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Original Research Paper

Pathology

OVEREXPRESSION of HER2/neu GENE: CORRELATION WITH HISTOLOGICAL GRADING IN BREAST CARCINOMA

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ABSTRACT

Background: Carcinoma of the breast is the most common non- skin malignancy in women. Prognosis and management of breast cancer are influenced by variables such as stage, grade, hormone receptor status of oestrogen(ER), progesterone(PR) and Human epidermal growth factor receptor2 (HER2/neu) overexpression. Aim to correlate grade of tumour with HER2/neu receptor status of breast carcinoma.

Methods: The present study has been conducted in Pathology department, MLB Medical College, Jhansi. The tissue material has been obtained from patients admitted in surgery department for breast conservation surgery or modified radical mastectomy and tissue blocks present in pathology department has been utilized. All samples were subjected for routine histological examination and immunohistochemical analysis.

Results: The patients were >20 years of age; 56% of tumours were right sided; 51% tumours were histopathologically grade 2 and 98% were invasive breast carcinoma. By immunohistochemistry, out of 45 cases, 19 was HER2/neu positive; Out of which the 6 cases (31.57%) with the score of 2+ (grade II), 9 cases (47.37%) with score 3+ (grade III) and 4 cases (21.06%) score of 0/1+ (negative) (grade I).

Conclusions: Assessment of hormone receptors for clinical management of breast cancer patients is strongly advocated to provide prognostic information and best therapeutic options. A significant correlation was observed between HER2/neu immunoreactivity and the histological grade of tumour.

KEYWORDS : Breast cancer, histological grade, HER2/neu overexpression, immunohistochemistry (IHC).

INTRODUCTION

Carcinoma of the breast is the most common non- skin malignancy in women. Breast cancer is more than 100 times more common in women than in men. In our country incidence of breast cancer is second only to that of cancer cervix (Jemal A et al 2003)[1].

Breast cancer is proportionately on the increase in a few metropolitan areas of India. This appear to be related to late marriage, late birth of first child, fewer children, shorter period of breast feeding, which are common among the educated urban women. Common denominator for most of these factors is strong and prolonged estrogen stimulation, operating on a genetically susceptible background. Genetic predisposition with familial breast carcinoma-

- BRCA-1 gene located on chromosome 17q
- BRCA-2 gene located on chromosome 13q

Other risk factors are familial relative like mother and sister, menstrual and reproductive history. Other physical and chemical factors are exogenous estrogens, contraceptive agents and ionizing radiation.

Commonest neoplasm of breast is tumour arising from epithelial component of glandular element of breast. Infiltrative ductal carcinoma being the most common type of carcinoma'(70%), lobular carcinoma is the second most common followed by smaller groups such as medullary, mucinous, comedo carcinoma, paget's disease, papillary, tubular and inflammatory carcinoma (Berg and Hutter 1995[2]). In recent years, interest in prognostic factors has been stimulated by the success of systemic adjuvant therapy for early stage, cancer breast. The important pathological prognostic factors in invasive breast carcinoma include patient's age, tumor size (Chervallier et at 1990[3], Galea et at 1992[4]), lymph node involvement + distant metastasis (Du-Toit et at 1990[5], Pisansky et al 1993[6], Ravdin et at 1994[7]), Beside it nuclear grade, histological grade, histological type, proliferative capacity, microvessel density, hormone receptors status Her2-neu, (Cohen 1978[8]). (Ullrich[9] and Yamamoto, et al[10]).

Cells have receptors on their surface and inside the cytoplasm and nucleus. Chemical messengers such as hormones bind to these receptors, causing changes in the cell. Breast cancer cells may or may not have three important receptors: estrogen receptor (ER), progesterone receptor (PR), and HER2/neu. HER2+ breast cancer had a worse prognosis, but HER2+ cancer cells respond to drugs such as the monoclonal antibody, trastuzumab, (in combination with conventional chemotherapy) and this has enhanced the prognosis significantly. Cells with none of these receptors are called basal-like or triple negative.

AIMS AND OBJECTIVES

Present study has been conducted with following aims and Objective:

1. To find out the incidence of invasive breast carcinoma in bundelkhand region
2. To assess the overexpression of her2neu in breast carcinoma by immunohistochernistry and its correlation with (A) Diagnosis (B) Prognosis (histological grade) in invasive breast carcinoma.

