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RESEARCH ARTICLE

FACTORS IN CARCINOMA BREAST PATIENTS POST-NEOADJUVANT THERAPY DETERMINING LYMPH NODE DISSECTION LEVELS: INSIGHTS FROM A REGIONAL CANCER CENTER IN EASTERN INDIA

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Abstract

Background and Objectives: Breast cancer is the most common cancer worldwide as well as India. According to Globocon 2020 data, breast cancer constitutes 11.7 % of all cancer cases and is the fourth most common cause for cancer mortality in the world. In addition, the situation is equally alarming in India with breast cancer constituting 13.5 % of all cancers and being the most common cause of cancer mortality in India.Primary objective of this article is to study factors in post Neoadjuvant Therapy patients of Carcinoma Breast to determine the level of Lymph Node Dissection.

Methods: A single institute prospective study that included all the patients who underwent definitive surgery (MRM with SLNB with Level 1,2 and 3 LN dissection) for Breast cancer (post Neoadjuvant Therapy) with clinically node negative axilla post Neoadjuvant Therapy from 1st March 2021- 28th February 2023 . At the end of study, patients with positive lymph nodes were analysed for the factors that can predict the lymph node positivity, sensitivity, and specificity of blue dye only technique for SLNB in post NAT patients Results: Total 48 patients underwent surgery. Mean age of the patients was 46.5±8.7 years and median age was 45 years. SLN identification rate was 89.6%. In this study, we found that pathological complete response in breast tumour was significantly associated with complete pathological response in axillary lymph nodes. Patients with non-luminal subtype such as triple negative breast cancer had significant association with axillary lymph node pathological complete response.

Conclusion: Patients who are clinically N1 disease and become clinically node negative after neoadjuvant therapy and belonging to non luminal subtype have more chances of nodal PCR and so can be considered for sentinel dye technique to determine the level of lymph

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node dissection and hence to taper the axillary surgery and reduce morbidity, however blue only dye technique is not recommended due to high false negative rates.

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Introduction:-

Breast cancer is the most common cancer worldwide as well as India. According to Globocon 2020 data, breast cancer constitutes 11.7 % of all cancer cases and is the fourth most common cause for cancer mortality in the world. In addition, the situation is equally alarming in India with breast cancer constituting 13.5 % of all cancers and being the most common cause of cancer mortality in India. Moreover, in India, most common affected age group is a decade earlier than that in west.

Due to this, the management of breast cancer has shifted from radical surgeries to more conservative approach [1]. However, it was presumed that complete axillary lymph node dissection should be the standard while breast conservation was the priority. Keeping in mind Halsted‘s principle of loco regional disease, even the node negative axilla underwent complete axillary nodal dissection to clear as much microscopic disease as possible [2,3]. The use of sentinel lymph node biopsy (SLNB) for axillary conservation was validated in 1994 by Guiliano et al. to stage the axilla in clinically node negative patients [4,5]. Since then, the landmark trials-NSABP-B32, IBCSG 23-01, ACOSOG Z0011-revolutionized conservative management of sentinel node-negative, microscopic node-positive, and sentinel node-positive axilla respectively [6-8]. However, this still has not been established in the management of axilla in locally advanced breast cancer after neoadjuvant chemotherapy. Due to abominably low identification rates (IR) and high false negative rates (FNR), conservative management of axilla in this group of patients has been controversial. The prospective trials ACOSOG-Z1071, SENTINA, SNFNAC, and GANEA 2 investigated patients with positive lymph nodes before neoadjuvant chemotherapy (NACT), who had a clinical complete axillary response and subsequently underwent SLNB and ALND [9-12]. The overall false-negative rates of the SLNB were between 11.9 and 14.2%, above the 10% rate that is considered oncologically safe. To this date, only one study investigated if ALND could be omitted when axillary nodes become negative after NAC, Galimberti et al [13]. The authors concluded that SLNB was a valid method to guide therapeutic decisions in this clinical setting, but recognized that their study, being small and retrospective, needed to be corroborated by additional investigations. There has been little data from Indian subcontinent regarding sentinel lymph node biopsy in locally advanced breast cancer (LABC) patients after neoadjuvant chemotherapy (NACT) where complete ALND remains the gold standard. In this study, we will evaluate factors in patient who underwent NACT, that can help in determining the level of axillary lymph node dissection and our other objective is to determine the prevalence of different molecular subtypes and their relation with lymph node positivity in this group of patients.

