

Pathological response of different molecular subtypes of locally advanced breast carcinoma to neoadjuvant chemotherapy



Nidhi Aley Alex¹, Sankar S², Saji Mary Varghese³, Suresh Kumar K⁴

¹Junior Resident, ²Professor, ³Assistant Professor, Department of Pathology, ⁴Professor, Department of Radiotherapy, Government Medical College, Kottayam, Kerala, India

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ABSTRACT

Background: This study was done on modified radical mastectomy (MRM) specimens of patients with locally advanced breast carcinoma (LABC) who were categorized into molecular subgroups based on Immunohistochemistry and underwent neoadjuvant chemotherapy (NACT). The purpose of the study was to look for the pathological response of the tumor to the NACT. The molecular subtypes include Luminal A, Luminal B, Human epidermal growth factor receptor 2 (HER2) enriched, and triple negative. For HER2 enriched subtype, trastuzumab was also given along with the chemotherapy regimen. **Aims and Objectives:** The primary objective of this study is to find out the pathological complete response (pCR) in various molecular subgroups of breast carcinoma following NACT. The secondary objective is to describe the cellular changes in tumor tissue following NACT. **Materials and Methods:** This is a descriptive study done on MRM specimens of patients with LABC received in histopathology lab of Government Medical College, Kottayam to look for the pCR of the tumor following NACT. Gross examination and histopathology study of the specimen including axillary lymph nodes were done. The trucut biopsies of these cases with immunohistochemistry study were reviewed for confirming the grade and molecular subtype. pCR was assessed based on Chevallier system. **Results:** Of the 131 MRM specimens studied, overall pathologic complete response rate was 24.4% and the pathologic complete response rate in Luminal A subtype, Luminal B subtype, HER2 enriched tumors, triple negative tumors were 23.6%, 24%, 33%, and 20%, respectively. **Conclusion:** 33% of HER2 enriched subtype showed a pCR, i.e., no residual foci of neoplasm seen in the MRM specimens following NACT. Following NACT, change in tumor cellularity, changes in the morphology of neoplastic cells, changes in the surrounding breast tissue, and a change in grade of tumor were also noticed.

Key words: Pathological complete response; Molecular subtypes; Locally advanced breast carcinoma

INTRODUCTION

Breast cancer is the second most common cancer in the world and ranks first in the frequency in women.¹ As per the Globocan data 2020, in India, breast carcinoma accounted for 13.5% of all cancer cases and 10.6% of all deaths with a cumulative risk of 2.81.¹ Breast cancer is divided into distinct molecular subtypes (Luminal A, Luminal B, human epidermal growth factor receptor 2 [HER2] enriched, and triple negative)

by the gene expression profiling with prognostic significance. For practical issue in the hospital setting, immunohistochemical surrogate panels of estrogen receptor, progesterone receptor, and HER2 is being used to potentially discriminate the subtypes instead of gene expression profiling.

Neoadjuvant chemotherapy (NACT) is being increasingly used for downstaging of large tumors. This had led to a significant decrease in morbidity.² There are

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Address for Correspondence:

Dr. Saji Mary Varghese, Assistant Professor, Government Medical College, Kottayam, Kerala, India. **Mobile:** +91-9946282136.

E-mail: sajivarghese36@gmail.com

several benefits to the use of NACT. It offers a unique opportunity for the evaluation of treatment response with complete pathologic response acting as a surrogate marker of survival and for a more rapid assessment of the efficacy of new therapeutic agents and early cessation of ineffective treatment.

Pathological complete response (pCR) is defined as the absence of residual invasive cancer on the evaluation of the complete resected breast specimen and all sampled regional lymph nodes following the completion of neoadjuvant systemic therapy.³ When there is pCR, it indicates better disease-free survival period for the patient. The pathological response gives an assessment of efficacy of new therapeutic agents, thereby helps to adjust the dose or to stop the usage of ineffective drugs. It provides individualized therapy and helps in translational research by the collection of tissue samples, before during and after the treatment.⁴⁻⁶

There have been varied NACT regimens which are crafted using the chemotherapeutic agents. The most commonly used regimens include.

