



CHEMOTHERAPY INDUCED HISTOPATHOLOGICAL CHANGES IN MASTECTOMY SPECIMENS - STUDY AT A TERTIARY CARE CENTRE

Dr. Gowri .M*

Senior Resident, Department of Pathology, Osmania Medical college, Hyderabad
*Corresponding Author

Dr. Triveni Bhopal

Professor, Department of Pathology, Osmania Medical college, Hyderabad

**Dr. G. J. Vani
Padmaja**

Professor &HOD, Department of Pathology, Osmania Medical college, Hyderabad

ABSTRACT **Background:** Pathological complete response (pCR) to Neo adjuvant chemotherapy (NACT) is a significant prognostic marker in breast cancer, so accurate diagnosis and grading of pathological chemo response of tumour is needed for an effective planning of treatment regimen. **Aims:** To study the various histopathological changes in Mastectomy specimens following NACT and to evaluate the pathological response of tumour to NACT, also to compare the expression of ER, PR, and HER2 in biopsies and post NACT mastectomy specimens by Immunohistochemistry (IHC). **Methods And Materials:** 50 breast carcinoma cases, who was diagnosed on trucut biopsies and treated with preoperative NACT followed by mastectomy was included. In post NACT mastectomy specimens, the chemo-response was classified according to Union for International Cancer Control (UICC) guidelines. ER, PR, and HER2/ neu receptor expression was compared between trucut biopsy specimens (pre- NACT) and resection specimens (post- NACT). Results were analysed by Chi square test and McNemar chi square test. **Results:** Among 50 cases, patients presented with lump, involved more on the right and in upper outer quadrant. Most common stage at presentation is stage III A. Histologically all belong to IDCC -NST histological subtype. Significant reduction in mean tumour size by 52.7% after NACT was observed. pCR, pPR and pNR was observed in 20 %, 58 % and 22 %of the cases respectively. Most common histological change seen in post NACT is necrosis, desmoplasia, fibrosis, lymphocytic inflammatory response. Discordance in ER expression (17.5 %), PR expression (20%) and HER2 /neu (12.5 %) before and after NACT. But these were statistically insignificant. **CONCLUSIONS:** Pathological evaluation of chemo response of tumour is the gold standard. The discordance rate of ER, PR and HER-2/neu expression following NACT was statistically insignificant, larger studies needed to analyse their impact in patient management.

KEYWORDS : Breast cancer, Neoadjuvant chemotherapy, IHC.

INTRODUCTION

Breast cancer is the most common cancer in females with an estimated 2 million new cancer cases diagnosed in 2018 (23% of all cancers), and ranks second overall in the world (10.9% of all cancers) [1]. Treatment options available for breast cancer includes surgery, chemotherapy, radiotherapy, hormonal therapy, targeted therapy and immunotherapy. Preoperative Neo adjuvant chemotherapy (NACT) is the standard care of therapy for locally advanced breast cancers. The major aims of NACT in these patients are to clinically downstage the tumour by eradicating the possible distant micro-metastatic disease and to decrease the tumour size [2], thus increasing the possibility for breast conservation therapy.

In the modern era, increased number of post NACT surgical resection specimens of tumours are being received in surgical histopathology labs. Pathological evaluation of chemo response on tumour is the gold standard, as the clinical and radiological responses do not correlate much with residual tumour after NACT. Pathological complete response to NACT carries prognostic significance, independent of other common prognostic biological markers [3] and is also considered the best predictor of overall survival [4].

Pathologist plays vital role in accurate diagnosis and grading of pathological chemo response of tumour which is needed for an effective planning of treatment regimen, thus increasing the prognosis and survival chance of the cancer patient. The aim of the study was to study the various histopathological changes in tumour tissue and adjacent normal tissue, following NACT in Mastectomy specimens and to evaluate the pathological response of tumour to chemotherapy, also to compare the expression of ER, PR, and HER2 in pre NACT biopsies and post neo adjuvant chemotherapy mastectomy specimens by Immunohistochemistry (IHC).

