N0 M0 breast cancer were the subject of a multicenter predictive analysis. Patients had bilateral iliac crest BM aspiration and SNB with breast-conserving surgery. IHC evaluations of BM and histologically negative SN were performed.

This study assessed the prognostic importance of metastases found in the SLN by conventional histology and IHC as well as metastases found in the bone marrow by IHC. In a multivariate analysis, there was no difference between patients who tested negative for SLN metastases by both IHC and histology and positive for metastases by IHC in terms of overall survival (OS), disease-free survival, or loco-regional recurrence.

The rate of SLN identification with the use of blue dye alone, radio colloid alone, or a combination of the two was not shown to differ significantly in a subgroup analysis. This report

casts doubt on the typical IHC assessment of SN[90].

2. ACOSOG Z –0011

This research was created to examine the issue of whether a patient with fewer than three SLN positives has to finish ALND. All patients had full breast radiation treatment as well as breast-conserving surgery[92]. Women who had clinically positive sentinel nodes, T1, T2, and N0 were randomly divided into two arms: ALND and No ALND. Overall Survival (OS) was the main outcome, whereas axillary recurrence and surgical morbidities were the secondary outcomes.

At a median follow-up of 6.3 years, the trial's findings showed no discernible differences in loco-regional recurrence or survival between the SLND plus ALND group and the SLND alone group. Women treated with SLND with ALND or SLND alone experienced identical five-year overall survival rates (91.9 vs. 92.5%) (HR 0.79, 90% CI 0.56-1.10). Additionally comparable across the two arms was the five-year disease-free survival rate (82.2 versus 83.9 per cent, respectively). The recurrence rates in the ipsilateral axilla were comparable across the two arms, with four recurrences (0.9%) in the SLND alone arm compared to two recurrences (0.5%) in the SLND plus ALND component.

Women in the ALND group had a significantly greater incidence of postoperative complications than did the SLN-only group (70% vs 25%). The ACOSOG Z-0011 trial was criticized for a number of performance deficiencies, including the target accrual for the ACOSOG Z-0011 study was 1900 patients. The study closed prematurely because of low accrual and low event rate after enrolling 436 patients in the SLND alone arm and 420 in the SLND plus ALND component. Due to low accrual, the trial was not adequately powered to meet the predetermined statistical survival primary endpoint. Eleven patients assigned to the SLND-only arm did have an ALND, and 32 patients assigned to the SLND plus ALND arm did not have an ALND. Almost 20 per cent of patients were lost to follow-up, and 7 per cent

of patients in the SLND arm were found to be node negative compared with 1 per cent of the ALND arm. There was no breakdown of the numbers of patients with isolated tumor cell clusters, micro-metastasis or macro-metastasis in the two arms. Most patients had T1 (almost 70 per cent) and hormone receptor-positive tumors (85 per cent). Estrogen receptor status and adjuvant systemic therapy were independent predictors of survival.

Based on the trial's apparent lack of regional benefit and low risk of events, completion ALND may not be requisite for all women with T1 tumors who have undertaken breast conservation surgery, are clinically node-negative, have much less than three positive SLNs, and will receive whole breast radiation, particularly in those with estrogen receptor-positive tumors. This was further demonstrated in a retrospective analysis of 242 patients in a row who satisfied the ACOSOG Z0011 criteria[92]. When the ALND is not completed in individuals who have a positive SLND, whole breast irradiation is advised. ALND should be finished if partial breast radiotherapy is intended.

3. IBCSG 2301 trial- the international Breast Cancer Study group trial 2301

This trial analyzed patients with micro-metastases in SLNs -ALND vs No ALND Patients with SLN micro-metastasis (<2 mm) and primary tumors <5 cm in size were randomized to completion for ALND Vs. No additional axillary surgery[93]. The primary end-points were five-year disease-free survival (DFS) and overall survival (OS) rates.

The trial results showed that with a median follow-up of 49 months, there was no significant difference in DFS rate for patients treated with an ALND compared with those treated with a SLND (87 versus 92 per cent). There was no significant difference in OS rate for patients treated with an ALND compared with those treated with a SLND (97.6 versus 98.0 per cent). The results of this trial, when considered in the context of the ACOSOG Z0011 trial, offer additional support to the concept that a subset of patients with metastases to sentinel nodes may, in fact, do well with SLND alone as compared with a completion ALND. However, the

data are derived from studies that need to be more experienced and have a relatively short follow-up.

4. AMAROS trial (After Mapping of the Axilla: Radiotherapy or Surgery)

Following a positive SLND for breast cancer, the axilla may require radiotherapy or surgery (EORTC 10981-22023AMAROS) [94]. A computer-generated allocation plan randomly allocated each patient (1:1) to undergo either axillary radiation or, in the event of a positive sentinel node, ALND. The 5-year axillary recurrence was the main outcome, and it was estimated to be less than 4% for the axillary radiation group against an anticipated 2% in the ALND group.

Following ALND, the 5-year axillary recurrence rate was 0.43% (95% CI 0.00-0.92), compared to 1.19% (0.31-2.08) after axillary radiotherapy. The low number of occurrences made the intended non-inferiority test underpowered. At one year, three years, and five years, the arm of the same side containing edema due to accumulation of lymph was observed substantially more frequently following ALND than after axillary irradiation. Results demonstrate that for patients with T1-2 primary breast cancer and no palpable lymphadenopathy, ALND and axillary radiation following a positive SLN give good and similar axillary control. Significantly reduced morbidity is caused by axillary radiation.

5. NCCN guidelines version 3.2014– recommendation

If all the following conditions are satisfied, ALND may be avoided in patients with positive SLN.

1. T1 or T2 tumors
2. 1 or 2 positive sentinel nodes
3. No neoadjuvant chemotherapy
4. Breast conservation therapy

5. Whole breast radiation therapy is planned

ALND should be performed even if one of the above criteria is not met

6. ASCO guidelines 2014

ALND completion is not required in patients with fewer than three SLN-positive nodes if the patient is receiving whole breast radiation therapy and there is no sign of bulky metastatic disease or extensive extra-capsular extension[79].

Complications of axillary lymph node dissection:

1. Seroma
2. Arm morbidity
3. Infection
4. Hematoma
5. Nerve injury

1. **Seroma –** After axillary dissection, the normal lymphatic drainage of the breast to the axilla will be disrupted due to axillary dissection which will lead to seroma formation [95, 96]. They are avoided by using drains and drained via percutaneous aspiration.
2. **Arm morbidity –** Post-ALND swelling and Lymphedema of the arm is the most potential and serious complication causing morbidity. The rate varies substantially with the level of dissection and whether or not postoperative radiation therapy is used. Shoulder stiffness, numbness, and paresthesia's in the upper arm are common complaints following an ALND. Although these symptoms do not usually interfere with daily living, they may reduce the quality of life [97, 98].
3. **Infection –** As per literature, 3 to 15 % of patients who have undergone ALND shows postoperative wound infection [99]. The most common organisms are usually gram-positive (streptococcal or staphylococcal species) and will respond to treatment with appropriate

oral antibiotics. If there is an underlying seroma, it should be aspirated and cultured to direct antibiotic treatment.

4. **Hematoma –** Around 2-10% of cases develop hematoma during the post-operative period and early recognition and management are necessary.
5. **Injury to the nerves -** There is a 1% chance of suffering a serious motor nerve injury after an ALND. The scapula wings when the long thoracic nerve is injured. Internal rotation and shoulder abduction are weakened by thoracodorsal nerve injury. Transection of the intercostobrachial nerve causes numbness and paresthesia on the inner upper arm. Injury to the medial pectoral nerve may result in atrophy of the lateral aspect of the pectoralis major muscle, which may affect the overall cosmetic result.

Complications of sentinel node biopsy

1. Axillary paresthesia
2. Seroma
3. Lymphedema
4. Decreased range of motion
5. Hematoma
6. Axillary wound infection
7. Axillary web syndrome.

8.6% of patients had axillary paresthesia, most likely as a result of intercostal brachial nerve injury. 3.8% of people reported limited mobility, while 6.9% of people had lymphedema[100]. The formation of palpable, sensitive cords in the upper arm or axilla antecubital fossa is known as axillary web syndrome. It follows SLNB but is less frequent than ALND. The process put forth by experimental teams includes tissue disorders and injuries brought on by the damage of the lymphatic system which was superficial and vessels while

performing surgery to the axilla, as well as lymphovenous damage, hyper-coagulation, and stasis of lymph. Physiotherapy and massage can be used to address the issue[100].

Compared to ALND, SLNB is strongly related to lower subjective and objective long-term morbidity [102]. In comparison to a lifetime risk of lymphedema with an ALND of 25%, the risk of lymphedema reported with SLN biopsy is 2% to 7%. In the ACOSOG Z0011 research, Lucci et al., 91 evaluated the postoperative complication rate of women having SLN vs. SLN plus ALND. They discovered that women in the ALND group experienced postoperative problems at a rate that was noticeably higher than that of the SLN-only group (70% vs. 25%). "Axillary seromas, axillary paresthesia, brachial plexus injuries, and lymphedema" were among the complications. Subjectively indicated by research participants, 13% of those in the ALND group had lymphedema at one year compared to 2% of those in the SLN- only group.

Axillary recurrence after SNB

Rarely occurs axillary recurrence following a negative SLN biopsy. The first prospective report of patients with negative SLNs receiving treatment without ALND was reported in 2000 by Giuliano and colleagues. Following surgery, all selections for radiation and systemic adjuvant therapy were made using traditional standards. There was no axillary recurrence after a median follow-up of 39 months[103]. 1.4% of patients who received SNB were found to have axillary recurrences, according to research by Naik and colleagues[104]. Patients with positive SLNs who did not receive ALND had a greater recurrence rate. With ALND[105], axillary recurrence can be addressed.

In a prospective experiment by **Young Woo Chang et al., [106]** fluorescein and 99mTc were intradermally injected into the areola of each patient's afflicted breast in 61 patients with breast cancer who required SLN biopsy. When fluorescein-dyed SLNs were illuminated with

blue LED light, it was found that 93.4% of them could be seen. Neither the use of fluorescein nor the blue LED light caused any side effects in any of the patients. The researchers came to the conclusion that it was possible and secure to identify SLNs in breast cancer patients using fluorescein and blue LED light. With this method, SLNs may be seen with the unaided eye and are measurable, simple to utilize, and affordable.

According to **Lidong Ren et al**[107] investigations, SLN detection rates were shown to be greater in the fluorescein sodium group compared to the isosulphan blue group. Regarding the distances between the detected sentinel lymph nodes, the number of detected SLN and second lymph nodes, the average dyeing time of the SLN and second lymph nodes, and the average fading time of the second lymph nodes, there were no discernible differences between the fluorescein sodium group and the isosulphan blue group. Fluorescein sodium is a promising novel tracer for breast sentinel lymph node biopsy, it was determined.

Khadka S et al.,[108] in their study among 130 patients > 18 years presenting with early breast cancer T1, T2, N0 breast carcinoma, were randomized to undergo SLN biopsy by either fluorescein + methylene blue or Tc-99 m sulfur colloid + methylene blue. It was observed that the SLN was identified in 89% of the Fluorescein + methylene blue group and 90.9% with the Tc-99 m Sulphur colloid + methylene blue group. The trial demonstrated non-inferiority of fluorescein + methylene blue as compared to isotope + methylene blue with effect size = 1; 95% confidence interval of 9.54 to + 11.54. The fluorescein + methylene blue was more cost-effective than isotope-guided SLN. It was concluded that Fluorescein guided SLN biopsy is non-inferior and more cost effective than isotope guided SLN biopsy.

In their prospective clinical investigation, **Liang Li et al.,** [109] split their 123 breast cancer patients into groups A (n = 67) and B (n = 56). In SLNB of breast cancer, the effectiveness of fluorescein and methylene blue was compared to that of indocyanine green

(ICG) and methylene blue (group A). There were no systemic or local responses seen in either group. SLN were detected in group A with a detection rate of 94.0% (63/67) and a false-negative rate of 7.5% (4/53). SLN detection in group B had a FN rate of 7.5% (3/40) and a detection rate of 92.9% (52/56). The findings of the two groups' biopsies did not significantly differ. The study found that SLNB for breast cancer patients utilizing fluorescein and UV LED light is possible.

MATERIALS AND METHODS

MATERIAL AND METHODS

Source of Data: Department of Surgery, R.L. Jalappa Hospital and Research Centre, Sri Devaraj Urs Medical College, Tamaka, Kolar.

Study Population: Patients diagnosed with carcinoma Breast in R.L. Jalappa Hospital and Research Centre, Tamaka, Kolar, attached to Sri Devaraj Urs Medical College.

Inclusion Criteria:

All patients who were diagnosed to have carcinoma breast aged>18 years with no clinically identifiable axillary lymph nodes.

Exclusion Criteria:

1. Patients with metastatic disease of carcinoma breast.
2. Patients with carcinoma breast.
3. Patients with allergic reactions to methylene blue or fluorescein sodium dye.

Duration of study: January 2021 To June 2022.

Study Design: Prospective study

Sampling technique: Convenient sampling

Sample size: 30

The sample size was calculated using the formula.

$$\square = \frac{\square^2 \times \square \times \square}{\square^2}$$

Based on the previous study by **Ramya et al.[110].** P=92%, d=10, with a 95% confidence interval and the sample size was calculated as 28, which was rounded off to 30.

The sample was calculated using the software nMaster 2.0

Method of Data Collection:

- Data was collected using proforma. From the patients with carcinoma breast, thorough history was taken, and relevant investigations were done.
- All patients with early and large operable breast cancer who had a clinical stage of N0, and who agreed to participate in the study after receiving full disclosure.
- Patients were scheduled for validation SLNB for axilla combined with either breast-conserving surgery or a mastectomy.
- The test dose was given in the pre-operative set-up and looked for reactions.
- The dye is injected around the areola intra-dermally and underneath the areola. Economic methylene blue, (2ml i.e-10mg) was infiltrated after induction of general anesthesia, and the infiltration area was compressed gently so the dye will distribute for 5 mins towards the axilla. This was followed by an infiltration of 2ml of 10% Fluorescein sodium in 1:5 dilution with distilled water and massaged for another 5 mins.
- The axilla was entered through a skin crease incision, and the blue nodes were identified, following which the room was darkened, and fluorescent lymph nodes were identified using blue light from a blue LED lamp (wavelength 480nm).
- SLNs were removed, and depending on the tracer used to identify them, they were either labelled as blue or fluorescent depending upon their combinations. Furthermore, all palpable lymph nodes were harvested and categorized as SLN.
- These lymph nodes were sent to the pathology department for histopathological analysis and evaluated for any evidence of metastasis.
- If more than one tracer may be used to identify a certain SLN, all of those tracers were included in the identification.

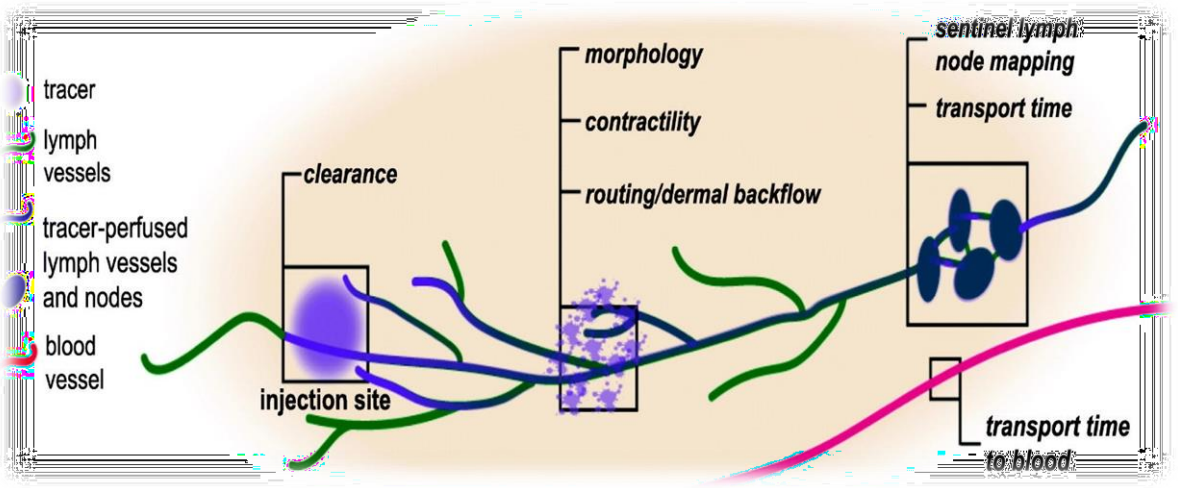
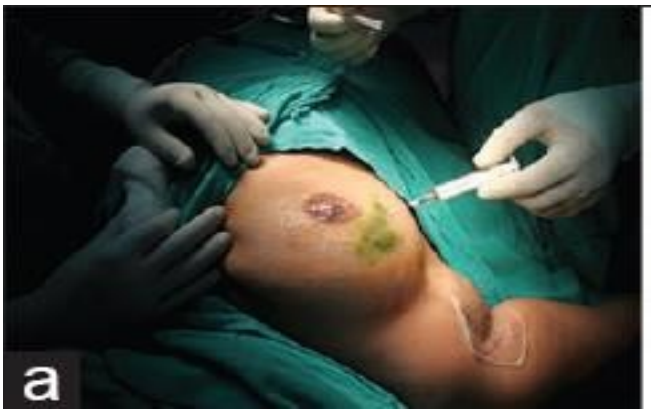
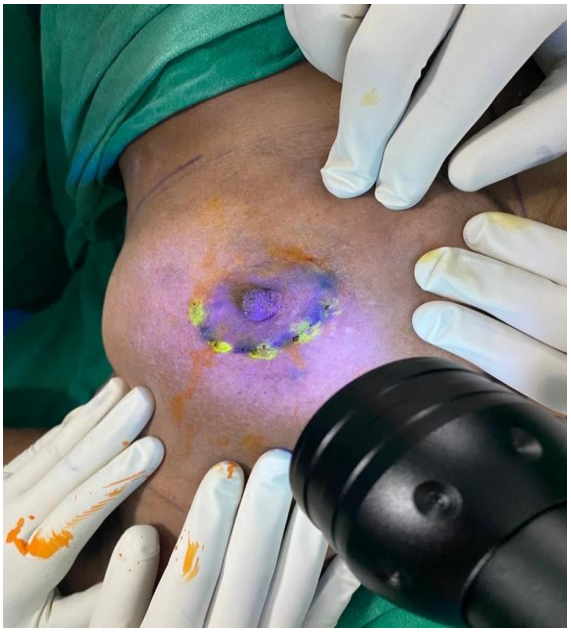


Figure 16: Imaging technology of the lymphatic system

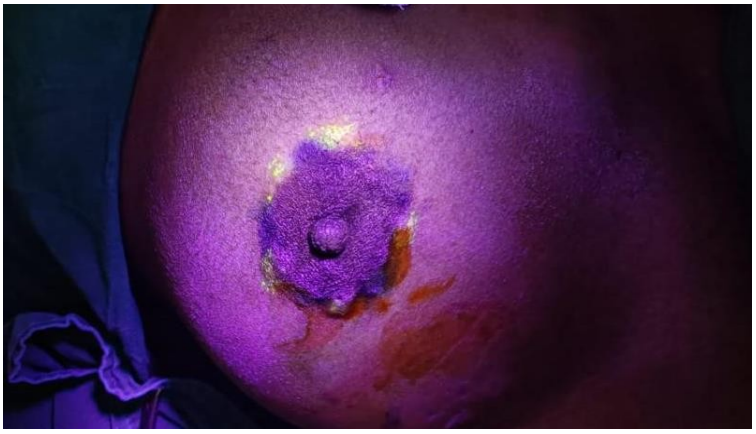
Figure 17: Operative illustration of fluorescein-guided sentinel node mapping.



