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IMMUNOHISTOCHEMICAL EXPRESSION OF HORMONE RECEPTORS AND HER-2/neu IN CARCINOMA BREAST: A CORRELATION STUDY

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ABSTRACT

Incidence of breast cancer is increasing in developing countries. The chances of cure are related to early diagnosis. In recent years there has been increased use of core needle biopsy for preoperative diagnosis of breast lesions. Seventy one patients, who were diagnosed to have infiltrating ductal carcinoma on fine needle cytology and for whom core needle biopsy was done were taken up for the study. A detailed histopathological examination and immunohistochemical profile using the antibodies against estrogen receptors(ER), progesterone receptors (PR) and HER-2/neu were done. Finally, histological grade, clinical stage and immunohistochemical profile were correlated with each other. Core needle biopsy yielded sufficient material for making reliable preoperative histopathological diagnosis. Of the six parameters studied, histological grade, hormone receptor expression and HER-2/neu overexpression correlated significantly with other parameters. Clinical stage correlated only with histological grade. Menopausal status did not correlate with any parameter.

KEYWORDS: Carcinoma breast, Core needle biopsy, Immunohistochemistry

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INTRODUCTION

Carcinoma breast is the second most common carcinoma in Indian women. In developing countries the incidence is increasing especially in India. It has become the leading factor for premature mortality. Diagnosis and treatment at the early stage of cancer can reduce the morbidity and mortality of carcinoma breast[1]. Core needle biopsy is currently used as the initial reliable and effective procedure for evaluation of palpable and symptomatic lesions of breast. It has largely replaced Fine needle cytology for the evaluation of carcinoma breast[2].

Since breast cancer is a heterogeneous disease, it has prompted many researchers to identify prognostic and predictive factors with therapeutic importance. Many authors have studied one or two IHC markers in isolation without correlating with prognostic markers like clinical stage, histological grade and clinical parameters. Controversy also exists in the correlation of new markers and clinicopathological variables. Hence, a correlation study of multiple markers and parameters in the same study is important to identify prognosis and appropriate therapy for an individual patient.

The present study was designed with this in view. Seventy one patients, who were diagnosed to have infiltrating ductal carcinoma on fine needle cytology and for whom core needle biopsy was done were taken up for the study. A detailed histopathological examination and immunohistochemical profile using the antibodies against estrogen receptors(ER), progesterone receptors (PR), and HER-2/neu were done. Finally, histological grade, clinical stage and immunohistochemical profile were correlated with each other.

MATERIALS AND METHODS

This study on breast carcinoma was carried out in the Department of Pathology in collaboration with the Department of Surgery, JIPMER. Seventy one patients, who were diagnosed to have infiltrating ductal carcinoma on fine needle cytology and for whom core needle biopsy was done were included in the study. The patients who had received any kind of chemotherapy or endocrine therapy, and patients with recurrent breast carcinoma were excluded from the study. Core needle biopsy specimens were studied in detail by histopathological examination. Immunohistochemical stain was done for ER, PR and HER-2/neu.

(i) Clinical stage:

Patients were staged according to TNM staging system determined by clinical examination and radiological examination[3].

(ii) Core needle biopsy:

Core needle biopsies received in our laboratory had 3 to 5 cores for each patient. The size of the cores ranged from 0.3 to 1.5 cm in length.

(iii) Fixation and processing:

The core needle biopsies were fixed in 10% buffered formalin and processed in an automated tissue processor (Histokinette) which had pre-programmed timings for each step. The tissue was then embedded in paraffin blocks. Four-six micron sections were cut by HM 320 micro rotary microtome. The biopsy slides were stained for haematoxylin and eosin (H&E).

(iv) Histological grading:

The H&E sections of core needle biopsies were independently seen by two authors and the findings were compared. There was >90% agreement between these two authors. Nottingham's modification of Bloom Richardson system[4] was used to grade the tumours. Tumours were categorized as grade1, 2 and 3. Only invasive carcinomas, not otherwise specified (NOS) were included in the study, and specific types were excluded.