MATERIALS AND METHODS:

A cross sectional study was conducted on 50 breast carcinoma cases for a period of 2 years from June 2018 – June 2020, in the Department of Pathology, MNJ Institute of Oncology and Regional Cancer Centre, Hyderabad. Breast carcinoma patients with adequate clinical and radiological data, who was diagnosed on trucut biopsies and treated with preoperative NACT followed by mastectomy were included. Breast carcinoma patients who underwent mastectomy without NACT and non-neoplastic conditions were excluded.

The tissue specimens were fixed in 10% neutral buffered formalin.

Close examination of fresh MRM specimens is done and the specimen is cut into 5-mm sections or smaller and the tumour bed is identified. Sections were then processed and paraffin block preparation is done. 4micron thick sections obtained, were stained with Haematoxylin and Eosin (H&E). Histopathological findings and diagnosis noted. In post NACT mastectomy specimens, the pathological response to chemotherapy was classified according to “Union for International Cancer Control (UICC) guidelines.

Estrogen receptor (ER), Progesterone receptor (PR), and HER2/ neu receptor expression was evaluated on all cases of invasive breast carcinomas, both on trucut biopsy specimens (pre- NACT) and on resection specimens (post- NACT) by standard IHC technique and evaluated for any discordance between pre NACT and post NACT specimens.

Institutional ethics committee, Osmania medical college has granted the clearance for the study. Correlation between pathological tumour response [pCR, pPR, pNR] and other pathological factors was evaluated using Chi square test. McNemar chi square test was used to analyse any change in ER, PR, HER 2neu status in pre NACT and post NACT breast.

RESULTS

50 female cases with pre-operative NACT treated breast carcinoma were studied, showed slight predominance on right side (26 cases). Age of patients ranged from 30 to 75 years. Mean age is 48 and median age is 48 years. Majority of cases were seen in the 4th and 5th decades. All the patients presented with complaints of lump in the breast, a few in addition had pain and nipple discharge.

Most common quadrant involved is upper outer quadrant (48%) cases. Most common stage at presentation is stage III A (42%) cases. All the 50 cases belong to IDCC -NST histological sub type. The mean size of tumour calculated before NACT was 36 cm² and after NACT it was 17 cm² indicating a significant reduction in mean tumour size by 52.7%.

Pathological response after NACT was assessed by UICC guidelines. pCR was observed in 10 (20 %) cases, pPR in 29 (58 %) cases and pNR in (11) 22 % cases. Most common change seen in tumour cells post NACT breast carcinoma is tumour necrosis, followed by nuclear enlargement [Table 1]. Most common stromal change observed is desmoplasia, seen in 52% cases followed by Fibrosis (44%) [Table 2].

Most common inflammatory response is lymphocytic response, seen in 60% of cases followed by giant cells response (18%), mixed inflammatory response (10%), plasmacytic response (8%) and prominent histiocytic response (4%) cases.

Most common histopathological change seen in adjacent normal breast parenchyma of post NACT mastectomy specimen is thickening of basement membrane (14%) followed by atrophy (12%), Sclerosis (8%).

10 (20 %) cases show presence of DCIS component in post NACT breast carcinoma, the p value is 0.204 which is statistically insignificant.

Table 1: Nuclear changes and cytoplasmic changes in tumor tissue after NACT in Breast Carcinoma

TUMOR CELLS	NO OF CASES	PERCENTAGE (%)	pCR	pPR	pNR
Nuclear enlargement	30	60	0	18	12
Hyperchromasia	29	58	0	20	09
Tumor necrosis	39	78	10	28	01
Nuclear & cytoplasmic Vacuolation	26	52	0	18	08
Increased Nuclear: Cytoplasmic ratio	26	52	0	22	04
Prominent nucleoli	27	54	0	18	09
Pleomorphic nuclei	22	44	0	17	05
Pyknosis	12	24	0	07	05

Table 2: Stromal changes / response after NACT in breast carcinoma.

