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EXPRESSION OF ESTROGEN, PROGESTERONE AND HER2/NEU RECEPTORS IN BREAST CARCINOMA: STUDY IN A TERTIARY CARE HOSPITAL

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ABSTRACT: Breast cancer is the most common cancer among women in the urban Indian population and second only to cervical cancer in the rural population. It has been shown that the presence of hormone receptors in the tumor tissue correlates well with response to hormone therapy and chemotherapy. At present the estrogen receptor status is considered the most powerful predictive marker in breast cancer management. Over-expression of HER2/neu is a poor prognostic factor in patients with early breast cancer. In the present day it is standard practice to look at the hormone receptor status in histologically proven breast malignancies. **AIMS AND OBJECTIVES:** To assess ER PR and HER2/neu status in breast malignancies and to correlate the IHC result with modified Bloom and Richardson grading. **MATERIALS AND METHODS:** This was a retrospective study carried out over a period of four years in the department of pathology at Kamineni Academy of Medical Sciences and Research Centre Hyderabad from September 2010 to August 2014. In this period 413 breast malignancies were reported out of which 391 invasive ductal carcinomas were not otherwise specified. These 391 samples were studied which included trucut biopsies resection specimens and paraffin blocks from referral hospitals. For all the cases routine hematoxylin and eosin staining was done and modified Bloom Richardson grading was applied. The three immunohistochemical markers were studied with monoclonal antibodies on 200 cases and the Allred score was performed. On 191 cases only ER and PR markers were studied. **RESULTS:** For the 391 cases there were 387 female and only 4 male patients. The age of the patients ranged from 20 to 84 years. Out of 413 cases 391 (94.6%) were invasive ductal carcinomas NOS type followed by 7 cases (1.6%) of lobular carcinomas. According to the histologic grading more cases were observed in Grade II category (57.8%) followed by Grade III category (26.3%) and least number were in the Grade I category (15.8%). **CONCLUSIONS:** In breast cancers histologically invasive ductal carcinoma NOS was the most common type encountered. According to grading more number of cases was found in the grade II and III categories. In our study we found an inverse correlation between higher grade lesions and positivity for ER PR and HER2. Triple negativity increased as the grade of the tumor increased. **KEYWORDS:** ER PR HER 2/neu markers carcinoma breast immunohistochemistry.

INTRODUCTION: Breast cancer is the most common cancer among women in the urban Indian population and second only to cervical cancer in the rural population.¹ It has been shown that the presence of hormone receptors in the tumour tissue correlates well with response to hormone therapy and chemotherapy.² At present the estrogen receptor status is considered the most powerful predictive marker in breast cancer management.³ In addition many studies have shown that over-expression of HER2/neu is a poor prognostic factor in patients with early breast cancer.⁴

In the present day it is standard practice to look at the hormone receptor status in histologically proven breast malignancies.

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AIMS AND OBJECTIVES: To assess estrogen receptor progesterone receptor (ER PR) and HER2/neu status in breast malignancies and correlate the immunohistochemistry results with the modified Bloom and Richardson grading system.

MATERIALS AND METHODS: This was a retrospective study carried out over a period of four years in the Department of Pathology at Kamineni Academy of Medical Sciences and Research Centre Hyderabad from September 2010 to August 2014. The study had 391 patients with the age ranging from 20 years to 84 years. (Table 1). Out of 391 patients there were 389 female and only 4 male patients. The four male patients were 45 47 49 and 61 years old.

A total of 413 breast malignancies were reported during this period out of which 391 cases (94.7%) were reported as invasive ductal carcinomas not otherwise specified (IDC NOS). As modified Bloom-Richardson grading is not applied to the other histologic types of carcinoma breast like medullary carcinoma etc. only the IDC NOS samples are considered for present study. Also cases with HER2 having 1+ and 2+ score were excluded. The material included trucut biopsies and modified radical mastectomy specimens from the Department of Oncology and General surgery. In addition as ours is a tertiary care Centre a few formalin fixed paraffin blocks for IHC studies were included from referral cases. (Table 2).

The trucut biopsies were fixed for minimum 8 hours and the resection specimens were sliced and fixed overnight in 10% buffered formalin.

