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IMPACT OF NEOADJUVANT CHEMOTHERAPY ON IMMUNOHISTOCHEMISTRY PROFILE OF BREAST CARCINOMA

Histopathology

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ABSTRACT

Introduction- Management of Breast Carcinoma is multidisciplinary and has come a long way. Chemotherapy and Hormonal therapy decisions for breast cancer management are based on expression of tumour biomarkers: Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal Growth Receptor 2 (HER 2 neu) and Ki-67 index. The impact of Neoadjuvant Chemotherapy (NACT) on the immunohistochemical (IHC) profile remains controversial with wide variation in results across studies. **Objectives-** To determine the immunohistochemical changes in post Neoadjuvant Chemotherapy breast cancer specimens and to study the histopathological findings after NACT. **Materials and Methods-** A cross sectional study was done using fifty-one (51) diagnosed cases of breast cancer after having received at least 4 cycles of NACT. Immunohistochemical study was done using monoclonal antibodies. The values of ER, PR, HER 2 neu & Ki-67 were evaluated & compared to pre-NACT scores. The histopathological findings were assessed. Statistical analysis was performed using MedCalc and Omni calculator. Associations between IHC markers before and after NACT were evaluated using the McNemars's test. **Results-** Out of 51 cases of breast carcinoma, majority (96.07%) were of Invasive Breast Carcinoma, no special type (IBC NST) and majority of the patients were in the age range of 41 to 50 years. 6 patients (11.76%) underwent pathological complete response. Change in HER 2 neu receptor expression post NACT was found to be statistically significant (p value- 0.016). There was an overall trend towards low Ki-67 observed after NACT which was also statistically significant (p value- 0.00004). The changes in ER & PR were not statistically significant. **Conclusion-** It was observed that biomarkers and histopathological response of breast cancer may change after NACT. Changes in HER 2 neu & Ki-67 expression were significant.

KEYWORDS

Neoadjuvant Chemotherapy, Invasive Breast Carcinoma, Immunohistochemistry

INTRODUCTION

Globally, Breast cancer is the commonest malignancy among women. From being fourth in the list of most common cancers in India (during the 1990s), it has become the first[1].

The pathological features, biological characteristics and prognosis of breast cancer are closely related to the expression of ER, PR, Her 2 neu and KI-67.

Nowadays, neoadjuvant chemotherapy (NACT) is frequently used for inflammatory, locally advanced, and operable breast cancers. Large tumours can also be down staged using NACT to make way for breast conservation surgery.

However, little is currently understood about the frequency of ER and/or PR conversion, HER2-neu, Ki-67 status, and the relationship to prognosis in patients with breast cancer following NACT.

Following NACT, changes in the biomarker profile could be brought on by intra-tumour heterogeneity or the chemotherapeutic effect [2]. Following surgery, decisions about treatment are based on these changes.

Additionally, cytomorphological alterations in malignant cells, such as dyscohesion, cytoplasmic and nuclear vacuolization, multinucleation, pyknosis, karyorrhexis, etc., have been noted as a result of NACT-induced histopathological changes[3]. Stromal alterations, such as necrosis, hyalinization, and extensive collagenization are also observed.

These morphological changes may affect prognostic evaluation and interfere with post-NACT grading of the tumour.

The size of invasive carcinomas that respond poorly may not change at all. Higher response levels result in less cellularity in the carcinoma, which can manifest as sporadic, tiny foci of invasion.

Sometimes a post-NACT sample will reveal no invasive carcinoma left behind i.e. Pathological Complete Response (PCR). Compared to patients whose tumours showed a probable or definite response to pre-surgical therapy, or no definite response at all, these patients were found to have a statistically improved [4] survival outcome.

The purpose of this study was to determine how NACT affected these variables as well as the overall histopathological effect by comparing the expression of ER, PR, HER 2 neu, and Ki-67 in breast tumour tissues before and after NACT.

Objectives

- To determine immunohistochemical marker changes after neoadjuvant chemotherapy in breast cancer
- To determine the histopathological findings in post NACT breast cancer specimens.

MATERIALS AND METHODS

A cross-sectional observational study was conducted at the Department of Pathology in collaboration with the Department of General Surgery of R.G. KAR MEDICAL COLLEGE and HOSPITAL, Kolkata over a period of eighteen months. Fifty-one (51) samples diagnosed as breast cancer were taken as per convenience sampling.

Inclusion Criteria

- Patients having breast tumour with known Estrogen, Progesterone, HER 2neu, Ki 67 receptor expression.
- Patient having received at least 4 cycles of Neoadjuvant Chemotherapy for the breast tumour.
- Patients having undergone Modified Radical Mastectomy (MRM), MRM with axillary clearance or Radical Mastectomy as decided by the surgeon followed by Histopathological examination and immunohistochemical study.

Exclusion Criteria

- Patients with benign breast lesion.
- Patients with stromal tumors of breast.

Breast carcinoma specimens were taken for histopathological and IHC examination after being fixed in 10% buffered formaldehyde solution for 6-72 hrs. Paraffin blocks were prepared using routine histopathological techniques.