(v) Immunohistochemistry (IHC):

The monoclonal antibodies used in the present study were ER, PR and HER-2/neu. All the kits were obtained from Novocastra. The technique used was Streptavidin biotin

method using di-amino-benzidine (DAB) as chromogen. Sections were made from paraffin blocks on silane coated slides. As a routine departmental protocol, all core needle biopsies were fixed in 10% formalin. Antigen retrieval was attempted with microwave. 30 minutes heat retrieval time was used for ER and HER-2/neu, 8 minutes was used for PR. Endometrial biopsy served as a positive control for ER and PR, previously diagnosed HER-2/neu 3+ breast carcinoma sections was used as a positive control for HER-2/neu.

(vi) Evaluation of immunoreactive scores for ER, PR [5]:

Only unequivocal nuclear staining was accepted as a positive reaction for ER and PR. The degree of expression of these receptors was expressed semi quantitatively by calculating immunopositive score method. With this method the intensity of the immunohistochemical reaction was recorded as 0 to 3. The proportion of tumour nuclei showing positive staining was also scored as 0 to 4. The score for intensity was multiplied by the score for proportion, giving the final score, with the range of 0 to 12 for each individual tumour.

(vii) Evaluation of immunoreactive score for HER-2/neu expression:

Membrane staining was interpreted as HER-2 protein overexpression according to an established HercepTest scoring system[6] and scored as 0, 1+, 2+ and 3+. Immunostaining was considered positive when more than 10% of all cells had 2+ or 3+ staining intensity.

RESULTS

This study was carried out over a period of two years on a total of 71 patients with cytologically proven carcinoma of the breast. Core needle biopsies done on 65 patients showed infiltrating ductal carcinoma – NOS (not otherwise specified). Six cases were excluded from the study due to inadequate biopsy sample, for another 3 patients the tissue got exhausted while taking additional sections for IHC profile. All patients in the study group were females. The age ranged from 23 to 76 years with a mean age of 48.2. It was seen that the majority of patients (25) were in the age group of 41-50 years. It was observed that 42% of patients were premenopausal and 58% were postmenopausal according to the patient's clinical history.

TNM staging was used to stage the patients. Patients in stage II and III were further subdivided into sub stages. As can be observed, 3(4.8%) cases presented with stage I, 24 (38.7%) cases with stage II and 35 (56.5 %) with stage III. There was no patient with stage IV disease. The maximum number of cases (22) presented with stage IIIB disease. Sixteen (26%) of the core needle biopsies studied were grade 1 tumours, 32(51%) cases were found to be complying with grade 2 and 14 (23%) were grade 3. (Figure 1, 2, 3)

H&E stained Core needle biopsy slides showing different grades of tumor

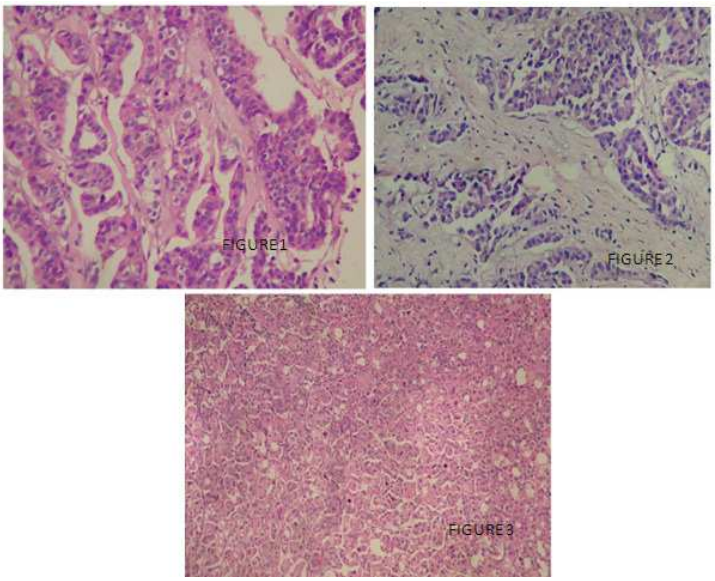


***Figure 1 – monomorphic tumor cells with tubule formation- grade 1 & stage 1, Figure 2 – tumor cells with moderate pleomorphism and tubule formation – grade2 & stage IIIB, Figure 3 – tumor cells with marked* pleomorphism with evidence of tubule formation score 8, grade 3 & stage IIIC (H&E, 400X)**

(i)Estrogen receptor (ER) status:

37 (59.6%) cases showed ER immunopositivity and 25 (40.3%) cases were negative. Eighteen cases (48.6%) out of the 37 ER positive cases showed highest positive score of 12 points (Figure 4, 5, 6). Maximum number of positive cases (27, 43.5%) was seen in the age group of 41 to 60 years, 11 out of 15 (75 %) cases in the age group of 51 to 60 years showed ER positivity.

