

The Predictive and Prognostic behavior of Breast Cancer by NACT in Relation to Tumor Grade, ER/PR and Her- 2/neu Expression- Study in a Tertiary Care Hospital of Eastern India

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ABSTRACT

Introduction: Neo-adjuvant chemotherapy (NACT) is considered as initial management of all patients with locally advanced breast cancers and followed by surgery. NACT enables reduction of breast tumor size- thereby prolonging survival and altered the expression of steroid receptor (ER/PR) and Her-2/NEU in the cancer cells. Our aims and objective was to look into the association of patient age, tumor grade, expression status of ER/ PR / Her-2/neu receptor status before and after NACT in Indian women patients.

Material and methods: The study was conducted over a period of 4 years. Hormone receptor and Her-2/neu status was determined by IHC over 412 samples. The patients receiving NACT and having both core needle biopsy and surgery (MRM) are considered as NACT group. The other group did not receive NACT but have core needle biopsy and surgery are taken as non NACT group. The discordance was compared between these two groups.

Results: Patients of more than 40years of age had predominantly favorable hormone receptor status expression. In the NACT group favorable receptor status was 60.35% and discordance was higher in ER, PR and HER- 2/neu in comparison to non-NACT group.

Conclusions: Significant alteration of hormone receptors and Her-2/neu expression was found in biopsy of the patients receiving NACT. So reevaluation of the hormonal status and Her 2 neu receptor in residual invasive carcinoma may be considered as it has advantage on chemotherapy after final surgery.

Keywords: Estrogen Receptor, Progesterone Receptor, HER-2/neu Expression, Breast Cancer, Neoadjuvant Chemotherapy

It is postulated that there may be alteration in hormone and Her 2 Neu expression status following NACT. There are several studies in the Indian population^{3,4} in this regard but those studies used different cut off, criteria for NACT and non-uniform study design. Our study was designed to re-evaluate the alteration of HR status and Her-2/ neu status so that patient will be benefited by specific adjuvant therapy. This study also found out the association of patient's age, histologic grade of tumor with hormone and Her- 2/ neu expression.

MATERIAL AND METHODS

The present study was retrospective study conducted at Nil Ratan Sircar Medical College and Hospital which is a tertiary referral Institution in Kolkata, India over a period of 4 years from December 2014 to November 2018. Clinical and relevant information were collected from Surgery and Radiotherapy department.

Inclusion Criteria: Our study includes 285 LABC (locally advanced breast carcinoma) who had received 4-6 cycles of NACT followed by Surgery (MRM) and had a residual tumor with Miller –Payne grade ≤ 3 considered as NACT group. The NACT was applied by a combination of 5- fluorouracil, doxorubicin and cyclophosphamide. The other group of 127 patients who did not receive any NACT, where mastectomy was performed for recurrence were also included as non NACT group. All patients in NACT and Non-NACT group had previous trucut biopsy with HR and Her- 2/ neu report before surgery. The specimens from both group were grossed as per protocols and section from paraffin blocks were stained by Hematoxyline and Eosin (H &E) for histo-pathological

INTRODUCTION

Cancer incidence among Indian women is increasing day by day, the breast cancer is being most common¹ cancer in India and the foremost cause of cancer death in female. Early diagnosis and appropriate therapy can prolong the survival of the patient. Recently neoadjuvant chemotherapy (NACT) is considered as initial management of all patient with locally advanced breast cancers and followed by surgery (MRM/ breast conservative surgery).

The kind of NACT depends upon the ER, PR and HER- 2/ Neu expression of cancer cells and has great influences on survival. ER/PR positive tumors are treated by Tamoxifen in premenopausal patient and Aromatase inhibitors are used in postmenopausal patient whereas Her 2 Neu positive tumors are treated by Trastuzumab (Herceptin). Now a days Adjuvant therapy is available in triple negative cases.²

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grading as per Nottingham's histologic score (Table 1). The response to NACT was categorized according to the Miller – Payne classification (Table 2).

Exclusion criteria: The cases showing significant reduction of residual tumor after NACT (Miller – Payne grade ≥ 4) were excluded from our study.

Appropriate blocks with adequate viable, residual tumor tissue were taken for immuno-histochemical (IHC) examination for study of ER, PR, Her-2/neu status. The ER/ PR scoring was done as per Allred scoring system (Table 3). Her 2/neu scoring was done as per standard four graded reporting protocols (Table 4). We did repeat IHC in the residual tumor of discordant cases. Appropriate positive and negative controls were also run with each batch.