Histopathological grading and tumour staging were noted as per CAP protocol after being concurrently reviewed by at least 2 histo-pathologists.

IHC was performed using commercially available monoclonal antibodies for ER, PR, HER2/Neu (pre-diluted Rabbit Monoclonal Antibody- ER-6ml, PR-6ml, HER 2-neu-25ml)

Immunostaining was evaluated by counting 500 tumour cells for each case at high power magnification. Sections from fibroadenoma were taken as positive control to show reliability of the staining in cases of ER, PR, HER2/NEU. Sections from germinal centers of lymph nodes were taken as positive controls of Ki-67.

Estrogen receptor and Progesterone receptor expressions were measured with the help of Allred [5] scoring method which is based on the assessment of the proportion and intensity of staining. Her2-neu scoring [6] was done based on the staining pattern. A Ki-67 cut-off point of 14 % was defined for breast cancer [7].

Statistical Analysis

Data were entered in Microsoft Office Excel spreadsheet. Quantitative and Qualitative data were described by estimating mean, standard deviation and proportion as applicable. Associations between IHC markers before and after NACT were evaluated by McNemar test using Omni calculator and Med Calc Software (version 22.009). P-value < 0.05 was considered to be statistically significant.

RESULTS

In our study, out of 51 cases, 41% were in the age range of 41 to 50 years. 96.07% of the study population had Invasive Breast Carcinoma, no special type (IBC NST).

6 cases underwent complete pathological response (pCR) following NACT (ypT0N0). Since there was no residual invasive tumour, those cases were not subjected to further IHC studies. The calculations were done out of 45 cases with residual tumour. ER positivity dropped from 36 to 34 following NACT. There was a corresponding increase in ER negativity from 9 to 11. (p value= 0.56).

30 patients were initially PR positive and 15 patients were PR negative. After NACT, PR negative cases increased to 19. (p value=0.20)

HER 2 neu receptor positivity was increased from 11 to 22, while negativity decreased from 34 to 23. This change in HER 2 neu receptor expression was found to be statistically significant.

There was an overall trend towards low Ki-67 observed after NACT. Initially 40 cases were having high Ki-67 index which reduced to 20 after NACT. This change was statistically significant.

DISCUSSION

Effect of NACT on Estrogen Receptor & Progesterone Receptor

De la Cruz et al [8]- Concluded that hormone receptor status changed from positive to negative in two cases and from negative to positive in one case.

Renu Gahlaut et al [9]- came to the conclusion that a change in ER/PR status occurred in 12%, 14.5% respectively, predominantly as a switch from negative to positive status for ER.

Gabriela Candás et al [10] found that the observed variability for estrogen receptors (ER) was 8.9% & for progesterone receptors (PR), 29.9%.

S van de Ven et al [11] found that ER and/or PR receptor remained stable after NACT.

Our study of ER & PR receptors was in concordance to this result.

Mary Diane Kinsella et al [12] studied 38 breast carcinomas, of which 45 % were positive for ER by IHC both pre- and post- neoadjuvant treatment. IHC studies for PR in these 38 patients showed 37% positivity for PR pre-neoadjuvant therapy and 21% positivity post-treatment.

Laura Rey-Vargas et al [13] reported that PR expression can be altered by NACT administration.

Ho-Chang Lee et al [14] Concluded that PR expression decreased after NACT.

Our results were comparable to the studies conducted by both Laura Rey-Vargas et al & Ho-Chang Lee et al.

In our study, initially 80% of cases out of those with residual tumour were ER positive, which dropped to 75% after NACT. This result was statistically insignificant. The PR positivity changed from 66.6% to 57.7% which was also statistically insignificant. These results were to some extent similar to the study conducted by BS Subhendu et al [15] where ER positivity decreased from 80 (80%) to 78 (78%) (p = 0.67). PR positive dropped from 66 to 62% (p = 0.002).

Effect of NACT on HER 2 neu & Ki-67 Status

Nilufer Avci et al [16] reported that NACT reduces the expression of HER2 and Ki-67 in breast cancer specimens.

Jiang Heng Peng et al [17] reported that the smallest and largest changes occurred in HER2 (20.5%) and Ki-67 (40.2%), respectively.

In our study, initially 24.4 % of cases out of those with residual tumour were HER 2 neu positive, which increased to 48.8 % after NACT. This result was statistically significant. This result was in concordance to the study conducted by Ayaka Katayama et al [18] which showed HER 2-positivity increasing from 62.9% to 77.7%.

In our study, initially 88.8 % cases were having high Ki-67 index which reduced to 44.4 % after NACT. This change was statistically significant & was similar to the study conducted by Chatterjee et al [19] which found that post NACT the cases with high Ki-67 reduced from 71 % to 49%.

We have observed the significant range of results after reviewing the earlier research. There is no clear agreement among the various authors who have reported a change in IHC status.[20]

According to Pedrini JL et al. [21], chemotherapy may cause clonal selection of tumor cells, which would explain the variations in IHC expression between pre- and post-NACT cells. There is little information available on subclonal evolution during primary breast cancer treatment.

