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Evaluation of lymph node ratio in breast carcinoma and its correlation with pathological and immunohistochemical prognostic markers

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Abstract

Introduction: Breast carcinoma is considered the most common malignancy among females worldwide. An early accurate diagnosis remains the most important determinant in the treatment and outcome. Pathological and immunohistochemical markers are the important prognostic factors with axillary lymph node status being the most significant prognostic marker.

However, there exists a heterogeneity, if only positive lymph nodes are used to classify breast carcinoma patients with different prognoses. Therefore, to improve the efficiency of the prognostic system and to provide appropriate treatment, not only positive lymph node but also the total number of lymph nodes dissected needs to be considered.

Hence, this study was undertaken to evaluate lymph node ratio (LNR) in breast carcinoma and to examine its association with significant prognostic markers.

Objectives

1. To evaluate lymph node ratio in resected specimens of breast carcinomas with axillary lymph node clearance.
2. To correlate LNR with pathological prognostic markers such as pathological T Stage, N Stage, Tumor Stage, SBR grade, histological tumor type.

3. To correlate LNR with immunohistochemical expression of ER, PR, Her2neu and Ki67.

Materials and methods: This cross-sectional study was conducted on 85 resected specimens of breast carcinomas with axillary lymph node clearance received over a period of 2 years (October 2019 to September 2021). In each case, LNR was calculated and correlated with pathological T stage(pT), pN classification, tumor stage, tumor grade, tumor type, ER, PR, HER2, and Ki67status.

Results: Of the 85 cases received, 56 cases showed nodal metastasis and LNR was evaluated in these cases. The majority of the cases exhibited low-risk LNR (57.1%) followed by intermediate-risk (30.4%) and high-risk LNR (12.5%). There was a statistically significant correlation noted between low and intermediate-risk LNR tumors with SBR grades 1 and 2 and high-risk LNR with grade 3 tumors ($p<0.001$). A statistically significant correlation of LNR with N stage and TNM stage was also seen ($p<0.001$). There was no significant association of LNR with histological tumor type, ER, PR, HER2, and Ki67status.

Conclusion: This study provides information about the association between LNR in breast carcinoma and other classic risk factors. Because of a statistically significant correlation of LNR with SBR grade, N stage, and TNM stage, we suggest that LNR can be used as a prognostic marker in node-positive breast carcinoma patients.

Novelity: This study helps to improve the efficiency of the prognostic system and to provide appropriate treatment, in patients with breast carcinoma.

Keywords: Axillary lymph node clearance, breast carcinoma, prognostic markers, lymph node ratio.

Introduction

Breast carcinoma is the commonest cancer among Indian females with age adjusted rate of 25.8 per 1,00,000 women and mortality rate of 12.7 per 1,00,000 women. There is significant increase in its incidence, mortality and morbidity globally and also in Indian subcontinent.[1]

It is a disease which consists of various histological patterns. Histopathological factors such as tumor size, grade and status of axillary lymph node along with histopathological biomarkers, oncogenes and tumor suppressor genes are considered as important prognostic markers.[2]

The status of axillary lymph node is considered among the most significant prognostic markers for breast carcinoma. An increase in the positive number of lymph nodes is associated with poor clinical outcome and increase rate of recurrence.[3]

Assuming that all axillary dissections are same, the American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) stages patients based on absolute number of lymph nodes which are positive and do not include the total number of removed lymph nodes or the number of negative lymph nodes. However, the extent of axillary dissection of lymph nodes varies widely between countries, centres, surgeons and researchers.[3,4]

Thus, there exist a heterogeneity, if only positive lymph node number is used to classify breast carcinoma patients with different prognosis. Researchers have suggested that to improve the efficiency of prognostic system in breast carcinoma and to aid in providing individualized and appropriate treatment, not only positive lymph node numbers but also the total number of lymph nodes dissected needs to be considered.[4]

Recent studies conducted abroad, have shown that Lymph Node Ratio (LNR) is defined as the number of lymph nodes which are positive over the total number of lymph nodes excised, and it is an useful predictor of breast

carcinoma recurrence and survival. Further different studies have also shown that LNR is a superior prognostic marker and superior indicator of axillary tumor burden over total number of lymph nodes which are positive (pN).[3]

Present approach for therapy of breast carcinoma is based on combination of surgery, radiation therapy, hormonal therapy and chemotherapy. The clinician's choice of treatment between hormonal therapy with minimal side effects and chemotherapy with high morbidity and risk, is very essential. Therefore, an accurate assessment of LNR, Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal Growth Factor Receptor 2 (HER2) and Ki67 status of breast carcinoma by the pathologist is necessary.[5]

There is paucity of such studies in India. Hence, this study is undertaken to evaluate LNR in breast carcinoma and to examine its association with significant prognostic markers like pathological T stage (pT) classification, pN classification, tumor stage, tumor grade, tumor type, ER, PR, HER2 and Ki67status.[3]

Aims and objectives

1. To evaluate lymph node ratio in resected specimens of breast carcinomas with axillary lymph node clearance.
2. To correlate LNR with pathological prognostic markers such as pathological T Stage, N Stage, Tumor Stage, SBR grade, histological tumor type.
3. To correlate LNR with immunohistochemical expression of ER, PR, Her2neu and Ki67.