Immunohistochemistry staining for Estrogen Receptors

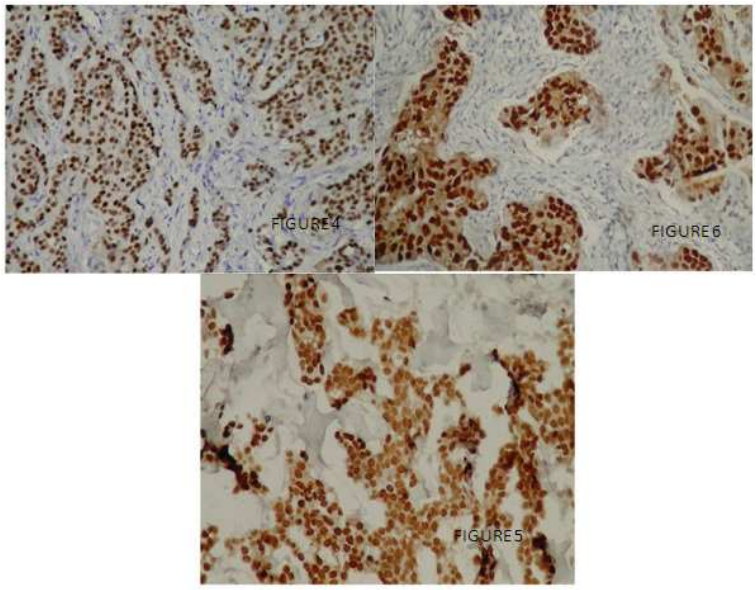


Figure 4 – grade 1, score 12, Figure 5 – grade 2, score 12, Figure 6 – grade 3, score 12. (H&E, 400X)

Correlation of estrogen receptors with clinical stage, grade and IHC markers: (Table 1) Estrogen and progesterone receptors were intimately associated with one another. It was observed that 32 (86.4%) ER positive cases were also positive for PR immunopositivity and 23(92%) ER negative cases also showed PR negativity. ER immunopositive score negatively correlated with the histological grade (p=0.03). A high immunopositive score was found among low grade tumours and absent or low estrogen receptor immunoscore was seen in the high grade tumours. Fifteen (40.5%) of ER positive tumours were found to be grade 1 whereas only 5(13.5%) of the ER positive tumours observed were grade 3. ER immunopositive score negatively correlated with HER-2/neu overexpression (p=0.00). 16(62%) ER negative cases were positive for HER-2/neu overexpression, whereas only 9(36%) ER negative cases showed negative HER-2/neu status. ER negative tumours showed high frequency of HER-2/neu overexpression. The number of cases with ER immunopositive score was seen more in the postmenopausal group when compared with premenopausal group in the present study. No correlation was found between clinical stage of the patients and estrogen receptor status. (p=0.60)