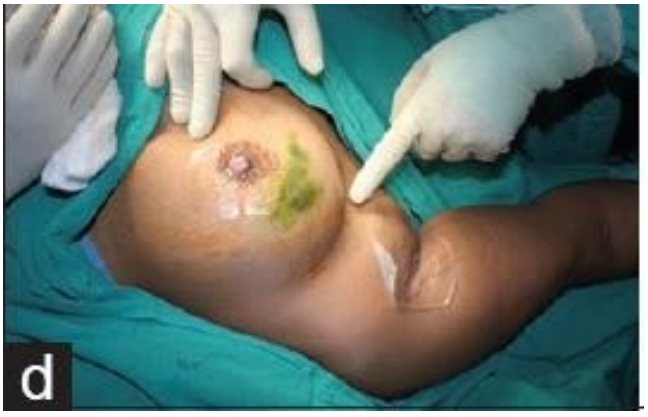
(a) Injection of combined tracer fluorescein 0.125 ml plus methylene blue one ml diluted in 4 ml of normal saline, injected half in the intradermal plane and half in the subareolar plane.



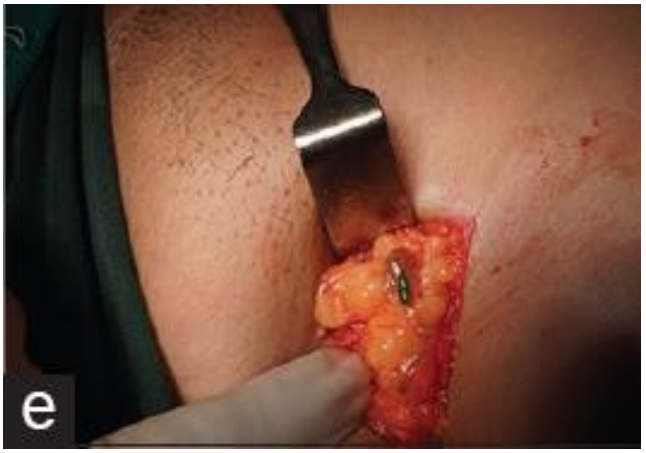
(b) Dermal lymphatic plexus delineated with blue and yellow dyes seen on white light.



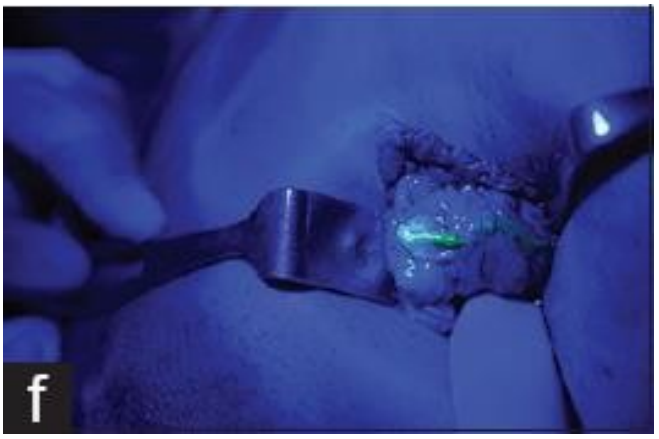
(c) Fluorescent lymphatics shining with blue LED light (480 nm wavelength).



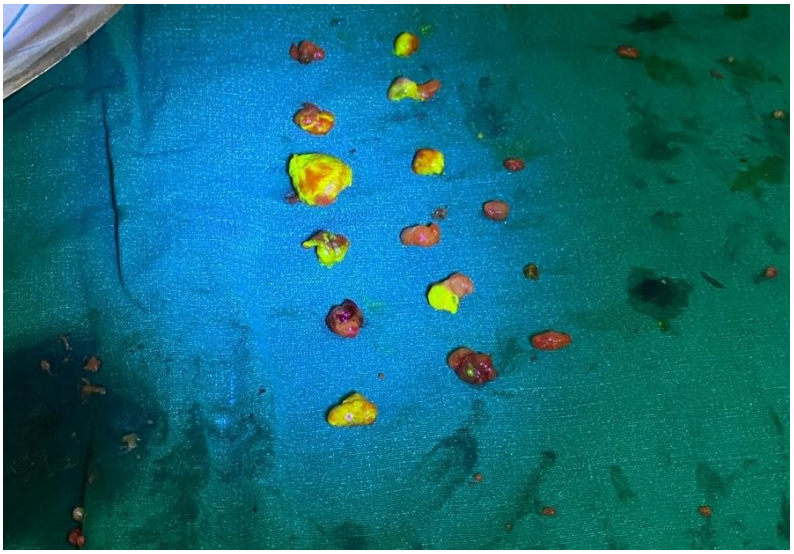
(d) Incision along a lateral mammary skin crease.



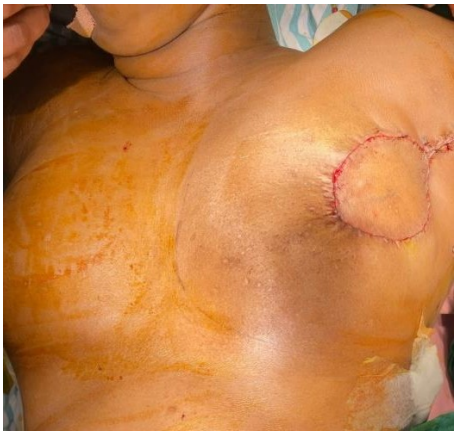
(e) Greenish-blue lymphatics and lymph nodes are seen with white light.



(f) Fluorescent lymphatic duct and lymph node shining with blue LED light.



(g)Nodes retrieved using fluorescein-guided sentinel node mapping



(h) Post-operative scar of a patient who has underdone BCS + fluorescein-guided sentinel node mapping

All patients underwent the following investigations:

- CBC
- Renal function tests
- Liver function tests
- Serum electrolytes
- Chest radiograph/ CT thorax
- FNAC/TRUCUT biopsy of breast tissue with ER, PR, and Her2neu Status.
- USG/MAMMOGRAPHY of breast and axilla.

Financial burden: All the investigations involved were part of the routine management of breast carcinoma. Hence it was borne by the patient party. The costs for the dye and the torch were borne by the investigator.

Statistical analysis:

Using the SPSS 22 version of software, data were analyzed after being input into a Microsoft Excel datasheet. Data that was categorical was shown as frequencies and proportions. For testing the importance of qualitative data, the chi-square test was employed.

Mean and standard deviation was used to depict continuous data.

Validity of a test in Screening of Disease:

Screening test results	Diagnosis		Total
	Diseased	Healthy	
Positive	a (True postive)	b (False Postive)	a+b
Negative	c (False Negative)	d (True Negative)	c+d
Total	a + c	b + d	a+b+c+d

- Sensitivity = a/ (a+c) x 100 = True positive / True positive + False Negative.
- Specificity = d/ (b+d) x 100 = True Negative / True Negative + False Positive.
- Positive predictive value = a/ (a+b) x 100 = True Positive / True positive + False positive.
- Negative predictive value = d/ (c+d) x 100 = True Negative / True Negative + False Negative.
- Diagnostic accuracy = a + d / a + b + c + d = True positive + True Negative / Total.

Graphical representation of data: MS Excel and MS word were used to obtain various graphs, such as bar diagrams and Pie diagrams. A **P-value** (Probability that the result is accurate) of <0.05 was considered statistically significant after assuming all the rules of statistical tests. **Statistical software:** MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers, NY, USA) was used to analyse data.

Ethical consideration:

1. Institutional ethical approval was acquired well before the research starts.
2. Before the trial began, every patient who was included provided their informed permission.
3. Throughout the research and follow-up, all patients received the Standard of Care

RESULTS

RESULTS

Table 1: Age distribution

		Count	%
Age	30 to 40 years	6	20.0%
	41 to 50 years	7	23.3%
	51 to 60 years	8	26.7%
	61 to 70 years	6	20.0%
	71 to 80 years	3	10.0%
	Total	30	100.0%

The mean age was 53.13 ±14.180 years. The majority of patients were in the age group 51 to 60 years (26.7%).

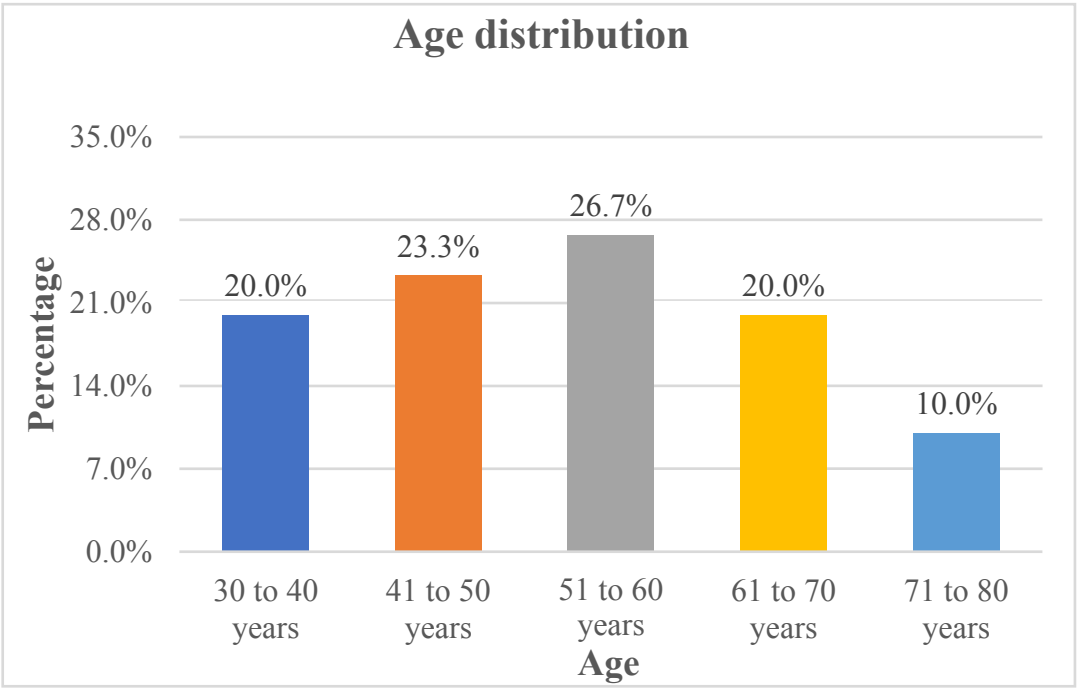


Figure 18: Bar diagram showing the Age distribution

Table 2: Occupation distribution

		Count	%
Occupation	Housewife	22	73.3%
	Labourer	8	26.7%
	Total	30	100.0%

In the study, 73.3% were housewives, and 26.7% were labourers.

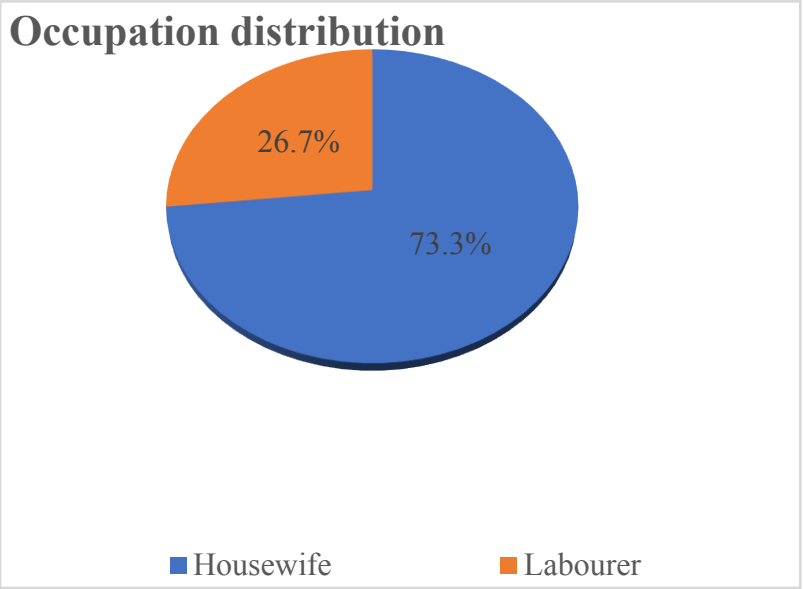


Figure 19: Pie diagram showing Occupation distribution

Table 3: Presenting Complaint distribution

		Count	%
Presenting Complaint	Lump in Left Breast	9	30.0%
	Lump in Right Breast	21	70.0%
	Total	30	100.0%

The mean duration of symptoms was 4.30 ±2.588 months. In the study majority of them had Lumps in the right breast (70%), and 30% had a lump in the Left breast.

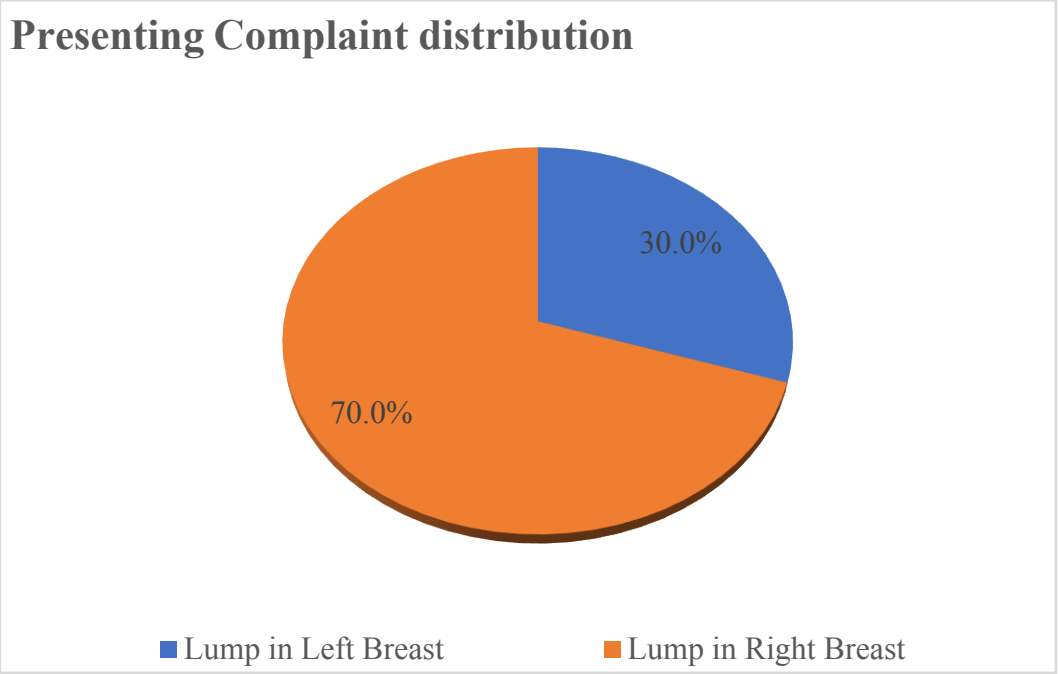


Figure 20: Bar diagram showing Presenting Complaint distribution

Table 4: Associated Complaints distribution

		Count	%
Associated Complaints	Nil	27	90.0%
	Pain	3	10.0%
	Total	30	100.0%

In the study, 10% had pain.