Study Type: Cross sectional study

Study duration: October 2019 to September 2021 (2 years)

No. of cases: 85

Specimen type: Breast carcinomas with axillary lymph node clearance (ALND).

Source of material: 10% Formalin Fixed Paraffin embedded (FFPE) tissue blocks.

Inclusion Criteria

All resection specimens of breast carcinomas with axillary lymph node clearance (level 1-3) from female patients of age group 18 or above.

Exclusion Criteria

- Cases where only biopsy or limited surgery without axillary lymph node clearance was done.
- Cases where there is extensive tumor necrosis without sufficient viable tumor cells for accurate evaluation of the immunohistochemical results.

Statistical Analysis

- Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software.
- All the categorical variables were expressed as frequency, mean, standard deviation and percentage.
- Chi-square test or Fischer's exact test (for 2x2 tables only) was used as test of significance for qualitative data.

Materials and method

Method of collection of data

The resected specimens of breast carcinomas with axillary lymph node clearance received in 10% formalin. In every case the standard protocol for surgical grossing of specimens was followed. Axillary lymph node was fixed in Carnoy's solution. The H & E-stained slides were studied for the tumor histology, grade, lymph vascular invasion, lymph node metastasis and other features. The tumor staging was done according to American Joint Committee on Cancer (8^{th} edition-2017) staging system.[6]

LNR was calculated and categorized as follows- Low risk ≤ 0.2; Intermediate risk 0.2-0.65; High risk > 0.65.[3,7]

Processing for immunohistochemistry

Immunohistochemical detection of ER, PR, Her2neu and Ki67 was done on 4-5µm thick sections, cut from a paraffin block of tumor tissue and taken on a glass slide coated with adhesive (poly L lysine).

The technique for IHC using "DAKO REALTM Envision TM detection system" includes antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibodies against ER (cloneEP1 DAKO), PR (clonePgR636 DAKO), Her2neu (cloneCB11 BIOGENEX) and Ki67 (clone MIB-1) proteins, enzyme labelling with horseradish peroxidase, developing chromogen with diaminobenzidine (DAB) and counterstaining with hematoxylin. Positive and negative controls wil run with each batch of the slides. Allred scoring system was used for ER and PR reporting and ASCO/CAP guidelines was followed for Her2neu reporting. Ki67 index, calculated as percentage, counting the Ki67 labelled tumor cells out of 1000 tumor cells.

Scoring system used in immunohistochemistry (according to 2018 guidelines)

Allred Scoring system was used for ER and PR. It is a semi quantitative method which considers the proportion of positive cells (scored between 0-5) and staining intensity (scored between 0-3). The sum of proportion score and intensity score gives a total allred score between 0 – 8 range. A score of 0 -2 is taken as negative whereas a score of 3 - 8 is taken as positive. ASCO/CAP guidelines were used for HER2 reporting.[66] Ki-67 values are determined by using MIB1, anti-human Ki-67 monoclonal antibody. Percentage score of Ki-67 is expressed as the positively stained tumor cells among the total number of malignant cells. Ki-67 cut-off is considered as 15 % according to the national and international recommendations at present. Ki-67-labeling index is considered as the percentage of cells with Ki-67-positive nuclear immunostaining.[8-12]

Result

We studied 85 cases of breast carcinomas, received over a duration of 2 years from 2019 to 2021. The mean age of the patients was 51.6±11.96 years (range, 29 to 85 years). The median age was 50 years with an interquartile range (IQR) of 16. Majority of the patients (84.7%) were in >40 years of age.

The most commonly received specimen type was Modified Radical Mastectomy (MRM) 51.8% followed by Mastectomy + Axillary Lymph Node Dissection (31.8%) and Wide Local Excision + Axillary Lymph Node Dissection (16.5%). The number of lymph nodes isolated from any specimen ranged from 7 to 33 in number. The most common histological type was invasive ductal carcinoma (85.9%) followed by Mucinous Carcinoma (7.1%). The single case of mixed carcinoma comprised of 12% invasive ductal carcinoma (NST), 70% invasive cribriform and 18% papillary carcinoma. Majority of the tumors were of grade II (moderately differentiated) (65.8%), followed by grade III (poorly differentiated) (20%) and grade I (well differentiated) (14.1%). Figure 1 shows the percentage of patients in each N stage; 29 (34%) in N0, 35 (41%) in pN1, 12 (14%) in pN2 and 9 (11%) in pN3. Figure 2 shows percentage of patients according to pathological T stage majority being in T2 stage (67.1%) followed by T3 stage (21.2%). Figure 3 shows percentage of patients according to LNR, evaluated in 56 node positive cases; 32 (57.1%) in low risk LNR (≤ 0.2)**,** 17 (30.4%) in intermediate risk LNR (>0.2 and ≤ 0.65) and 7 (12.5%) in high risk LNR (>0.65).

The pathological and immunohistochemical prognostic markers of node-positive breast carcinoma patients according to the lymph node ratio are shown in Table.

Table: Pathological and immunohistochemical prognostic markers of 56 women with node-positive breast carcinoma according to the lymph node ratio.