Table 1
Correlation of Estrogen Receptor expression with other parameters

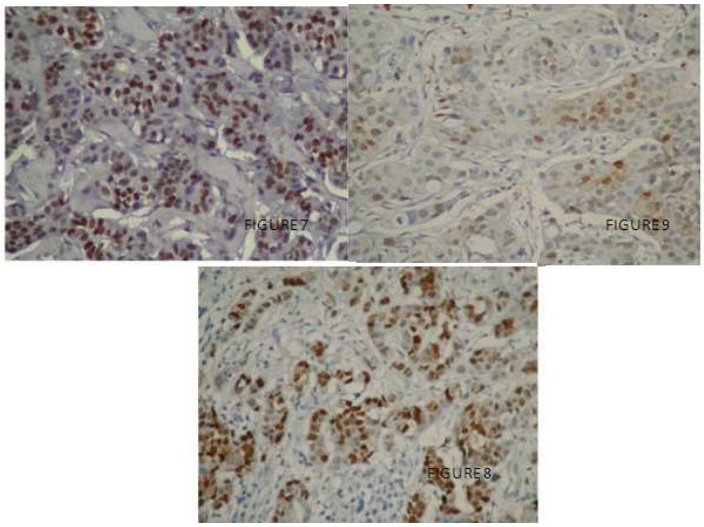
Parameters		ER +	ER –	p value
PR +		32 (86.4)	2 (8.0)	**0.00**
PR -		5 (13.5)	23 (92.0)	
HER-2/neu +		7 (18.9)	16 (64.0)	**0.00**
HER-2 /neu -		30 (81.0)	9 (36.0)	
Menopausal status premenopausal		12 (32.4)	14 (56.0)	0.06
Postmenopausal		25 (67.5)	11 (44.0)	
Grade	**G1**	15 (40.5)	1 (4.0)	**0.03**
	G2	17 (45.9)	15 (60.0)	
	G3	5 (13.5)	4 (36.0)	
Stage	**I**	3 (8.1)	0	0.60
	II A	6 (16.2)	3 (12.0)	
	II B	9 (24.3)	6 (24.0)	
	III A	6 (16.2)	6 (24.0)	
	III B	12 (32.4)	10 (40.0)	
	III C	1 (2.7)	0	
	IV	0	0	

p <0.05 is considered significant, Parenthesis shows percentage

(ii) Progesterone receptor status

35 (56.5%) cases showed PR immunopositivity (Figure 7, 8, 9) and 27 (43.5%) cases were negative for progesterone receptors. Four (11.4%) out of the 35 PR positive cases showed the highest positive score of 12 points. Maximum number of positive 24 (38.7%) cases was seen in the age group of 41 to 60 years, 15(68.8 %) cases in the age group of 51 to 60 years showed PR positivity. Maximum number of negative cases was detected in the age group of 61 to 80 years followed by the age group 21 to 30 years.

Photomicrographs showing Immunohistochemistry of Progesterone Receptors



Immunohistochemistry staining for PR, Figure 7 – grade 1, score 12, Figure 8 – grade 2, score 9, Figure 9 – grade 3, score 6

Correlation of Progesterone receptors to clinical stage, grade and IHC markers: (Table 2) A highly significant positive correlation was found between ER and PR immunoscore (p=0.00). It was observed that 32(86.4%) PR positive cases were also positive for ER immunoscore and 23(67.64%) PR negative cases also showed ER negativity. A highly significant negative correlation of PR expression was seen with HER-2/neu over expression (p=0.00). 29(85.2%) PR positive cases were negative for HER-2/neu status and 18(64.25 %) PR negative cases showed HER-2/neu over expression. PR immunopositive showed negative correlation with grade of the tumours. It was observed that low immunopositive score was seen in the high grade (Grade 3) tumours when compared with low grade (Grade 1) tumours. Progesterone expression did not significantly correlate with clinical stage (p value 0.27). Similarly there was no correlation with menopausal status of the patients. (p=0.5)

Table 2
Correlation of Progesterone Receptor expression with other parameters

Parameters		PR +	PR –	p Value
ER +		32 (86.4)	2 (5.88)	**P = 0.00**
ER -		5 (13.5)	23 (67.64)	
HER-2/neu +		5 (14.7)	18 (64.25)	**P = 0.00**
HER-2/neu -		29 (85.2)	10 (35.71)	
Menopausal status				
	Premenopausal	13 (38.23)	13 (46.42)	P = 0.5
	Postmenopausal	21 (61.76)	15 (53.7)	
Grade	**G1**	14 (41.17)	2 (7.14)	**P = 0.04**
	G2	16 (47.05)	16 (57.14)	

	G3	4 (11.76)	10 (35.75)	
Stage	**I**	3 (8.82)	0	P = 0.27
	II A	5 (14.70)	4 (14.28)	
	II B	10 (29.11)	5 (17.85)	
	III A	7 (20.58)	5 (17.85)	
	III B	9 (26.47)	13 (46.42)	
	III C	0	1 (3.57)	
	IV	0	0	