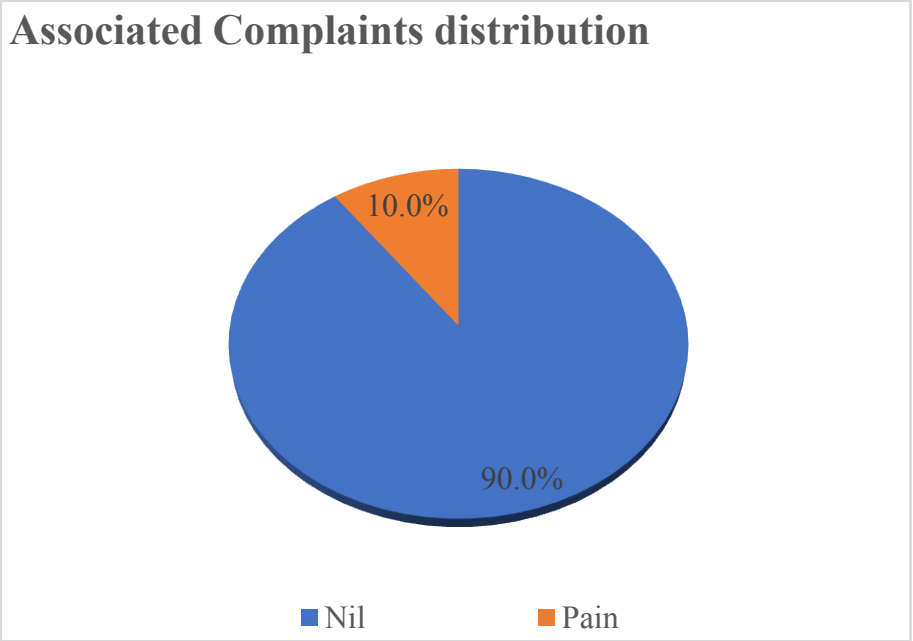


Figure 21: Pie diagram showing Associated Complaints distribution

Table 5: Past History distribution

		Count	%
Past History	DM	1	3.3%
	HTN	3	10.0%
	HTN/DM	2	6.7%
	Nil	24	80.0%
	Total	30	100.0%

In the study, 3.3% had DM, 10% had HTN, and 6.7% had HTN/DM.

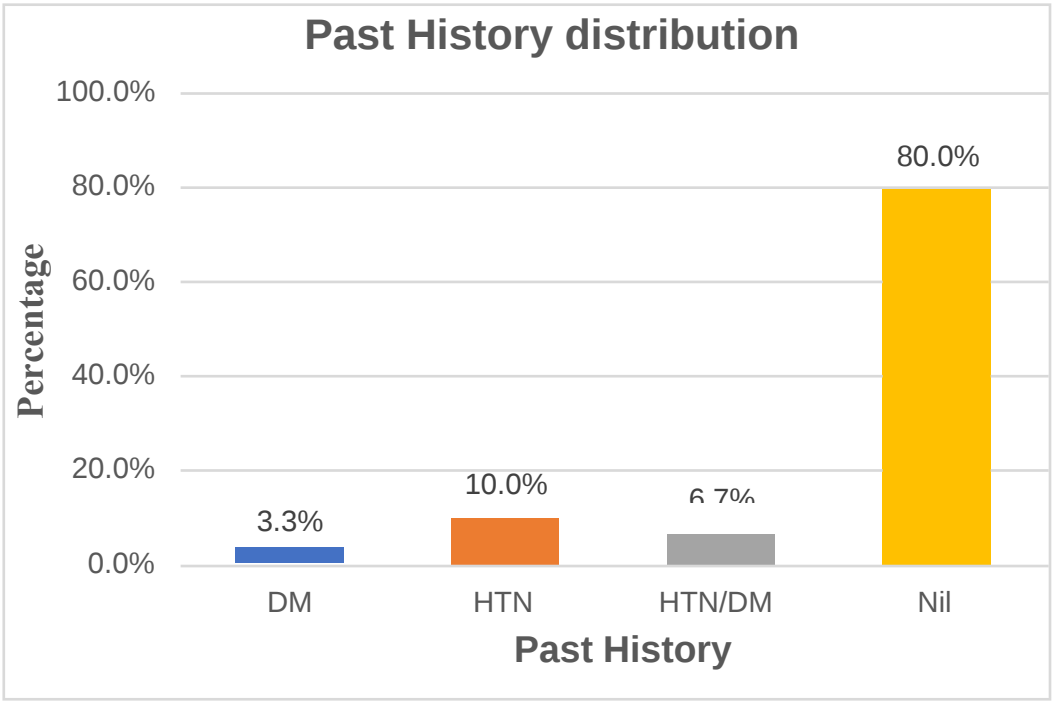


Figure 22: Bar diagram showing Past History distribution

Table 6: Menstrual History distribution

		Count	%
Menstrual History	Irregular Cycles	2	6.7%
	Post-Menopausal	19	63.3%
	Regular Cycles	9	30.0%
	Total	30	100.0%

In the study, 6.7% had irregular cycles, 63.3% were post-menopausal, and 30% had a regular cycle.

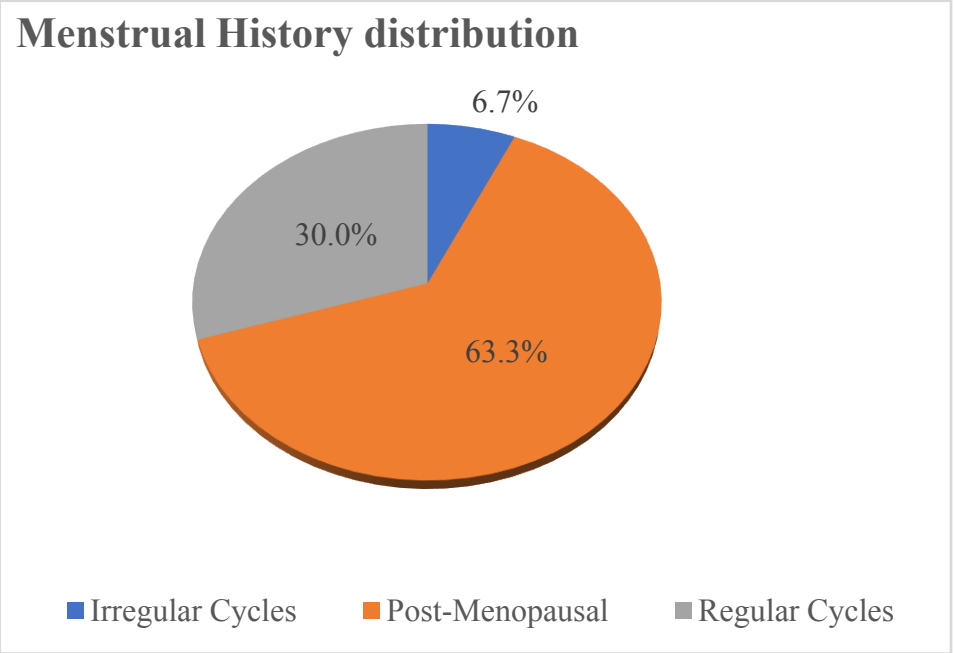


Figure 23: Pie diagram showing Menstrual History distribution

Table 7: Obstetric History distribution

		Count	%
Obstetric History	P0L0	1	3.3%
	P1L1	4	13.3%
	P2A2	1	3.3%
	P2L1D1	1	3.3%
	P2L2	7	23.3%
	P3L2	3	10.0%
	P3L2A1	2	6.7%
	P3L3	6	20.0%
	P3L4	1	3.3%
	P4L3A1	1	3.3%
	P4L4	2	6.7%
	P5L5	1	3.3%
	Total	30	100.0%

The majority of the study had an obstetric score of P2L2 (23.3%).

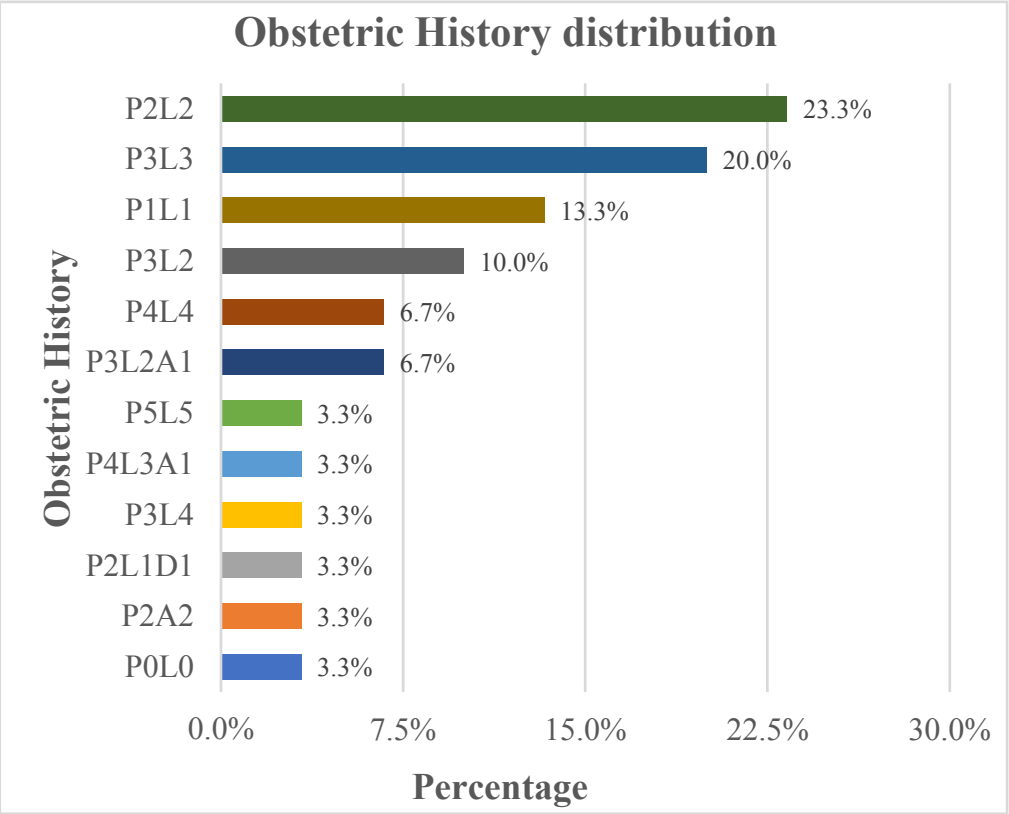


Figure 24: Bar diagram showing Obstetric History distribution

Table 8: Built and Nourishment distribution

		Count	%
Build	Moderate	30	100.0%
Nourishment	Moderate	30	100.0%

In the study, 100% had moderately built and nourished.

Table 9: General Physical Examination

	Absent		Present	
	Count	Row N %	Count	Row N %
Pallor	25	83.3%	5	16.7%
Icterus	30	100.0%	0	0.0%
Cyanosis	30	100.0%	0	0.0%
Clubbing	30	100.0%	0	0.0%
Gen. Lymphadenopathy	30	100.0%	0	0.0%
Edema	30	100.0%	0	0.0%

In the study, 16.7% had pallor, no other signs were present on GPR, and 100% had a normal spine.

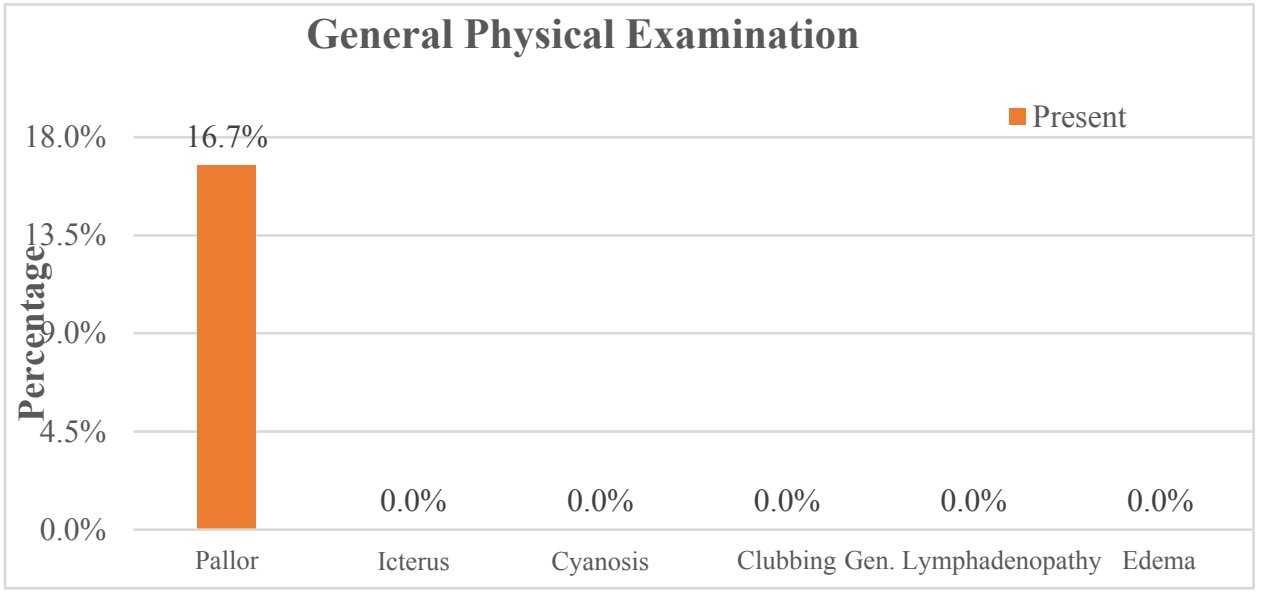


Figure 25: Bar diagram showing General Physical Examination

Table 10: Breast examination findings

		Count	%
Breast Symmetry	Asymmetrical	27	90.0%
	Symmetrical	3	10.0%
Nipple Retraction	Absent	19	63.3%
	Present	11	36.7%
Nipple Discharge	Absent	30	100.0%
Site	L-All Quadrants	1	3.3%
	L-UI	3	10.0%
	L-UO	5	16.7%
	R-All Quadrants	3	10.0%
	R-LI	4	13.3%
	R-LO	2	6.7%
	R-U 1/2	1	3.3%
	R-UI	6	20.0%
	R-UO	5	16.7%
Borders	Ill Defined	25	83.3%
	Well Defined	5	16.7%
Surface	Irregular	8	26.7%
	Smooth	22	73.3%
Skin Changes	None	14	46.7%
	PDO	5	16.7%
	Skin Nodules	1	3.3%
	Tethering	5	16.7%
	Ulcer	5	16.7%
Lymphadenopathy	Nil	30	100.0%
Contralateral Breast	Normal	30	100.0%

Temperature	Local Rise +	2	6.7%
	Normal	28	93.3%
Tenderness	Non-Tender	23	76.7%
	Tender	7	23.3%
Number	Single	30	100.0%
Size	3x2	2	6.7%
	4X2	1	3.3%
	4X3	3	10.0%
	4X4	1	3.3%
	5x3	2	6.7%
	5X4	2	6.7%
	5X6	2	6.7%
	6X5	4	13.3%
	6X6	4	13.3%
	7X5	2	6.7%
	7X7	1	3.3%
	8X5	1	3.3%
	8X6	2	6.7%
	8X7	2	6.7%
	9X7	1	3.3%
Borders	Ill Defined	4	13.3%
	Well Defined	26	86.7%
Surface	Irregular	10	33.3%
	Smooth	20	66.7%
Consistency	Firm	2	6.7%
	Hard	28	93.3%
Mobility	Fixed to Breast Tissue	29	96.7%

	Fixed to Chest Wall	1	3.3%
Axilla	Nil	27	90.0%
	Multiple	1	3.3%
	Single	2	6.7%
Supraclavicular Lymphadenopathy	Absent	29	96.7%
	Present	1	3.3%