Prognostic markers	LNR			Total	P value
	Low risk LNR ≤0.20	Intermediate risk LNR >0.20-≤0.65	High risk LNR >0.65		
Histological Type					
Invasive Ductal Carcinoma (NST)	29 (90.6%)	15 (88.2%)	5 (71.4%)	49	p=0.175
Invasive Lobular Carcinoma	0 (0%)	1 (5.9%)	1 (14.3%)	2	
Mixed Carcinoma	1 (3.1%)	0 (0%)	0 (0%)	1	
Mucinous Carcinoma	2 (6.3%)	1 (5.9%)	0 (0%)	3	
Invasive micropapillary Carcinoma	0 (0%)	0 (0%)	1 (14.3%)	1	
SBR Grade					
Grade 1	20 (62.5%)	3 (17.6%)	1 (14.3%)	24	p<0.001
Grade 2	10 (31.2%)	12 (70.6%)	1 (14.3%)	23	
Grade 3	2 (6.3%)	2 (11.7%)	5 71.4%)	9	
TOTAL	32 (100%)	17 (100%)	7 (100%)	56	
T Stage					
T1	3 (9.4%)	0 (0%)	1 (14.3%)	4	p=0.188

T2	21 (65.6%)	13 (76.5%)	2 (28.6%)	36	
T3	7 (21.9%)	3 (17.6%)	4 (57.1%)	14	
T4	1 (3.1%)	1 (5.9%)	0 (0%)	2	
Total	32 (100.0%)	17 (100.0%)	7 (100.0%)	56	
N Stage					
N1	31 (96.9%)	4 (23.5%)	0 (0%)	35	p<0.001
N2	1 (3.1%)	9 (52.9%)	2 (28.6%)	12	
N3	0 (0%)	4 (23.5%)	5 (71.4%)	9	
TOTAL	32 (100%)	17 (100%)	7 (100%)	56	
Tumor Stage					
I	3 (9.4%)	0 (0%)	0 (0%)	3	p<0.001
II	22 (68.8%)	4 (23.5%)	0 (0%)	26	
III	7 (21.9%)	13 (76.5%)	7 (100%)	27	
TOTAL	32 (100%)	17 (100%)	7 (100%)	56	
ER/PR					
NEGATIVE	10 (31.3%)	5 (29.4%)	4 (57.1%)	19	p=0.372
POSITIVE	22 (68.8%)	12 (70.6%)	3 (42.9%)	37	
Total	32 (100%)	17 (100%)	7 (100%)	56	
HER2 Status					
NEGATIVE	24 (77.4%)	15 (88.2%)	6 (85.7%)	45	p=0.783
POSITIVE	7 (22.6%)	2 (11.8%)	1 (14.3%)	10	
Total	31 (100%)	17 (100%)	7 (100%)	55	
Ki67					
≤15	13 (40.6%)	5 (29.4%)	2 (28.6%)	20	p=0.725
>15	19 (59.4%)	12 (70.6%)	5 (71.4%)	36	
Total	32 (100%)	17 (100%)	7 (100%)	56	

A statistically significant correlation between low and intermediate risk LNR tumors with SBR grades 1 and 2 and high risk LNR with grade 3 tumors ($p<0.001$) was noted with low and intermediate risk LNR tumors being associated with SBR grades 1 and 2 and high risk LNR being associated with grade 3 tumors. There was also a statistically significant correlation between N stage and LNR ($p<0.001$) with Low risk LNR being associated with low (N1) N stage in contrast to high risk LNR being associated with high (N3) N stage. Similarly, a statistically significant correlation of LNR with TNM stage was noted ($p<0.001$), with Low risk LNR tumors being associated with early TNM stage and high risk LNR tumors being associated with advanced TNM stage.

There was no significant association of LNR with histological tumor type ($p=0.175$) and pathological T stage ($p=0.188$). Positive correlation could not be established between LNR and immunohistochemical expression of ER/PR ($p=0.372$), Her2neu ($p=0.783$) and Ki67 proliferation index ($p=0.725$). The one case which exhibited equivocal staining for Her2neu was excluded from statistical analysis.