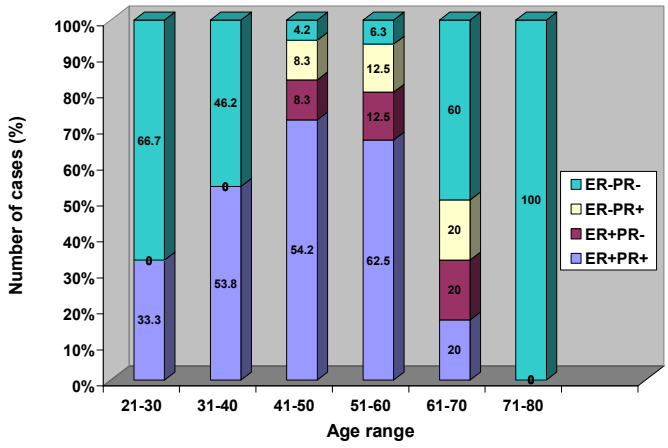
p <0.05 is considered significant, Parenthesis shows percentage

(iii) Combined receptor status:

A combination ER, PR expression revealed four different phenotype groups namely ER+PR+, ER+PR-, ER-PR+ and ER-PR-. It was found that 32(51.61%) tumours showed positivity for both receptors (ER+PR+) and 2(3.22%) tumours were negative (ER-PR-). 23(37.09%) tumours showed only PR positivity (ER-PR+) and 5(8.06%) cases expressed only ER (ER+PR-). Distribution of the combined receptor status based on the age group is shown in Figure 10**.** 9(62.5%) out of 15 cases in the age group 51 to 60 years showed strong positivity.

Figure 10

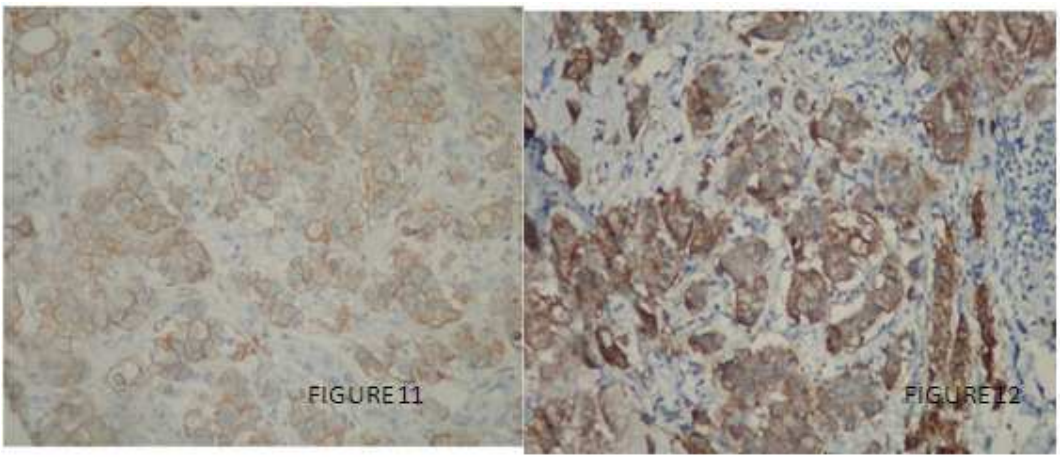
Breakup of the cases (%) based on Combined ER, PR status



(iv) HER-2/neu status:

In this study HER-2/neu protein was found to be over expressed in 23 out of 62 (37.1%) cases. 11(17.7%) cases showed moderate positivity (2+) for HER-2/neu expression (Figure 11) and 12(19.4%) cases showed high (3+) HER-2/neu staining intensity (Figure 12) . There were 11(17.7%) additional cases which showed only focally stained membrane positivity and cytoplasmic staining (1+), and 28(45.2%) cases were negative for HER-2/neu expression.

Photomicrographs showing Immunohistochemistry of HER-2/neu



Immunohistochemistry of HER-2/neu, Figure 11 – grade 2, 2+ expression, Figure 12 – grade 3, 3+ expression

Correlation of HER-2/neu status with other parameters: (Table 3) The frequency and distribution of HER-2/neu expression with ER, PR positivity was studied. 7(30.4%) HER-2/neu positive cases showed ER positivity and 16(69.5%) HER-2/neu positive cases showed ER negativity. Difference between ER+/ HER-2/neu- and ER-/HER-2/neu+ groups showed significant p value (p=0.00). Similarly women with PR+ tumours over expressed HER-2/neu protein in 5(21.7%) cases whereas PR- tumours expressed HER-2/neu positivity in 18(78.2%) cases (p=0.00). Thus a highly significant negative correlation was found with hormone receptors expression. HER-2/neu positive tumours were more often negative for hormone receptors than HER-2/neu non expressing tumours.