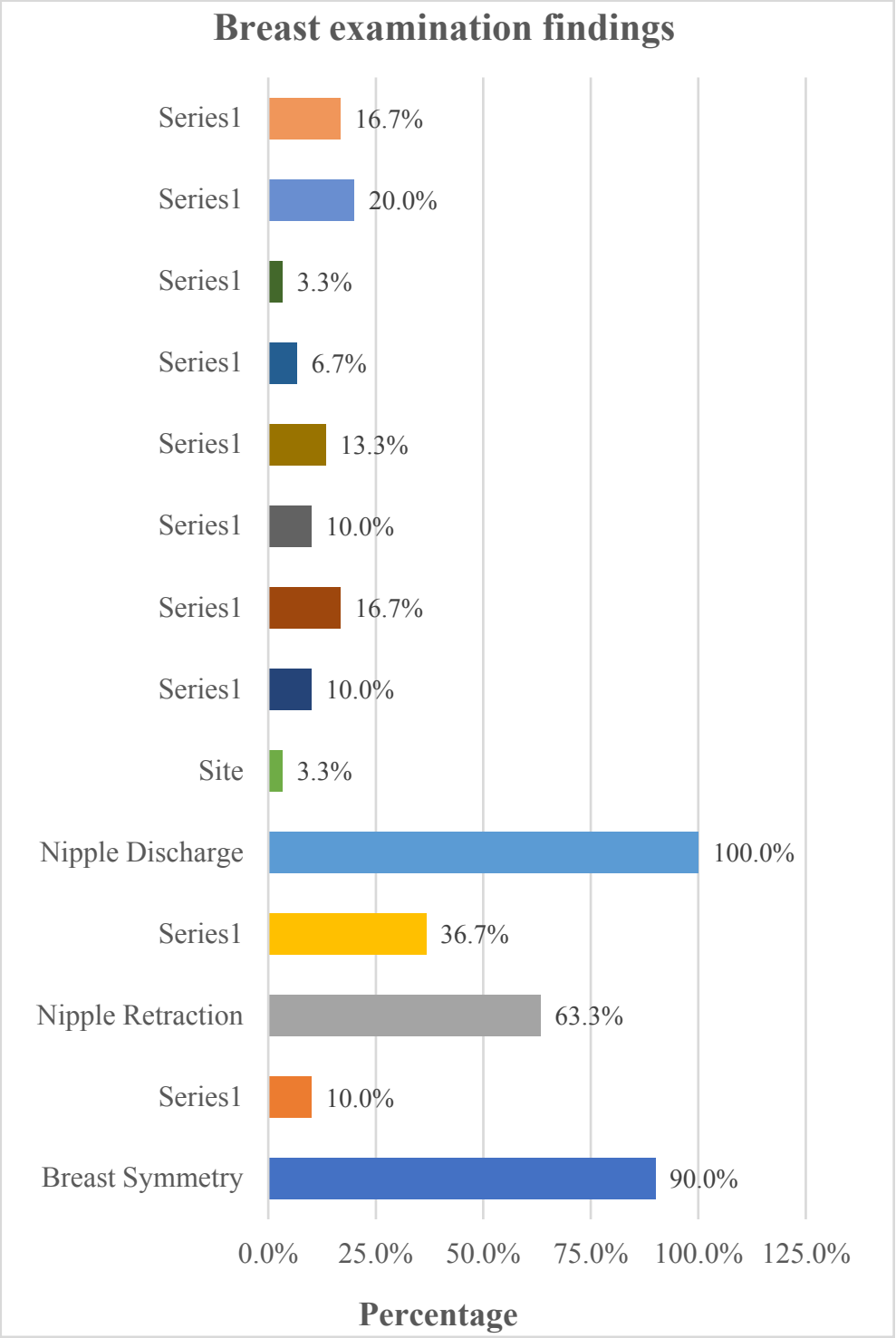


Figure 26: Bar diagram showing Breast examination findings

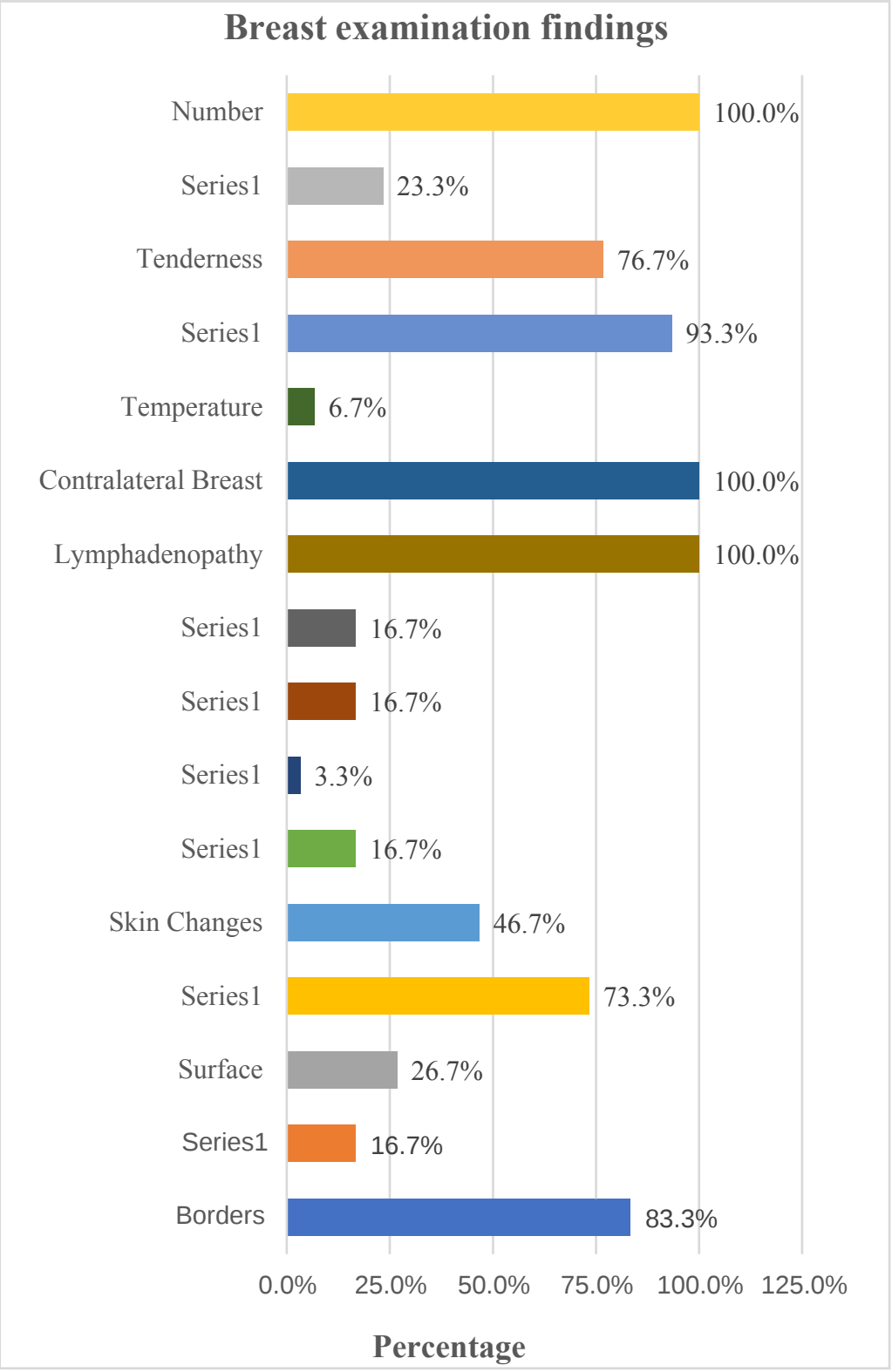


Figure 27: Bar diagram showing Breast examination findings

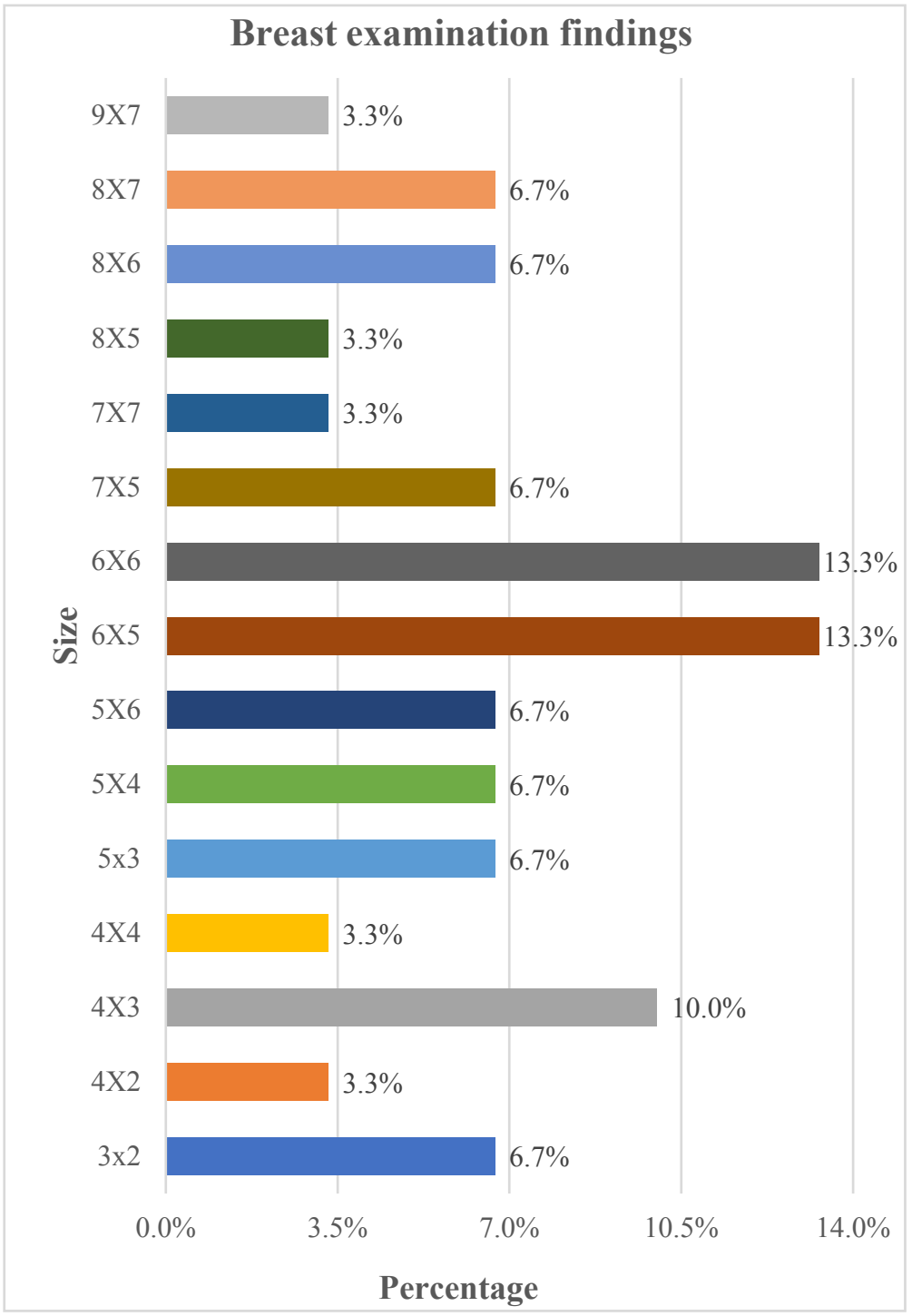


Figure 28: Bar diagram showing Breast examination findings

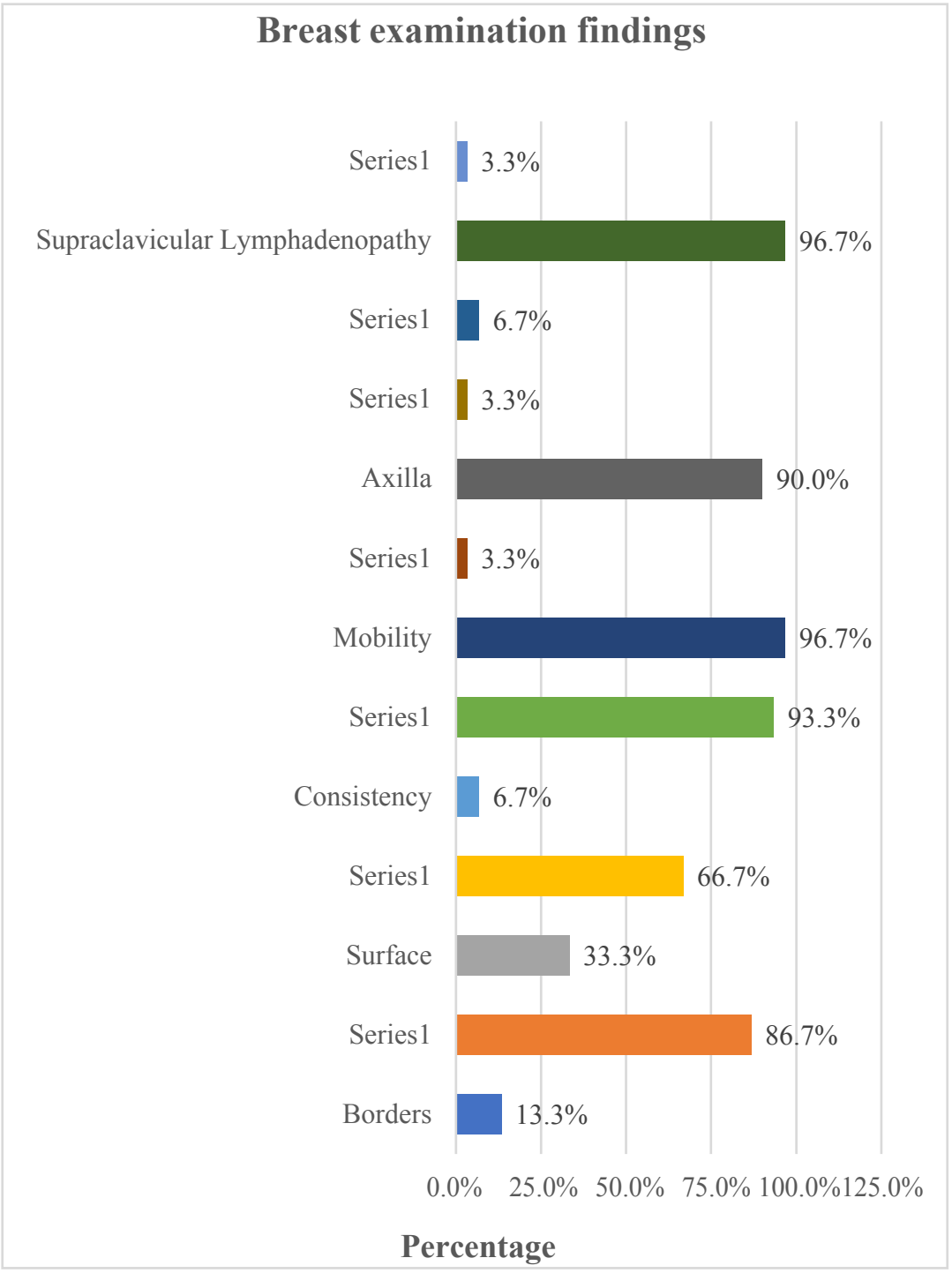


Figure 29: Bar diagram showing Breast examination findings

Table 11: Systemic examination findings

		Count	%
CVS	S1 S2 HEARD	30	100.0%
RS	B/L NVBS +	30	100.0%
PA	NAD	30	100.0%
CNS	NAD	30	100.0%

On systemic examination, no abnormal findings were found.

Table 12: Diagnosis

		Count	%
Diagnosis	Ca Left Breast	10	33.3%
	Ca Right Breast	20	66.7%
	Total	30	100.0%

In the study, 66.7% had Ca Right breast, and 33.3% had Ca left breast.

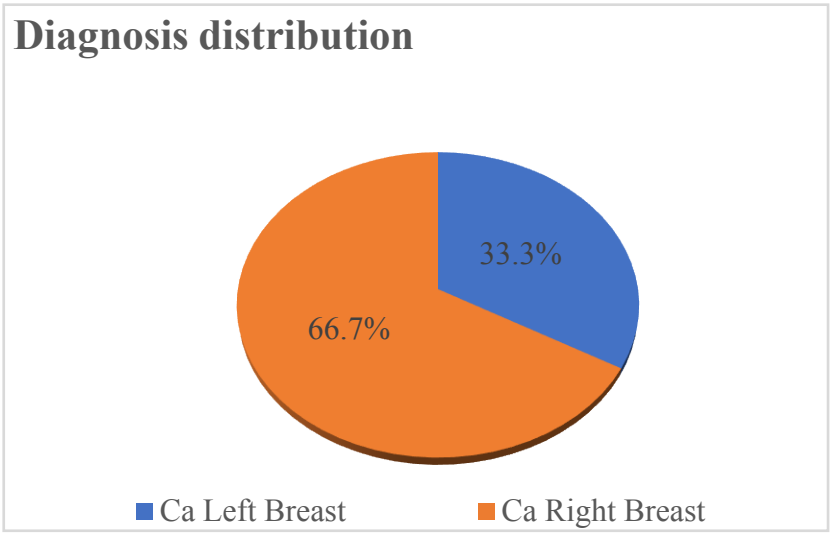


Figure 30: Pie diagram showing Diagnosis distribution

Table 13: Stage of Tumor

		Count	%
Stage	T2N0M0	6	20.0%
	T3N0M0	9	30.0%
	T4BN0M0	15	50.0%
	Total	30	100.0%

In the study, 20% were in stage T2N0M0, 30% were in T3N0M0, and 50% were in T4BN0M0.

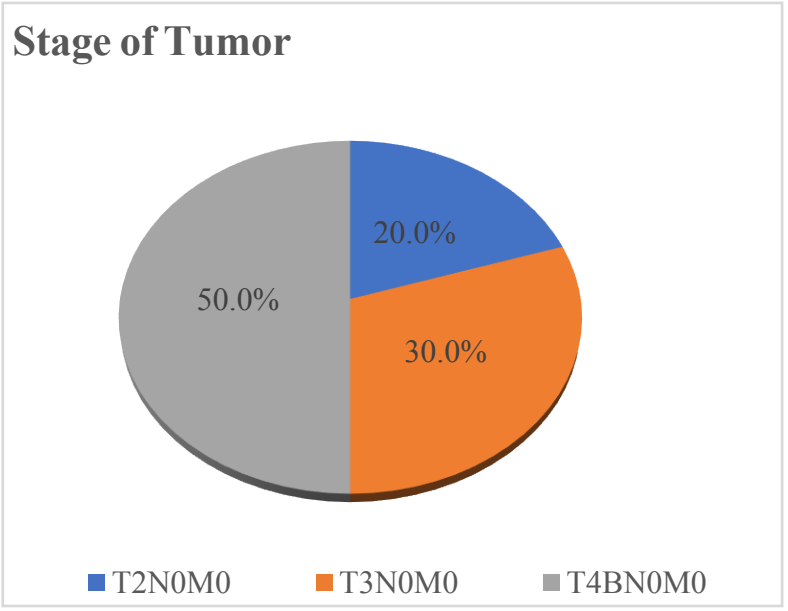


Figure 31: Pie diagram showing the Stage of the Tumor.

Table 14: Surgery Done

		Count	%
Surgery Done	Lumpectomy	10	33.3%
	Mastectomy	20	66.7%
	Total	30	100.0%

In the study, 66.7% underwent a mastectomy, and 33.3% underwent Lumpectomy.

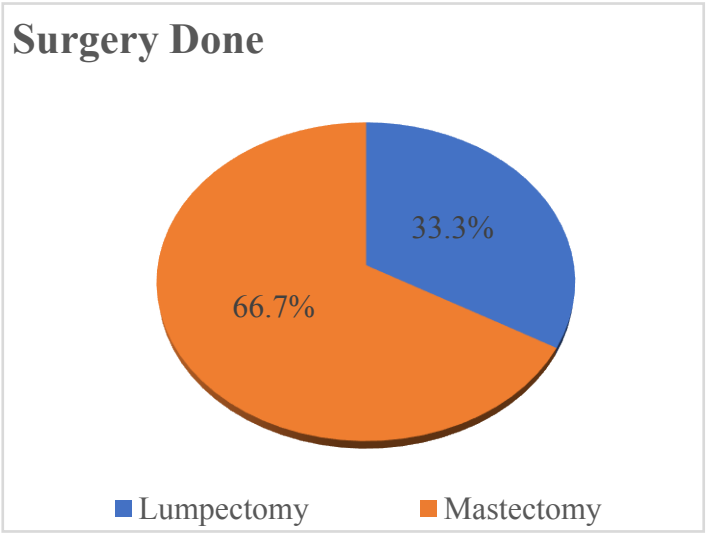


Figure 32: Pie diagram showing Surgery Done.

Table 15: Histopathology diagnosis

		Count	%
Histopathology	Infiltrating Ductal Ca	26	86.7%
	Lobular Ca	3	10.0%
	Squamous Cell Ca	1	3.3%
	Total	30	100.0%

In the study on HPE, 86.7% had Infiltrating Ductal Ca, 10% had Lobular Ca, and 3.3% had Squamous Cell Ca.

