The Clinical Pattern of HER - 2/neu Oncogene Overexpressing Breast Cancer in Pakistani Patients at initial presentation: An Analysis of HER - 2/neu Positive versus Negative Disease - A Preliminary Report

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Abstract

Background: HER 2/new oncogene is an important prognostic marker in Breast Cancer and has implications in therapy planning.
Objective: To describe the clinical features of HER 2/new positive and negative Breast Cancer in the Pakistani patient population and note clinical differences between the two groups if any.
Design: A retrospective analysis of Breast Cancer cases at the Aga Khan University, Hospital. Method: Immunochemical stainning on formation fixed paraffin embedded tissue using oxidase antiperoxidase method. A total of 152 Breast cancer tissue samples were tested for HER-2/neu gene presence. Of these 43 (39%) samples tested positive and 109 (61%) tested negative. A comparison of the two groups revealed that only a few factors tested for either significance or borderline statistical significance between the two groups. These factors included the estrogen receptor status and the number of lymph nodes involved in the axilla. The progesterone receptor status was of borderline significance.
Conclusion: Given the large number of factors tested it appears that there is no consistent defining feature which helps to separate HER-2/neu positive versus HER-2/neu negative cases with Breast Cancer (JPMA 49:294,1999).

Introduction

The HER-2/neu (C- erb B2) Protooncogene belongs to the family of human Epidermal growth factor receptor genes[1]. There are four such known genes at this time. The HER-2/neu oncogene is localized to chromosome 17q and encodes a transmembrane tyrosine kinase receptor called p 185 HER-2/neu[2,3]. Breast cancer may be associated with an over amplification of this gene in 25 - 30% in western population, who have been reported so far[3,4]. HER-2/neu over amplification results in increased messenger RNA production (m RNA) with increased protein expression on the cell surface and a more aggressive biological behavior[5].
An important reason to know the HER-2/neu status in Breast cancer are the evolving therapeutic ramifications of HER-2/neu positivity. Some of these ramifications are based on information which suggests that chemotherapy with[6,7] cyclophosphamide, methotrexate, flouracil (CMF)[6,7] and the antiestrogen tamoxifen[2,8] are not very effective as therapeutic agents when Breast cancer cells express the
HER-2/neu gene. More recently a monoclonal anti-body (Herceptin) has been released for therapeutic use in the west. In order to use this anti-body it is important that the HER-2/neu status of the tumor should be documented. Other wise treatment with the antibody would be ineffective[9].
We would like to present data on the clinical and pathological features of patients whose breast cancer

was tested for the HER-2/neu gene product. This we feel is an addition to the knowledge base of Breast cancer in our patient population and will help in guiding treatment issues in the future.

Materials and Methods

As a part of our ongoing database in Breast cancer ad the inpatient records of patients with breast cancer who had their tumor tested for the HER-2/neu gene product and additional molecular markers were the subject of this analysis. Outpatients cannot be captured in our data base system at this time. Nevertheless these numbers include all patients who are admitted to our day care unit for chemotherapy and therefore represents most of the patients seen with Breast cancer at the Aga Khan Hospital in Karachi, Pakistan. In our study not all the clinical and pathological features were present in each and every case.

Immunohistochemical staining was performed on formalin fixed paraffin embedded tissue using oxidase, antiperoxidase method. Microwave oven pretreatment was performed for HER-2/neu protein product for immuno staining. Rabbit anti-human HER-2/neu (Dako, Denmark) oncoprotein was used. Paraffin sections were cut from tissue blocks. The sections were incubated at room temperature (22-25°C) for 10 minutes with 1:25 ul of primary antibody10. After extensive washing slides were incubated for 45 minutes with 1:100 ul of secondary antibody. After second washing the slides were incubated with PAP complex. 3,3 diaminobenzadine tetrachioride was used as the final chromogen. Nuclei were stained with hematoxylin. Positivity for HER-2/neu overexpression was documented if the membrane was stained and it was measured as a product of percent of positive cells and intensity of positivity. This was expressed semiquantitatively on a scale of 1+ to 4+. Both negative and positive controls were run with each batch concurrently.

Results were expressed as means, standard deviation, odds ratio and 95% confidence intervals. Univariate analysis was performed by using the two independent sample t-test, chi-square and Fisher's exact two tailed test whenever appropriate. Analysis were carried out using the statistical software SPSS 8 (standard version software). A p-value of <0.05 was taken as statistically significant and less then 0.1 was taken as a borderline significant. Analysis was carried out by a qualified statistician (AS).

Results

There were total of 152 breast cancer patients in out data bank whose tumors were analysed for HER-2/neu oncogene. HER-2/neu positivity was documented in 43 cases whereas 109 were negative.

Table 1. Univariate Analysis of different factors in Breast Cancer.