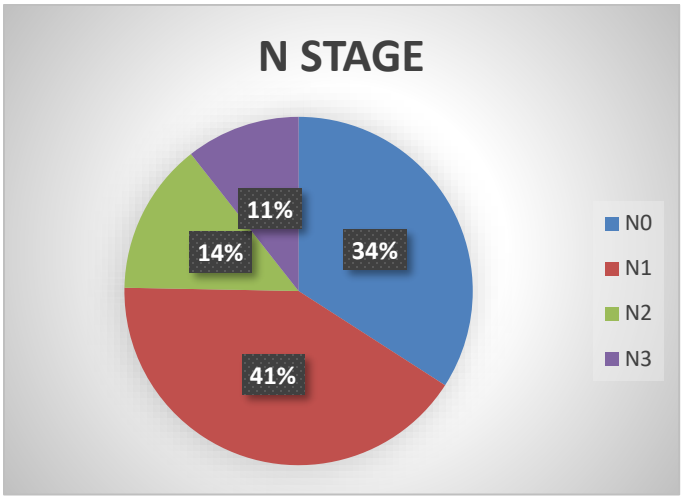

Figure 1: Distribution of patients according to N stage

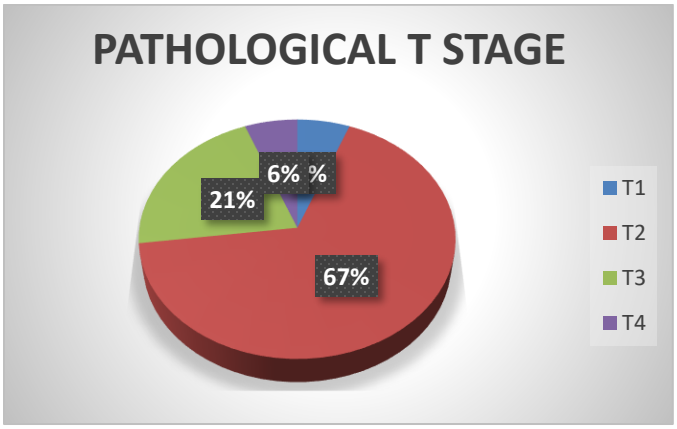

Figure 2: Distribution of patients according to pathological T Stage

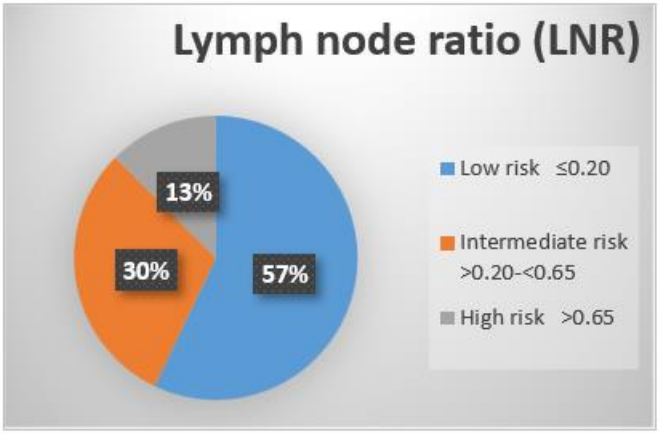

Figure 3: Distribution of patients according to LNR

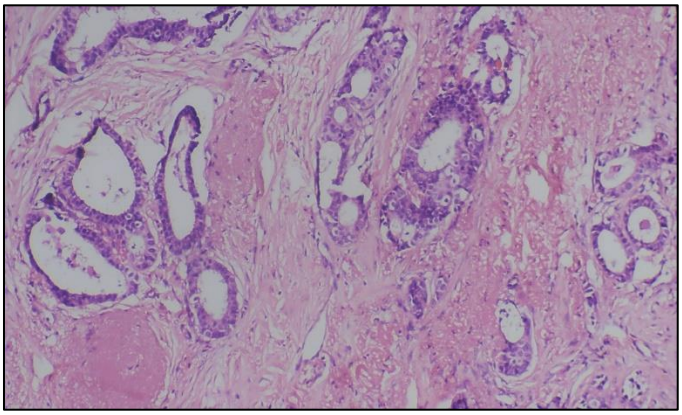

Figure 4A : Infiltrating Ductal Carcinoma (NST) Grade I – Microphotograph shows prominent gland formation. The tumor cells show mild nuclear pleomorphism with hyperchromatic nuclei (H&E, X100).

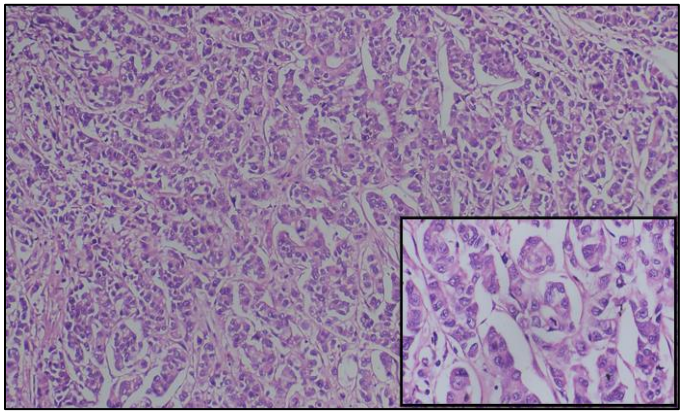

Figure 4B: Infiltrating Ductal Carcinoma (NST) Grade (H&E, X100)

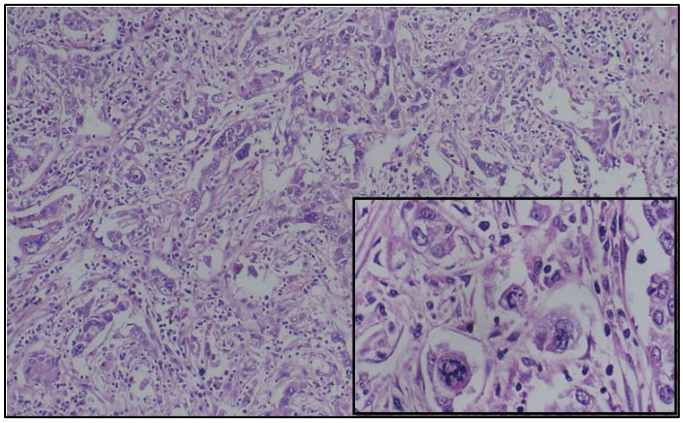

Figure 4C: Infiltrating Ductal Carcinoma (NST) Grade III (H&E, X100).