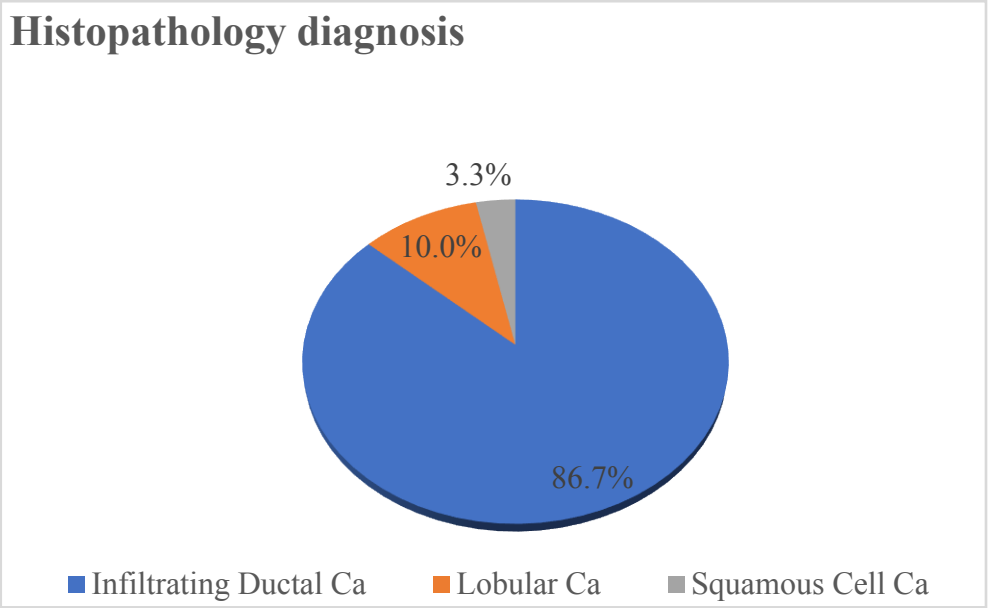


Figure 33: Pie diagram showing Histopathology diagnosis.

Table 16: Lymph Nodes Identified

		Count	%
Lymph Nodes Identified	1	1	3.3%
	2	5	16.7%
	3	8	26.7%
	4	8	26.7%
	5	6	20.0%
	6	2	6.7%
	Total	30	100.0%

In the study, in 26.7% of patients, three lymph nodes and four lymph nodes were identified; in 20% of patients, five lymph nodes, in 16.7% of patients, two lymph nodes; in 6.7% of patients, six lymph nodes and 3.3% of patients one lymph node were identified.

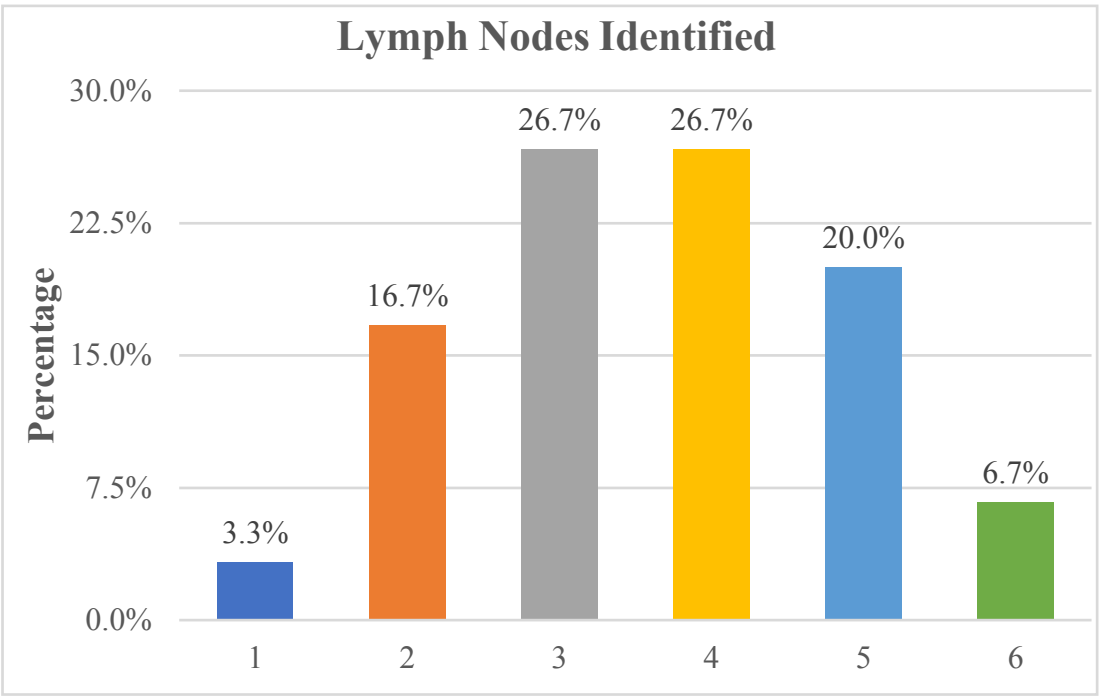


Figure 34: Bar diagram showing Lymph Nodes Identified

Table 17: Lymph Nodes Identified

		Count	%
Lymph Nodes Identified with Fluorescein and Methylene Blue Dye	0	3	10.0%
	1	14	46.7%
	2	11	36.7%
	3	2	6.7%
Lymph Nodes with Fluorescein Dye Only	0	3	10.0%
	1	16	53.3%
	2	10	33.3%
	3	1	3.3%
Lymph Nodes with Methylene Blue Only	0	16	53.3%
	1	7	23.3%
	2	7	23.3%
Lymph Nodes with No Dye	0	23	76.7%
	1	7	23.3%
Fluorescein Dye and Methylene Blue Dye Positive Nodes	0	18	60.0%
	1	11	36.7%
	2	1	3.3%
Fluorescein Dye Positive Nodes	0	13	43.3%
	1	15	50.0%
	2	2	6.7%
Methylene Blue Dye Positive Nodes	0	26	86.7%
	1	4	13.3%
Positive Lymph Nodes with No Dye	0	28	93.3%
	1	2	6.7%

In the study, Lymph Nodes Identified with Fluorescein and Methylene Blue Dye were 1 in 46.7%, 2 in 36.7% of patients and 3 in 6.7% of patients, and 10% of patients them had no

lymph nodes in both staining.

On Fluorescein Dye, 10% had no lymph nodes, 53.3% had one lymph node, 33.3% had two lymph nodes, and 3.3% had three lymph nodes.

On Methylene Blue staining, 53.3% had no lymph nodes, 23.3% had one lymph node, and 23.3% had two lymph nodes.

In the study, out of 30 patients, seven had no lymph nodes stained with dye, and 23 had staining of lymph nodes.

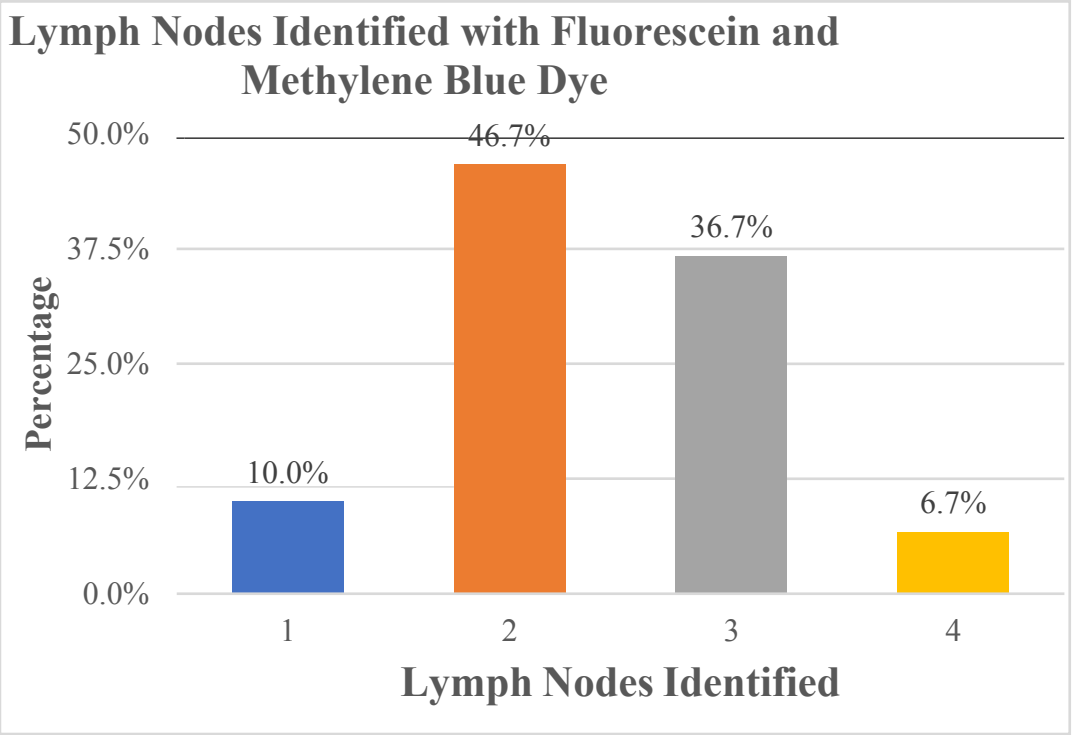


Figure 35: Bar diagram showing Lymph Nodes Identified with Fluorescein and Methylene Blue Dye.

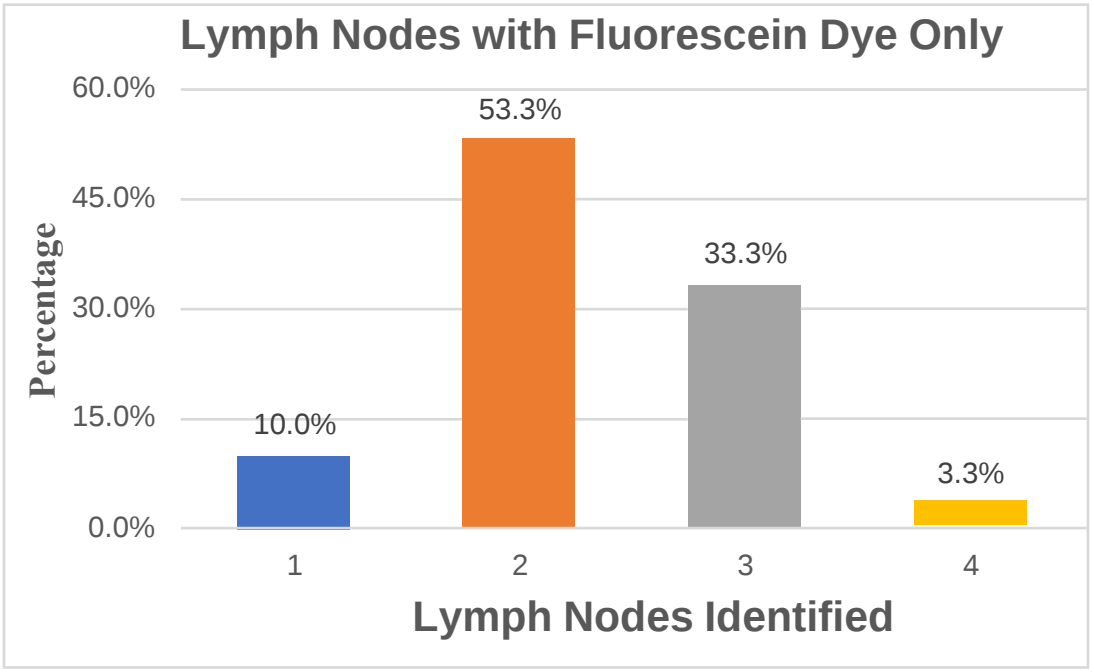


Figure 36:Bar diagram showing Lymph Nodes with Fluorescein Dye Only.

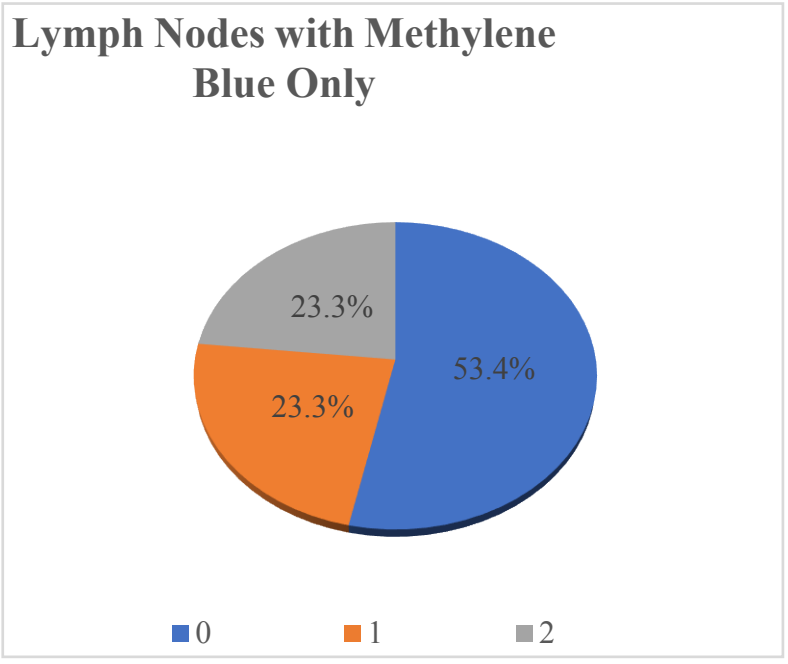


Figure 37: Pie diagram showing Lymph Nodes with Methylene Blue Only.

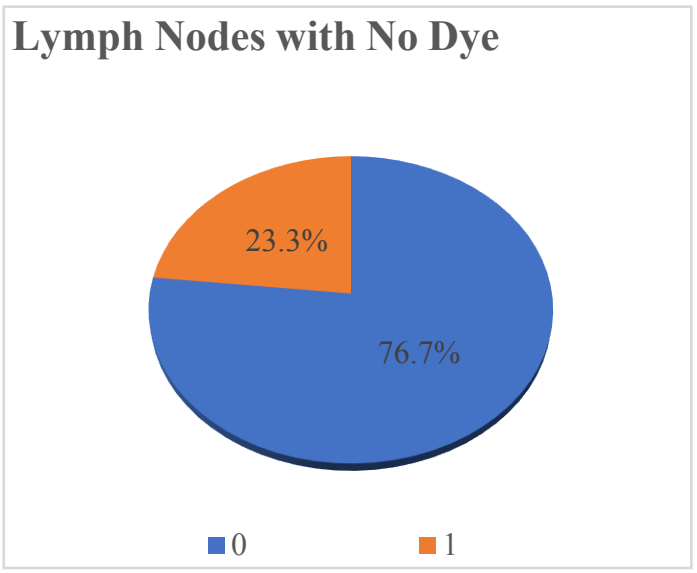


Figure 38: Pie diagram showing Lymph Nodes with No Dye.

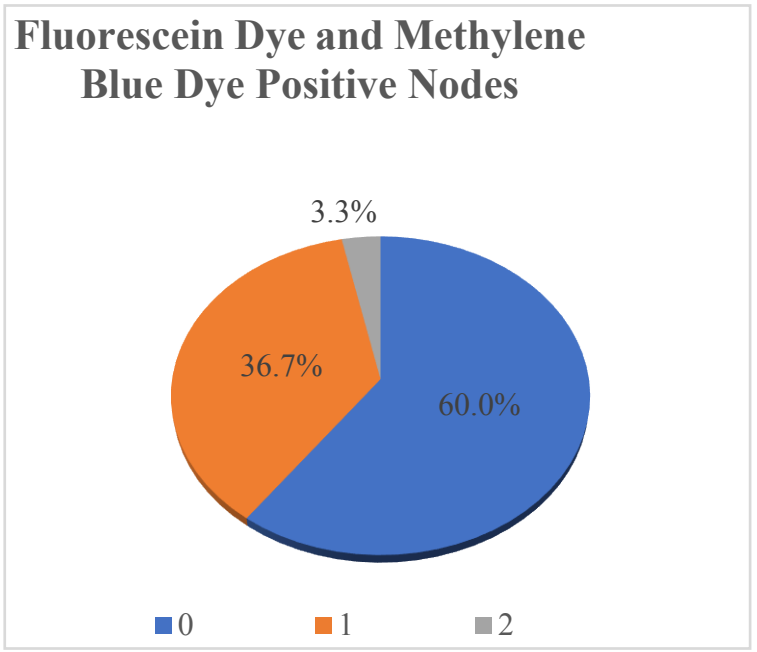


Figure 39: Pie diagram showing Fluorescein Dye and Methylene Blue Dye Positive Nodes.

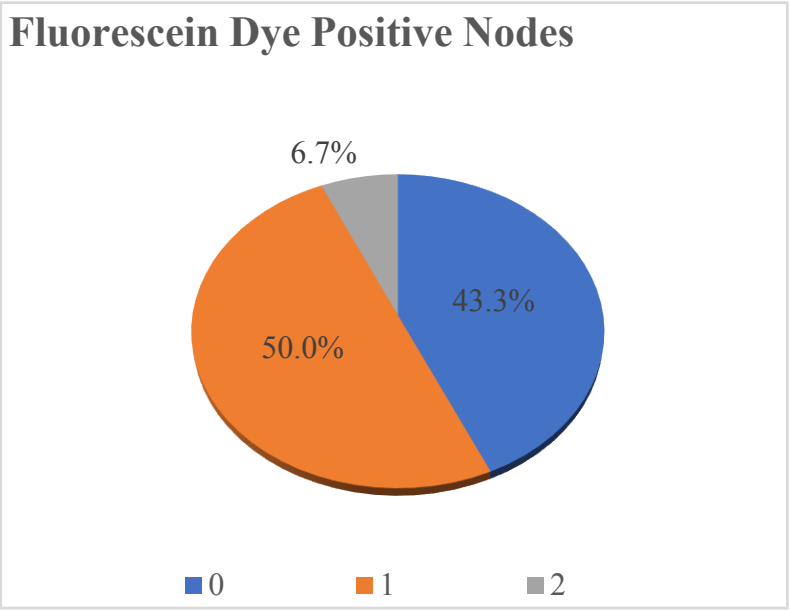


Figure 40: Pie diagram showing Fluorescein Dye Positive Nodes.