It would be possible to identify the molecular mechanisms behind treatment resistance if subclonal redistribution and the emergence or disappearance of genomic aberrations during treatment were better understood [22]. More precise decisions in personalized medicine will ultimately be defined by a deeper understanding of breast cancer at the molecular level (multiomics) [23].

Table -01: Distribution of Histological Types of Breast Carcinoma

Histological Type	Frequency	Percentage
IBC NST	49	96.07%
IBC WITH AREAS OF MUCINOUS & LOBULAR DIFFERENTIATION	1	1.96%
MUCINOUS BREAST CARCINOMA	1	1.96%

Table- 02: McNemar's test Contingency Table for ER Status Pre & Post NACT

Change of ER status before & after NACT (n=45)				
ER status before NACT	ER status after NACT TAL		Total	P Value
POSITIVE	29	7	36	0.56
NEGATIVE	5	4	9	
Total	34	11	45	

Table- 03: McNemar's Test Contingency Table for PR Status Pre & Post NACT

Change of PR Status Before & After NACT (n=45)				
PR status before NACT	PR status after NACT Positive	Negative	Total	P Value
POSITIVE	23	7	30	0.205
NEGATIVE	3	12	15	
Total	26	19	45	

Table- 04: McNemar's Test Contingency Table for HER 2 Neu Status Pre & Post NACT

Change of HER 2 neu status before & after NACT (n=45)

HER 2 neu status before NACT	HER 2 neu status after NACT Positve	Negative	Total	P Value
POSITIVE	6	5	11	0.016
NEGATIVE	16	18	34	
Total	22	23	45	

Table- 05: McNemar's Test Contingency Table for Ki-67 Status Pre & Post NACT

Change of Ki-67 index before & after NACT (n=45)				
Ki-67 index before NACT	Ki-67 index after NACT Low	High	Total	P Value
LOW	3	2	5	0.00004
HIGH	22	18	40	
Total	25	20	45	

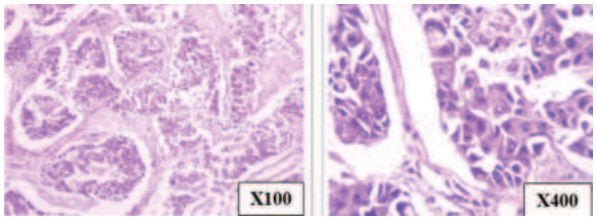

Figure-01: Photomicrograph Depicting Invasive Breast Carcinoma, No Special Type {Ibc Nst} (H&E)

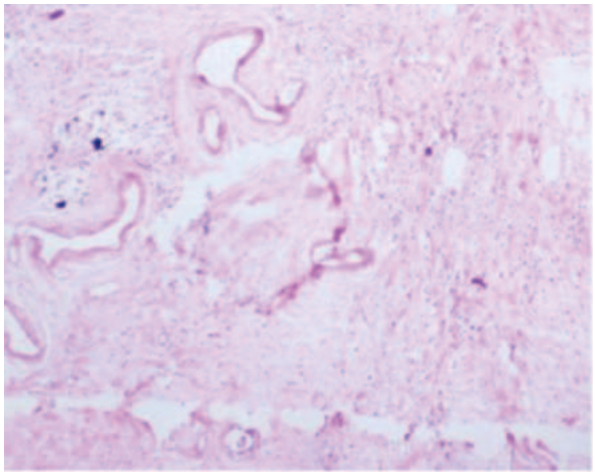

Figure-02: Photomicrograph Depicting stromal response-fibrosis (H&E, X100)

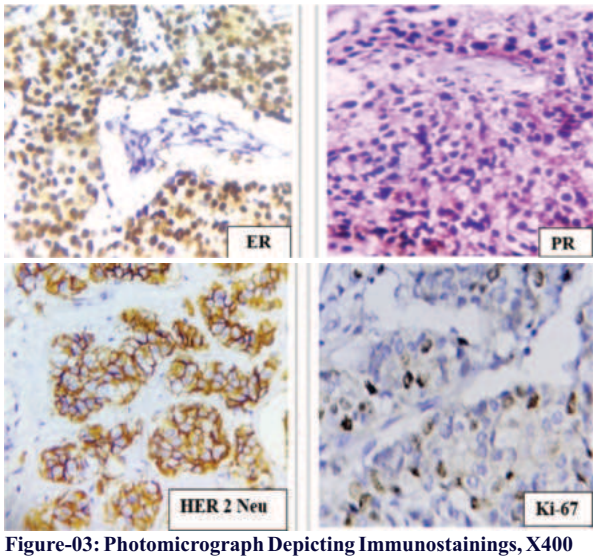

Figure-03: Photomicrograph Depicting Immunostainings, X400

CONCLUSION

Breast cancer is a very complex and heterogeneous illness with unique molecular and morphological features. This study revealed significant changes in molecular markers following NACT. The changes were highly variable & as such each & every patient should have individualized treatment approach following NACT. Conventional therapeutics where one medicine fits all will not be beneficial in this regard.

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