STATISTICAL ANALYSIS

Data was entered in MS excel. For descriptive purposes percentages were calculated. Intergroup p value was calculated by Fisher Exact test for 2x2 contingency table. Parameters were studied on categorical scale between two or more groups, using SPSS-18. Significance level was considered at p value < 0.05.

RESULTS

In our four years of study period we found total 412 cases of invasive breast carcinoma. The 29.36% of breast cancer were ≤ 40 years (n=291) of age while 70.63% of patients were above 40 years (n=295) of age. The age range was 17 years to 77 years. Patients of > 40years of age had predominantly favourable hormone receptor status expression (Table 3).

Among all the patients, grade 2 tumor were more in both NACT (65.61%) and Non NACT patients (57.4%). But

compared to NACT group grade 3 tumor were much more in non NACT group (39.37%). The NACT group of patient had more favourable receptor status (60.35%) than non NACT group (table-4).

The details of hormone receptor status and Her- 2/ neu expression are shown in table 5. Among the NACT group significant alteration of ER status (p= 0.010), PR status (p= 0.003) and also in Her-2/neu status (p= 0.016) was found

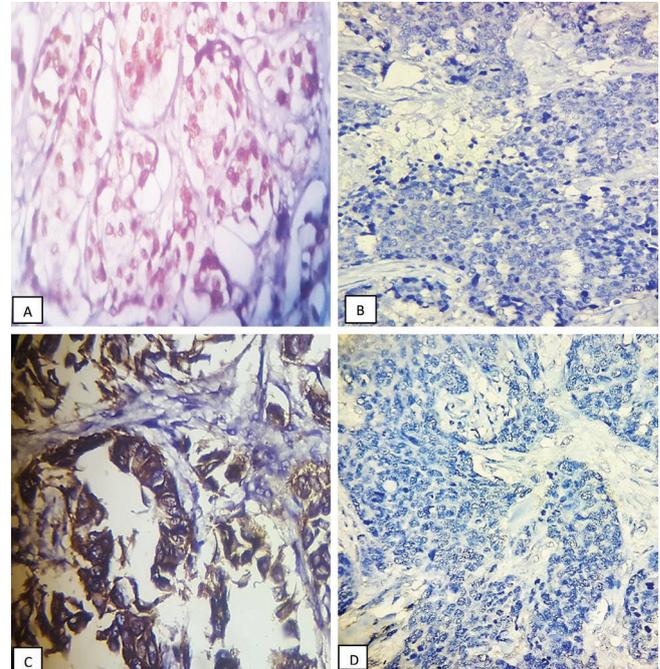


Figure-1: Microphotograph depicting A) ER Positive, B) ER Negative C) Her-2/ Neu Positive D) Her-2 /Neu negative tumors. (400X Magnification)

	The amount of gland formation/ tumor differentiation	The nuclear features of tumor	The mitotic activity (counted in 0.58mm field diameter and 0.264 sq. mm area of microscope)
Score 1:	>75% of tumor forming glandular/ tubular structures	Small nuclei with little variation / increase in size, regular outlines	≤ 9 mitoses/ 10 hpf
Score 2:	10% to 75% of tumor forming glands/ tubules	Larger nuclei with open vesicular chromatin, visible nucleoli, moderate variation of size/ shape	10-19 mitoses/ 10 hpf
Score 3:	< 10% of tumor area forming glands/ tubules	Marked variation of size/ shapes, ve- sicular chromatin, prominent nucleoli	≥ 20 mitoses/ 10 hpf
Score 3-5=	Grade 1		
Score 6-7=	Grade 2		
Score 8-9=	Grade 3		

Table-1: Histological grade of tumor as per the Nottingham's histologic score

Grade	Tumor percentage
Grade 1	No change or some alteration to individual malignant cells, but no reduction in overall cellularity
Grade 2	A minor loss of tumor cells, but overall cellularity is still high; up to 30% loss
Grade 3	Between an estimated 30% and 90% reduction in tumor cells
Grade 4	A marked disappearance of tumor cells such as small clusters of widely dispersed individual cells remain ; > 90% loss of tumor cells
Grade 5	No malignant cell identifiable in sections from the tumor; only vascular fibroelastic stroma remains often containing macrophages. DCIS may be present.

Table-2: The Miller –Payne system for classification for neo-adjuvent chemotherapy treated breast cancers.

