



A PROSPECTIVE STUDY ON RELATION OF HISTOLOGICAL GRADE AS WELL AS HORMONE RECEPTOR PROFILE IN LOCALLY ADVANCED BREAST CARCINOMA PATIENTS IN RESPONSE TO NEOADJUVANT CHEMOTHERAPY

General Surgery

Dr. Samir Ranjan

Assistant Professor, Department Of General Surgery, R.g. Kar Medical College & Hospital.

Dr. Arun Gupta*

Department Of General Surgery, R.g. Kar Medical College & Hospital.*Corresponding Author

ABSTRACT

Introduction: Infiltrating ductal carcinoma being the most common type of carcinoma (70%), lobular carcinoma is the 2nd most common followed by smaller groups such as medullary, mucinous, comedo, paget's disease, papillary, tubular and inflammatory carcinoma.

Aims and objectives: To establish incidence and correlation between ER, PR, HER 2 neu receptor status, histology with different patient profiles. Assessment of therapeutic effectivity and its relation with receptor status and histology after neoadjuvant chemotherapy.

Material and methods: This will be an institution-based observational and prospective study. Lab studies- hemoglobin, total count, fasting and postprandial blood sugar, urea, creatinine, albumin and total protein Pretested and predesigned perform

Conclusion: This study shows that age group is an important factor for developing breast carcinoma, which was more prevalent in premenstrual population.

KEYWORDS

neoadjuvant chemotherapy, Tamoxifen and cyclophosphamide.

INTRODUCTION

Infiltrating ductal carcinoma being the most common type of carcinoma (70%), lobular carcinoma is the 2nd most common followed by smaller groups such as medullary, mucinous, comedo, paget's disease, papillary, tubular and inflammatory carcinoma.

A single parameter with strongest prognostic significance is hormone receptor status.

Intracellular steroid hormone receptor protein primarily estrogen receptor(ER) and progesterone receptor (PR) have received intensive study both are indicator of prognosis and as guides to hormone and endocrine therapy. It has been standard practice for 25 years to analyze on invasive breast cancers for estrogen receptor(ER) and progesterone Receptor (PR) contain as a means of estimating prognosis and predicting responsiveness to endocrine treatment and recurrence after initial treatment Endocrine treatment were found to prevent or delay recurrence.

HER2/neu is a trans-membrane glycoprotein with tyrosine kinase activity which belongs to the of epidermal growth factor receptors (EGFR). Determination of tumor HER2/neu expression in all newly diagnosed patients with breast cancer is now recommended.

The estrogen receptor (ER), progesterone receptor (PR), and proto-oncogene HER2/neu (HER2) profile of a female primary breast carcinoma plays a significant role as a predictive marker in patient management. In addition to factors such as age, tumor size, lymph node involvement, histologic type, and tumor grade, the hormone receptor and HER2 status at the time of initial diagnosis has been established as a clinically useful, standard-of-care parameter in determining treatment options and subsequent patient response. Current therapeutic strategies for management of primary breast carcinomas rely on the accurate immune histo-chemical (IHC) determination of hormone receptor status in order to determine the clinical utility of hormone-directed therapies such as selective estrogen receptor modulators (SERMs). For example, multiple major clinical trials conducted by the National Surgical Adjuvant Breast and Bowel Project have shown that addition of Tamoxifen, a SERM, to conventional chemotherapeutic and surgical treatment protocols consistently improves disease free survival in women with hormone receptor-positive tumors¹.

Preoperative (neoadjuvant) chemotherapy with agents such as doxorubicin and cyclophosphamide had historically been offered to patients with locally advanced disease with a goal of reducing tumor size to enable surgical resection. In recent years, neo adjuvant chemotherapy has additionally become an option for patients with operable tumors who desire breast conservation therapy. Several randomized clinical trials have demonstrated no statistically significant difference in disease-free survival or overall survival in the

patients receiving preoperative therapy as opposed to those receiving postoperative chemotherapy².