Materials and Methods:-

Study design

Single institute Prospective study

Study population

The study population was taken from patients that underwent definitive surgery for breast cancer (Post Neoadjuvant therapy) at the Department of Surgical Oncology at AHPGIC, Cuttack.

Sample size and study period

All patients who underwent definitive surgery for Breast cancer (post Neoadjuvant Therapy) with clinically node negative axilla post Neoadjuvant Therapy from 1st March 2021- 28th February 2023 were included in the study.

Informed consent

Patients diagnosed with breast cancer, who had received Neoadjuvant therapy and planned for Modified Radical Mastectomy, satisfying inclusion criteria were invited to take part in the study and the informed consent was presented in the native language explaining about the surgical procedure and usage of methylene blue dye for SLNB.

Inclusion criteria
Patient with Breast Cancer with clinically node negative axilla post Neoadjuvant therapy, which were clinically node positive on Fine needle aspiration cytology before receiving Neoadjuvant therapy

Informed consent
Age > 18 years

Patients medically fit for surgery

Exclusion criteria
1. Patients not medically fit for surgery.
2. Patients with metastatic disease.
3. Patient undergoing palliative surgery.
4. Patients with palpable or radiologically detected axillary lymphadenopathy after neoadjuvant therapy.
5. Previous axillary dissection or mastectomy.
6. Any scar of previous surgery within the natural pathway of lymphatic drainage to block the lymphatics going towards axilla.
7. Patients received radiation therapy to chest wall for any other reason.
8. Male patients with breast cancers.
9. Pregnancy.
10. Inflammatory breast cancer.

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Methods:-

All patients presenting to the department of Surgical Oncology with breast cancer were staged according to AJCC 8th edition and all locally advanced breast cancer patients were sent for neoadjuvant treatment in the form of systemic chemotherapy or combination of chemotherapy and immunotherapy according to Estrogen receptor, Progesterone receptor and Her2neu status and institute protocol. Metastatic work up including Contrast Enhanced Computed Tomography scan (CECT) of thorax, abdomen and pelvis and immunohistochemistry including ER, PR, and HER2 neu and ki67% status was done for all patients. Some of the patients of early stage breast cancer but with high-risk features such as her2 positivity or triple negative breast cancer were also sent for neoadjuvant therapy after

discussion in multidisciplinary tumour board. After receiving NAT, all the patients satisfying the inclusion criteria were included in the study. Clinically and radiologically negative axilla was included in the study.

All patients were evaluated in the outpatient department. Detailed history and clinical examination was done for each patient with physical examination including systemic examination. Workup included routine blood investigations, bilateral Mammography and with Ultrasound bilateral breast and axilla and metastatic work up in the form of USG abdomen and pelvis and chest x-ray was done. CECT abdomen pelvis thorax was done as indicated. Preoperatively all patients underwent cardiology evaluation. Additional investigations were done based on co-morbidities. All patients with uncontrolled co morbidities were optimized after getting consultations from the concerned departments. Informed and written consent was taken regarding the procedure and its risks and patients were planned for surgery.

Preoperative Preparation:
An overnight 6-8hr fasting was done. Prophylactic antibiotic prior to surgery was given according to the institutional protocol.

Procedure:
All patients were operated under general anaesthesia. Modified radical mastectomy was done with level 1, 2 and 3 lymph node dissection for all patients. All patients underwent Sentinel Lymph node biopsy using methylene blue dye. Three mL of 1% methylene blue was administered in retroareolar region and massage was done for 5 minutes. Then mastectomy incision was kept and upper flap raised. After raising upper flap, axilla was entered. Efferent, blue-stained lymphatic channel(s) were identified and traced to sentinel lymph nodes, defined as any blue-stained lymph node package into which a blue lymphatic vessel drained and were sent to frozen section. Irrespective of the result of frozen section, in all the patients, complete modified radical mastectomy with axillary dissection of level 1, 2 and 3 was done. Level 3 lymph nodal tissue was sent separately. An ex-vivo search of sentinel lymph nodes was again done to find any other sentinel lymph node, and if found was sent separately for histopathological examination. Negative suction was kept and closure was done. Postoperative antibiotics were given based on the institute‘s antibiotic policy. After discharge from hospital, patients were advised to come to Out Patient Department with final Histopathology report. Detailed pathological assessment was done for histological type, grade, extent of tumour, margin status, total number of lymph node retrieval ,number of positive lymph nodes, number of positive level 3 lymph nodes, extra nodal extension, lymphovascular and perineural invasion, in-situ component, pathological response grade, presence of necrosis, calcification, desmoplasia and lymphocyte response.