For all the cases routine hematoxylin and eosin staining was done and the histological type of tumor was defined according to the World Health Organisation classification.⁵ (Table 3). (Figure 1) Grading of tumor was done as per modified Bloom Richardson grading system.⁶ Of the 391 cases all three IHC markers were studied in 200 cases and in 191 cases only ER and PR markers were done. (Figure 2).

The three immunohistochemical markers were studied with monoclonal antibodies on a 4 micron tissue section. For ER clone 1D5 for PR clone PR88 and for HER2/neu clone Ep1045y antibodies were used in the immunoperoxidase method. As far as possible blocks for IHC were selected in such a way that they contained normal breast parenchyma in addition to tumor so as to give an in-built positive internal control. The IHC slides were scored according to the Allred⁷ scoring system which considers both proportion and intensity of the stained cells. The proportion score (PS) estimates the proportion of positive tumor cells and ranges from 0 to 5 with 0 being non-reacting 1 for 1% reacting tumor cells 2 for 10% 3 for one-third 4 for two-thirds and 5 if 100% of tumor cells show reactivity. The intensity score (IS) ranges from 0 to 3 with 0 being no staining 1 weak staining 2 intermediate staining and 3 intense staining. The PS and IS are added to obtain a total score (TS) that ranges from 0 to 8. Tumor cells with a total score of 3 to 8 were considered positive whereas those with TS less than 3 were considered negative cases.

Her-2/neu was scored on a 0 to 3 scale.⁷ The staining was scored as negative (0) when no membrane staining was observed or when membranous staining was observed in less than 10% of the tumor cells; weak positive (1+) if weak focal membrane staining was seen in more than 10% of the tumor cells; intermediate (2+) if weak to moderate complete membrane staining was seen in more than 10% of the tumor cells; and strongly positive (3+) if intense membrane staining with weak to moderate cytoplasmic reactivity was seen in more than 10% of the tumor cells.

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RESULTS: The study indicated that invasive ductal carcinoma not otherwise specified (NOS) was the commonest histologic type encountered (94.6%) cases followed by invasive lobular carcinoma (1.6%). (Table 3) According to the histologic grading more cases were observed in Grade II category (57.8%) followed by Grade III category (26.3%) and least number were in the Grade I category (15.8%). (Table 4)

Correlation with IHC for ER only (391 cases) showed that in the higher grade tumors (grade II and III combined) 239 (87.5%) were negative for ER (Table 5) whereas individually grade II and III respectively were 65.1% and 26% negative. In the grade I tumors only 12.4% were negative for ER.

In 3 cases out of 191 (1.5%) an estrogen receptor negative and progesterone receptor positive result was seen. (Table 6) In 200 cases where all three markers were studied triple negative ones were 16.5%. 113 cases (56.5%) were HER2 positive. In higher grades (Grade II and III) triple negative cases were more as compared to grade I tumors. (Table 7).

DISCUSSION: In the present day scenario estrogen receptor (ER) and progesterone receptor (PR) tests are routinely performed in the evaluation of breast cancers. While the clinical utility of ER as a predictive biomarker to identify patients likely to benefit from hormonal therapy is well-established the added value of PR is less well-defined.⁸ Studies have shown that hormone receptor positive tumours are responsive to adjuvant hormonal or chemotherapeutic regimens and give a better survival advantage.⁹

The median age of patients in our study was 41 to 50 years which is approximately a decade younger than the western population.¹⁰

In our study we found 118 cases (30.17%) positive for ER which is similar to the figure reported by Desai et al ¹¹ who found 32.6% cases positive for estrogen receptor in the Indian population whereas Ghosh et al ¹² in their study found ER positivity in 51.2% patients. Correlating the IHC results with the grade of the tumor we found that the higher grade tumours (Grade II and III combined) showed more of ER/PR negative status 87.4% which is similar to the findings of Rotaru et al¹³ who observed an inverse correlation between hormone receptor immunoreactivity and histological grading.

Approximately 25-30% of all breast cancers have been reported to over-express HER2 in the western literature.¹⁴ in our study we found the expression of HER2 in 113 cases (56.5%) which is higher than that reported by Ghosh et al¹² as 16.7%. The triple negative cases comprised 16.5% in our study which is slightly less than as reported in Ghosh et al ¹² study where they found 29.8% of triple negative cases. The triple negative breast cancers are more common in younger age patients whereas in our study more number of patients were above 40 years. The medullary carcinomas which are triple negative were excluded from our IHC study which could have contributed to the overall less number of triple negative cases. We encountered 3 cases (1.5%) which were ER-/PR+.