Deaths from breast cancer have increased approximately 3- fold over the last 30 years in Japan, which historically has been one of the countries with the lowest incidence of breast cancer.³ Increasing numbers of patients are being diagnosed with invasive breast cancer all over the world, and much work is being done to develop medicines and operations tailored to the individual characteristics of patients. However, standardization of the assays relating to Herceptin (Her-2), estrogen receptor (ER), and progesterone receptor (PR) is insufficient.⁴ After NAC, patients with complete remission may still have residual carcinoma cells in the bone marrow.⁵ Either tumor resistance or a negative influence on survival may occur because of NAC.

- 1) To establish incidence and correlation between ER,PR,Her 2 neu receptor status, histology with different patient profiles.
- 2) Assessment of Therapeutic effectivity and its relation with receptor status and histology after neoadjuvant chemotherapy.

MATERIAL AND METHODS

STUDY DESIGN:-

This will be an institution-based observational and prospective study.

STUDY TOOLS:-

- Pretested and predesigned proforma.
- Lab studies- hemoglobin, total count, fasting and postprandial blood sugar, urea, creatinine, albumin and total protein,
- Ultrasound imaging of breasts.
- Mammography of Bilateral breast.
- MRI of Bilateral Breast
- USG Whole Abdomen.
- X-ray of Lumbar Spine.
- Core needle (TruCut) biopsy of breast tissue with ER, PR, HER2neu status.

STUDY TECHNIQUES:-

- Individual interview (one to one history taking based on predesigned and pretested case record proforma.)
- including their age, sex, smoking habits, any precancerous lesions. Previous medical and surgical history, whether elective or emergency operation undertaken, with nutritional status, contraceptive study.
- Lab studies- haemoglobin, total count, fasting and postprandial blood sugar, urea, creatinine, albumin and total protein, Ultrasound imaging of breasts
- Truecut biopsy from mass lesion for ER, PR, HER 2neu status and ki-67.
- Mammography of Bilateral Breasts.
- Mir of Bilateral Breasts with Axilla
- USG Whole Abdomen.

- Digital X-ray of lumbar Spine.

STATISTICAL ANALYSIS:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate. p -value ≤ 0.05 was considered for statistically significant for this study.

RESULT AND ANALYSIS

Our study showed that in our study, 17(34.0%) patients were ≤ 40 years old, 19(38.0%) patients were 41-50 years old and 14(28.0%) patient were 51-60 years old. Minimum age was 36 years and maximum was 60 years. p -value calculated by using single sample t-test was <0.0001 showing statistical significance. In our study, 22(44.0%) patients were of post-menopausal Status and 28(56.0%) patients were of pre-menopausal Status. In our study, 48(96.0%) patients had IDC(NOS), 1(2.0%) patients had Medullary and 1(2.0%) patient had Invasive Lobular Carcinoma. In our study, 12(24.0%) patients had Pre-NACT Histological grade I, 22(44.0%) patients had Pre-NACT Histological grade II and 16(32.0%) patient had Pre-NACT Histological grade III. In our study, 13(26.0%) patients had Negative Pre Neoadjuvant CT-ER and 37(74.0%) patient had Positive Pre Neoadjuvant CT-ER. In our study, 13(26.0%) patients had Negative Post NACT-ER and 37(74.0%) patient had Positive Post NACT-ER. In our study, 3(6.0%) patients had ER change to negative (i.e. deterioration), 2(4.0%) patients had ER change to positive (i.e. improvement) and 45(90.0%) patient had no change in ER Status (i.e. same). In our study, 19(38.0%) patients had Negative Pre-neoadjuvant CT PR and 31(62.0%) patient had Positive Pre-neoadjuvant CT PR. In our study, 21(42.0%) patients had Negative Post -PR and 29(58.0%) patient had Positive Post -PR. In our study, 4(8.00%) patients had PR change from positive to negative (i.e. deterioration), 2(4.0%) patients had PR change from negative to positive (i.e. improvement) and 44(88.0%) patient had no PR change (i.e. same). In our study, 40(80.0%) patients had Negative Pre-neoadjuvant CT Her-2 neu and 10(20.0%) patient had Positive Pre-neoadjuvant CT Her-2 neu.