Table 3
Correlation of HER-2/neu expression with other parameters

Parameters		HER-2/ neu+	HER-2 /neu-	p value
ER +		7 (30.4)	30 (76.9)	**P = 0.00**
ER -		16 (69.5)	9 (23.0)	
PR +		5 (21.7)	29 (74.3)	**P = 0.00**
PR -		18 (78.2)	10 (25.6)	
Menopausal status				
Premenopausal		10 (43.4)	16 (41.0)	P = 0.8
Postmenopausal		13 (56.5)	23 (58.9)	
Grade	**G1**	1 (4.3)	15 (30.4)	**P = 0.004**
	G2	13 (56.5)	19 (48.7)	
	G3	9 (39.1)	5 (12.8)	
Stage	**I**	0	3 (1.6)	P = 0.17
	II A	1 (4.3)	8 (20.5)	

II B	5 (21.7)	10 (25.6)
III A	6 (26.0)	6 (15.3)
III B	10 (43.4)	12 (30.7)
III C	1 (4.3)	0
IV	0	0

P <0.05 is considered significant,Parenthesis shows percentage

Distribution of HER-2/neu expression among the different ER, PR combination phenotypes showed in Table 4. Differences in HER-2/neu frequency between the combined hormone receptor phenotypes were highly significant (p=0.000). The frequency of HER-2/neu over expression decreased significantly from ER-PR- cases to ER+PR+ cases (60.9% to 13%). Tumour grade also predicted HER-2/neu status. It was observed that only 1(4.3%) case in grade 1 category over expressed HER-2/neu compared with 9(39.1%) cases in grade 3 tumours which over expressed HER-2/neu. There was a significant positive correlation found with the grade of the tumours (p=0.004). Statistically significant correlation was not found with either clinical stage or menstrual status of the patient (p=0.12, p=0.85 respectively).

Table 4
Distribution of combined ER, PR phenotypes based on HER-2/neu positive and negative cases

ER,PR combinations	HER-2/neu+	HER-2/neu-
ER+PR+	3(13%)	29(74.4%)
ER+PR-	4(17.4%)	1(2.6%)
ER-PR+	2(8.3%)	0
ER-PR-	14(60.9%)	9(23.0%)
Total	23	39

(v) Histological grade:

It was observed that 15(93.75%) grade 1 tumours, 17(53.1%) grade 2 tumours but only 5(35.7%) grade 3 tumours were ER positive. The difference in the positivity among different grades was statistically significant (p=0.03). Similarly 14(87.5%) grade 1 tumours and 16(50%) grade 2 tumours were positive for PR whereas only 4(20.5%) grade 3 tumours showed PR positivity (p=0.04). There was a significant negative association found between grade and hormone receptor expression. Positive correlation of grade with HER-2/neu over expression was statistically significant. Histological grade showed a positive correlation with clinical stage (p=0.02). Histological grade was not associated clinically with menopausal status of the patients (p=0.4). There was no significant correlation found when menopausal status was compared with stage, grade and all other immunohistochemical markers. (p>0.05).

(vi) Clinical stage:

Clinical stage showed no correlation with hormone receptors p value was not significant with ER, PR status (p=0.60, p= 0.2 respectively). No correlation was found with HER-2/neu expression (p=0.17). There was a significant positive correlation (p=0.02) found between clinical stage and histological grade. 12(91.6%) of the grade 3 tumours were found to be in stage III, and only 2(12.5%) grade 1 tumours were seen in stage III. No significant association was found with patient's menopausal status. (p=0.9).

DISCUSSIONS

The study of ER, PR status, tumour suppressor genes inactivation, oncogenes over expression in relation to tumor grade and clinical stage is proved to be valuable[7]. In the present study there were 62 female patients with the mean age at the time of diagnosis being 48.2 years which was comparable to another study with a mean age of 49 years[8]. In this study it was observed that 42% patients were premenopausal and 58% were postmenopausal according to patient's clinical history. This data was comparable with the study done by Pertschuk et al[9] who had studied 56% of the postmenopausal women with breast carcinoma.