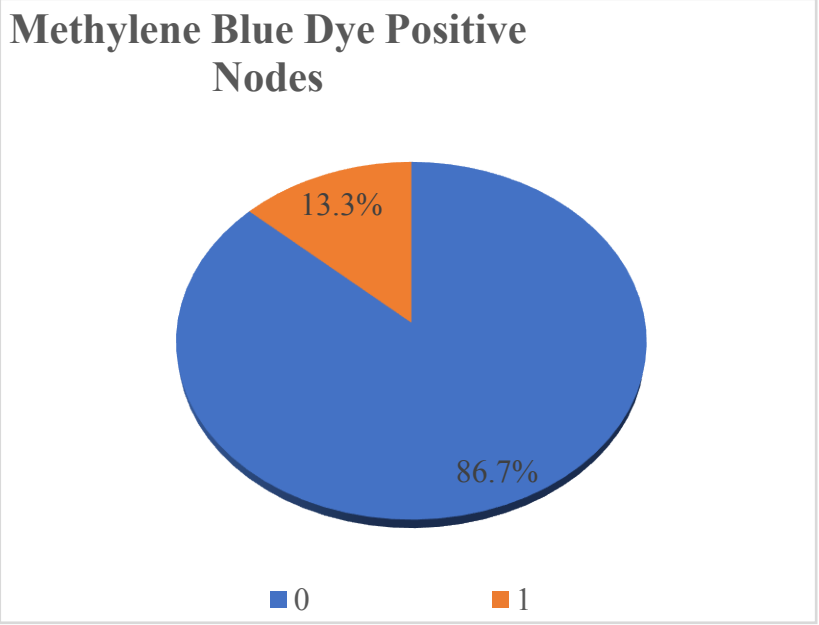


Figure 41: Pie diagram showing Methylene Blue Dye Positive Nodes.

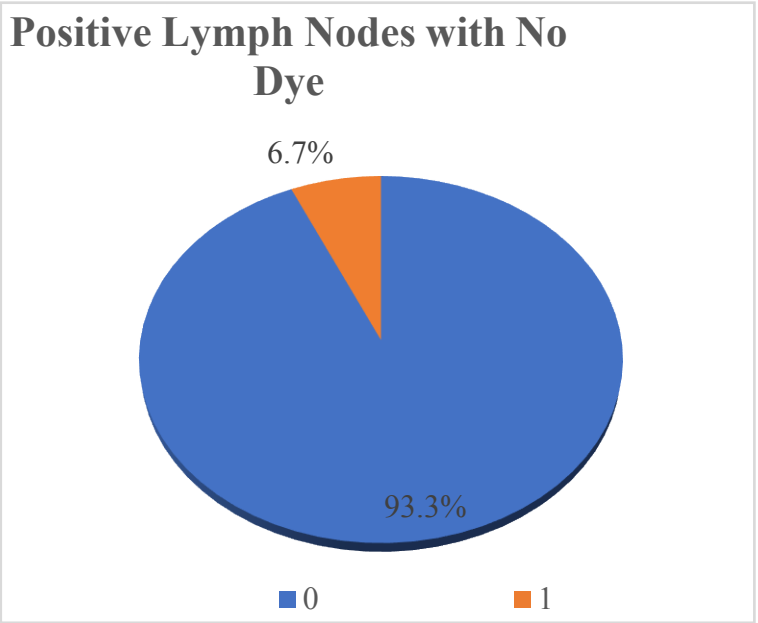


Figure 42: Pie diagram showing Positive Lymph Nodes with No Dye

Table 18: No of Lymph Nodes Identified

	Median	Maximum	Minimum
Lymph Nodes Identified	4	6	1
Lymph Nodes Identified with Fluorescein and Methylene Blue Dye	1	3	0
Lymph Nodes with Fluorescein Dye Only	1	3	0
Lymph Nodes with Methylene Blue Only	0	2	0
Lymph Nodes with No Dye	0	1	0
Fluorescein Dye and Methylene Blue Dye Positive Nodes	0	2	0
Fluorescein Dye Positive Nodes	1	2	0
Methylene Blue Dye Positive Nodes	0	1	0
Positive Lymph Nodes with No Dye	0	1	0

Table 19: Validity of Methylene Blue in stationing Lymph nodes in comparison with Fluorescein Dye

		Fluorescein Dye				
		Positive		Negative		Total
		Count	%	Count	%	
Methylene Blue	Positive	42	51.9%	21	75%	63
	Negative	39	48.1%	7	25%	46
	Total	81	100%	28	100%	109

In the study, among 81 nodes with Fluorescein Dye positive lymph nodes, 51.9% were positive in Methylene blue, and 48.1% were negative. Among 28 nodes negative for Lymph node in Fluorescein Dye, 75% were positive for Methylene Blue, and 25% were negative for both.

Validity of Methylene Blue in comparison with Fluorescein Dye

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	51.85%	41.14, 62.4
Specificity	25%	12.68, 43.36
Positive Predictive Value	66.67%	54.37, 77.05
Negative Predictive Value	15.22%	7.572, 28.22
Diagnostic Accuracy	44.95%	35.95, 54.31

Lymph nodes identified:

Methylene + Fluorescein Dye = 42 nodes

Fluorescein Dye = 39 nodes

Methylene Dye = 21 nodes

No Dye = 7 nodes

Total number of nodes = 109

Total by Methylene Dye = 63

Total by Fluorescein Dye = 81

Table 20: **Validity of Fluorescein Dye in stationing Lymph nodes in comparison with HPE**

		HPE				Total
		Positive		Negative		
		Count	%	Count	%	
Fluorescein Dye	Positive	32	84.2%	49	69.1%	81
	Negative	6	15.8%	22	30.9%	28
	Total	38	100%	71	100%	109

Validity of Fluorescein Dye in comparison with HPE

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	84.21%	69.58, 92.56
Specificity	30.99%	21.44, 42.48
Positive Predictive Value	39.51%	29.57, 50.39
Negative Predictive Value	78.57%	60.46, 89.79
Diagnostic Accuracy	49.54%	40.33, 58.78

Table 21: **Validity of Methylene Blue in stationing Lymph nodes in comparison with HPE**

		HPE				Total
		Positive		Negative		
		Count	%	Count	%	
Methylene Blue	Positive	17	44.7%	46	64.8%	63
	Negative	21	55.3%	25	35.2%	46
	Total	38	100%	71	100%	109

Validity of Methylene Blue in comparison with HPE

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	44.74%	30.15, 60.29
Specificity	35.21%	25.12, 46.82
Positive Predictive Value	26.98%	17.58, 39.03
Negative Predictive Value	54.35%	40.18, 67.85
Diagnostic Accuracy	38.53%	29.93, 47.91

HPE Positive Nodes:

Methylene + Fluorescein Dye = 13 nodes

Fluorescein Dye = 19 nodes

Methylene Dye = 4 nodes

No Dye = 2 nodes

Total number of nodes = 38

Total by Methylene Dye = 17

Total by Fluorescein Dye = 32

DISCUSSION

DISCUSSION

MBD is a widely accessible, secure, affordable, and equally effective dye for SLN identification in EBC. The SLN procedure consists of recognizing and removing the first LNs that filter the lymphatic field from the tumor. One main pitfall is the failure to visualize the SLN, resulting in incorrect tumor staging leading to sub-optimal treatment or axillary recurrence. Careful intra-operative digital examination, SLN identification, frozen section and HPE are keys to successful management in EBC. Thus, the technique of SLNB, which is less invasive and with the least morbidity, is recommended toavoid unnecessary axillary dissections if SLN is negative. SLNB is a relatively new surgical technique that has its own learning curve, which is highly multidisciplinary and needs to be standardized. Although MBD aids in a better knowledge of SLN anatomic placement and raises the detection rate in the axilla, the paradigm of surgical care of ALN in patients with breast cancer has changed from major invasive surgery to minimally invasive and selective surgery.

SLN biopsy has acquired mainstream popularity as a minimally invasive replacement for ALND for nodal staging of breast cancer. Compared with developed countries, in underdeveloped countries, more breast carcinoma cases we're presented late and were diagnosed in advanced stages. Axillary dissection is a regular technique among us owing to this. In the mapping of sentinel nodes in different tumors, FS has been described as an alternate tracer with some tangible benefits. Indocyanine green (ICG), a fluorescent dye, has been touted as a novel technique in SLNB for breast cancer.

Although the standardway of lymphatic mapping is by combination technique, hence both fluorescein dye and methylene blue in SLNB need to be assessed. Therefore a Prospective study was conducted among 30 patients with carcinoma of the breast aged >18 years with no palpable or visible lymph nodes of axilla at the Department of General Surgery, R.L. Jalappa

Hospital and Research Centre, Sri Devaraj Urs Medical College, Tamaka, Kolar. This research was carried out for a year. First, before research begins, institutional ethics review committee approval was acquired. Prior to the study's commencement, informed permission was acquired from every assist in the process.

General Profile:

The mean age was 53.13 ±14.180 years. Most patients were in the age group 51 to 60 years (26.7%). 73.3% were housewives, and 26.7% were laborers. The capacity to recognize the SLN is adversely linked with patient age. This previously reported observation might be explained by the blue dye's failure to enter the system of lymphatics after being infiltrated into the breast of postmenopausal women that had fat restored. 32 individuals with a median age of 50 were investigated by Altan zdemir et al. in [111]. With a median calculated age of 43, Arindam Mukherjee et al. [112] assessed 27 cases.

Symptoms:

The average timeframe of symptoms was 4.30 ±2.588 months. Most of them had a Lump in the right breast (70%), and 30% had a lump in the Left breast. 10% had pain. According to research by Altan zdemir et al., 2013, the RT side and upper outside quadrant were the most frequent side and site of tumor location, respectively. In the study conducted by Arindam Mukherjee et al., the upper outer quadrant accounted for 44% of all tumor sites in 112 patients.

Comorbidities and history:

In the present study, 3.3% had DM, 10% had HTN, and 6.7% had HTN/DM. 6.7% had irregular cycles, 63.3% were post-menopausal, and 30% had a regular cycle. In the present

study, 16.7% had pallor, no other signs were present on GPR, and 100% had a normal spine.

Carcinoma of Breast features:

In the present study, 66.7% had Ca Right breast, and 33.3% had Ca left breast. 20% were in stage T2N0M0, 30% were in T3N0M0 and 50% were in T4BN0M0. 66.7% underwent a mastectomy, and 33.3% underwent Lumpectomy. On HPE, 86.7% had Infiltrating Ductal Ca, 10% had Lobular Ca, and 3.3% had Squamous Cell Ca.

Gipponi M et al.,[113] detected invasive ductal carcinoma in 87 cases, invasivelobular carcinoma in 6 cases, mucinous carcinoma in 2 patients, medullary carcinoma in 2 cases, tubular carcinoma in 1 case and high-grade ductal carcinoma in situ with micro invasion in 2 cases. **Sadat pusina et al.,**[114] studied 50 patients. The HPE analysis revealed that the most common cancers were ductal carcinoma in 27, lobular in 12 and other types in 11 patients, with one each apocrine, mucinous, neuroendocrine, and papillary.

Carole Methelin et al.,[115] studied 100 patients, and the histological types were ductal carcinoma 83, lobular carcinoma 14 and mixed carcinoma in 4 patients they observed low-grade tumors in 38 patients, intermediate-grade 42 patients and high-grade 22 patients with carcinoma. Our results with 37 patients were almost similar, with most tumors being invasive ductal carcinoma. This study's findings are comparable to those reported by Nano MT et al., [116] who researched the clinical and histological parameters connected to the identification of SLN and found that clinical features had no impact on sentinel node identification.

Sentinel Lymph nodes:

In the present study, in 26.7% of patients, three lymph nodes and four lymph nodes

were identified; in 20% of patients, five lymph nodes, in 16.7% of patients, two lymph nodes; in 6.7% of patients, six lymph nodes and 3.3% of patients one lymph node were identified.

In the current research, Lymph Nodes Identified with Fluorescein and Methylene Blue Dye were 1 in 46.7%, 2 in 36.7% of patients and 3 in 6.7% of patients, and 10% of patients had no lymph nodes in both staining. On Fluorescein Dye, 10% had no lymph nodes, 53.3% had one lymph node, 33.3% had two lymph nodes, and 3.3% had three lymph nodes. On Methylene Blue staining, 53.3% had no lymph nodes, 23.3% had one lymph node, and 23.3% had two lymph nodes. Out of 30 patients, seven patients had no lymph nodes stained with dye, and 23 had staining of lymph nodes.

Brahma et al., [117] in their study observed that the mean number of the SLN which were noticeable was 2; there was no anaphylactic reaction seen; however, some patients had skin necrosis. In the current research, there was no anaphylactic reaction to methylene blue dye and no skin necrosis.

Validity of Fluorescein Dye and Methylene Blue in the identification of SLN:

In the present study, among 81 nodes with Fluorescein Dye positive lymph nodes, 51.9% were positive in Methylene blue, and 48.1% were negative. Among 28 nodes negative for Lymph node in Fluorescein Dye, 75% were positive for Methylene Blue, and 25% were negative for both. Methylene blue staining had a sensitivity of 51.85%, specificity was 25%, PPV was 66.67%, NPV was 15.22%, and Diagnostic Accuracy was 44.95% in comparison with Fluorescein Dye. Fluorescein Dye staining had a sensitivity of 84.21%, specificity was 30.99%, PPV was 39.51%, NPV was 78.57%, and Diagnostic Accuracy was 49.54% in comparison with HPE.

Methylene Blue staining had a sensitivity of 44.74%, specificity was 35.21%, PPV was

26.98%, NPV was 54.35%, and Diagnostic Accuracy was 38.53% in comparison with HPE. In their study, **Ren L et al.** [118] observed showed SLN detection rates in the fluorescein sodium group outperformed those in the isosulphan blue group. ICG's detection rate in their research was estimated to be 80%, which is considerably lower than FS's.

Thongvitokomarn S et al., [119] in their meta-analysis, it was noted that there was a statistically significant difference between "ICG (Fluorescein dye) and BD (Blue dye)" methods in terms of SLN detection rate (OR, 6.73; 95% CI, 4.20-10.78). The number of SLNs removed per patient was "2.35 (1.46-5.4), 1.92 (1.0-3.64)", and "1.72 (1.35-2.08)" for ICG, BD, and RI (Radioisotope), respectively.

IN THEIR STUDY, Chang YW et al., [106] observed that SLNs dyed with fluorescein were visualised in 57 of 61 patients (93.4%). Neither of the patients experienced adverse reactions from the usage of fluorescein. **Li L et al.,**[109] in Indocyanine green (ICG) combined with methylene blue (group A), SLN of carcinoma of the breast were noticed in 63 patients, with an incidence of 94.0% (63/67), a false-negative rate of 7.5% (4/53). In methylene blue (group B), SLN of carcinoma of the breast were noticed in 52 patients, with an incidence of 92.9% (52/56) and a false-negative rate of 7.5% (3/40). The outcomes of the two groups biopsies weren't significantly different. As demonstrated by this prospective clinical trial, SLNB for breast cancer patients using fluorescein and UV LED light is possible. In this study, there were no negative outcomes.

Comparing our study to a SLN biopsy done by **Somashekhar et al.,**[6] where he did SLNB in 135 patients using a blend of methylene blue dye and radioactive technetium- 99m Sulphur colloid, the sensitivity, specificity, PPV, NPV and accuracy of SLNB was 96.2%, 100%, 100%, 98.6% and 99% respectively. The validity of Methylene blue was low in the present study.

Brahma et al[117] study, which is consistent with our result obtained, employed just methylene blue dye to determine SLN in 96 patients. He identified Sentinel lymph nodes in 91.7% and metastasis in 53.4%.

In a study by **Goel et al.,** [33,] he stated that suspicious lymph nodes might obstruct the lymphatic channel hindering sampling of sentinel lymph nodes and leading to inadequate nodal staging. With this belief, he used axillary ultrasound first to investigate nodal status. Suspicious nodes were subjected to image-guided FNAC, and non-suspicious and FNAC-negative nodes proceeded to SLNB. The sensitivity and specificity of SLNB were 56.5% and 97.56%, respectively. In another study by **Orr RK et al.,** [120,] he concluded that all surgeons should perform full ALND while learning node biopsy technique to ensure accurate staging of a patient with breast carcinoma. He also stated that after documentation of the accuracy of SLNB (sensitivity > 90%) should, all ALND be omitted for patients with a negative SLN.

Similarly, in other studies done rate of negative SLN and the axillary node was reported at 62% by "**Kebudi et al.,**[121], 70% by **Zaman et al.,**[122] and 53.3% by **Vohra et al.,**[123]". Likewise, in our study, 10.81% of patients in which a sentinel lymph node was not detected and all the 10.81% cases were injected with methylene blue dye by a surgery resident who had no experience with injecting methylene blue dye. **Chung A et al.**[103] proposed two possible explanations for this variation: the surgeon's early practice or the broad tumor penetration that caused the lymph fluid to be redistributed to non-sentinel nodes.

Madhivadhanam et al., [124] conducted a comprehensive evaluation regarding the use of methylene blue dye in SLNB in assessing breast cancer. They came to the conclusion that methylene blue is the most effective substitute for other dyes in identifying breast cancer patients' SLN. This study is similar as regards the efficacy of methylene blue with our research, where the identification rate of SLN by methylene blue dye was 89.1%. This was supported

by the studies done by "**Golshan and Nakhils[125], Carole methelin et al.,[115], Chen X et al., [126], Sohail et al.[127], Ozdemir et al.[128], Fattahi et al.[129], Lyman GH et al.[130], Paulinelli et al.[131]**" showing identification rate 68-96.5%. This dye might also be utilized with confidence because it is more complicated, inexpensive, widely and easily accessible. However, our study showed a lower sensitivity for Methylene blue compared to Fluorescein staining.