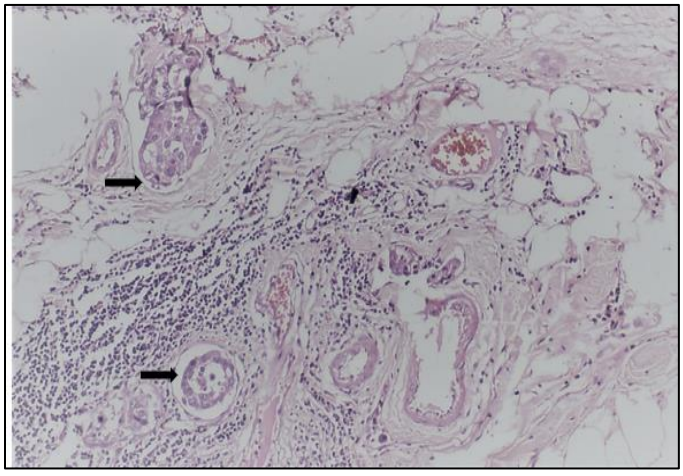

Figure 5: Microphotograph shows lymphatic permeation by IDC (black arrows) (H&E, X400).

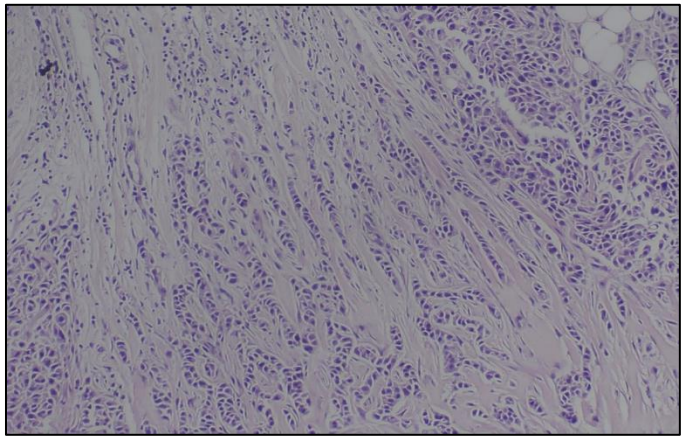

Figure 6: Infiltrating Lobular Carcinoma (H&E, X100).

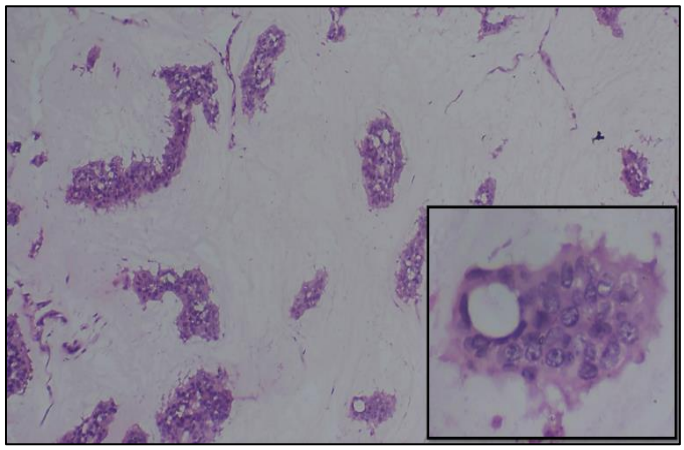

Figure 7: Mucinous Carcinoma (H&E, X400).

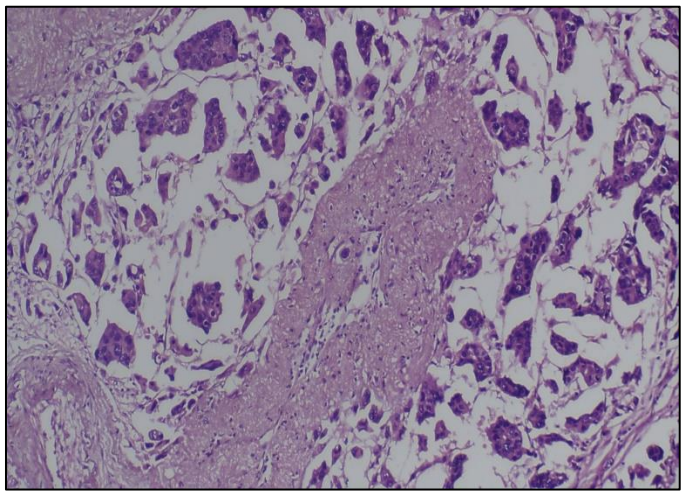

Figure 8: Micropapillary Carcinoma (H&E, X100).