STROMAL CHANGE	NO OF CASES	PERCENTAGE (%)	pCR	pPR	pNR
Elastosis/ Collagenization	19	38	07	08	04
Fibrosis	22	44	08	11	03
Desmoplasia	26	52	03	16	07
Hyalinization of the walls of blood vessels	06	12	03	02	01
Calcification	08	16	04	02	02
Mucinous change	02	04	01	01	00
Angiogenesis	10	20	04	02	04
Hemosiderin-laden Macrophages	06	12	03	02	01

ER, PR and HER2/ neu receptor changes post NACT in breast carcinoma [Table 3,4,5]:

In the present study total number of cases studied is 50 (n=50), but comparison of expression of ER, PR and HER2/neu receptor by IHC before and after NACT was done on 40 cases (n=40) as 10 cases (20%) had complete pathological response (pCR), with no tumour tissue left to perform IHC on Modified Radical Mastectomy (MRM)specimens .

In post NACT breast carcinoma, discordance in ER expression, PR expression and HER2 neu expression was observed in 7 (17.5 %) patients, 8 (20%) patients and 5 (12.5 %) patients respectively.

ER positivity decreased from 45 to 32.5 % following NACT. There was a corresponding increase of ER negativity from 55% to 67.5 % (p = 0.130). This result was statistically insignificant p>0.05). PR positivity decreased from 37.5 to 32.5 % following NACT. There was a corresponding increase of PR negativity from 62.5 % to 67.5% (p = 0.72). This result was statistically insignificant p>0.05). HER2/ neu positivity is decreased from 37.5 to 25 % following NACT. There was a corresponding increase of HER2/neu negativity from 62.5 % to 75% (p=0.07). This result was statistically insignificant p>0.05)

Table 3 : Changes in ER expression before and after NACT (n=40)

ER STATUS (PRE NACT)	ER STATUS POST NACT IN BREAST CARCINOMA		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	12 (30%)	06(15%)	18(45%)
NEGATIVE	01(2.5%)	21(52.5%)	22(55%)
TOTAL	13(32.5%)	27 (67.5%)	40(100%)

Table 4: Changes in PR expression before and after NACT (n=40)

PR STATUS (PRE NACT)	PR STATUS POST NACT IN BREAST CARCINOMA	TOTAL

	POSITIVE	NEGATIVE	
POSITIVE	10(25%)	05(12.5%)	15(37.5%)
NEGATIVE	03(7.5%)	22(55%)	25(62.5%)
TOTAL	13(32.5%)	27(67.5%)	40(100%)

Table 5: Change in HER2/NEU expression before and after NACT (n=40)

HER2 / NEU STATUS(PRE NACT)	HER2/ NEU STATUS POST NACT IN BREAST CARCINOMA		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	10 (25%)	05(12.5%)	15(37.5%)
NEGATIVE	00(0%)	25(62.5%)	25(62.5%)
TOTAL	10(25%)	30(75%)	40(100%)

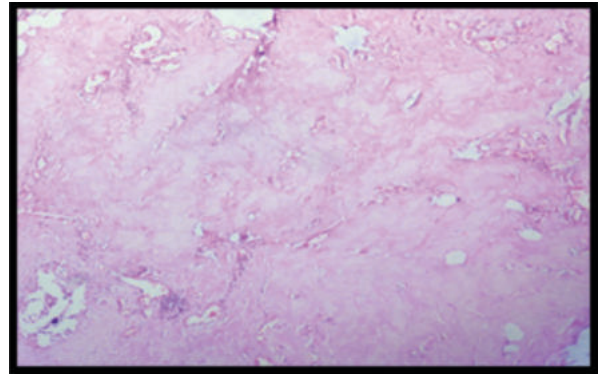


Figure 1: Breast carcinoma, IDCC -NOS Subtype, Post-NACT: Residual tumor cells showing areas of fibrosis (H & E, 10X)