Table 22: SLN identification by using either combination or blue dye alone

Study/Author	No. of Cases	SLN Identification Rate (%)	FN Rate (%)
Canavese (2009)[132]	202	97.1%	6.5%
Krag (1998)[133]	443	91.0%	11.0%
Tafra (2001)[134]	529	87.0%	13.0%
Gill G. [135]	1,080	94.5%	5.5%
Harlow SP et al.[136]	5,611	97.1%	9.8%
Mansel RE.,[8]	803	96.1%	6.7%
Zavagno G et al.[7]	697	95.0%	6.7%
Rama Mani et al., 2014 [137]	96	72%%	14.5%
Altan Özdemir et al.,[111]	32	94%	15%
This study	30	57.8%	55.3%

There is disagreement regarding the ideal MBD dosage for SNB. MBD is often administered in doses of 2 and 5 ml for SNB. In our trial, we administered 3 ml of MBD to each patient. The amount of MBD used in the 18 papers that **Jiyu Li et al. [138]** considered in their meta-analysis ranged from 0.1 ml to 10 ml. The trials that employed 5-ml doses of MBD as opposed to 2-ml injections of MBD did not vary in their identification rates or FNR. Future research must be well-designed to discover the ideal MBD dosage for SNB.

CONCLUSION

CONCLUSION

The study findings show that the detection of SLNs using fluorescein was high compared to methylene blue; both methods were efficient, acceptable and secure in breast cancer patients. Fluorescein dye had better validity in the diagnosis of Sentinel lymph node compared to Methylene blue in the Carcinoma of the breast. Fluorescein dye had higher sensitivity, specificity, PPV and NPV when compared to Methylene blue in identifying SLN in Breast cancer. Methylene blue findings also showed moderate validity when compared with Fluorescein dye findings. Hence use of fluorescein dye in place of methylene blue for SLN can be considered due to its higher validity and low side effects profile.

RECOMMENDATIONS

The study recommends using fluorescein dye for the detection of the sentinel lymph nodes sentinel lymph node in carcinoma of the breast due to its higher diagnostic ability than Methylene blue. The study also suggests using “Methylene blue dye for SLN identification” in breast carcinoma when fluorescein dye is unavailable or in case of contraindications to Fluorescein dye. Hence Fluorescein dye is recommended as the first choice for sentinel surveillance due to better validity than Methylene blue. The study also suggests re-examining findings by surveying a large sample size and in multiple centers.

LIMITATIONS

1. The number of patients involved in this study was few, and as a result, the results might well be biased. Hence future studies can be done on larger samples.
2. Convenient sampling technique – This can lead to selection bias. Hence random selection methods are to be used in future studies.
3. Training status – Technicians performing dye injections and other procedures can also hinder the results. Hence training of technicians can play a role in sentinel lymph node identification.
4. The cost of both dyes for SLN identification was not assessed.

SUMMARY

SUMMARY

A Prospective study was conducted among 30 patients with breast carcinoma aged >18 years who are having no identifiable axillary lymph nodes on palpation at the Department of General Surgery, R.L. Jalappa Hospital and Research Centre, Sri Devaraj Urs Medical College, Tamaka, Kolar. The investigation lasted for a total of 1 year. Obtained institutional ethical approval before the start of the study. Before the study began, informed permission and written consent were gathered from each patient who was enrolled.

1. The mean age was 53.13 ± 14.180 years. Most patients were in the age group 51 to 60 years (26.7%).
2. In the present study, 73.3% were housewives, and 26.7% were laborers.
3. The average number of months the symptoms persisted was 4.30 ± 2.588 months. 70% of them had a lump in their right breast, 30% had one in their left, and 10% complained of pain.
4. In the present study, 3.3% had DM, 10% had HTN, and 6.7% had HTN/DM.
5. In the present study, 66.7% had Ca Right breast, and 33.3% had Ca left breast.
6. In the present study, 20% were in stage T2N0M0, 30% were in T3N0M0, and 50% were in T4BN0M0.
7. In the present study, 66.7% underwent a mastectomy and 33.3% experienced Lumpectomy.
8. In the present study on HPE, 86.7% had Infiltrating Ductal Ca, 10% had Lobular Ca, and 3.3% had Squamous Cell Ca.
9. In the present study, in 26.7% of patients, three lymph nodes and four lymph nodes were identified; in 20% of patients, five lymph nodes, in 16.7% of patients, two lymph nodes; in 6.7% of patients, six lymph nodes and 3.3% of patients one lymph node were identified.

10. In the present study, Lymph Nodes Identified with Fluorescein and Methylene Blue Dye were 1 in 46.7%, 2 in 36.7% of patients and 3 in 6.7% of patients, and 10% of patients had no lymph nodes in both staining.
11. On Fluorescein Dye, 10% had no lymph nodes, 53.3% had one lymph node, 33.3% had two lymph nodes, and 3.3% had three lymph nodes.
12. On Methylene Blue staining, 53.3% had no lymph nodes, 23.3% had one lymph node, and 23.3% had two lymph nodes.
13. In the present study, out of 30 patients, seven had no lymph nodes stained with dye and 23 had staining of lymph nodes.
14. In the present study, among 81 nodes with Fluorescein Dye positive lymph nodes, 51.9% were positive in Methylene blue, and 48.1% were negative. Among 28 nodes negative for Lymph node in Fluorescein Dye, 75% were positive for Methylene Blue, and 25% were negative for both.
15. Methylene blue staining had a “sensitivity of 51.85%, specificity was 25%, Positive Predictive Value was 66.67%, Negative Predictive Value was 15.22%, and Diagnostic Accuracy was 44.95% ’’ in comparison with Fluorescein Dye.
16. Fluorescein Dye staining had a “sensitivity of 84.21%, specificity was 30.99%, Positive Predictive Value was 39.51%, Negative Predictive Value was 78.57%, and Diagnostic Accuracy was 49.54%” in comparison with HPE.
17. Methylene Blue staining had a “sensitivity of 44.74%, specificity was 35.21%, Positive Predictive Value was 26.98%, Negative Predictive Value was 54.35%”, and Diagnostic Accuracy was 38.53% in comparison with HPE.

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ANNEXURES

ANNEXURE – I: PROFORMA

FLUORESCEIN SODIUM -AN ALTERNATIVE TO METHYLENE BLUE IN DETECTING SENTINEL AXILLARY LYMPH NODES IN CARCINOMA BREAST.

Name: Phone number:
Age: Address:
Sex: DOA:
Occupation: DOS:
UHID number: DOD:
Presenting complaints:
Past history:
Family history:
Menstrual history:
Obstetric history:

Year of 1st and last childbirth: Duration of breastfeeding:

GENERAL PHYSICAL EXAMINATION:

- Built and nourishment:
- Pallor/Cyanosis/Icterus/Clubbing/oedema/Generalized lymphadenopathy

VITAL DATA:

- Pulse:
- Temperature:
- BP:
- Respiration rate :

Systemic examination

- Per abdomen:
- Respiratory system:
- Cardiovascular system:
- Central nervons system:

LOCAL EXAMINATION:

Inspection :

- Site:
- Size:
- Symmetry: symmetrical/asymmetrical
- Number:
- Borders: well-defined/ill-defined
- Surface: smooth/irregular
- Skin changes:
  - Peau d'orange : yes / no
  - Dimpling: yes/no
  - Ulceration and fungation: yes/no
  - Nipple discharge: yes/no
  - Scars: yes/no

Palpation :

- Local rise of temperature: present/absent
- Tenderness: present/absent
- Number:
- Size:
- Borders: well-defined/ill-defined
- Consistency: soft/firm/hard
- Fluctuation: present/absent
- Transillumination: present/absent
- Surface: smooth/irregular
- Axillary lymphadenopathy:
  - Location:
  - Number:
  - Consistency:
  - Fixity
- Supraclavicular lymphadenopathy:
  - Number
  - Consistency
  - Fixity

Investigations:

- FNAC:
- TRUCUT biopsy:

- CECT thorax:
- USG(ABD+PELVIS):
- MAMMOGRAPHY:
- LFT:
  - IHC: ER: positive /negative
  - PR: positive /negative
  - Her2neu: positive /negative

Intra-operative findings:

- Time of injection:
- Site of injection:
- No. of lymph nodes identified by-Methylene blue:
- Fluorescein sodium:
- Surgical technique:
- Interpretation:

ANNEXURE – II: PATIENT INFORMATION SHEET

Study title: "**Fluorescein Sodium -A Convenient Alternative to Methylene Blue in Detecting Sentinel Axillary Lymph Node in Carcinoma Breast.**"

GUIDE: DR. SREERAMULU P. N.

STUDY CONDUCTED BY: DR. VEDANTH M.

Study location: R L Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar. The purpose of the study is explained in detail to us and all information collected is for study purposes only. The data collected is submitted to the department of surgery, SDUMC, Kolar and confidentiality are ensured. The merits and demerits were explained briefly to us.

All Patients diagnosed with carcinoma breast will be included in this study. Patients in this study will undergo routine investigations, CBC, RFT, FNAC/Biopsy of breast tissue, ER, PR, and Her2neu. Patients planned for either breast-conserving surgery or a mastectomy, along with validation SLNB for axilla, will be selected for the study. Two dyes (methylene blue dye and Fluorescein sodium) will be injected through the intradermal periareolar route. Then the sentinel lymph nodes will be identified and sent for biopsy.

The benefits of the study were avoidance of post-operative pain, impaired shoulder mobility, arm weakness, lymphangitis, lymphedema, and lymphangiosarcoma. The risks of the study such as allergic reactions to the dye, anaphylactic shock, staining of the breast tissue, and the need for ventilatory support. Please read the following information and discuss it with your family members. You can ask any question regarding the study. If you agree to participate in the survey, we will collect information (as per proforma) from you, a person responsible for

you, or both. Relevant history will be taken. This information collected will be used only for dissertation and publication.

All information collected from you will be confidential and will not be disclosed to any outsider. Your identity will not be revealed. The Institutional Ethics Committee has reviewed this study, and you are free to contact the member of the Institutional Ethics Committee. There is no compulsion to agree to this study. The care you will get will not change if you don't wish to participate. You must sign/ provide a thumb impression only if you voluntarily agree to participate in this study.

For further information contact:
Dr Vedanth M [postgraduate]
Department of General Surgery
SDUMC, Kolar
Phone number
9880012233.

left thumb impression/signature of the patient

Left thumb impression/signature of the witness.

ANNEXURE – III: CONSENT FORM

Study title: "**Fluorescein Sodium -A Convenient Alternative to Methylene Blue in Detecting Sentinel Axillary Lymph Node in Carcinoma Breast.**"

Principal investigator: Dr.Vedanth. M

I, Mrs. …….., have been explained in my understandable language that I will be included in a study, "**Fluorescein Sodium -A Convenient Alternative to Methylene Blue in Detecting Sentinel Axillary Lymph Node in Carcinoma Breast.**" being conducted in RL JALAPPA HOSPITAL.

1. I have been explained that my clinical findings, investigations, and preoperative and postoperative findings will be assessed and documented for study purposes.
2. I have been explained that my participation in this study is entirely voluntary, and I can withdraw from the study at any time. This will not affect my relationship with my doctor or treatment for my ailment.
3. I have explained the study's risks/benefits, such as allergic reactions to the dye, anaphylactic shock, staining of the breast tissue, and the need for ventilatory support.
4. I understand that the medical information produced by this study will become part of institutional records and will be kept confidential by my said institute.
5. I agree not to restrict the use of any data or results that arise from this study, provided such service is only for scientific purpose(s).
6. I have the principal investigator's mobile number for enquiries.
7. I have been informed that the standard of care will be maintained throughout the treatment period.
8. I give full consent to be added to this study.

Investigator: Dr Vedanth M.

Participant's signature/ thumb impression

Name:

Date:

Signature/thumb impression of the witness:

Name:

Relation to patient:

Date:

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[illegible]: "[illegible] - [illegible] [illegible]

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ANNEXURE – IV: MASTER CHART

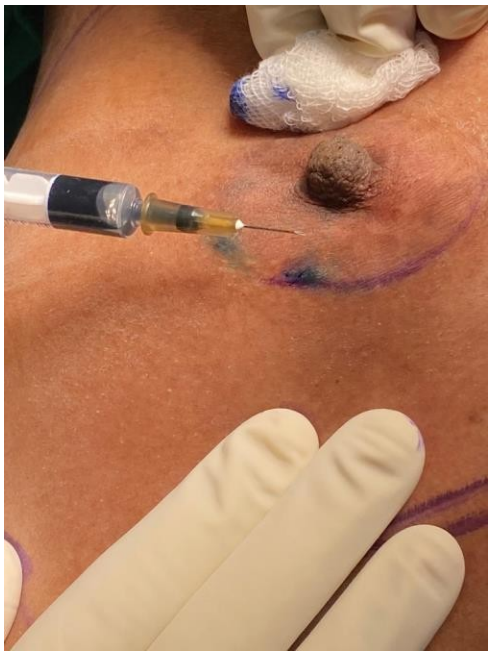
S.No.	UHID	AGE	OCCUPATION	PRESENTING COMPLAINT	DURATION	ASSOCIATED COMPLAINTS	PAST HISTORY	FAMILY HISTORY	MENSTRUAL HISTORY	OBSTETRIC HISTORY	BUILD	NOURISHMENT	PALLOR	ICTERUS	CYANOSIS	CLUBBING	GEN. LYMPHADENOPATHY	EDEMA	SPINE	PULSE	BP
1	68097	58	HOUSEWIFE	LUMP IN LEFT BREAST	5 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L2A1	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	110/80
2	58769	65	HOUSEWIFE	LUMP IN RIGHT BREAST	2 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L3	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	120/80
3	62864	35	HOUSEWIFE	LUMP IN RIGHT BREAST	4 M	NIL	NIL	NIL	REGULAR CYCLES	P2L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	120/80
4	946403	55	HOUSEWIFE	LUMP IN LEFT BREAST	5 M	PAIN	HTN	NIL	POST MENOPAUSAL	P2L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	110/70
5	941032	33	HOUSEWIFE	LUMP IN LEFT BREAST	3 M	NIL	NIL	NIL	REGULAR CYCLES	P2L1D1	MODERATE	MODERATE	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	72	110/70
6	923327	40	HOUSEWIFE	LUMP IN RIGHT BREAST	4 M	NIL	HTN	NIL	POST MENOPAUSAL	P4L3A1	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	110/70
7	921954	45	HOUSEWIFE	LUMP IN RIGHT BREAST	12 M	NIL	NIL	NIL	IRREGULAR CYCLES	P3L3	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	110/70
8	903629	42	HOUSEWIFE	LUMP IN LEFT BREAST	6 M	NIL	NIL	NIL	IRREGULAR CYCLES	P3L3	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	78	120/80
9	941346	65	LABOURER	LUMP IN LEFT BREAST	1 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	110/70
10	948715	30	HOUSEWIFE	LUMP IN RIGHT BREAST	3 M	NIL	NIL	NIL	REGULAR CYCLES	P2L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	83	110/70
11	888033	80	HOUSEWIFE	LUMP IN RIGHT BREAST	2 M	NIL	NIL	NIL	POST MENOPAUSAL	P1L1	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	68	110/70
12	907808	55	HOUSEWIFE	LUMP IN RIGHT BREAST	1 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L4	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	120/80
13	923478	72	HOUSEWIFE	LUMP IN LEFT BREAST	2 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L2A1	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	110/70
14	938269	42	LABOURER	LUMP IN RIGHT BREAST	3 M	NIL	NIL	NIL	REGULAR CYCLES	P2L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	110/70
15	922183	34	LABOURER	LUMP IN RIGHT BREAST	2 M	NIL	NIL	NIL	REGULAR CYCLES	P2A2	MODERATE	MODERATE	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	84	100/60
16	883577	42	HOUSEWIFE	LUMP IN RIGHT BREAST	3 M	NIL	NIL	NIL	REGULAR CYCLES	P1L1	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	120/80
17	915766	70	HOUSEWIFE	LUMP IN RIGHT BREAST	6 M	NIL	NIL	NIL	POST MENOPAUSAL	P5L5	MODERATE	MODERATE	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	68	110/70
18	933465	45	LABOURER	LUMP IN LEFT BREAST	3 M	NIL	NIL	NIL	REGULAR CYCLES	P3L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	76	140/90
19	876387	42	HOUSEWIFE	LUMP IN RIGHT BREAST	6 M	NIL	NIL	NIL	REGULAR CYCLES	P2L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	82	130/80
20	860129	76	HOUSEWIFE	LUMP IN RIGHT BREAST	5 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	88	130/80
21	891674	65	HOUSEWIFE	LUMP IN RIGHT BREAST	6 M	NIL	NIL	NIL	POST MENOPAUSAL	P2L2	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	78	110/70
22	887908	31	LABOURER	LUMP IN RIGHT BREAST	4 M	NIL	NIL	NIL	REGULAR CYCLES	P1L1	MODERATE	MODERATE	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	84	120/80
23	897214	60	HOUSEWIFE	LUMP IN RIGHT BREAST	6 M	NIL	HTN	NIL	POST MENOPAUSAL	P3L3	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	140/80
24	879122	65	HOUSEWIFE	LUMP IN RIGHT BREAST	5 M	NIL	NIL	NIL	POST MENOPAUSAL	P4L4	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	84	120/80
25	897018	59	LABOURER	LUMP IN LEFT BREAST	3 M	NIL	NIL	NIL	POST MENOPAUSAL	P0L0	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	110/80
26	897153	70	HOUSEWIFE	LUMP IN LEFT BREAST	4 M	NIL	DM	NIL	POST MENOPAUSAL	P2L2	MODERATE	MODERATE	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	83	140/90
27	892991	55	HOUSEWIFE	LUMP IN RIGHT BREAST	12 M	NIL	HTN/DM	NIL	POST MENOPAUSAL	P4L4	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	140/80
28	879823	50	LABOURER	LUMP IN RIGHT BREAST	5 M	PAIN	HTN/DM	NIL	POST MENOPAUSAL	P1L1	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	72	130/80
29	815423	53	HOUSEWIFE	LUMP IN RIGHT BREAST	4 M	NIL	NIL	NIL	POST MENOPAUSAL	P3L3	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	81	110/70
30	845877	60	LABOURER	LUMP IN RIGHT BREAST	2 M	PAIN	NIL	NIL	POST MENOPAUSAL	P3L3	MODERATE	MODERATE	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	NORMAL	80	100/60