Factor studied (Numbers)	Oncogene		P-value
	Positive	Negative	
Number of patients (152)	43 (39%)	109 (61%)	
Age of Patients - years (151)	48.05 ± 10.92	49.45 ± 13.37	0.546
Height of Patients - cms (131)	156.01 ± 8.23	153.43 ± 6.58	0.69
Wt. Of Patients - Kg (140)	64.81 ± 13.34	61.17 ± 12.18	0.131
Menarche - years (111)	14.03 ± 1.07	13.60 ± 1.49	0.144
Age of First Pregnancy -years (91)	21.04 ± 3.58	22.03 ± 4.59	0.311
Parity (125)	3.82 ± 1.86	4.45 ± 3.00	0.167
Size of tumor - cms (136)	5.04 ± 3.35	4.18 ± 3.09	0.157
Intraductal Component - percentage (61)	14.54 ± 25.53	18.48 ± 28.11	0.589
Length of Breast feeding - month (38)	12.12 ± 8.75	10.56 ± 8.78	0.597
No. of Lymphnodes Resected (139)	14.84 ± 6.11	14.24 ± 6.72	0.629
No. of Lymphnodes Involved (139)**	3.84 ± 4.30	2.37 ± 3.85	0.001
Estrogen Receptors by Immunohistochemistry (141)**	50.73 ± 53.35	84.19 ± 53.17	0.001
Progesterone Receptor by Immunohistochemistry (141)*	25.48 ± 39.87	38.83 ± 50.87	0.098

Results are expressed as Mean ± St. Deviation.
* = Significant (p-value < 0.10)
** = Significant (p-value < 0.05)

Table 1 compares the factors which were measurable quantitatively both in those who were positive for HER-2/neu oncogene and those cases which were not. The only two factors which achieved statistical significance between the two groups were the number of lymph nodes involved in the axilla and the estrogen receptor status. The progesterone receptor status achieved only borderline significance (P0.098). All other factors such as the age, height, weight, age of menarche, age of first pregnancy, parity, size of the tumor, numbers of lymph nodes resected, the intraductal component of the tumor, the length of time of breast feeding were all of no statistical significance between the HER-2/neu positive and the HER-2/neu negative Breast cancer in this population.

There were other factors evaluated but none of these achieved significance in univariate analysis. These were the presence or absence of menopause, family history of breast cancer, palpable nodes in the axilla, skin or nipple involvement, martial status, pan chewing habits, prior cancer, ovarian cancer, supraclavicular lymph node involvement and inflammatory breast cancer. Likewise right versus left sided breast cancer, inner versus outer quadrant sites were of no significance. Tumor size and stage did not correlate with the presence or absence of oncogene positive versus negative disease.

Table 2. Univariate Analysis of different pathological factors in Breast Cancer.

Factor studied	Oncogene		Odds Ratio	95% CI for Odds Ratio
Numbers	Positive	Negative		
Histological Grade (125)				
· Well	4	9	1	1
· Intermediate	25	59	0.95	(0.24-4.64)
· Poor	8	20	0.90	(0.18-5.20)
Vessel Invasion (100)				
· No	19	48	1	1
· Yes	12	21	1.44	(0.54-3.8)
Lymphatic Invasion (105)				
· No	19	47	1	1
· Yes	13	26	1.24	(0.48-3.20)
Estrogen Receptor Status (150)**				
· Positive	20	78	1	1
· Negative	23	29	3.09	(1.39-6.9)
Progesterone Receptor Status (149)				
· Positive	9	36	1	1
· Negative	33	71	1.86	(0.75-4.70)
Ploidy (92)				
· Diploid	19	37	1	1
· Aneuploid	9	27	0.65	(0.23-1.80)
P53 (150)				
· Negative	14	45	1	1
· Positive	28	63	1.43	(0.64-3.23)
Cathepsin D (149)				
· Negative	15	54	1	1
· Positive	27	56	1.64	(0.74-3.7)
PCNA (141)				
· Low	7	20	1	1
· High	32	82	1.11	(0.40-3.23)
S Phase (57)				
· Low	8	18	1	1
· High	13	18	1.63	(0.48-5.63)

Results are expressed as numbers, Odds ratio and 95% confidence interval (CI) for Odds ratio.

* = Significant (p-value <0.10)

** = Significant (p-value <0.05)

Table 2 compares the various histopathological features and the molecular markers which were tested. As noted earlier the presence of Estrogen receptor positivity was the only factor significantly associated with HER-2/neu negative disease.

Discussion

Over expression of the HER-2/neu oncogene is reported in 25 -30% of all cases of Breast cancer in the western population[3,4]. In this report we have noted HER2/neu positivity of 39%. With the small numbers involved in this study are own figures of 39% are not far removed from the published western figures. Clearly as the number of cases continues to increase these figures of positivity will need to be adjusted.

What may all of this mean in our poor south Asian setting, is a question that can only be answered as more information becomes available here and from the west. In our setting it would be extremely useful, to have some clinical information and guidelines which helps predict whether a tumor is positive for the HER-2/neu oncogene or not. Unfortunately there is no way to assess over expression of HER-2/neu oncogene at this time except by actually testing for the gene or its products as in our cases. In our cases more axillary lymph nodes were liable to be involved with tumor when its expressed the HER-2/neu oncogene. In addition the absence of estrogen receptor positivity was more likely to be associated with HER-2/neu positive tumor. However these findings are preliminary and probably should not be used to make any predictive clinical comment. Nevertheless the number of cases in this study are still increasing and these observation should still be considered preliminary.

When one looks at the overall clinico pathological pattern of breast cancer in Pakistan a discouraging picture emerges. In this study 39% of the patients were HER-2/neu positive, the mean size of the tumor were large, axillary lymph nodes were often involved. This coupled with the fact that Breast cancers which are HER-2/neu positive do not respond well to chemotherapy with CMF and tamoxifen[6,7,11] means that more aggressive treatment program may have to be undertaken in the adjunctive setting of Breast cancer in Pakistan. Therefore it would be expected that anthracycline based or more intense therapies may have to be applied. In our socio economic setting this means the acceptance of more cost, clinical toxicity and social problems which arise thereof.

This paper hopefully adds some knowledge to the descriptive elements of Breast cancer in Pakistan and our growing belief that not only must we treat Breast cancer when it is detected but also try and find it early by teaching Breast self examinations, mammography and finally by education and public awareness.

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