MATERIALS AND METHODS

The present study has been conducted in Pathology department, MLB Medical College, Jhansi. The tissue material has been obtained from patients admitted in surgery department for breast conservation surgery or modified radical mastectomy and tissue blocks present in pathology department has been utilized.

- Biopsies and mastectomy specimens were fixed in 10% formalin.
- Detailed history about age, residence, clinical diagnosis and chief complaints was enquired.
- Gross appearance of mastectomy specimen/biopsy was

noted.
- Paraffin blocks after through tissue processing were prepared.
- Sections were cut 3-4 micron thick and subjected to following:
- Routine haematoxylin and Eosin staining has been done for histomorphological typing according to WHO and grading of all cases.
- Immunohistochemistry has been done using monoclonal antibodies obtained from 'BIOGENEX' company. HER2/neu (clone EP3) immunohistochemistry marker was used.

Histological grading of invasive carcinoma:

Inavasive ductal carcinomas and all other invasive tumors are graded based on an assessment of tubule/gland formations, nuclear pleomorphism and mitotic counts. Assessment of histological grade has become more objective with modifications of the Patley and Scarff method first by Bloom and Richardson (1957) and more recently by Elaston and Ellis (1991.

Feature	Score
Tubule and gland formation	
Majority of tumor (>75%)	1
Moderate degree (10-75%)	2
Little or more (<10%)	3
Nuclear pleomorphism	
Small, regular uniform cell	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic counts per 10 high power fields	
Dependent on microscopic field area	1-3

Examples of assignment of points for mitotic counts for 3 different field areas.

Field diameter (mm)	0.44	0.59	0.63
Field area (mm^2)	0.152	0.274	0.312
Mitotic count			
1 point	0-5	0-9	0-11
2 point	6-10	10-19	12-22
3 point	>11	>20	>23

The three values are added together to produce score of 3 to 9, which the grade in assigned as follows:

Grade I	Well differentiated	3-5 points
Grade II	Moderately differentiated	6-7 points
Grade III	Poorly differentiated	8-9 points

Immunohistochemical detection of HER2/neu immunoreactivity:

Representative sections of tumour were further processed for immunohistochemistry using immunoperoxidase technique. Sections were taken on readymade poly-L Lysine coated slide. Antigen retrieval was done in pressure cooker using citrate buffer solution at pH 6.0-6.2 with washing using PBS (Phosphate buffer solution) at pH=7.2-7.6 and slides were stained with monoclonal antibodies obtained from 'BIOGENEX' company. HER2/neu (clone EP3) immunohistochemistry markers were used. HER2/neu positivity was interpreted and reported using ASCO 2007 guidelines (American Society of Cancer Oncology) which takes into account the cytoplasmic membrane staining and the proportion of immuno-positive tumour cells.

REUSLTS:

Parameters [N=45]	No. of Cases	Percentage
Table 1: Age wise distribution of cases		
21-30	02	4.44%
31-40	03	6.67%
41-50	16	35.55%
51-60	14	31.11%
>60	16	22.22%
Table 2: Clinical presentation wise distribution of cases		
Left breast lump	25	55.56%
Right breast lump	06	13.33%
Pain	07	15.56%
Axillary mass	06	13.13%
Nipple discharge	01	02.22%
Biopsy	05	11.11%
Table 3: Distribution of cases according histological type		
Invasive ductal carcinoma (not otherwise specified)	41	91.12%
Mucinous	02	04.44%
Medullary	01	2.22%
Sarcoma phylloidis	01	2.22%
Table 4: Distribution of invasive ductal carcinoma according to tubular components		
>75% (Score 1)	12	29.27%
10-75% (Score 2)	26	63.41%
<10% (Score 3)	03	7.32%
Table 5: Distribution according to nuclear pleomorphism		
Small, uniform cells (Score 1)	15	36.59%
Moderate variation (Score 2)	16	39.02%
Marked variation (Score 3)	10	24.39%
Table 6: Distribution according to mitotic counts/10HPF		
0-5/10HPF (Score 1)	05	12.20%
6-10/10HPF (Score 2)	26	63.41%
>10/10HPF (Score 3)	10	24.39%
Table 7: Histological grade wise distribution of cases		
Grade 1 (Score 3-5)	8	19.51%
Grade 2 (Score 6-7)	21	51.22%
Grade 3 (Score 8-9)	12	29.27%
Table 8: Correlation of HER2/neu immunoreactivity with histological grade		
Grade 1	04	21.06%
Grade 2	06	31.57%
Grade 3	09	47.37%

DISCUSSION:

Lesions of the breast constitute a very important chunk of the causes of morbidity and mortality in women worldwide. Recently there has been increasing interest in the early detection of the malignant breast lesions as early therapeutic intervention improves survival.