At the end of study, patients with positive lymph nodes were analysed for the factors that can predict the lymph node positivity, sensitivity, and specificity of blue dye only technique for SLNB in post NAT patients.

Statistical analysis
Statistical analysis was done by 2x2 contingency table and sensitivity, specificity, positive and negative predictive value and false negative rates were calculated. Chi square test and fisher exact test were used according to variables and cell size. Other tests used included Wilcoxon signed rank test.Unpaired t test was used to calculate the difference between means of two groups. p value of <0.05 was considered as significant.

Results:-
Total cases initially identified for the study and underwent sentinel lymph node biopsy and subsequent MRM were 48. Mean age of the patients was 46.5±8.7 years and median age was 45 years. Clinical Characteristics of all the patients in the study was as described in Table 1 and tumour characteristics as described in Table 2 and Table 3.

Study Parameters		Mean/Total Number	Percentage
Laterality	Right	23	47.9
	Left	25	52.1
Tumour quadrant	UOQ	21	43.6

	UIQ	7	14.6
	LOQ	2	4.2
	LIQ	2	4.2
	Central	5	10.4
	Multiple	11	23
Tumour size	Pre NAT	5.42±1.3 cm	
Histology	IDC NST	48	100
Grade	1	1	2.08
	2	34	70.83
	3	13	27.08
Luminal A	ER +,PR+,Her2neu-, ki67<20%	3	6.25
Luminal B	ER+,PR-,Her2neu-	1	25
	ER+,PR+,Her2neu- , ki67≥20%	11	
Her 2 neu enriched	ER-,PR-,Her2neu+	6	41.67
	Triple positive	10	
	ER+,PR-,Her2nue +	4	
TNBC	ER-,PR-,Her2neu-	13	27.08
Pre NAT Stage	Ia	0	0
	Ib	0	0
	IIa	1	2.08
	IIb	4	8.3
	IIIa	24	50
	IIIb	18	37.5
	IIIc	1	2.08
Nodal staging	N1	41	85.42
	N2	6	12.5
	N3	1	2.08

Table 1:- Tumour characteristics.

Study Parameters		Mean/Total Number(n=48)	Percentage	Median
Age at Diagnosis		46.5±8.7 years		45 years
Menopausal status	Pre/peri	25	52.1	
	Post	23	47.9	
Age of Menarche		13.43 years		13 years
Age of Menopause		44.04 years		45 years
History of Tubal ligation		14	29.2	
History of OCP		1	2.1	
History of HRT		0	0	
Number of full term pregnancies		2.3		2
Duration of Breast feeding		1.8 ± 0.8 years		2 years
Body mass index		25.4 ±1.66		26

Table 2:- Clinical characteristics.

	Mean tumour size (cm)	Standard deviation
Pre NACT	5.42	1.29
Post NACT	2.08	1.74

Table 3:- Pre NACT vs Post NACT tumour size , NACT- neoadjuvant chemotherapy.

Methylene blue dye was injected in 48 cases. However, in 5 cases, there was no uptake of blue dye and sentinel lymph nodes could not be identified. Therefore, identification rate was 43 out of 48 cases, i.e., 89.6%. Whole statistical analysis for SLN technique was done for 43 cases.

The average number of sentinel lymph node identified was 1.85; with median number of lymph node identified was 2.Sentinel Lymph nodes were positive in 3 patients whereas negative in 40 patients. However, in final histopathology, lymph nodes were positive in 8 patients.

All those patients (3 patients) with positive sentinel lymph node had positive lymph nodes on final histopathology report, accounting to specificity of 100 % with positive predictive value of 100%. Out of 40 patients, that had negative sentinel lymph node, 35 were negative in final histopathology whereas 5 were positive on final histopathology report, leading to negative predictive value of 81.4%, but true positivity rate that is sensitivity of 37.5% and false negativity rate of 62.5%. (Table 4 and 5).