- a. 3 cycles of 5- fluorouracil, epirubicin, and cyclophosphamide followed by four cycles of docetaxel (3FEC+ 4DOCE)
- b. 6 cycles of 5-fluorouracil, adriamycin, and cyclophosphamide (6FAC)
- c. 6 cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (6 FEC).⁷

In addition to these therapeutic agents, trastuzumab has been added as an adjunct for patients with HER2-positive breast cancers.⁸

Aims and objectives

The primary objective of this study is to find out the pathological complete response in various molecular subgroups of LABC following neoadjuvant chemotherapy. Secondary objective is to describe the cellular changes in tumor tissue following NACT.

MATERIALS AND METHODS

The approval of institutional ethical board was obtained from Government Medical College Kottayam Institutional Review Board (IRB) on January 21, 2021 (IRB no. 17/2021). The sample size calculated was 144 which include modified radical mastectomy (MRM) specimens of patients who have been clinically diagnosed as locally advanced breast carcinoma (LABC) and who has received appropriate standard of care NACT depending upon the molecular subtypeclassification based on NCCN Guidelines Version 5.2020 during the study periods of 18 months. The NACT regimens given were as follows

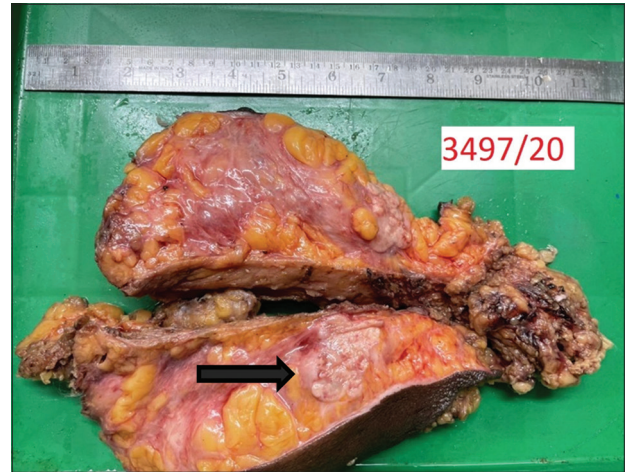


Figure 1: Gross: Mastectomy specimen, on cut section showing a grey white lesion



Figure 2: Gross: Slice taken from specimen revealing the largest cross-section of pre-treatment area of involvement. The whole slice was submitted in representative blocks

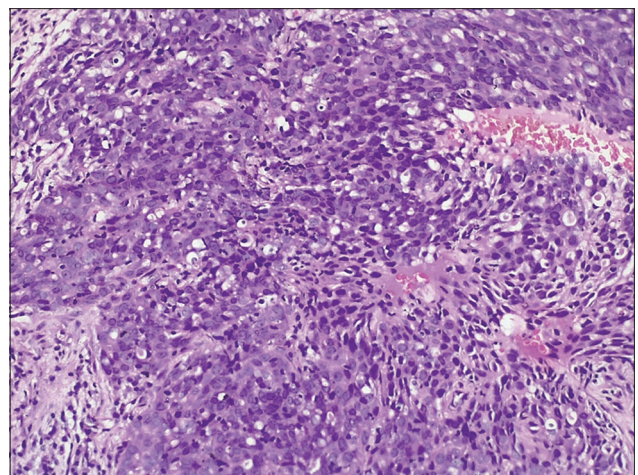


Figure 3: H&E: Invasive carcinoma breast no special type - High grade- x20

1. Luminal A and Luminal B subtype patients received Anthracyclines (A), and cyclophosphamide (C) followed by Docetaxel(T)- (AC followed by T or TAC regimen