	Favourable (ER+,PR+,HER2+, ER+PR-HER2-, ER+PR-HER2+, ER+PR+HER2-)	Unfavourable (ER-PR-HER2-, ER-PR+HER2-, ER-PR-HER2+)
Age ≤40 [n=121]	56(46.28%)	65(53.72%)
Age >40[n=291]	192(65.97%)	99(34.03%)

P value 0.003. The result is significant at p<0.05

Table-3: Association study of patient age with hormone receptor status.

		NACT (n=285)	Non NACT(n=127)
1.	Mean Age		
2.	Age ≤40 [n=121]	84(29.47%)	37(29.13%)
3.	Age >40[n=291]	201 (70.53%)	90 (70.87%)
4.	Histologic grade		
	Grade1	31(10.87%)	4(3.14%)
	Grade2	187(65.61%)	73(57.40%)
	Grade3	67(23.50%)	50(39.37%)
5.	Favourable Receptor status	172(60.35%)	27(21.25%)
6.	Unfavourable Receptor status	113(39.65%)	100(78.75%)
7.	Trucut biopsy ER positive	170 (59.64%)	27 (21.25%)
8.	ER+ on residual tumor	152(53.33%)	25 (19.68%)
9.	ER- to +	27	8
10.	ER discordance present	15.78%	7.87%
11.	Trucut biopsy PR positive	146	19
12.	PR+ on residual tumor	129	16
13.	PR – to +	18	1
14.	PR discordance present	12.28%	3.14%
15.	Trucut biopsy Her2 neu +	146	23
16.	Her2 neu + on Residual tumor	125	21
17.	Her2neu – to +	29	8
18.	Her 2neu discordance	21.1%	7.87%

Table-4: Clinical, histo-morphological features and hormone receptor status of NACT and Non NACT group

	Markers	Positive to positive	Negative to negative	Positive to negative	Negative to positive	Total discordance	Intergroup p value
1.	ER						
	NACT(285)	159	81	18(6.31%)	27(9.47%)	51(17.89%)	0.010
	Non NACT(127)	89	28	2	8	10(7.87%)	
2.	PR						
	NACT(285)	129	121	17	18(6.31%)	35(12.28%)	0.003
	Non NACT(127)	102	21	3	1	4(3.14)	
3.	HER 2 Neu						
	NACT(285)	120	115	21	29(10.17%)	50(17.54%)	0.016
	Non NACT(127)	84	33	2	8	10(7.87%)	

Significance level: p value<0.05. Statistical test: Fisher Exact test for 2x2 contingency table.

Table-5: Hormone receptor and Her/2neu receptor changes detailed after NACT

compared to Non NACT controls. (Inter group p value <0.05).

Control group showed discordance in 10 cases for ER expression. The PR status changes found about 12.28% among the NACT group. 17(5.96%) cases NACT group showed loss of PR immune-expression in residual tumor and 18(6.31%) cases showed PR immune-expression in residual tumor after NACT.

There was no changes of Her-2/neu immune-expression in 235 (82.45%)cases out of total 285 NACT cases and 117(92.15%) cases out of 127 non-NACT cases. Total discordance of 17.54% Her-2 expression was found in

NACT group which was statistically significant(p=0.016) compared to controls. Also in Non NACT group discordance (7.87%) was found in HER-2 expression.

DISCUSSION

Breast cancer is the most common cancer in women in India and accounts for 14% of all cancers in women.^{5,6} According to the Union health ministry, breast cancer ranks as the number one cancer among Indian females with rate as high as 25.8 per 100,000 women and mortality of 12.7 per 100,000 women.⁷ These data clearly point toward a steady rise in the cases. According to estimates, at least 17, 97,900 women in

India may have breast cancer by 2020.

The management of breast cancer patient has evolved from radical mastectomy to breast conserving surgeries after the introduction of NACT. It has long term disease free survival with the institution of timely and appropriate chemotherapy.^{8,9} Overall benefit of adjuvant therapy can be determined by the expression of ER, PR, HER-2/Neu on the breast cancer cells. It is also an important determinant of patient survival and prognosis.¹⁰ Determination of factors that can affect the expression of these receptors may actually have role in patient prognosis hence essential.

This study with a large sample size of 412 has been able to make some important revelations. Determination of alteration in HR and Her-2 Neu receptor status following NACT was done and also compared with patients without NACT. We found more breast cancer patients belonged to peri-menopausal and post-menopausal age group i.e. patients older than 40 years. However these older patients (age >40years) have more favourable hormone receptor status (65.97%) in comparison to younger patients (age ≤40 years: unfavorable hormone receptor status=53.72%). This observation is in concordance with previous other studies by Sinha, Sofi GN and Thangjam S.^{11,12,13}

The most common histological grade of the tumors is grade 2 in both groups(65.61% and 57.40%) in this study. It means necessity for regular self-breast examination by women and urgent consultation with physician before tumor progresses to higher grade.