No. of SLN nodes identified	No. of patients	%
0	5	10.4
1	10	20.83
2	23	47.9
3	8	16.6
4	1	2.08
5	1	2.08
Sentinel LN negative	40	83.3
Sentinel LN positive	3	6.25
Sentinel LN failed	5	10.4
ALND positive	11	22.9
ALND negative	37	77.1
Positive Level 3 Axillary Lymph node	0	0%

Table 4:- Sentinel and Axillary Lymph Node Status.

Frozen	Positive lymph node in final HPR	Negative lymph node in final HPR	Number of patients
Positive Sentinel Lymph node	3	0	3
Negative sentinel lymph node	5	35	40
	8	35	43

Table 5:- Sensitivity and Specificity of SLNB using Methylene Blue.
Specificity: 35/35 x 100=100%
PPV= 3/3 x 100= 100%
NPV= 35/43 x 100= 81.4%
False negativity rate = 5/8= 62.5%

In all of 43 patients, level three axillary lymph nodes were negative for metastasis.

Of the 3 sentinel node positive patients, one was cN3, rest two were cN1. Out of these 8 patients who were true node positive, one was cN3, four were cN2 and three were N1. On further analysis, it was found that, out of 5 false negative cases, 3 of the cases were cN2 on presentation and 2 were N1, before starting chemotherapy.

Therefore, on calculating for only those cases, which were cN1, total were 39 cases, false negative were 2 cases. Therefore, sensitivity increased to 50% with false negative rate of 50% and NPV of 89.7 %.(Table 6)

Frozen	Positive lymph node in final HPR	Negative lymph node in final HPR	Number of patients
Positive Sentinel Lymph node	2	0	2
Negative sentinel lymph node	2	35	37
	4	35	39

Table 6:- Sensitivity and Specificity of SLNB using Methylene Blue in cN1 patients.
Sensitivity: 2/4 x 100= 50%
Specificity: 35/35 x 100=100%
PPV= 2/2 x 100= 100%
NPV= 35/39 x 100= 89.7%
False negativity rate = 2/4= 50%

On further sub analysis, the sensitivity and specificity was calculated for the patients who were clinical N1 pre NAT and had either her2 positive or triple negative IHC. Luminal type A and B were excluded.

Total 28 patients fulfilled the above criteria. Sentinel lymph node was identified in 25 cases. And out of 25, sentinel node was positive in 1 patient, and in final histopathology, same patient had positive lymph node. None of the other patients had positive lymph node. For these patients, sensitivity increased to 100% with NPV of 96.42 %.(Table 7).

Frozen	Positive lymph node in final HPR	Negative lymph node in final HPR	Number of patients
Positive Sentinel Lymph node	1	0	1
Negative sentinel lymph node	0	27	27
	1	27	28

Table 7:- Sensitivity and Specificity of SLNB using Methylene Blue in non-luminal subtype patients.
Sensitivity: 1/1 x 100= 100%
Specificity: 27/27 x 100=100%
PPV= 1/1 x 100= 100%
NPV= 27/28 x 100= 96.42%
False negativity rate = 0/1= 0%

Further analysis was done to identify the factors that can predict the lymph node positivity.

Patient characteristics:
Age and Menopausal status: - not significant
To investigate whether patient characteristic was predictive of axillary LN pCR in response to NAT, the patients were assigned to groups that did and did not achieve LN pCR. The patient characteristics in the form of age and menopausal status were compared with axillary lymph node pathological complete response. 37 patients had axillary nodal pCR, out of which, 23 of these 37(62.2%) patients belonged to <50 years age group, however, p value was not

significant (p = 0.7). 20 of these 37 (54.1%) patients were postmenopausal; again p value was not significant. (p=0.2).

Tumour Characteristics:
Comparison was done between the axillary LN pCR and no axillary LN pCR group on the basis of pre NAT clinical T staging, pre NAT nodal staging, histological grade, post NAT clinical T staging, post NAT complete clinical response in breast tumour, post NAT complete pathological response in breast tumour, hormone receptor, her2 neu status and ki67% levels, luminal vs. non luminal subtypes, sentinel lymph node positivity. (Table 8)