We found that in our study, 36(72.0%) patients had Negative Post-neoadjuvant CT HER-2/neu and 14(28.0%) patient had Positive Post-neoadjuvant CT HER-2/neu. In our study, 4(8.00%) patients had HER 2/neu status change to positive and 46(92.0%) patients had no change in HER 2/neu status. In our study, 12(24.0%) patients had T2N2M0, 17(34.0%) patients had T3N1M0, 7(14.0%) patients had T3N2M0, 6(12.0%) patients had T4N0M0, 6(12.0%) patients had T4N1M0 and 2(4.0%) patients had T4N2M0 before receiving NACT. In our study, 10(20.0%) patients had T0N0M0, 17(34.0%) patients had T1N0M0, 6(12.0%) patients had T1N1M0, 6(12.0%) patients had T2N0M0, 3(6.0%) patients had T2N1M0, 1(2.0%) patients had T2N2M0, 3(6.0%) patients had T3N0M0, 2(4.0%) patients had T3N1M0, 1(2.0%) patients had T4N1M0 and 1(2.0%) patients had T4N2M0 after receiving NACT. In our study, 2(4.0%) patients had deterioration in TNM Staging, 45(90.0%) patients had improvement (i.e., down staging of tumor) and 3(6.0%) patient had no change in TNM staging after receiving NACT. In our study, 10(20.0%) patients had CR Response, 2(4.0%) patients had PD Response, 32(64.0%) patients had PR Response and 6(12.0%) patient had SD Response. Total number of Pre-neoadjuvant CT ER negative cases were 13 (26%), among which 2 cases (4%) turned positive after NACT. Similarly, total number of Pre-neoadjuvant CT ER positive cases were 37 (74%), among which 35 cases (70%) remained positive after NACT, however 2 cases (4%) turned negative. This was statistically significant in Chi-Square test, the p -value calculated was <0.0001 . Total number of Pre Neoadjuvant CT PR negative cases were 19(38%) among which 2 cases (4%) turned positive after NACT. Similarly, total no. of Pre-Neoadjuvant CT PR positive cases was 31(62%) among which 27 cases (54%) remained positive after NACT, however 4 cases (8%) turned negative. This was Statistically significant in Chi-square test, the p -value calculated was <0.0001 .

It was found that total number of Pre Neoadjuvant CT Her 2 neu negative cases were 40(80%) among which 4 cases (8%) turned positive after NACT. Similarly, total no. of Pre-Neoadjuvant CT Her 2 neu positive cases were 10(20%) which remained positive after NACT. This was Statistically significant in Chi-square test, the p -value calculated was <0.0001 . The pre-NACT TNM stages are depicted along the X-axis and they are namely T2N2M0 (24%),

T3N1M0 (34%), T3N2M0 (14%), T4N0M0 (12%), T4N1M0 (12%) and lastly T4N2M0 (4%). After neoadjuvant chemotherapy the TNM staging was changed to T0N0M0 (20%), T1N1M0 (34%), T1N1M0 (12%), T2N0M0 (12%), T2N1M0 (6%), T2N2M0 (2%), T3N0M0 (6%), T3N1M0 (4%), T4N1M0 (2%), T4N2M0 (2%). After neoadjuvant chemotherapy, the treatment responses were assessed among the 3 age groups, namely ≤ 40 years, 41-50 years and 51-60 years. Partial response (PR) was the most frequently encountered outcome in all the 3 age groups, 22%, 20% and 22% respectively. Next was complete response (CR), which was 6%, 8% and 6% respectively. Stable disease (SD) and Progressive disease (PD) were less frequently seen after NACT. However, this data was not statistically significant in Chi-Square test. After Neoadjuvant Chemotherapy, responses were assessed in relation to two groups namely, Pre-menopausal and Post-menopausal. CR 6%, PR 38%, SD 10%, PD 2% was noted in pre-menopausal group whereas CR 14%, PR 26%, SD 2% and PD 2% was noted in Post-menopausal group. However, this data was not statistically significant in Chi-square test. Response to NACT was noted in each Histopathological subtypes namely IDC(NOS), Invasive Lobular Carcinoma, and medullary type of Infiltrating ductal carcinoma. CR 20%, PR 60%, SD 12%, PD 4% was noted in IDC(NOS). Only 2% cases showed Partial Response in Invasive Lobular Carcinoma and Medullary type of IDC respectively. However, this data was not statistically significant in Chi-Square test. Shows association of Pre-Neoadjuvant CT histological Grading Namely Grade I, Grade II, Grade III with Responses to NACT. CR 12%, PR 8%, SD 4% and no PD was noted in Pre NACT histological grade I. CR 4%, PR 34%, SD 4% and 2% PD was noted in Pre NACT histological grade II. CR 4%, PR 22%, SD 4%, PD 2% was noted in Pre NACT histological grade III. However, this data was not statistically significant in Chi-Square test.