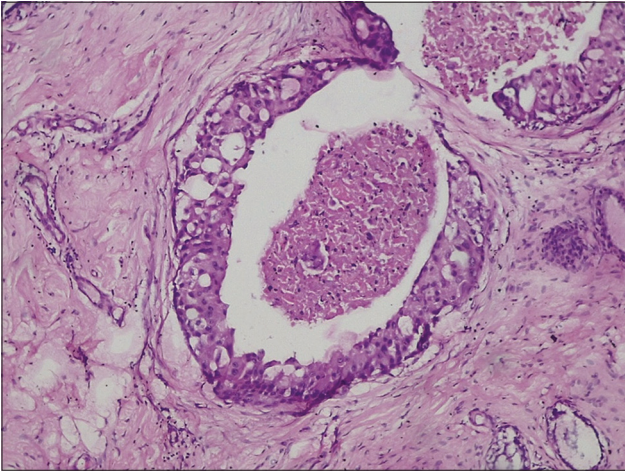


Figure 4: H&E: Ductal carcinoma *in situ* with comedonecrosis- x10

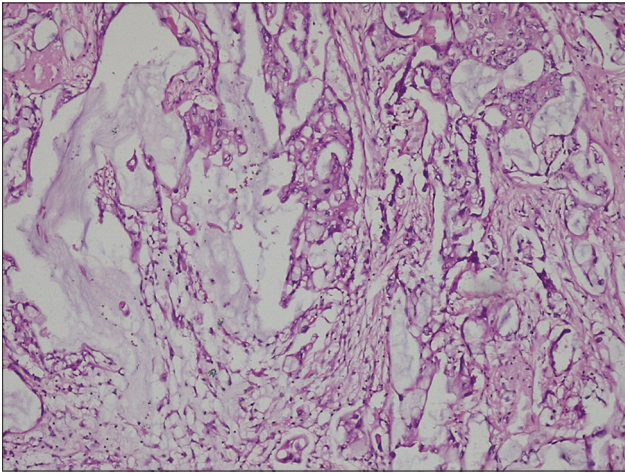


Figure 5: H&E: Invasive carcinoma breast with mucinous component- x10

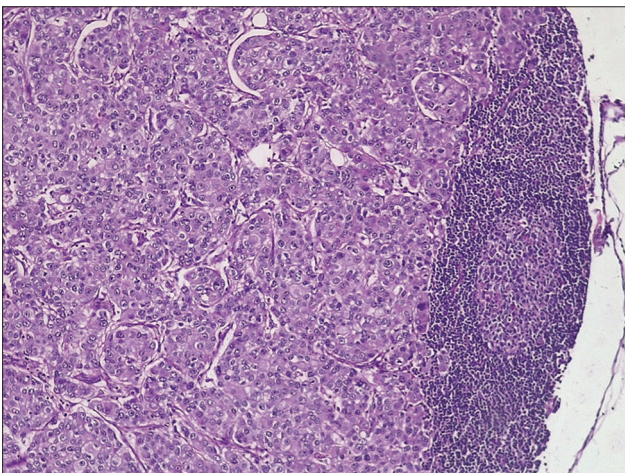


Figure 6: H&E: Section from Lymph node with metastasis from invasive breast carcinoma- x10

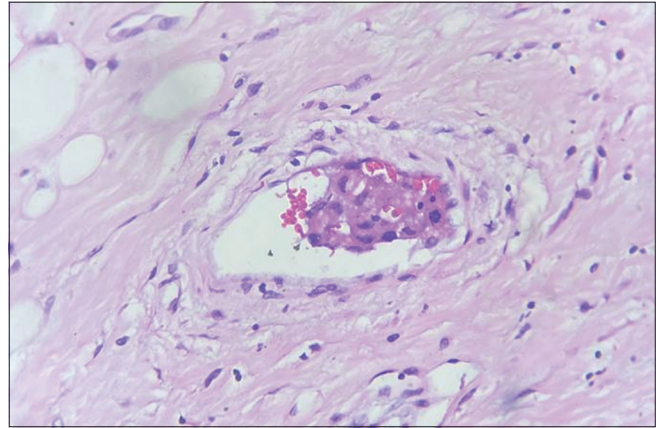


Figure 7: H&E: Lymphovascular tumor emboli- x20

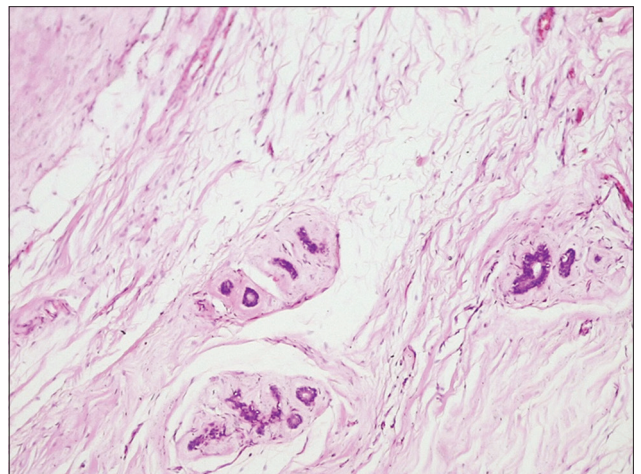


Figure 8: H&E: Non neoplastic breast parenchyma showing alterations following chemotherapy such as atrophy of glandular elements-x10