Tumour characteristic	Axillary LN PCR	
	Yes (n=37) no. (%)	No (n=11) no. (%)
Clinical Tumour stage		
T1	0(0)	1(9.1)
T2	4(10.8)	1(9.1)
T3	20(54.1)	4(36.4)
T4	13(35.1)	5(45.4)
Clinical Nodal Stage		
N1	35(94.6)	6(54.5)
N2	2(5.4)	4(36.4)
N3	0(0)	1(9.1)
Primary Tumour Size (cm)	5.6±1.05	4.9±1.8
Post NACT Tumour Size(cm)	2.0±1.8	2.4±1.6
Post NACT clinical T stage		
ycT0	10	4
ycT1	13	1
ycT2	11	4
ycT3	1	0
ycT4	2	2
Final T stage		
ypTis	1	0
ypT0	17	1
ypT1	8	3
ypT2	10	6

ypT3		1	1
Histological Grade			
G1		1(2.7)	0(0)
G2		25(67.6)	9(81.8)
G3		11(29.7)	2(18.2)
ER			
Negative		17(45.9)	2(18.2)
Positive		20(54.1)	9(81.8)
PR			
Negative		21(56.8)	3(27.3)
Positive		16(43.2)	8(72.7)
Her2neu			
Negative		21(56.2)	8(72.7)
Positive		16(43.2)	3(27.3)
Ki67			
<20%		5(13.5)	2(18.2)
≥20%		32(86.5)	9(81.8)
Subtype			
Luminal A	ER +,PR+, Her2neu-, ki67<20%	1(2.7)	2(18.2)
Luminal B	ER+,PR-,Her2neu-	0 8(21.6)	1(9.1) 4(36.4)
	ER+,PR+,Her2neu- , ki67≥20%		
Her2 neu +	ER-,PR-,Her2neu+	5(13.5) 7(51.6) 4(10.9)	1(9.1) 2(18.2) 0(0)
	Triple positive		
	ER+,PR-,Her2nue +		
TNBC		12(32.4)	1(9.1)

Table 8:- Comparison of tumour characteristics between the axillary LN pCR and non-axillary LN pCR.

Pre NAT and post NAT clinical T staging, average tumour size and histological grade: - not significant
In terms of pre NAT clinical T staging and grade of tumour, there was no significant difference between the two groups. There was no difference in the mean tumour size between the axillary LN pCR and no LN pCR group, both

pre NAT and post NAT setting (p=0.11, 0.53,respectively).There was also no statistical difference in post NAT clinical T staging with respect to both groups and there was statistical significant difference in reduction of tumour size after NAT in both axillary LN pCR and no LN pCR,(p=<0.0001,p=,0.005 respectively), however sample size in no LN pCR group was less. (Table 9)

Tumour characteristic	Axillary LN PCR		P value
	Yes (n=37) no. (%)	No (n=11) no. (%)	
Grade			0.7
1 or 2	26(70.3)	9(81.8)	
3	11(29.7)	2(18.2)	
Clinical N stage			0.004
N1	35(94.6)	6(54.5)	
N2/N3	2(5.4)	5(45.5)	
Clinical T Stage			0.56
T1/T2	4(10.8)	1(9.1)	
T3	20(54.1)	4(36.4)	
T4	13(35.1)	5(45.5)	
Post NACT cT stage			0.36
cT0	10(27)	4(36.4)	
cT1	13(35.1)	1(9.1)	
cT2	11(29.7)	4(36.4)	
cT3/cT4	3(8.1)	2(18.2)	
Primary Tumour Size (cm)	5.6±1.05	4.9±1.8	0.11
Post NACT Tumour Size(cm)	2.0±1.8	2.4±1.6	0.53
Pathological T stage			0.10

ypTis/ypT0	18(48.6)	1(9.1)	
ypT1	8(21.6)	3(27.3)	
ypT2	10(27)	6(54.5)	
ypT3	1(2.7)	1(9.1)	
ER			0.09
Negative	17(45.9)	2(18.2)	
Positive	20(54.1)	9(81.8)	
PR			0.08
Negative	21(56.8)	3(27.3)	
Positive	16(43.2)	8(72.7)	
Her2neu			0.34
Negative	21(56.2)	8(72.7)	
Positive	16(43.2)	3(27.3)	
Ki67			0.91
<20%	5(13.5)	2(18.2)	
≥20%	32(86.5)	9(81.8)	
Subtype			0.02
Luminal subtype	9(24.3)	7(63.6)	
Non luminal subtype	28(75.7)	4(36.4)	
SLN			0.07

Identified	35	8	
Not identified	2	3	
SLN			0.004
Positive	0	3	
Negative	35	5	

Table 9:- Comparison of patient baseline characteristics between the axillary LN pCR and non-axillary LN pCR.