In our study, the patients who were receiving NACT had more favorable receptors status (60.35%). We found alteration in ER, PR, and Her-2/neu status between trucut biopsy and residual in NACT group. Significant discordance (17.89%) was found in ER status in NACT group. Among these 6.31% patients who were ER positive on trucut biopsy were found to be ER negative upon resection and 9.47 % were ER negative on trucut biopsy were found to be ER positive upon resection. 12.28% patients shows discordances in expression of PR receptor. Discordance of HR in this study could be due to sampling error within the heterogenous tumor or may be due to effect of NACT targeting sensitive tumor cells. Previous similar studies have found significant changes in the HR status after NACT.^{14,15} Ramteke P, Seenu V, Prashad R et al. in their work on Alteration in steroid hormone and Her-2/neu receptor status following neoadjuvant chemotherapy in locally advanced breast cancer have found notable alterations in hormonal and Her-2/neu receptors in patients receiving NACT. Larger studies analyzing the impact of these alterations in patient survival need to be undertaken to know the significance of these alterations in patient management.²

A similar study conducted by Quddus RM on HER-2/neu expression in locally advanced breast carcinomas: Pre- and post-neoadjuvant chemotherapy¹⁴ showed that HER-2/neu IHC scores decreased in 28.5% of patients receiving neoadjuvant chemotherapy compared to 11.7% (of patients in the control (p < 0.013). HER-2/neu IHC status changed from strongly positive to negative (3+ to 0) in 12.5% in the study

group and in 3.3% in control group (p = 0.104).¹⁴

Hans Neubauer et al of University of Tübingen in their work on Changes in Tumour Biological Markers during Primary Systemic Chemotherapy (PST) concluded that Her2/neu status as well as ER and PR status should be reevaluated on post-chemotherapy surgical specimens since changes can be observed.¹⁵

We found alteration of ER, PR, and Her-2/neu status between trucut and RS in Non-NACT group also. Discordance of 7.87% in ER, 3.14% in PR and 7.87% in Her-2 neu were noticed. These discordance in Non NACT group may be due to intratumoral heterogeneity or intra/ inter observer variability.

There are many previous studies addressed the effect of NACT on Her-2/neu status.^{15,16,17} We use Her-2/neu positivity of 2+ as cut off value after NACT. The present study showed 17.54% alteration in Her-2/neu expression. We found 10.17% increase in the expression of of Her-2/neu in patients after NACT. These alteration may be due to anthracycline/taxane based chemotherapy.

In contrast to our study Adams AL¹⁶ in the study on the effect of neoadjuvant chemotherapy on histologic grade, hormone receptor status, and HER2/neu status in breast carcinoma found that there was no significant difference between pre- and post-treatment specimens for hormone receptor status. However, there were more patients with HER2/neu overexpression after receiving neoadjuvant therapy (p = 0.027). Neoadjuvant therapy resulted in a significant decrease in mitotic count and an increase in the proportion of patients with HER2/neu overexpression. No significant changes were noted for the degree of tubule formation, nuclear pleomorphism, overall Bloom-Richardson score, and hormone receptor status Similarly previous studies did not find any significant alteration in Her-2/ neu expression after NACT.^{18,19} Like our study, in several other studies FISH was not performed in equivocal (2+) cases and was considered positive. FISH should be done in every Her-2/neu equivocal case.¹⁹

Guangchao Jin, and Yu Han²¹ in their study on Evaluation of biomarker changes after administration of various neoadjuvant chemotherapies in breast cancer also observed that there were significant differences between the groups regarding ER and Ki-67 status changes, and these changes can be used to inform treatment strategies.

CONCLUSION

This study, done on large samples had been able to focus important interferences. This study showed breast cancer has higher incidence but more favorable hormonal receptor expression in older women of more than 40 years.

A statistically significant alteration of intergroup hormone receptors and Her-2/neu receptors has been found in NACT group in comparison to non-NACT group. Therefore, reevaluation of hormonal and Her-2/neu status in resected specimens should be done even after NACT, though current guidelines did not recommend it. For the management of breast cancer patients, alteration of Hormone Status and

its impact on patients' survival can be analyzed by larger sample studies.

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