Earlier studies have suggested that such type of expression can be found in about 10% of all cases but more recent studies suggest that this group probably represents false-negative ER studies and that this should be in fact near zero or zero.¹⁵ There could have been a technical problem for these three cases and probably repeat IHC would have helped. There are several factors that affect the ER and PR status as determined by IHC like tissue fixation time (Minimum recommended fixation time is 6-8 hours for biopsies and 6-18 hours for adequately trimmed blocks of approximately 2.0 mm thickness) prolonged fixation over a few days can lead to reduced sensitivity of staining choice

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of anti-ER or anti-PR antibodies and thresholds for reporting positive results.^{16,17} In referral centres where already fixed paraffin blocks are received there is little control over these pre-analytic factors.

CONCLUSIONS: In breast cancers histologically invasive ductal carcinoma NOS was the most common type encountered. According to grading more number of cases was found in the grade II and III categories. In our study we found an inverse correlation between higher grade lesions and positivity for ER and PR and HER2. Triple negativity increased as the grade of the tumor increased.

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Age (Years)	No. of Cases	Percentage
1-10	-	-
11-20	1	0.25
21-30	16	4.09
31-40	72	18.41
41-50	130	33.24
51-60	99	25.31
61-70	53	13.55
71-80	17	4.34
81-90	3	0.76
Total	391	100

Table 1: Age Distribution of Cases

	No. of Cases	Percentage
Trucut biopsies	77	19.69
Resection specimens	290	74.16
Referral Paraffin blocks	24	6.13
Total	391	100

Table 2: Specimen-wise distribution

Histological type	No. of Cases	Percentage (%)
Invasive ductal carcinoma NOS	391	94.6
Invasive lobular carcinoma	7	1.6
Medullary carcinoma	5	1.2
Mucinous carcinoma	5	1.2
Papillary carcinoma	4	0.9
Metaplastic carcinoma	1	0.2
Total	413	100

Table 3: Histopathology-wise distribution of cases

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Grade of the Tumor	No. of Cases	Percentage (%)
Grade I	62	15.8
Grade II	226	57.8
Grade III	103	26.3
Total	391	100

Table 4: Modified Bloom Richardson Grading of Tumors

	ER + Cases	%	ER - Cases	%
Grade I	28	23.7	34	12.4
Grade II, III	90	76.2	239	87.5
Total (391)	118	100	273	100

Table 5: Only ER status (391 cases)

	No. of Cases	%	ER+/PR+	%	ER-/PR-	%	ER+/PR-	%	ER-/PR+	%
Grade I	32	16.7	10	31.2	18	56.2	4	12.5	-	-
Grade II	116	60.7	18	15.51	86	74.1	10	8.6	2	1.7
Grade III	43	22.51	7	16.27	34	79.0	1	2.3	1	2.3

Table 6: Cases where only ER and PR were done (191 cases)

Histologic Grade	No. of Cases	%	ER/PR+ HER2-	%	ER/PR- HER2+	%	Triple+	%	Triple-	%
Grade I	30	15	10	33.3	14	46.6	4	13.3	2	6.6
Grade II	110	55	22	20	60	54.5	8	7.27	20	18.1
Grade III	60	30	22	36.6	25	41.6	2	3.3	11	18.3

Table 7: Immunohistochemistry Triple Marker Results (200 cases)

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Figure 1a: Invasive ductal carcinoma not otherwise specified. (H&E 100X).

1b: Invasive ductal carcinoma showing glandular and trabecular pattern. (H&E 400X).

1c: Invasive ductal carcinoma showing a typical mitotic figure. (H&E 400X).

Figure 1a, 1b & 1c

Figure 2a: Invasive ductal carcinoma IHC for HER2 strongly positive. (IHC 100X).

2b: Invasive ductal carcinoma IHC for HER2 showing membrane positivity. (IHC 400X).

2c: Invasive ductal carcinoma IHC for ER showing strong positivity. (IHC 100X).

2d: Invasive ductal carcinoma IHC for ER showing strong nuclear positivity. (IHC 400X).

Figure 2a, 2b, 2c & 2d

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