Pre NAT clinical N staging: - significant

35 of 41 patients (85.3%) who were clinical N1 had axillary LN pCR while 2 of the 7 patients (28.6%) who were either N2 or N3 had axillary LN pCR, and this difference was statistically significant (p=0.004).(Table 9)

Complete clinical response in breast tumour and complete pathological response in breast tumour: - CCR not significant, PCR significant

Comparison was made between complete clinical response to axillary pathological response, and out of 37 cases of axillary LN pCR, 10 cases (27%) had clinical complete response(CCR), while 4 out of no LN pCR group that is out of 11 patients, had CCR(36.3%), however this difference was not statistical significant (p=0.71). But, when comparison was done between pathological complete primary breast tumour response to LN pCR, 17 out of 37(45.9%) had between pathological complete primary breast tumour response in axillary LN pCR group while it was in only 1 patient out of 11(9.1%) in no axillary LN pCR group and this difference was statistically significant (p=0.03).(Table 10,11,12)

	PCR present (breast plus axilla)	PCR absent	
CCR present	7	7	14
CCR absent	10	24	34
	17	31	48

Table 10:- Clinical complete response and pathological complete response rates.

Clinical complete response rates: 14/48 (29.2%)

Pathological complete response: 17/48(35.4%)

7 patients who had CCR had PCR

While rest of the 7 patients, did not have PCR

Clinical complete Primary breast tumour response	Axillary LN pCR	
	Yes	No
Yes	10	4
No	27	7

	37	11

Table 11:- Comparison of CCR between the axillary LN pCR and non-axillary LN pCR.
p value= 0.71, statistically not significant

Pathological complete Primary breast tumour response	Axillary LN pCR	
	Yes	No
Yes	17	1
No	20	10
	37	11

Table 12:- Comparison of PCR in breast tumour between the axillary LN pCR and non-axillary LN pCR.
p value= 0.03, statistically significant

Hormonal receptor status, Her2 neu status and ki67% levels: not significant
Comparison was done between the axillary LN pCR and no axillary LN pCR group on the basis of hormone status, 17(45.9%) and 2(18.2%) patients were ER negative in the LN pCR group and no LN pCR group respectively, while 20(54.1%) and 9(81.8%) patients were ER positive in LN pCR and no LN pCR group respectively, and p value was not significant (p=0.09).

21 patients (56.8%) and 3(27.3%) were PR negative in Axillary LN pCR and no LN pCR group respectively , while 16(43.2%) and 8(72.7%) patients were PR positive in axillary LN pCR and no LN pCR group respectively, and p value was not significant(p=0.08).

21 (56.2%) and 8 (72.7%)patients were Her 2 neu negative in axillary LN pCR and no LN pCR group respectively, 16(43.2%) and 3(27.3%) patients were Her 2 neu positive in axillary LN pCR and no LN pCR group respectively and p value was not significant (p=0.34).

5 (13.5%) and 2(18.2%) patients had ki67% <20% in axillary LN pCR and no LN pCR group respectively, while 32(86.5%) and 9(81.8%) respectively in axillary LN pCR and no LN pCR respectively were ≥20% and p value was not significant (p=0.91). (Table 9)

Luminal and non-luminal subtypes: significant
9 (24.3%) and 7 (63.6%) patients belonged to luminal subtype (A and B) in axillary LN pCR and no LN pCR group respectively while 28(75.7%) and 4(36.4%) patients belonged to non luminal subtype in axillary LN pCR and no LN pCR group respectively and this difference was statistically significant . (p=0.02), that is patient who had non luminal subtype were more likely to attain axillary LN pCR as compared to luminal A or B subtype. (Table 9)

Positive Sentinel LN: significant
None of the patient who had positive sentinel lymph node had axillary LN pCR while 35 patients out of 40 who had negative sentinel lymph node had axillary LN pCR and this difference was statistically significant with p value of 0.004.(Table 9)

Discussion:-

Axillary lymph node status is one of the important prognostic factors in patient with carcinoma breast. It remains important factor in post neoadjuvant therapy cases also. However, residual axillary nodal disease is found in only around 50-60% of breast cancer patients initially presenting with node-positive disease (cN1) who receive neoadjuvant chemotherapy. Therefore, determination of axillary involvement post neoadjuvant chemotherapy can

determine the level of lymph node dissection and can reduce the morbidity of complete axillary dissection. Sentinel lymph node surgery is the method to identify those with negative axilla and hence to avoid complete axillary dissection.

