

Original Research Article

Evaluation of expression of Her-2/neu in breast carcinoma - An immunohistochemistry study

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Abstract

Background: Assessment of Her-2/neu is important in targeted therapies for breast cancer notably including Herceptin/Trastuzumab. Thus, the present study was undertaken to find expression of Her-2/neu in breast carcinoma and to correlate expression of this tumor markers with clinical parameters.

Materials and methods: The present retrospective study was carried out on 50 cases of breast cancer received as lumpectomy or mastectomy specimens. All the cases were subjected to immunohistochemistry for Her-2/neu expression. Only membrane staining of tumor cells was considered as positive. For scoring of Her-2/neu only staining intensity was taken into account.

Results: Her-2/neu positivity was seen in 3 cases comprising 6% of the total cases. Percentage of positive cells varies from 30 to 53% with moderate intensity. 66.7% Her-2/neu positive cases was of grade II and (33.3%) was of grade I. All Her-2/neu positive cases showed Lymph node involvement. A definite correlation was observed between Her-2/neu positivity and tumor size. All cases showed size varying from 2 to 5 cm.

Conclusion: The present study concluded that Her-2/neu positivity is associated with aggressive nature of breast carcinoma. Hence, it is of utmost importance to correctly identify the subset of

patients with positive cases so that they can avail benefit from this novel mode of therapy targeted therapy against the Her-2 gene.

Key words

Biomarkers, Prognostic factors, Women, Antigen, Antibody, Breast carcinoma.

Introduction

Over 50,000 women are estimated to die of Breast Carcinoma annually in India. According to the Delhi Cancer Registry data the number of cancer cases in rural areas is 14.5 per 100,000, whereas in urban areas it is 26.9 per 100,000. In India, almost 80% of patients are in advanced stages when they come to hospitals whereas in the West the number is just 20%. According to National Cancer Research Program Projections the breast cancer death in India will climb to 106,124 in 2015 and 123,634 in 2020 [1]. Carcinoma breast is found to be the most common malignancy in women between 45-55 years of age, more common in nulliparous women than multiparous, in women with family history of carcinoma breast and who have their first child after 30 years of age. Late menopause after 50 years of age, early menarche before 13 years of age are also commonly associated factors [2].

Immunohistochemistry can be used to assess which tumors are likely to respond to therapy by detecting the presence or elevated levels of the molecular target [3]. With the newer understanding of the disease pattern, tumor grading, immunohistochemistry and genetic study, various other factors have become more important than size of tumor and clinical staging. These are called prognostic factors. Knowledge of these prognostic factors is very important as they help in deciding the type of surgical requirement and whether adjuvant therapy is required or not and what type of drug can give best results in a particular case. Among these prognostic factors Estrogen receptor (ER), Progesterone receptor (PR), Her-2/neu and BRCA 1 are the most important and studied factors. Hormonal receptor status is used to select adjuvant treatment [4]. Typically Her-2

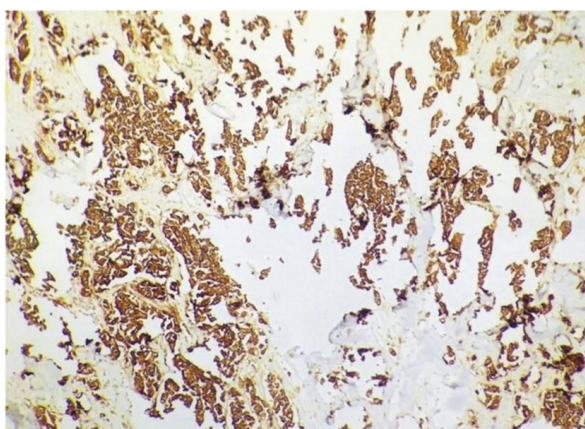
amplified tumors are associated with high grade disease i.e. high grade often extensive ductal carcinoma in situ and grade 2 and 3 infiltrating ductal carcinomas. Assessment of Her-2/neu is important in targeting therapies for breast cancer notably including Herceptin/Trastuzumab [5]. Thus, the present study was undertaken to find expression of Her-2/neu in breast carcinoma and to correlate expression of this tumor markers with clinical parameters.