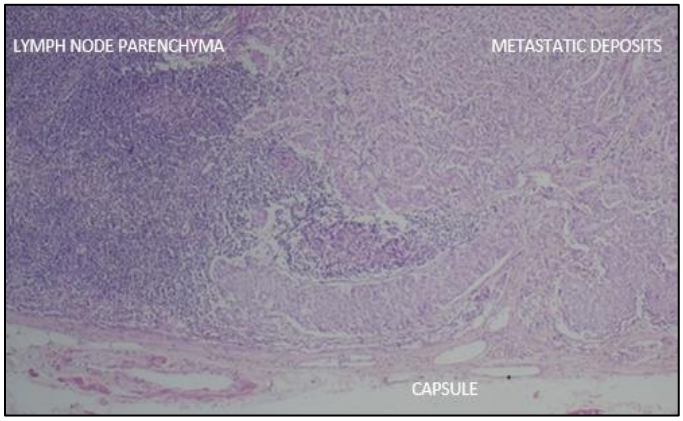

Figure 9A: Microphotograph shows lymph node metastasis (H&E, X40).

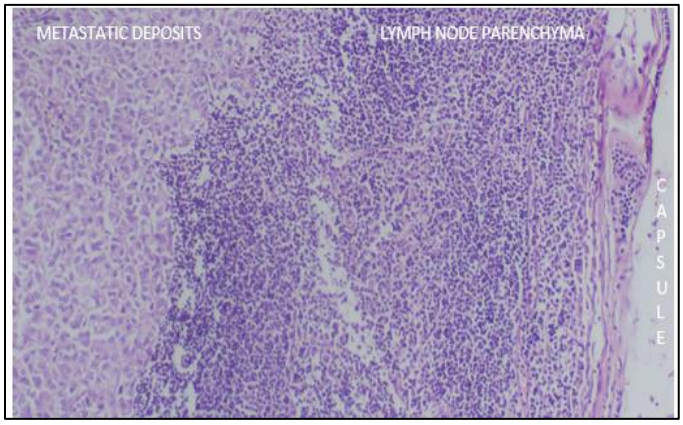

Figure 9B: Microphotograph shows lymph node metastasis (H&E, X100).

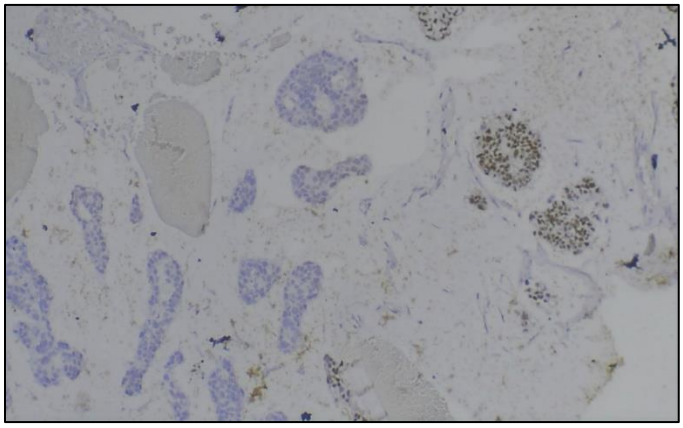

Figure 10A: ER positive 2+, in 10 % 0f tumor cell nuclei (Anti-ER poly horseradish- DAB chromogen, (X100).

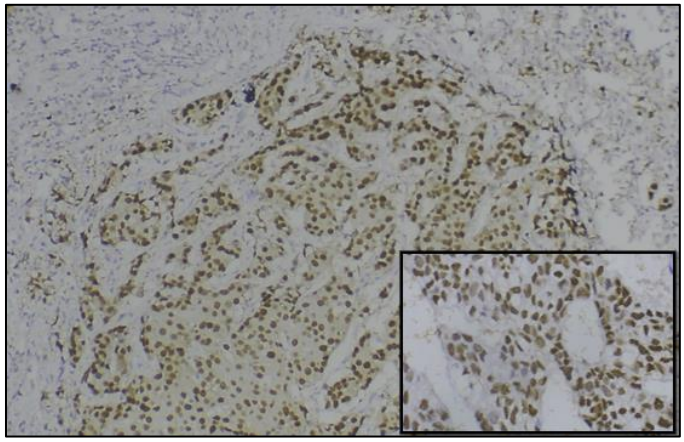

Figure 10B: ER positive 3+ in 80% of tumor cell nuclei (Anti-ER poly horseradish- DAB chromogen, 100X). Inset shows tumor cells exhibiting intense nuclear staining (x400).

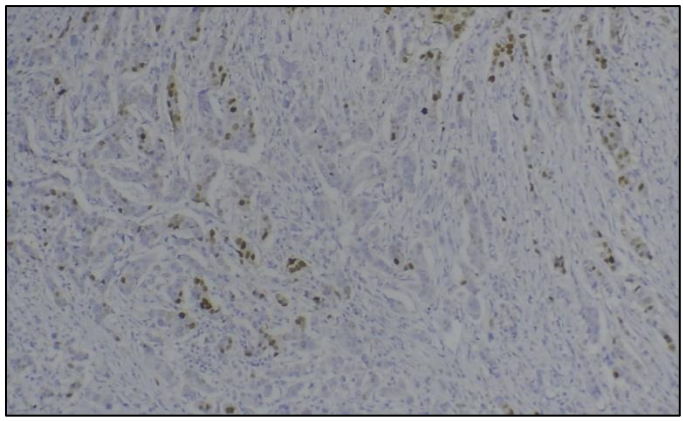

Figure 11A: PR positive 2+ in 10 % of tumor cell nuclei (Anti-PR poly horseradish- DAB chromogen, X100).