Identification rate:- In our study, blue only dye method was used to identify sentinel nodes. The identification rate was 89.6% in our study. This rate is comparable to other studies that used blue only dye method or dual tracer technique. In one of the metaanalysis by Siyang Cao et al, published in October 2021, identification rate for post NACT SLNB ranged from 72 to 100 % with a pooled IR of 91% [14]. (Table 13)

Method	No. Of studies	No. Of patients SLN identified	No. Of patients SLN attempted	IR
BD	3	102	108	89%
RI	4	397	432	96%
BD + RI	4	511	599	92%

Table 13:- IR of SLNB according to mapping technique in metanalysis by Siyaang cao et al.

False Negativity Rate:-
Another indicator for reliability for SLNB technique is false negativity rate.

In our study, overall false negativity rate was 62.5%. Nevertheless, when cases with cN2 and cN3 were omitted, and only those who were cN1 prechemotherapy were taken, false negativity rate reduced to 50%. This was much higher than that reported in studies in the literature. This may lead to under staging and so it is necessary to identify those factors leading to high false negativity rate.

Reason for high FNR in our study can be explained by:
All the patients, which were node positive before chemotherapy and became node negative, were taken into the study irrespective of stage, grade or hormone status.

Single, blue dye technique was used.
IHC was not used for identification of metastasis in lymph node on frozen section.

Overall, lymph node positivity rate was low and sample size was smaller, leading to more false negativity rate.

Median number of lymph node removed was 2.

However, when sub analysis was done, according to the luminal subtype, and luminal subtypes A and B were excluded, false negativity rate came down to 0 % in our study with negative predictive value of 96.42%. But the sample size was small (28 patients), and though false negativity rate was 0%, but overall out of 28 patients, only one patient had nodal positivity. This shows that the nodal response was better in this subgroup of patients.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-27 trial published in 2005, one of the largest studies published on SLNB after NACT included total of 428 patients underwent SLNB with concomitant ALND after NACT with an identification rate of 78.1% using the blue dye isosulfan blue alone and FNR of 14% with lymphazurin blue dye alone whereas 9.3% with dual dye.

In SENTINA study [10] published in 2013, in patients who converted after neoadjuvant chemotherapy from cN+ to ycN0 (arm C), the detection rate was 80·1% (95% CI 76·6-83·2; 474 of 592) and false-negative rate was 14·2% .it used radioactive tracer alone or tracer with blue dye. Use of additional blue dye with radio colloid lowered the false-negative rate. On further analysis, it was seen that FNRs of SLNB after chemotherapy were 24.3% for women who had one sentinel node removed and 18.5% for those who had two nodes removed.

In another study, (ACOSOG) Z1071 Clinical Trial [9], published in 2014, FNR of SLN surgery after neoadjuvant chemotherapy in patients with cN1 breast cancer and at least 2 SLNs identified at the time of surgery was

12.6%.FNRs were even higher when only one SLN was dissected with a 31% rate. It was 20.3% with single mapping method while 10.8% with dual agent mapping technique.

In another study by Sanchez et al [15], published in 2021, regarding Sentinel Node Biopsy after Neoadjuvant Chemotherapy for Breast Cancer: Preliminary Experience with Clinically Node Negative Patients after Systemic Treatment, blue dye only technique was used and identification rate was 95.2% for cN1/cN2 patients who converted to ycN0. FNR was not calculated in this study, however along with blue nodes; suspiciously nodes were also analyzed and removed. This study found that three-year OS, DDFS and RDFS rates in patients who were cN1/2 were were comparable to those registered in cN0 patients (93%, 84.8% and 87.9%, respectively).