Materials and methods

The present retrospective study was carried out on 50 cases of breast cancer received as lumpectomy or mastectomy specimens in the Department of Pathology, of our institute. Ethical clearance was obtained for the commencement of the study. History was taken as per the proforma attached. The tissue was formalin fixed and paraffin embedded and was then stained for Haematoxylin and Eosin for histopathological typing and grading. The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system. All the cases were subjected to immunohistochemistry for Her-2/neu expression. The antigen retrieval was done by using pressure cooker method with 10 mmol citrate buffer at pH 6.0. This buffer was used as the wash buffer and Diaminobenzene tetrahydrochloride (DAB) was used as the chromogen. The endogenous activity was blocked by using hydrogen peroxide. After protein blocking, the slides were incubated overnight with the available Her-2/neu primary antibodies and they were conjugated with streptavidin Horse Radish Peroxidase (HRP). Target antigen retrieval was done by heat induced epitope retrieval technique. Antigen retrieval was followed by avidin biotin method of immunostaining. 3-5 μ m sections were cut and

mounted on poly-l-lysine coated slides. Slides were dried overnight at 37°C and dewaxed in xylene and hydrated. The slides were counterstained with hematoxylin and were examined by light microscopy. Positive and negative controls were run with every batch of the IHC. Only membrane staining of tumor cells was considered as positive. For scoring of Her-2/neu only staining intensity was taken into account. Photomicrograph of stained section was as per **Figure - 1**.

Figure - 1: Her-2/neu positivity (100X).



For Her-2/neu receptor, results were interpreted according to following scores.

Score 0: No evident staining or membrane staining in less than 10% tumor cells.

Score 1: Partial staining in more than 10% tumor cells.

Score 2: Complete weak to moderate staining of more than 10% tumor cells.

Score 3: Strong complete staining of more than 10% tumor cells.

Results

The demographic data of the present study revealed that all patients were female with 61% of patients were in the age group of 41-60 years. Of all the 50 cases, it was Infiltrating Duct Carcinoma (NOS) type. Nottingham modification of RBB scoring was done and 62% cases were reported as Grade II and 38% cases as Grade III. There was no case in Grade I. (**Table - 1**).

Table - 1: Grade of tumor.

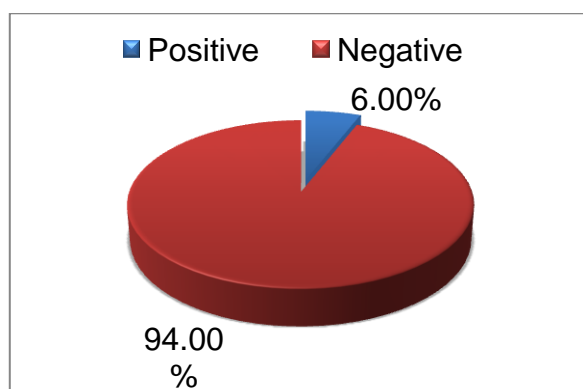
Grade	No. of cases	Percentage
I	0	0%
II	31	62%
III	19	38%
Total	50	100%

Her-2/neu positivity was seen in 3 cases comprising 6% of the total cases. Percentage of positive cells varies from 30 to 53% with moderate intensity (**Table - 2, Graph - 1**).

Table - 2: Her-2/neu positivity.

Her-2/ neu receptor	No. of cases	Percentage
Positive	3	6%
Negative	47	94%
Total	50	100%

Graph - 1: Her-2/neu positivity.



Correlation of Her-2/neu with tumor grade

2 cases (66.7%) were of grade II and 1 (33.3%) was of grade I.

Correlation of Her-2/neu with lymph node

All Her-2/neu positive cases showed Lymph node involvement (**Table - 3**).

Correlation of Her-2/neu with tumor size

In the present study, majority of cases (38) were having size varying from 2 to 5 cm and a definite correlation was observed between Her-2/neu

positivity and tumor size. All cases showed size varying from 2 to 5 cm.

Table - 3: Correlation of Her-2/neu with lymph node.