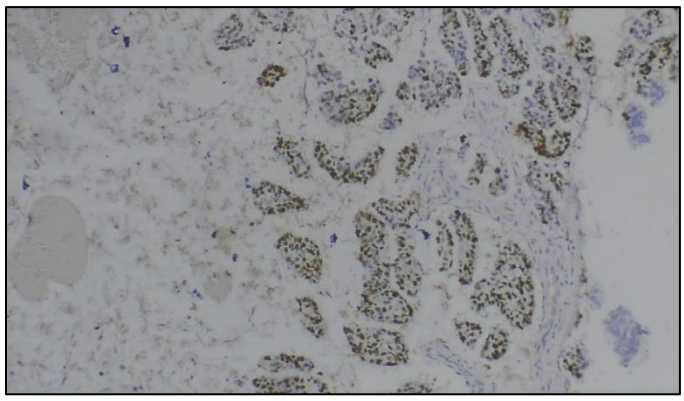

Figure 11B: PR positivity 3+, in 25% of tumor cell nuclei (Anti-PR poly horseradish- DAB chromogen, X100).

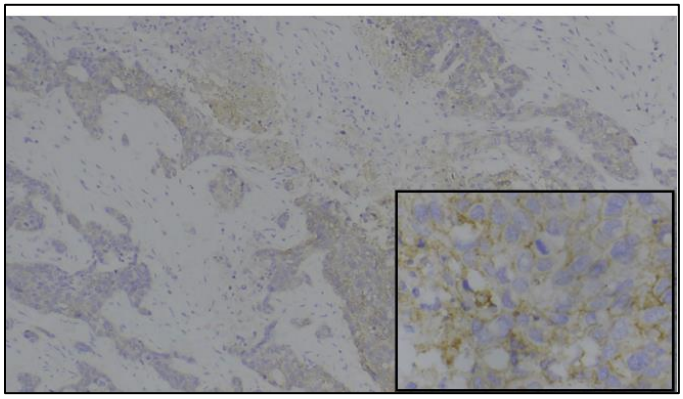

Figure 12A: HER2 positive 2+, with staining in >10% of tumor cells (Anti-HER2 poly horseradish- DAB chromogen, X100). Inset shows tumor cells exhibiting incomplete membranous staining of weak to moderate intensity (X400).

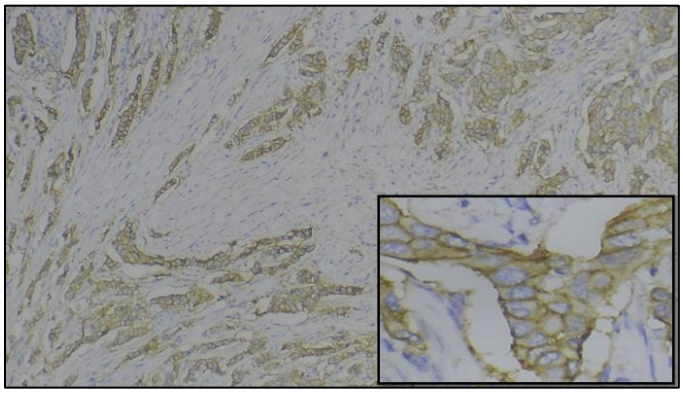

Figure 12B: HER2 positive 3+ with staining in >10% of tumor cells (Anti-HER2 poly horseradish- DAB chromogen, X100). Inset shows complete membranous staining of strong intensity (x400).

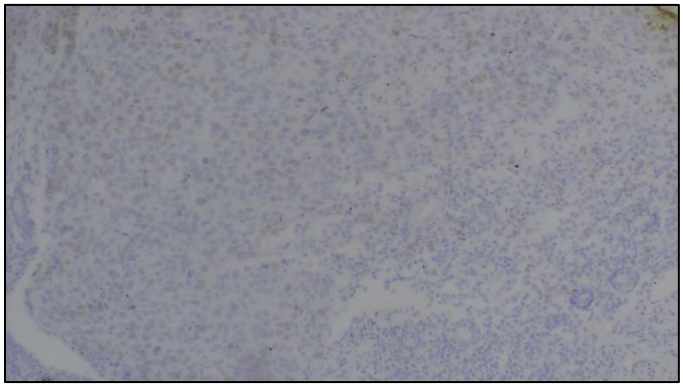

Figure 13A: Ki67 positivity in <15% of tumor cells nuclei (MIB 1 Antibody- poly horseradish- DAB chromogen, X100).