Parker et al showed that the diagnostic accuracy of core needle biopsy for the detection of breast cancer was higher than the accuracy of FNAB[3]. Only ductal carcinoma NOS (not otherwise specified) were taken for evaluation, as invasive ductal carcinoma NOS tumours are more common and yield a significantly higher number of samples with less than 10% hormone positive cells than the other histological tumour types[10].

Clinical stage was the single consistent prognostic factor that helps in selection of adjuvant therapy[11]. The presentation of number of cases in different stages correlates with study done by Linjawi[8]. It was observed in the present study that only 12.5% in grade 1 tumours were seen in higher stages (II and III). Conversely 79.70% of the grade 3 tumours were seen in patients with higher stages (II and III). These results were comparable to the study done by Gurjeet et al[12]. HJ Huang et al[13] showed positive correlation between clinical stage and HER-2/neu expression. Higher stage (stage III) tumours showed 3+ HER-2/neu expression in their study. In contrast to that study no association was found in the present study between clinical stage and the intensity of HER-2/neu expression.

Michael et al showed a significant negative association between the grade and the hormone receptor expression[14]. A similar result was found in the present study also. It has been shown that higher histological grade was positively associated with 3+ HER-2/neu expression[15]. A similar positive correlation was found in the present study also. Therefore, the histological grading may be considered a substitute for the molecular analysis of many genes.

Many studies indicate that ER is not only predictive marker but also a prognostic index. When PR is accurately calculated, it's an important predictive factor for endocrine therapy[16]. Combined measurement of PR along with ER is more predictive than ER alone. ER, PR immunoscore results were similar to the study done by James et al[17]. ER, PR immunopositive score was higher in the older age group compared to the younger patients. Michael et al[14] showed that ER yielded a significant positive correlation with a bend towards higher positivity for older women, but progesterone receptor distribution revealed no significant association. Thus ER and PR immunopositive score negatively correlated with histological grade and HER-2/neu over expression. Both receptors showed no association with clinical stage. ER and PR were intimately associated with one another. More ER positive tumours were found in postmenopausal age group than premenopausal women; in contrast to this PR immunoscore did not show any association with menopausal status of the study group[18].

An interesting result reported at the consensus development conference was that highly differentiated (grade1) tumours appeared to contain higher levels of hormone receptors than poorly differentiated tumours[19]. Similar negative correlation was observed in the present study also. Our results closely related to study done by Thorpe et al[20.] In the present study showed higher score for ER in postmenopausal patients than premenopausal patients. There was no correlation found between PR and menopausal status of the patients. (p=0.5). Similar results were found with other studies also [21]. The higher frequency of ER/PR receptor positivity in the postmenopausal women can be explained on the basis of low levels of circulatory estrogen

and progesterone occurring to this group. These observations agree with previously published reports[7]. The inverse association between steroid hormone receptors and HER-2/neu has also been described in many studies[22]. This leads to lower or absent hormone receptors in women with HER-2/neu positive breast cancers. This may be one of the reasons for women with HER-2/neu over expression who are resistant to tamoxifen therapy[13].

In the present study 23(37.1%) cases showed HER-2/neu over expression. Wang and associates[23] found that 50% of the cases were positive for HER-2/neu over expression. The varied ranges of HER-2/neu expression in different studies suggest that the specificity of the primary antibody might be the key factor. In the present study menstrual status of the patient did not correlate with all other parameters studied. Josma Isola et al[24] also found that there was no significant association of menopausal status with HER-2/neu expression, histological grade and clinical stage. Reduction in the progesterone down regulation of ER related to unopposed stimulation causes higher level in postmenopausal women. Increased unoccupied cytosolic receptor level is suggested for increased ER levels in the patients[25].

CONCLUSION

Core needle biopsy yielded sufficient material for making reliable preoperative histopathological diagnosis of carcinoma breast. Of the six parameters studied, histological grade, hormone receptor expression and HER-2/neu over expression correlated significantly with other parameters. Clinical stage correlated only with histological grade. Menopausal status did not correlate with any parameter. It may thus be seen that a number of additional parameters are needed in the treatment protocol of carcinoma breast patients.

The study would be complete only with clinical follow up for a period of at least 5 years to know the prognostic significance of these parameters on breast carcinoma patients in our population.

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