Nodal positivity Rate and nodal pCR rates:-
In our study, nodal positivity rate was 22.9% (11 out of 48 patients), and pathological nodal pCR was 77.1 %(37 out of 48) which was higher than that reported in the studies. (Table 14)

Author year	Number biopsy proven pN+	Number who convert to ypN0 post-NAC	pCR rate
Boughey, 2014	525	215	41%
Boileua,2015	145	50	35%
Kim 2015	415	159	38%
Mamtani 2016	195	96	49%
Diego 2016	30	19	63%
Enokido 2016	143	68	48%

Table 14:- Rates of Nodal Pathologic Complete Response Following Neoadjuvant Chemotherapy in Biopsy Proven Node Positive Patients.

Factors predicting axillary LN pCR:-
In our study, there was no significant difference between the age and menopausal status of the patient between the two groups though patients who achieved axillary pCR tended to be younger (<50 years), 62.2%, this was similar to study done by Kim et al, 2017 [16].

In our study, grade of the tumour, Ki67, ER status, PR status, Her 2neu status and T staging did not significantly differ in both groups; however patients with clinical nodal staging cN1 preNACT were more likely to have LN pCR as compared to cN2/cN3 with significant p value (0.004). Another factor was that non luminal subtypes (ER-,PR-,Her2neu +,triple positive, triple negative) were significantly associated with axillary LN pCR (p=0.02) as compared to luminal A or B subtype. And all the patients who had positive SLN had positive axillary Lymph node in final histopathology.

In our study, although partial or complete clinical breast tumour response had no significant association, but pathological complete breast tumour response was significantly associated with axillary LN pCR with p value of 0.03. There was no significant association of complete clinical breast tumour response with axillary LN pCR (p=0.71). The plausible explanation behind this may be because for clinical response, clinical examination and ultrasound breast was used, while in other studies, MRI breast was used for assessing response.

In the study by Kim et al [16], negative ER and PR status, positive HER2 status, and tumors with early clinical and nodal stage did not differ significantly in the two groups while higher histological grade and higher (≥47.1%) tumor response rate were significantly associated with an increased probability of achieving axillary pCR.

In another study by Olga Kantor et al, 2018 [17], younger age, HR-negative/Her-2-negative, HR-positive/Her2-positive, HR-negative/Her2-positive, high grade, ductal histology, cN1 versus cN2 and extent of breast response were all significant independent predictors of pN0.

Reason for high FNR in our study can be explained by:
All the patients, which were node positive before chemotherapy and became node negative, were taken into the study irrespective of stage, grade or hormone status.

Single, blue dye technique was used.
IHC was not used for identification of metastasis in lymph node on frozen section.

Overall, lymph node positivity rate was low and sample size was smaller, leading to more false negativity rate.

Median number of lymph node removed was 2.

However, when sub analysis was done, according to the luminal subtype, and luminal subtypes A and B were excluded, false negativity rate came down to 0 % in our study with negative predictive value of 96.42%. But the sample size was small (28 patients), and though false negativity rate was 0%, but overall out of 28 patients, only one patient had nodal positivity. This shows that the nodal response was better in this subgroup of patients.

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Nodal positivity Rate and nodal pCR rates:-
In our study, nodal positivity rate was 22.9% (11 out of 48 patients), and pathological nodal pCR was 77.1 %(37 out of 48) which was higher than that reported in the studies. (Table 14)

Conclusions:-

This study evaluated the factors in post neoadjuvant therapy patients of carcinoma breast that can determine the level of lymph node dissection, and Sentinel lymph node technique using methylene blue was employed in this study to finally decide the level of lymph node dissection, though all of the patients underwent level 1, 2 and 3 lymph node dissection to find out the final lymph node pathological status.

In this study, we found that pathological complete response in breast tumour was significantly associated with complete pathological response in axillary lymph nodes. Clinical complete response in our study did not have significant association, though imaging in the form of only ultrasound was used to document clinical response. Apart from this, patients with non-luminal subtype such as triple negative breast cancer had significant association with axillary lymph node pathological complete response. In addition, the patients who had bulky axillary nodal burden before giving neoadjuvant therapy had less chances of lymph node pathological complete response. Moreover, none of the patient in the study had positive level 3 axillary lymph nodes.

In this study, false negative rate of sentinel blue dye technique was 62.5%, which further reduced to 50% on excluding cN2 and cN3 disease and further reduced to 0% when luminal subtypes A and B were excluded.

So, patients who are clinically N1 disease and become clinically node negative after neoadjuvant therapy , belonging to non luminal subtype, can be considered for sentinel dye technique to determine the level of lymph node dissection, however blue only dye technique is not recommended due to high false negative rates.

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