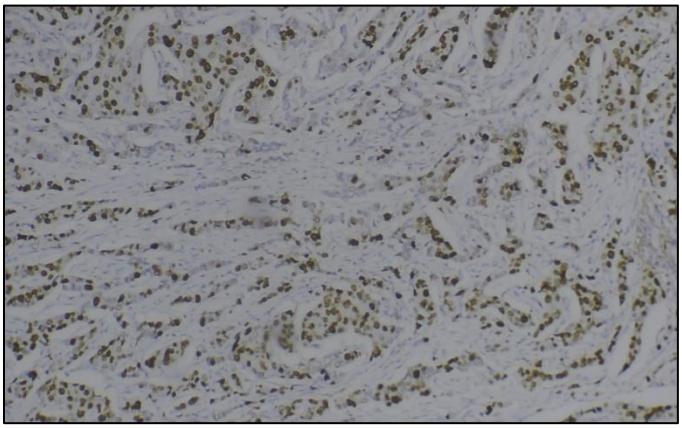

Figure 13B: Ki67 positivity in >15% of tumor cells nuclei (MIB 1 Antibody- poly horseradish- DAB chromogen, X100).

Discussion

Majority of the cases exhibited low risk LNR followed by intermediate risk LNR and high risk LNR. Similar results were seen in the studies done by Bansal GJ et al and Solak M et al. However, in a study done by Elkhodary TR et al intermediate risk LNR was the commonest followed by high risk and low risk LNR whereas in a study done by Asaad RA intermediate risk LNR was the commonest followed by low risk and high risk LNR.[3,13,14,15] Comparison of our data with other studies was difficult as literature review revealed only few studies with study design similar to ours. Some of the studies have used different cut off limits and criteria for classification of LNR. In a study by The et al the criteria for classification of LNR was ≤0.30 for low risk, 0.30-0.70 for medium risk and over 0.70 for high risk LNR. Another study done by Xiang-Sheng Xiao et al, the cut off limits for LNR were 0, 0.30 and 0.81.[16,17]

There is a statistically significant association of LNR with SBR grade, N stage and TNM stage. Low and

intermediate risk LNR are associated with SBR grades 1 and 2 and high risk LNR is associated with SBR grade 3. A progressively increasing trend in tumor grade was observed with increasing LNR. Only a few studies have investigated the association between LNR and SBR grading. Solak M et al [13] evaluated 1004 cases of lymph node positive breast cancer and observed that high risk LNR is significantly associated with high grade tumors (p = 0.026), which is in concordance with our study. Bansal GJ et al.[3] evaluated 150 cases with node positive breast cancer and observed that moderate and high risk LNR was significantly associated with grade 2 tumors (p=0.000). Asaad RA [15] evaluated 60 cases with node positive breast cancer and observed that all the patients having grade 3 tumors were associated with intermediate risk LNR. Low risk LNR is associated with low N stage (N1), and high risk LNR is associated with high N stage (N3). In a study by Asaad RA[15], low risk LNR was associated with low N stage (N1), whereas intermediate and high risk LNR were associated with N2 and N3 stage. In the study by Bansal GJ et al.[3] moderate and high risk LNR was significantly associated with pN2 and pN3 classification compared to low risk LNR (p=0.017). The authors concluded that higher the LNR, the more likely the patient is to have a high pN classification. Low risk LNR is associated with early TNM stage and high risk LNR is associated with advanced TNM stage. A study done by Assad RA [15] also showed strong significant correlation between LNR and TNM stage (p=0.001).

There is no statistically significant association of lymph node ratio with histological types of breast carcinoma, pathological T stage and immunohistochemical expression of ER, PR, Her2neu and Ki67 proliferation index. In the present study, the most common histological subtype of breast carcinoma was IDC (NST) and was commonly associated with low risk LNR. However, there was no significant correlation between LNR and histological subtypes of breast carcinoma (p=0.175). The study done by Solak M et al.[13] also showed no significant correlation between LNR and histological subtypes of breast carcinoma (p=0.132). In our study, there was no statistically important relation between LNR and pathological T stage (p=0.188), although 65.6% of patients with low risk LNR were seen in T2 stage while 57.1% of the patients with high risk LNR were seen in T3 stage. Similar results were shown by the study done by Assad RA[15] with no significant correlation between LNR and pathological T stage (p=0.239). In contrast, study done by Bansal GJ et al.[3] showed a statistically significant association of intermediate and high risk LNR with pT3 stage (p=0.001). There was no significant correlation between LNR and immunohistochemical prognostic markers such as ER, PR and HER2 status and Ki67 proliferation index. Literature review did not reveal any other study, wherein the association between Ki67 and LNR was studied.

Conclusion

Because of a statistically significant correlation between lymph node ratio and SBR grade, N stage and TNM stage we suggest that LNR can be used as a prognostic factor in node positive breast carcinoma patients. LNR may also act as a standardization factor against the variable nodes retrieved and assessed by surgeons and pathologists. However, further studies on a larger and varied sample size with standardization of LNR cut-off values, longitudinal follow up and survival data is required to expound the role of LNR in breast carcinoma and its inclusion in staging. Our sample size was relatively small, when compared with other studies, and follow up period was short, which may be the reason for some of the

insignificant results in this study. Overall survival or disease-free survival, mortality rate or recurrence rate of breast carcinoma were not studied because of difficulties regarding follow up of the patients.

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