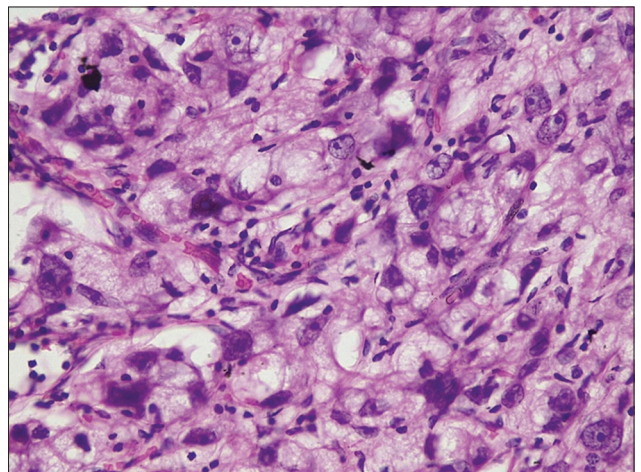


Figure 9: H&E: Morphological alterations in neoplastic cells following chemotherapy in the form of cytoplasmic vacuoles, nuclear enlargement, hyperchromasia and pleomorphism- x20

2. Triple-negative breast cancer patients received the same AC followed by T or TAC regimen
3. Patients with HER2 enriched subtype the AC followed by TH/TCH regimen (anthracyclines

and cyclophosphamide, followed by docetaxel with trastuzumab [Herceptin]) was given.

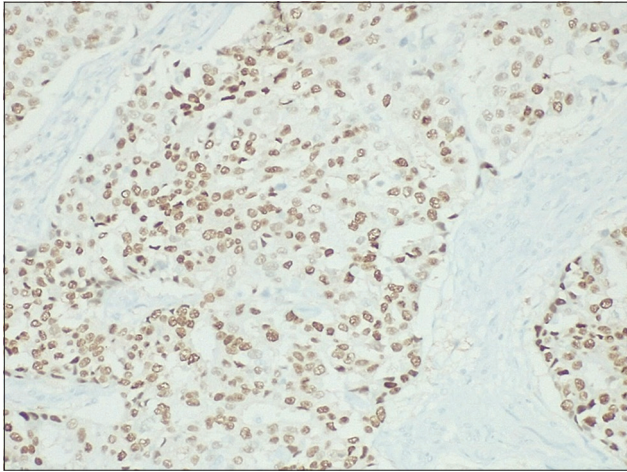


Figure 10: Immunohistochemistry: Neoplastic cells showing Estrogen receptor positivity- x20

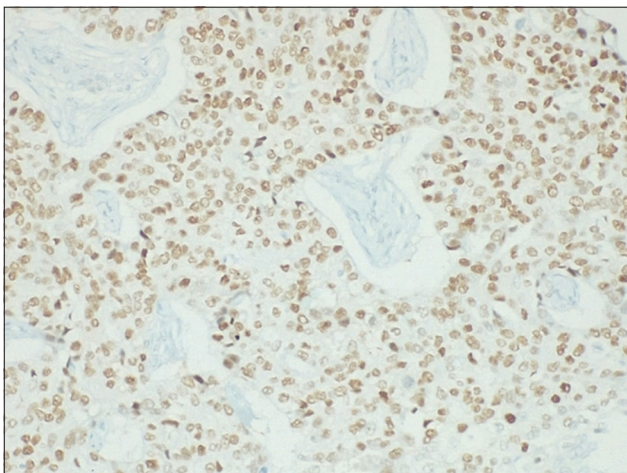


Figure 11: Immunohistochemistry: Neoplastic cells which are Progesterone receptor positive – x20

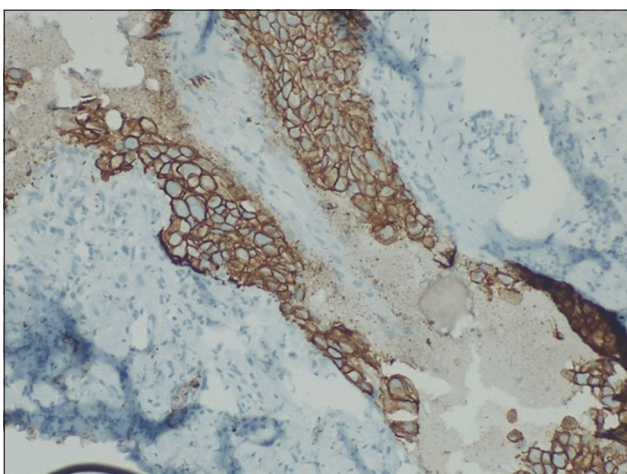


Figure 12: Immunohistochemistry: Neoplastic cells which are positive for Human epidermal growth factor receptor 2 – x20