Our study showed that association of Post-Neoadjuvant CT histological Grading Namely Grade I, Grade II, Grade III with Responses to NACT. CR 16%, PR 20%, SD 2% and no PD was noted in Post NACT histological grade I. CR 4%, PR 32%, SD 6% and 2% PD was noted in Post NACT histological grade II. No case showed CR, PR 12%, SD 4% and PD 2% was noted in Post NACT histological grade III. However, this data was not statistically significant in Chi-Square test. Shows comparison between pre and post NACT Histological grade. Majority of cases of Pre-NACT histological Grade I remained same after NACT. Out of 44% cases of Pre-NACT histological grade II, 30% of the cases remained same whereas 14% of the cases downgraded to histological grade II after receiving NACT. Out of 32% cases of Pre-NACT histological grade III, 18% of the cases remained same, however 12% of the cases downgraded to histological grade II and 2% case downgraded to histological grade I. This data was found to be statistically significant in Chi-Square test, with p -value being <0.0001 . The ER status change after NACT was depicted as same, deterioration (det) and improvement (imp). 8 cases (16%) with complete response (CR) had no change in ER status, however 2 cases (4%) showed deterioration in ER status. 30 cases (60%) with partial response (PR) showed no change in ER status i.e. same. This data was found to be statistically significant in Chi-Square test, with p -value of 0.013. The PR status change after NACT was depicted as same, deterioration (det) and improvement (imp). 8 cases (16%) with complete response (CR) and 28 cases (56%) with partial response (PR) showed no change in PR status. These data were not statistically significant in Chi-Square test. The HER 2 neu status change after NACT was depicted as same, deterioration (det) and improvement (imp). No cases showed deterioration in Her 2 neu status post NACT. 60% of the cases with partial response, 18% of the cases with complete response (CR), 10% of the cases with Stable Disease had 4% cases with Progressive Disease showed no change in Her 2 neu status. This data was not statistically significant in Chi-Square test. 32 cases (64%) showed improvement with partial response (PR) and 10 cases (20%) showed complete response after NACT. This data was found to be statistically significant in Chi-Square test with p -value being <0.0001 .

DISCUSSION

Breast cancer is considered as the most common type of female cancer accountable for nearly 23% of all cancers in women globally. As per National Breast Cancer Foundation, currently available treatments for breast cancer are based on a number of factors including size of tumor in relation to size of breast, result of specific pathology tests (hormone receptors, HER2 receptors), menopause, age, general health conditions, family history, or other risk factors. The treatments include surgery, chemotherapy, radiation therapy, hormone therapy, and

targeted therapy along with the involvement of nutrition and physical activity and follow-up care. This study focuses upon these factors and its intertwined relation in the context of LABC among 50 cases between the age of 15-75 years.

Al-Husban N et al⁶(2020) found that the correlation for ER and PR between cytology and histology was 72.3% and 74.5%. The grading along with ER and PR immunostaining of breast carcinoma on smears is advocated because of high concordance between cytology and histology. This allows the patient to be treated with hormonal therapy on the basis of FNAC alone.

Nishimura R et al⁷(2018) found that estrogen receptor, progesterone receptor, and HER2 protein in 3 (5.3%), 13 (22.8%), and 19 (33.3%) cases, respectively. The discrepant results for the HRs were attributed to the presence of a few positive cells or faintly stained cells. The use of categorical scoring into positive and negative is recommended for evaluating the HR expressions. Use of strict criteria for HER2 protein 2+ and 3+ cases is recommended, as HER2-positive cases should not be missed.