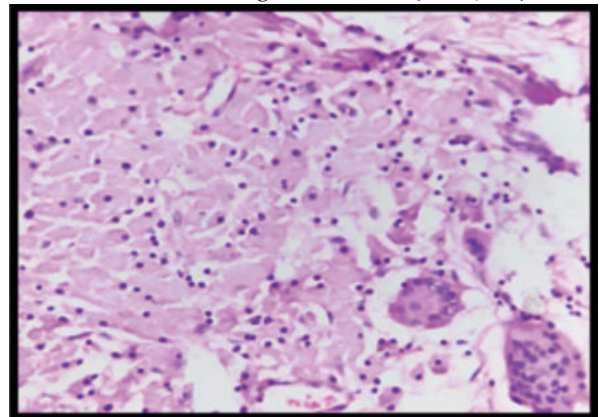


Figure 2: Breast carcinoma, IDCC -NOS Subtype, Post-NACT, showing, histiocytic giant cell response (H & E, 10X),

DISCUSSION

Age of the patients ranged from 30 to 75 years with majority of cases seen in 4th and 5th decade. The age range is comparable to the studies done by Cheryl Sarah Philipose et al [5], Ramana kumari P et al [6], Moon et al [7]. Dhanya et al [8] had a similar study group with the mean age group being 46.3 years and the range being 21-80 years.

Most of the studies showed slight increase in right breast involvement than the left breast similar to the present study, 52% cases on the right , Shazima sheereen et al [9] (66.7 %) cases and Burcombe et al [10] (54 %) cases. Present study showed a significant reduction in mean tumour size by 52.7% after NACT . It is comparable to Shazima sheereen et al [9] which showed 53% reduction and Sethi D et al [11] showed 47% reduction.

Union for International Cancer Control (UICC) guidelines of assessment of pathological response to NACT in breast carcinoma on MRM specimens includes Pathologic Complete Response (pCR) - Absence of residual viable tumour cells (DCIS: Ductal carcinoma in-situ included), Pathologic Partial

Response (pPR)- Greater than 50% reduction in the number of viable tumour cells and Pathologic No Response (pNR)- Less than 50% reduction in the number of viable tumour cells, stable disease (SD) and /or, no change and progressive disease (PD) as an increase of at least 25% of viable tumour cells. Comparison of percentage of pCR, pPR

and pNR in post NACT breast carcinoma cases in different studies tabulated in [Table 6]

Table 6: Comparison percentage of pCR, pPR and pNR in post NACT breast carcinoma cases in different studies:

STUDY	pCR (%)	pPR(%)	pNR(%)
Sethi D et al	20	37.5	42.5
Ramana kumari P et al	20	40	40
Shazima sheereen et al	17.9	15.4	66.7
Moon et al. et al	25.3	59.7	15
Present study	20	58	22

In the present study, most common nuclear and cytoplasmic changes in tumour tissue after NACT in breast carcinoma is tumour necrosis, seen in 78 % cases, Cheryl Sarah Philipose et al[5] observed in (72.7 %) cases. Increased Nuclear: Cytoplasmic ratio (52%) ,another most common change seen in the present study. Shazima sheereen et al [9] observed nuclear enlargement, hyperchromasia, and increased N: C ratio in 85% of the cases. Sethi D et al [11] observed dyscohesion and shrinkage of tumor as the most common histologic change, whereas Cheryl Sarah Philipose et al [5] observed pyknosis in 72.7% cases, 63.63% cases showed karyorrhexis and karyolysis. The cytoplasmic alterations included vacuolation and foamy cell change.