Anthracyclines used were doxorubicin (Adriamycin), they act by inhibiting DNA replication and creating

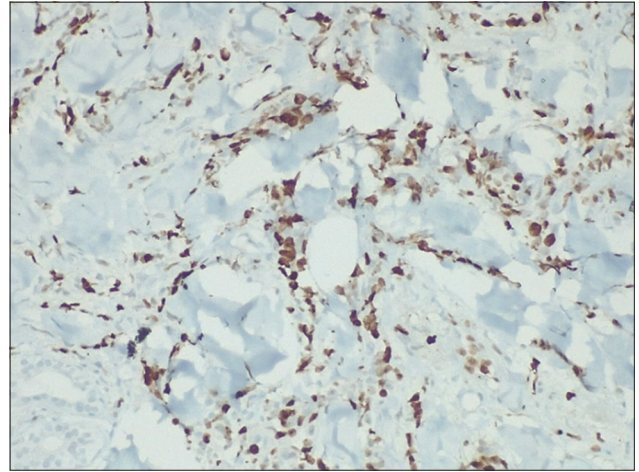


Figure 13: Immunohistochemistry: Ki-67 proliferation index- 65%- x20

free radical that damage cancer cells. Along with them, alkylating agents such as cyclophosphamide which covalently links to DNA and interfere with its function were given. Taxanes used were docetaxel and paclitaxel, which acts by inhibiting microtubule disassembly during mitosis and prevent cell division. HER2 enriched subtype patients were given ERBB2 targeted monoclonal antibodies such as Trastuzumab (Herceptin).

The initial tru-cut biopsy of the patient and the immunohistochemistry slides (Figures 10-13) were reviewed. For gross examination of MRM specimens, radiological correlation was helpful in assessing the site of the tumor in those cases where a grossly visible tumor was not evident and extensive sampling was done for such specimens along with image of sliced specimen being recorded (drawing) and used as a map for the sections taken (Figures 1 and 2), so that histopathological assessment of residual disease can be easily understood. Sections were taken and processed and the slides obtained were analyzed. Final microscopic reporting was done based on the suggested template given by the Residual Disease Characterization of Working Group of the Breast International Group-North American Breast Cancer Group.³ The pathological response by each molecular subtype (Luminal A+B, HER2 enriched, triple negative) was assessed.

Inclusion criteria

The inclusion criteria were as follows: MRM specimens of patients who have been clinically diagnosed as LABC and who has received appropriate standard of care NACT depending upon the molecular.

Exclusion criteria

The exclusion criteria were as follows: Patients whose IHC details are not available and patients who has undergone lumpectomy were excluded from the study.

Table 1: Microscopic features of molecular subtypes

Subtype	Grade	Histologic type (%)	pCR (%)	Lymph node metastasis (%)	DCIS (%)	LV emboli (%)
Luminal A (n=55)	Low grade-43 (78) High grade- 12 (22)	IDC- NST- 52 (94) IDC with mucin secretion- 2 (4) IPC- 1 (2)	13 (23.6)	25 (45.45)	13 (24)	14 (25.4)
Luminal B (n=25)	Low grade- 20 (80) High grade- 5 (20)	IDC- NST – 23 (92) IDC with mucin secretion – 1 (4) IPC- 1 (4)	6 (24)	7 (28)	8 (32)	8 (32)
HER2 enriched (n=21)	Low grade- 12 (57%) High grade- 9 (43)	IDC- NST- 21 (100)	7 (33)	6 (29)	6 (29)	6 (29)
Triple negative subtype (n=30)	Low grade – 14 (47) High grade – 16 (53)	IDC- NST- 30 (100)	6 (20)	9 (30)	9 (30)	6 (20)

pCR: Pathologic complete response, DCIS: Ductal Carcinoma *in situ*, LV emboli: Lymphovascular emboli, IDC: Invasive ductal carcinoma, IDC- NST: Invasive ductal carcinoma, no special type, IPC: Invasive papillary carcinoma