One of the most fundamental aspects of oncologic pathology has been the recognition that the morphologic appearance of tumour can be correlated with their degree of malignancy. HER2 amplified breast cancers have increased sensitivity to certain cytotoxic agents such as doxorubicin, relative resistance to hormonal agents, and propensity to metastasize to the brain and viscera. HER2-amplified tumors have an increased sensitivity to doxorubicin possibly due to co-amplification of the topoisomerase-2 gene which is near the HER2 locus on chromosome 17 and is the target of the drug. Half of HER2-positive breast cancers are ER-positive but they generally have lower ER levels, and many have p53 alterations. These tumors have higher proliferation rates, more aneuploidy, and are associated with poorer patient prognosis. The poor outcome is dramatically improved with appropriate chemotherapy combined with the HER2-targeting drug trastuzumab. (Ross JS 2003[11]; Gabos Z 2006[12]; Villman K 2009[13].

In the present study 45 cases of malignant lesions of the breast were included.

Out of the 45 cases studied, maximum patients were in age group more than 41-50 years (35.55%) followed by 31.11% patients from 51-60 years. 22.22% patients were >60 years and 6.67% patients from 31-40 years of age group (table I), 4.44% cases in age group 21-30.This is in accordance with study of (Hussain et al 1994[14]).

Out of 45 cases maximum 31 cases (68.89%) clinically presented with lump in breast followed by pain in 7 cases (15.56%), 6 cases (13.33%) presented with axillary lump and only 1 case (2.22%) presented with nipple discharge (Table II). These findings are in accordance with the study of (Blarney et al 1998[15]). In our study out of 31 cases presented with breast lump 25/31(55.56%) localized in left side and 6/31 (13.33%) localized in right side (Table II). This was accordance with the study of (Haagensen 1986[16]).

Out of 45 cases these 44 invasive carcinomas (epithelial), 41 (91.12%) were Invasive ductal CA (NOS), 2 case(4.44%) of mucinous carcinoma,) case(2.22%) of medullary carcinoma ,1 case (2.22%) of sarcoma phylloidis (Table III). Similar findings were noted by (Berg and Hutter 1995[2] and Hussain et at 1994[14]).

In this study, all invasive epithelial tumours were graded according to the Modified Bloom Richardson Grading by (Elaston and Ellis et al 1991[17]). Out of the 41 cases of invasive ductal carcinomas, 51.22% were Grade II, 29.27% were Grade III while 19.51% were Grade I (Table VII). Our results closely matched with the study of Doussal et al 1989[18] and Zubair Ahmad et al, 2009[19].

For all carcinomas, her2/neu was also studied using immunoperoxidase method and scoring was done. The immunostaining was read in a semiquantitative manner and graded as follows: 0, 1+, 2+ and 3+. Intensity scores of 0 or 1+ were designated as negative expression and 3+ were designated as positive expression for HER-2/neu. Scores of 2+ were taken as equivocal cases, which were further recommended for FISH analysis. Kalal Iravathy Goud et al 2012[20].

Out of 45 cases her2 positive immunostaining is seen in 19 cases. Out of those 6 cases (31.57%) were seen with the score of 2+ (grade II), 9 cases (47.37%) with score 3+ and 4 cases (21.06%) score of 0/1+ (negative) (Table VIII). Our results closely matched with the study of Kalal Iravathy Goud et al[20]. However the number of cases in present study is small and long follows up is required to reach any definitive conclusion. Ultimate goal of each study is how it can be beneficial to the society. Any research by itself on breast carcinoma is useful because of the sheer numbers of women worldwide who suffer morbidity & mortality due to this disease. Hence there is relevance of search for new prognostic markers which may help in stratifying patients.

CONCLUSIONS

The present study entitled "*Overexpression of HER2/neu gene: correlation with histological grading in breast carcinoma*" shows the conclusion, that HER2/neu immunoreactivity is directly related to histological grade. Staining is more intense in higher grade of malignancies. The number of cases in present study is small to reach any definitive conclusion. However results of present study did show concurrence with those of other researchers.

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