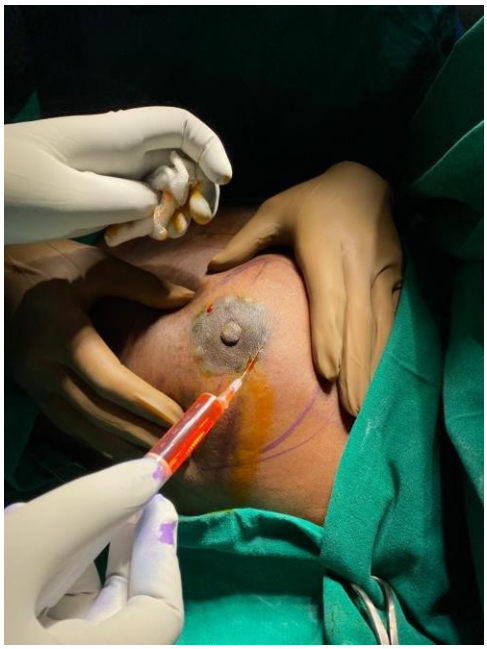
TEMPERATURE	RESP. RATE	BREAST SYMMETRY	NIPPLE RETRACTION	NIPPLE DISCHARGE	SITE	BORDERS	SURFACE	SKIN CHANGES	LYMPHADENOPATHY	CONTRALATERAL BREAST	TEMPERATURE	TENDERNESS	NUMBER	SIZE	BORDERS	SURFACE	CONSISTENCY
AFEBRILE	14	ASSYMMETRICAL	PRESENT	ABSENT	L-ALL QUADRANTS	ILL DEFINED	SMOOTH	PDO	NIL	NORMAL	NORMAL	TENDER	SINGLE	7X7	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	R-LO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	5X4	WELL DEFINED	SMOOTH	HARD
AFEBRILE	14	ASSYMMETRICAL	PRESENT	ABSENT	R-LI	ILL DEFINED	IRREGULAR	ULCER	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	8X5	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	L-UI	ILL DEFINED	SMOOTH	ULCER	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	4X4	WELL DEFINED	SMOOTH	HARD
AFEBRILE	14	SYMMETRICAL	ABSENT	ABSENT	L-UO	ILL DEFINED	IRREGULAR	PDO	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	7X5	ILL DEFINED	IRREGULAR	HARD
AFEBRILE	16	SYMMETRICAL	ABSENT	ABSENT	R-UI	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	5X6	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	R-ALL QUADRANTS	ILL DEFINED	SMOOTH	PDO	NIL	NORMAL	NORMAL	TENDER	SINGLE	8X7	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	L-UO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	5X6	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	L-UO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X5	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	18	ASSYMMETRICAL	ABSENT	ABSENT	R-UI	WELL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	3X2	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	R-UI	WELL DEFINED	SMOOTH	PDO	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	8X6	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	R-LO	WELL DEFINED	IRREGULAR	SKIN NODULES	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	4X3	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	L-UI	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	3X2	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	R-UO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	TENDER	SINGLE	6X5	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	R-U 1/2	ILL DEFINED	IRREGULAR	TETHERING	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X6	ILL DEFINED	IRREGULAR	HARD
AFEBRILE	14	ASSYMMETRICAL	ABSENT	ABSENT	R-UI	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	TENDER	SINGLE	4X3	ILL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	R-LI	ILL DEFINED	SMOOTH	ULCER	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X5	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	L-UO	WELL DEFINED	SMOOTH	ULCER	NIL	NORMAL	LOCAL RISE +	TENDER	SINGLE	7X5	WELL DEFINED	SMOOTH	HARD
AFEBRILE	18	ASSYMMETRICAL	ABSENT	ABSENT	R-UI	ILL DEFINED	IRREGULAR	TETHERING	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X5	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	17	ASSYMMETRICAL	ABSENT	ABSENT	R-UO	ILL DEFINED	IRREGULAR	TETHERING	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	5x3	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	R-UI	ILL DEFINED	IRREGULAR	TETHERING	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	8X7	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	R-LI	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	4X3	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	R-UO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X6	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	17	ASSYMMETRICAL	ABSENT	ABSENT	R-UO	WELL DEFINED	SMOOTH	TETHERING	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	5X4	WELL DEFINED	SMOOTH	HARD
AFEBRILE	16	SYMMETRICAL	ABSENT	ABSENT	L-UI	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	TENDER	SINGLE	4X2	ILL DEFINED	SMOOTH	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	L-UO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	5x3	WELL DEFINED	SMOOTH	HARD
AFEBRILE	18	ASSYMMETRICAL	PRESENT	ABSENT	R-ALL QUADRANTS	ILL DEFINED	SMOOTH	ULCER	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X6	WELL DEFINED	IRREGULAR	HARD
AFEBRILE	16	ASSYMMETRICAL	ABSENT	ABSENT	R-LI	ILL DEFINED	IRREGULAR	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	6X6	WELL DEFINED	SMOOTH	FIRM
AFEBRILE	17	ASSYMMETRICAL	ABSENT	ABSENT	R-UO	ILL DEFINED	SMOOTH	NONE	NIL	NORMAL	NORMAL	NON-TENDER	SINGLE	9X7	WELL DEFINED	IRREGULAR	FIRM
AFEBRILE	16	ASSYMMETRICAL	PRESENT	ABSENT	R-ALL QUADRANTS	ILL DEFINED	SMOOTH	PDO	NIL	NORMAL	LOCAL RISE +	TENDER	SINGLE	8X6	WELL DEFINED	SMOOTH	HARD

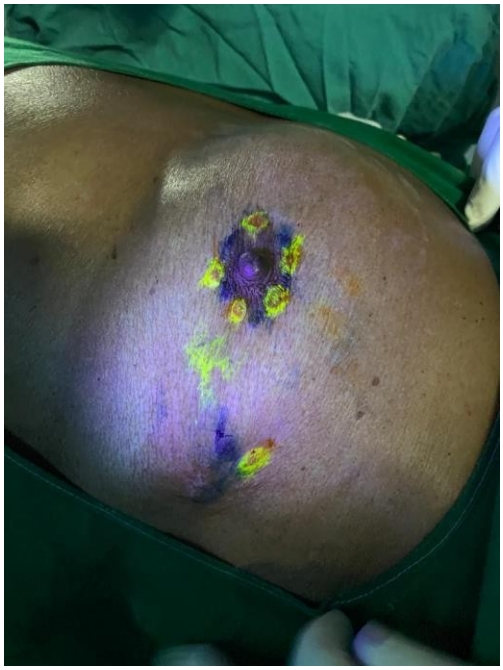
MOBILTY	AXILLA	SUPRACLAVICUAR LYMPHADENOPATHY	CVS	RS	PA	CNS	DIAGNOSIS	STAGE	SURGERY DONE	HISTOPATHOLOGY	LYMPH NODES IDENTIFIED	LYMPH NODES IDENTIFIED WITH FLUORESCEIN AND METHYLENE BLUE DYE	LYMPH NODES WITH FLUORESCEIN DYE ONLY	LYMPH NODES WITH METHYLENE BLUE ONLY	LYMPH NODES WITH NO DYE
FIXED TO BREAST TISSUE	MULTIPLE	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	4	2	1	1	0
FIXED TO BREAST TISSUE	SINGLE	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T3N0M0	MASTECTOMY	INFILTRATING DUCTAL CA	3	1	1	1	0
FIXED TO BREAST TISSUE	SINGLE	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	SQUAMOUS CELL CA	5	2	2	0	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	3	1	2	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T3N0M0	MASTECTOMY	INFILTRATING DUCTAL CA	5	0	3	2	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T3N0M0	MASTECTOMY	INFILTRATING DUCTAL CA	5	3	2	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	LOBULAR CA	4	1	2	1	0
FIXED TO CHEST WALL	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T3N0M0	MASTECTOMY	INFILTRATING DUCTAL CA	4	2	1	0	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T3N0M0	MASTECTOMY	LOBULAR CA	6	3	1	2	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T2N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	5	2	1	2	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	2	2	0	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	3	1	2	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T2N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	3	0	2	0	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T3N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	1	0	0	0	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	2	2	0	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T2N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	3	1	1	1	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	2	1	1	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	5	2	1	2	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	6	2	2	2	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	4	1	2	0	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	2	1	1	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T2N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	3	1	2	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T3N0M0	MASTECTOMY	INFILTRATING DUCTAL CA	2	1	1	0	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	4	2	1	1	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T2N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	4	1	1	2	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA LEFT BREAST	T2N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	4	1	1	1	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	5	2	2	1	0
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T3N0M0	LUMPECTOMY	INFILTRATING DUCTAL CA	3	1	1	0	1
FIXED TO BREAST TISSUE	NIL	ABSENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T3N0M0	LUMPECTOMY	LOBULAR CA	4	1	1	2	0
FIXED TO BREAST TISSUE	NIL	PRESENT	S1 S2 HEARD	B/L NVBS +	NAD	NAD	CA RIGHT BREAST	T4BN0M0	MASTECTOMY	INFILTRATING DUCTAL CA	3	2	1	0	0

CASE IMAGES

Figure 43: INJECTION OF DYES







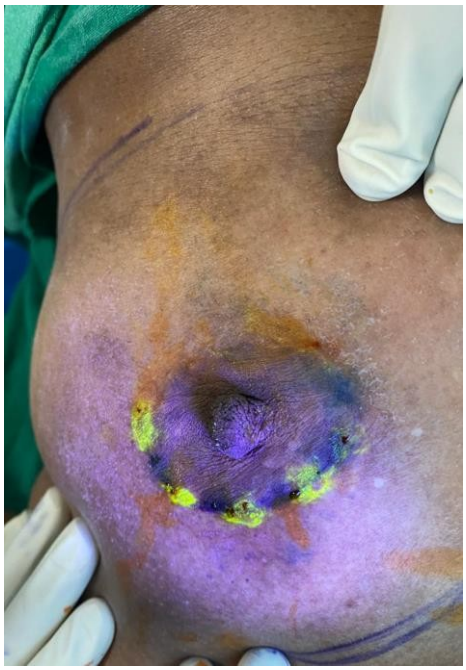
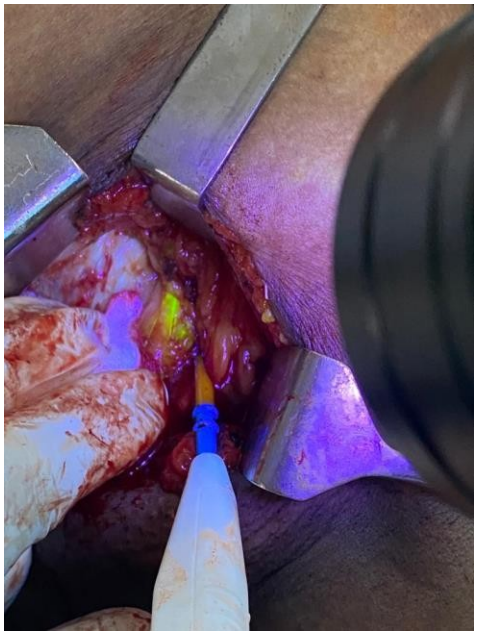
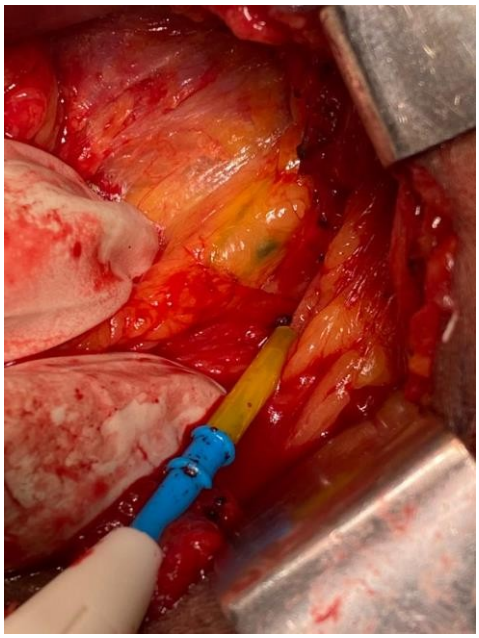
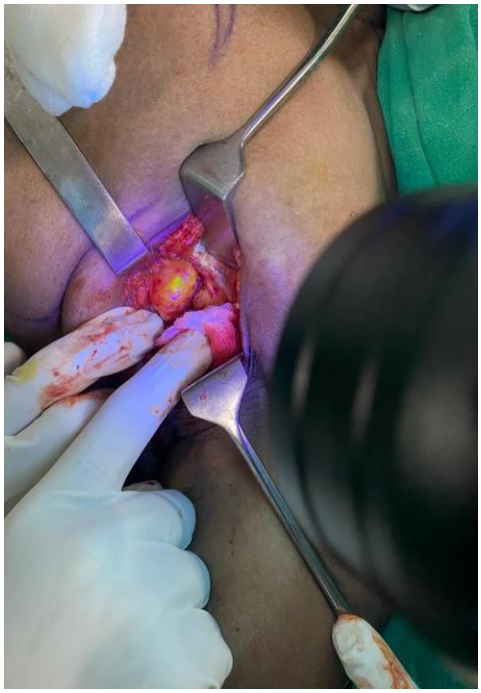


Figure 44: AXILLARY DISSECTION







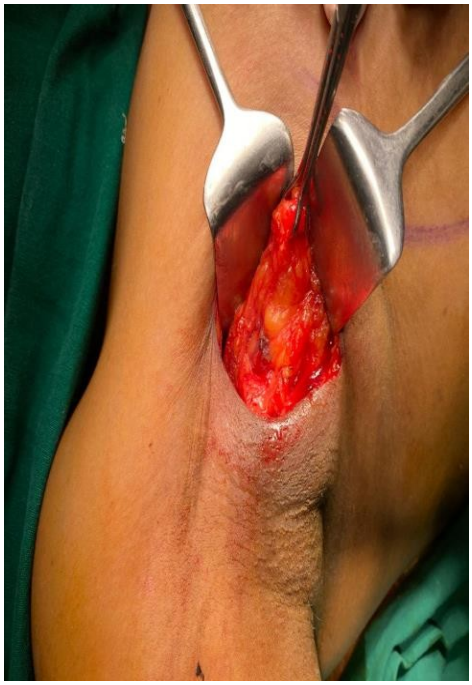
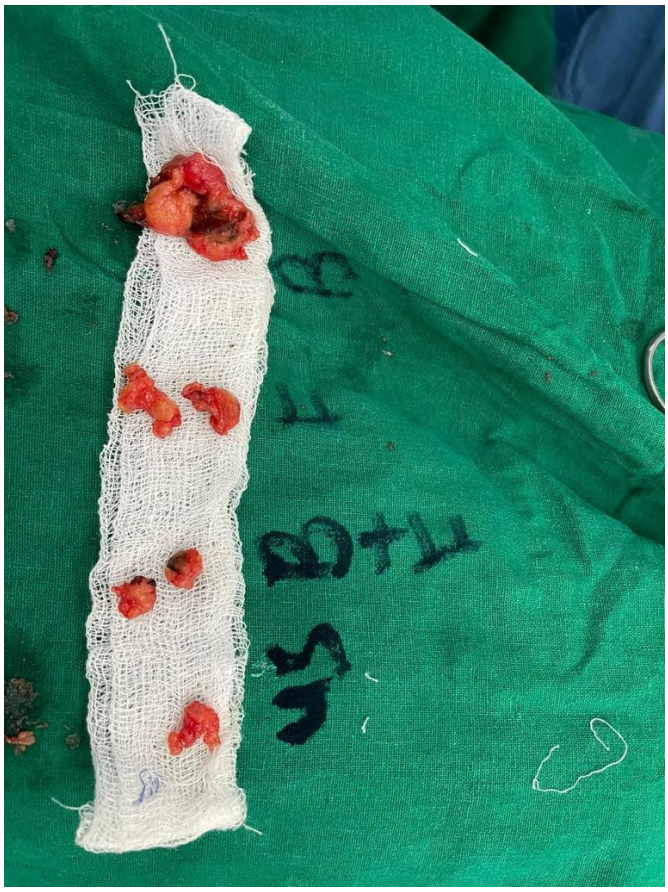


Figure 45: LYMPH NODES IDENTIFIED



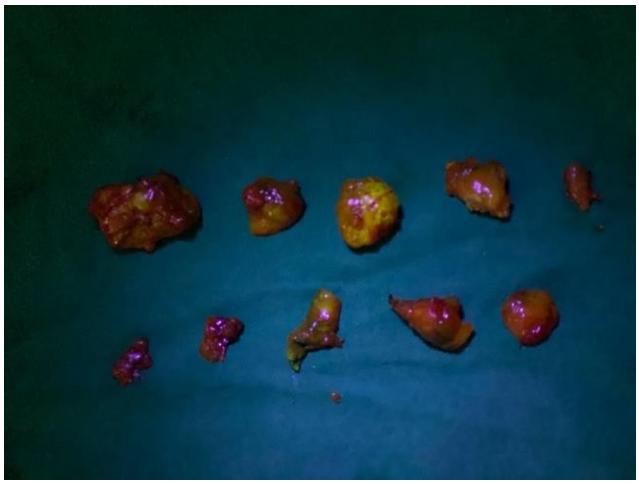


Figure 46: POST OPERATIVE PICTURES