Table 2: Chemotherapy induced changes in subtype (n=131)

Changes studied	Luminal A (n=55) (%)	Luminal B (n=25) (%)	HER2 enriched (n=21) (%)	Triple negative (n=30) (%)
Change in tumor cellularity	39 (71)	11 (44)	7 (33)	21 (70)
Chemotherapy induced changes in neoplastic cells	9 (16.3)	4 (26)	4 (19)	7 (23)
Chemotherapy induced changes in surrounding stroma	41 (74.5)	17 (68)	13 (62)	17 (56)
Change in tumor grade	6 (11) (Decrease in grade-4 Increase in grade-2)	3 (12) (Decrease in grade- 2 Increase in grade-1)	6 (28) (Decrease in grade- 5 Increase in grade-1)	6 (20) (Decrease in grade-4 Increase in grade – 2)

Table 3: Pathological complete response: comparison with similar studies

Pathological complete response rates	Our Study (n=131) (%)	Study by Subbiah et al. (n=60) ⁹ (%)	Study by Kim et al. (n=257) ¹¹ (%)	Study by Díaz-Casas et al. (n=414) ¹² (%)	Study by Haque et al. (n=13939) ¹⁰ (%)
Overall pCR	24.4	15	25.7	15.2	18.8
Luminal A- pCR	23.6	12.5	3.9	10.9	0.3
Luminal B- pCR	24	13.3	5	18.6	8.3
HER2-pCR	33	40	10	40.5	38.7
Triple negative -pCR	20	0	21.1	26.7	23.2

pCR: Pathological complete response, HER2: Human epidermal growth factor receptor 2

Data were entered in Microsoft Excel and analyzed using the IBM SPSS software version 26. The parameters analyzed were baseline clinicopathological parameters including the menopausal status, family history of carcinoma, history of breast feeding in the study population, distribution of molecular subtypes in study population, pathologic complete response rate to NACT among the molecular subtypes, and the chemotherapy-induced changes in the neoplastic cells and the surrounding breast tissue.

RESULTS

Among the 131 patients studied, 35 patients (27%) were premenopausal, rest were post-menopausal. Family history of breast carcinoma was observed in six patients (4%), history of gastric carcinoma in 1 patient (1%). One hundred and twenty-nine (98.5%) patients had a history of breast feeding, whereas 2 patients were nulliparous. The distribution of molecular subtypes in the study population

was 55 patients were Luminal A (42%), 25 were Luminal B (19%), 21 were HER-2 enriched (16%), and 30 were triple negative (23%) (Table 1).

Among the Luminal A subtype, 43 patients (78%) lesion were low grade nature by histology and the predominant histological type was invasive carcinoma breast no special type (NST) (94%) with 2 cases of IDC with mucin secretion (Figure 5). Residual neoplasm observed in 42 cases (76%), out of which microscopic foci of invasive carcinoma seen in 10 cases, thus pathologic complete response was observed in 13 cases (23.6%). Lymph node metastasis was seen in 25 cases (45.4%) (Figure 6). Ductal carcinoma *in situ* (DCIS) was observed in 13 cases (24%) (Figure 4) and lymphovascular tumor emboli was seen in 14 cases (25.4%) (Figure 7).

Out of the 25 Luminal B subtype cases, 20 cases (80%) cases were low grade nature by histology and the predominant histologic type was invasive carcinoma breast NST (92%). Residual neoplasm observed in 19 cases (76%)

out of which microscopic foci of invasive carcinoma seen in four cases. Pathologic complete response was observed in six cases (24%) and lymph node metastasis was seen in 7 cases (28%). DCIS was observed in 8 cases (32%) and lymphovascular tumor emboli were seen in 8 cases (32%).

Twenty-one cases of Her 2 enriched subtype were studied with majority (57%) cases being of low grade nature and all the cases studied were invasive carcinoma breast NST. Residual neoplasm was observed in 14 cases (67%), out of which microscopic foci of invasive carcinoma seen in 6 cases (29%) thus pathologic complete response was observed in 7 cases (33%). Lymph node metastasis was seen in six cases (29%); DCIS was observed in 6 cases (29%); and lymphovascular tumor emboli were seen in 6 cases (29%).