Lymph nodes	Her-2/neu+
N0	-
N1	2 (66.7%)
N2	-
N3	1 (33.3%)

Discussion

The outcome of breast cancer varies widely and it is widely acknowledged that the progression of breast cancer is partially dependent on interaction between hormones and growth factors with tumor cells. Hence immunohistochemical evaluation of hormone receptors has become a routine investigation to predict response to endocrine therapy. Among the various prognostic and predictive factors of breast cancer, the human epidermal growth factor receptor 2 (Her-2) gene, also referred to as ERBB2 or Her-2/neu, is a gene whose amplification is associated with the rapid progression of the disease, increased metastatic potential, increased resistance to tamoxifen and better response to anthracycline-based chemotherapy [6].

Her-2/neu over expression is associated with poorer survival [7]. Her-2/neu is a protein giving higher aggressiveness in breast cancers. This is a proto-oncogene located at the long arm of human chromosome 17 (17q11.2-q12) and exists on the surface of epithelial cells and functions in the normal cell as a receptor for cellular growth factor. In some cancerous cells this protein loses its usual response to other regulatory proteins and leads to unregulated growth of cells forming a cancer [8]. It is a member of the type 1 tyrosine kinase growth factor receptor growth family which is over expressed in high grade tumors and in the present study Her-2/neu was positive in 6% cases which was less as compared to other studies. Percentage of positive cells varied from 30 to 53% with moderate intensity. Studies by

Huang HJ, et al. [9] and Lal P, et al. [10] showed 17.7% and 26.89% positivity respectively. However in a study conducted by Vaidhyanathan, et al. [11], Her-2/neu positivity was found 43.2% and Munjal, et al. [12] found 40.2% positivity in their study. Ross, et al. [13] in their study reported over- expression of Her-2/neu protein to be in the range of 10 – 34% of breast cancer cases. Similarly, McCann, et al. [14] reported positive membrane staining for Her-2/neu in 17% of cases and also observed that expression of Her-2/neu oncoprotein was associated with poorer grade and shorter disease free survival.

In the present study, Her-2/neu positivity was observed in 66.7% cases of grade II and 33.3% cases of grade III. Bhagat Vasudha, et al. reported Her-2/neu positivity of 28% and 52.9% in grade II and grade III respectively [15]. The variation could be due to more number of cases of Grade II in the present study.

Her-2/neu positivity was seen more in cases with Metastatic lymph nodes suggesting poor prognosis. Similar results were observed by Azizun Nisa, et al. [16] and Bhagat Vasudha, et al. [15].

Tumors with Her-2/neu expression showed more tumor size. These results were supported by studies carried out by Maksimenko J, et al. [17] and Bhagat Vasudha, et al. [15].

Hudelist, et al. [18] found that Her-2/neu potentiated tumor genesis by inducing tumor cell resistance to host defense mechanisms as it induces resistance to tumor necrosis factor (TNF). Her-2-positive breast cancers tend to be more aggressive than other types of breast cancer. They're less likely to be sensitive to hormone therapy, and only treatments that specifically target Her-2 are effective [19].

The discovery of targeted therapy against the Her-2 gene in the form of the humanized anti-Her-2 monoclonal antibody trastuzumab (Herceptin, Genentech, South San Francisco,

CA) and Her-1/Her-2 dual receptor inhibitor, lapatinib, has brought forward an effective treatment modality for patients having the gene amplification. However, the treatment is expensive and carries certain serious adverse effects like cardiotoxicity with trastuzumab. Both drugs have been found to be effective only in tumours showing true gene amplification [6]. The limitation of the present study was the small sample size. Further studies with large sample size are required for the benefit of the mankind.

Conclusion

The present study concluded that Her-2/neu positivity is associated with aggressive nature of breast carcinoma. All cases showed lymph node metastasis and large tumor size. Moreover, Her-2/neu negative cases are less likely to be sensitive to hormone therapy and the treatments that specifically target Her-2 are effective. Hence, it is of utmost importance to correctly identify the subset of patients with positive cases so that they can avail benefit from this novel mode of therapy targeted therapy against the Her-2 gene.

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