Ramteke P et al⁸(2016) found that there was a significant alteration in ER and Her-2/neu status from the core biopsy to the treated respected tumor in the study group. As these changes may impact treatment, HR and Her-2/neu expression reanalysis in final surgical specimens is recommended.

Kanwar A et al⁹(2020) found that the immunohistochemical types ER/PR +ve Her2neu +ve and ER/PR -ve Her2neu -ve are significantly related to grading of tumours. From the results, it was concluded that ER, PR and HER-2 status correlates well with histopathological

grading. These results highlight the fact that molecular subtypes correlate with diagnosis and aid in targeted therapy.

Rao C et al¹⁰(2013) found that Estrogen receptor (ER) and progesterone receptor (PR) positivity decreased with increase in tumor grade. There was significant association between tumor size and ER positivity. Breast carcinoma in their population presents at younger age than Western population.

Singh M et al¹¹(2019) found that there is a positive correlation between histopathological grade and other prognostic factors including immunohistochemical markers. Immunohistochemical markers can be effectively used to predict prognosis and therapeutic management of patients with carcinoma breast.

CONCLUSION

Commonest subtype of LABC was Infiltrating ductal carcinoma-not otherwise specified.

There was little change in hormonal receptor status i.e. ER, PR or HER 2 neu status of cases before and after neoadjuvant chemotherapy, which was statistically significant.

However, the TNM staging of breast cancer was notably affected by NACT as 90% of tumor showed down staging.

Majority of the cases belonged to histological grade II and there was statistically significant down grading in histological grades after receiving NACT.

Table : Association between menstrual status, type, PRE-NACT histological grade, post-NACT histological grade, change in ER status, change in PR status, change in HER-2 NEU status, change in TNM staging vs. response to NACT

		Response					P-Value
		CR	PR	SD	PD	Total	
Menstrual status (n=50)	Pre-menopausal	3(6%)	19(38%)	5(10%)	1(2%)	28(54%)	0.191
	Post-menopausal	7(14%)	13(26%)	1(2%)	1(2%)	22(44%)	
Histopathological Type	IDC(NOS)	10(20%)	30(60%)	6(12%)	2(4%)	48(96%)	0.978
	Invasive Lobular Carcinoma	0	1(2%)	0	0	1(2%)	
	Medullary type	0	1(2%)	0	0	1(2%)	
Pre-NACT Histological Grade	I	6(12%)	4(8%)	2(4%)	0	12(24%)	0.099
	II	2(4%)	17(34%)	2(4%)	1(2%)	22(44%)	
	III	2(4%)	11(22%)	2(4%)	1(2%)	16(32%)	
Post-NACT Histological Grade	I	8(16%)	10(20%)	1(2%)	0	19(38%)	0.064
	II	2(4%)	16(32%)	3(6%)	1(2%)	22(44%)	
	III	0	6(12%)	2(4%)	1(2%)	9(18%)	
Change in ER status	Imp	0	1(2%)	0	1(2%)	2(4%)	0.013
	Det	2(4%)	1(2%)	0	0	3(6%)	
	Same	8(16%)	30(60%)	6(12%)	1(2%)	45(90%)	
Change in PR status	Imp	1(2%)	1(2%)	0	0	2(4%)	0.895
	Det	1(2%)	3(6%)	0	0	4(8%)	
	Same	8(16%)	28(56%)	6(12%)	2(4%)	44(88%)	
Change in HER 2 neu status	Imp	1 (2%)	2 (4%)	1 (2%)	0	4 (8%)	0.808
	Det	0	0	0	0	0	
	Same	9 (18%)	30(60%)	5 (10%)	2 (4%)	46 (92%)	
Change in TNM status	Imp	10 (20%)	32 (64%)	3 (6%)	0	45 (90%)	<0.0001
	Det	0	0	0	2 (4%)	2 (4%)	
	Same	0	0	3 (6%)	0	3 (6%)	

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