Among the 30 cases of triple-negative subtype, all the cases were invasive carcinoma breast NST, and predominantly high grade nature by histology (53%) (Figure 3). Residual neoplasm was observed in 24 cases (80%) out of which microscopic foci of invasive carcinoma seen in 5 cases. Pathologic complete response was observed in 6 cases (20%), lymph node metastasis was seen in 9 cases (30%). DCIS was observed in 9 cases (30%) and lymphovascular tumor emboli were seen in 6 cases (20%).

Chemotherapy induced changes were observed in all subtypes which includes change in tumor cellularity with a reduction in tumor cellularity following treatment, neoplastic cells also showed an increase in cell size with increased cytoplasmic volume/cytoplasmic vacuolations, nuclear enlargement, hyperchromasia, pleomorphism with multinucleated cells (Figure 9). The surrounding breast tissue also show alteration in the form of diffuse atrophy of glandular elements, loss of acinar luminal cells and lymphoplasmacytic infiltrate (Figure 8 and Table 2).

DISCUSSION

In this study, the incidence of breast carcinoma was predominantly in the post-menopausal age group which was in concordance with the study by Subbiah et al.,⁹ and Haque et al.¹⁰ All the patients studied were females and all of them were clinically diagnosed as LABC. A few patients (6 out of 131 cases) had a history of carcinoma breast in family and in 2 of them the diagnosis of carcinoma breast was known in first degree relative. One case had a history of Carcinoma Stomach in a 1st degree relative.

Among the 131 cases studied, 55 patients were Luminal A, 25 were Luminal B, 21 were HER2 enriched, 30 were Triple negative. Thus the predominant subtype in our study was Luminal A subtype which was in concordance with study

conducted by Kim et al.¹¹ In study conducted by Haque et al.,¹⁰ in a large sample size (13,939 patients), majority of the patients were in the Luminal B subtype.

Among the molecular subtypes studied, Luminal subtypes (Luminal A and B) and HER2 enriched subtype cases were predominantly of low histologic grade, while triple-negative cases showed an increased frequency of cases with high histologic grade.

Triple-negative subtype showed the maximum number of cases with residual neoplasm (80%), followed by Luminal A and Luminal B subtype showing 76% of cases each with residual neoplasm. Among the HER2-positive cases, 67% cases had residual neoplasm following NACT.

Twenty-one cases studied showed a change in tumor grade, out of which 15 cases showed a decrease in grade and 6 cases showed an increase in grade of the residual neoplasm as compared to the initial tru-cut biopsy. Following NACT, change in tumor cellularity, changes in the morphology of neoplastic cells, and changes in the surrounding breast tissue were seen.

Overall, pCR rate in our study was 24.4% cases and the pCR among the molecular subtypes was the highest for HER2-positive tumors (pure HER2) which was 33% in our study which was in concordance with the study by Subbiah et al.,⁹ Díaz-Casas et al.,¹² and Haque et al.¹⁰ (Table 3).

Limitations of the study

The limitations observed during the study were sample size could not be achieved and only MRM cases were studied, patients who underwent lumpectomy were not included in the study. Long-term follow-up of the patient was also not possible.

CONCLUSION

Among the different molecular subtypes, overall pathologic complete response was 24.4% and the pathologic complete response rate was the highest for HER 2 enriched subtype (33%) following NACT. Following NACT, changes in tumor cellularity, change in tumor grade, morphology of neoplastic cells, and changes in surrounding breast tissue were also noticed.

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Authors' Contributions:

NAA- Concept, design, clinical protocol, manuscript preparation, data collection, data analysis, result analysis, interpretation and literature survey; **SS**- Concept and design of the study, guiding the progress of the study, reviewed result analysis and interpretation, guidance and support; **SMV**- Concept and design of the study, reviewed the literature, result analysis and interpretation, review manuscript; **SKK**- Concept and design of the study. Reviewed result and interpretation, literature survey, guidance and support.

Work attributed to:

Government of Medical College, Kottayam, Pincode- 686008, Kerala, India.

Orcid ID:

Dr. Nidhi Aley Alex - <https://orcid.org/0009-0000-4586-1494>

Dr. Sankar S - <https://orcid.org/0000-0002-5707-6423>

Dr. Saji Mary Varghese - <https://orcid.org/0009-0007-1878-5482>

Dr. Suresh Kumar K - <https://orcid.org/0000-0002-0443-8857>

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