In the present study most common stromal change observed is desmoplasia (52%) followed by Fibrosis (44%), Shazima sheereen et al [9] observed desmoplasia in 59% of cases, fibrosis (64.1%), which is comparable to the present study. Sethi D et al observed elastosis/collagenization as the most common stromal change seen in 72.5% cases followed by microcalcification (17.5%), hyalinization of the walls of the blood vessels (10%). Similar changes were seen in the present study with micro calcification seen in 16% cases and, hyalinization of the walls of the blood vessels (12%). In present study mucinous change was observed in 4% cases whereas Sethi D et al [11] observed in 10% cases.

The most common inflammatory host response observed in the present study is lymphocytic response seen in 60% of cases, similar to Sethi D et al[11]. Cheryl Sarah Philipose et al [5] showed lymphoplasmacytic infiltrate in 86.3% cases in contrast present study showed only in 10% cases. Giant cell response was seen in 35.9 % cases in Shazima sheereen et al [9], 30 % by Sethi D et al [11], 22.7% of cases in Cheryl Sarah Philipose et al[5], whereas it is 18% cases in present study Foamy histiocytic response was observed in 4% cases in the present study, in contrast Cheryl Sarah Philipose et al[5] observed in 22.7% cases , Shazima sheereen et al [9] in 20.5 % cases and Sethi D [11] et al in 7.5% cases.

Most common change seen in adjacent normal breast parenchyma is thickening of basement membrane (14%) followed by atrophy (12%), Sclerosis (8%) in the present study. Shazima sheereen et al [9] observed atrophy of adjacent breast parenchyma (17.9% cases) and cancerisation of lobules in 25.6% cases, as also observed by Sethi D[11] et al

10(20 %) cases shows presence of DCIS component in post NACT breast carcinoma, Dhanya et al [8] observed DCIS in 13 cases (27.1%), and stated that DCIS was consistently present in 84% of the cases with pCR indicating that it was resistant to chemotherapy. Another study by Agarwal Savita et al [12], observed DCIS in 11 cases (35.4%) whereas Galal et al [13] observed 86% of tumors with DCIS had a poor response to treatment.

In the present study, discordance in ER expression was observed in 7 (17.5 %) patients and this discordance is comparable with P Ramteke et al[14], Shet T et al[15] and Jin G et al[16] [Table 7] . ER positivity decreased from 45 to 32.5 % following NACT. There was a corresponding increase of ER negativity from 55% to 67.5 % (p = 0.130). This result was statistically insignificant p>0.05).

In the present study, Discordance in PR expression was observed in 8 (20%) patients and this discordance is comparable with P Ramteke et al [14], Shet T et al[15] and Jin G et al[16] .PR positivity decreased from 37.5 to 32.5 % following NACT. There was a corresponding increase of PR negativity from 62.5 % to 67.5% (p = 0.72). This result is again statistically insignificant p>0.05).

In the present study, discordance in HER2 neu expression was observed in 12.5 % patients and this discordance is comparable with P

Ramteke et al [14], Jin et al [16] and Neubauer H et al [17]. HER2/neu positivity decreased from 37.5 to 25 % following NACT. There was a corresponding increase of HER2/neu negativity from 62.5 % to 75% (p = 0.07). This result was statistically insignificant p>0.05)

Table 7: Comparison of ER, PR expression in breast carcinoma specimens, before and after neoadjuvant chemotherapy

STUDY	PERCENTAGE % OF CASES SHOWING DISCORDANCE IN ER EXPRESSION	PERCENTAGE % OF CASES SHOWING DISCORDANCE IN PR EXPRESSION
P Ramteke et al	17%	13%
Shet T et al	13%	22%
Jin G et al	16.2 %	22.1%
Neubauer H et al	8 %	18%
Present study	17.5 %	20%

CONCLUSION

Pathological evaluation of chemo response of tumour is the gold standard. Pathological complete response (pCR) to NACT carries prognostic significance independent of other prognostic biological markers and considered as the best predictor of overall survival. In the present study, discordance rate of ER, PR and HER-2/neu expression following NACT was statistically insignificant, however larger studies are needed to analyse the impact of these alterations in prognosis and patient management.

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