ORIGINAL ARTICLE

Her-2/ c-erb B 2 receptors Expression in Benign Fibroepithelial Lesions – Fibroadenomas; with Realtion to age of patients

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ABSTRACT

Background: Fibroadenomas are benign breast tumours that are commonly diagnosed in young woman and are associated with slight increase in the risk of breast cancer. The lesions vary considerably in their histologic characteristics. Her-2/neu (c-erb-B2) oncogene is a member of epidermal growth factor receptor family and its amplification is one of the most common genetic alterations associated with human breast tumours.
Aim: To evaluate any correlation between HER-2 /neu/c-erb B2 immunostaining expression in benign fibroepithelial lesions - fibroadenoma and its correlation with specific age group.
Methodology: This study was based on the principle that Her-2 / neu antigen is demonstrated using Avidin –Biotin peroxidase complex method of immunostaining in 25 cases of fibroadenoma.
Results: The results showed that 56% cases took no immunostaining, 28% cases showed faint (+) level of immunostaining while 16% cases exhibited moderate (++) level of expression. No benign fibroepithelial lesion manifested intense (+++) level of staining.
Conclusion: The age range in 25 patients of fibroadenoma was 15-38 years with mean age 24.4 +/- 6.59 years. In fibroadenoma group, out of 25 cases, 4 cases (16%) showed a moderate (2+) level of Her-2 over-expression.
Keywords: Fibroadenoma; Her-2 (Herceptin); Immunostaining

INTRODUCTION

Fibroadenoma is the most common breast tumor in adolescent and young women. About one third of women are under 20 years and two thirds under 25 *(OA Egwuonwu et al 2017)*. Though fibroadenomas are benign breast tumours; however more aggressive lesions may mimic or arise within fibroadenomas *(Hubbard JL et al 2015).* The role of tumour markers in the clinical management of cancer is undergoing continuous reassessment. A large number of tumour markers have been described, a few became part of routine investigation. The ability for individual marker to provide a guide to diagnosis, to assess an extent of disease and monitor response to treatment became established after several years of clinical trials. Her-2/neu gene amplification and / or over expression in benign breast disease was associated with an increased risk of subsequent breast cancer *(Azadeh et al 2000)*. Major efforts in breast cancer research are directed at evaluating the correlation between gene alteration and clinical behavior of cancers. In particular, alterations of proto oncogenes in cancer care are of major interest *(Chen et al 2015).* Her-2 / neu amplification has greater prognostic value than most currently used prognostic factors, including hormonal receptor status. This gene may play a role in the biological behavior and / or pathogenesis of human breast cancer *(El Hadi et al 2017).* HER-2 over expression might also play some role in the etiology of breast fibroadenoma formation *(Zubor et al 2008).* Over expression and amplification of Her-2/neu oncogene in patients with breast cancer has correlated with early onset of metastasis, resistance to hormonal therapy, some forms of chemotherapy and shortened survival. Therefore evaluation of this putative prognostic or predictive factor seems critical *(Ding et al 2017).* The present study has been planned to determine cell membrane Herceptin receptors (Her-2/neu) over expression in 25 cases of fibroadenoma by immunohistochemistry. The purpose of this study is to examine the Her-2 /Neu over expression and finding any association with age of patients

MATERIALS & METHODS

The present study was based on immunohisto-chemical staining for Herceptin receptors (Her-2/neu, c erb B-2) in 25 patients of fibroadenoma. The tissue specimens were collected from Hijaz Hospital, Lahore during the period between November 2014 till December 2015. Clinicopathological data including case number, age, biopsy type were verified from operation notes and patients registration form. The

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age was double confirmed by ID card. The diagnosed cases of fibroadenoma were selected irrespective of specific age. Following criteria was fulfilled to include the cases in research study.

- Confirmed tissue blocks of fibroadenoma were included in the study.
- Mastectomy specimens and biopsy obtained by incisional or excisional means were included in the study.

Ductal carcinomas in situ (DCIS), paget's disease of the breast, borderline lesions, tissue sections showing extensive cellular distortion and cases in which diagnosis was based upon fine needle aspiration cytology (FNAC) were excluded from study. Selected portions of tissue specimens were processed in tissue processor. Paraffin embedded blocks were made and after trimming of tissue blocks, sections were cut and staining done. The following stains were performed:

1: Haematoxylin and Eosin

2: Immunohistochemical for Her-2/c-erb B-2

Immunohistochemical Protocol (*Bancroft and Gamble 2008) Beraki (2012) and Hsu et al (1981)* was based on the principal that)a certain antigen is demonstrated using the avidin-biotin-peroxidase complex (ABC) method of immunohistochemistry. Labelled avidin-biotin reagents were used with primary antibody from mouse. Bound primary antibody is visualized after incubation with a biotinylated secondary antibody followed by a preformed avidin-biotinylated horseradish peroxidase macromolecular complex and substrate. As it is well known fact that in paraffin embedded tissue, cross-linking bonds of the fixative with the protein are formed which masks the antigenic sites. Microwave heating is helpful for receptor antigenic sites *(pervaiz et al 1994).* Standard microwave protocol laid down by Biogenix was followed. Meyer's haematoxylin is used as a counter stain. The aqueous mounting medium was used for mounting immunohisto-chemically stained slides and non-alcoholic stain was used for counterstaining the immunohistochemically stained slides. The positive control tissue slide was obtained from Biogenex and prepared in the same manner as the specimens.

Assessment of Receptor Status: The slides were then examined under low and high power lens of microscope. The cases were considered positive as the positive control slides showed positive staining results and negative control showed no such staining *(Penault et al 1994).*

In the present study immunohistochemically stained slides were scored according to the criteria practiced in *North Shore Medical Centre, MA, USA (2015).* The results were evaluated qualitatively and divided into four groups.

- "0" or no staining=negative,
- "1+" or weak staining= faint partial staining of the membrane,
- "2+" or moderate staining= weak complete staining of the membrane, > 10% of cancer cells and
- "3+" or strong= intense complete staining of the membrane, > 10% of cancer cells.

Percentage cells positive were assessed in a semi quantitative fashion. Only membranous staining was considered as positive reactivity using the CB11 monoclonal antimouse antibody *(Lundy et al 1991).* The observations were tabulated. To test the significance of difference between two variables, we compared the two totals using cross-tabulations one dimensionally, i.e., columns. All the hypotheses were tested at 0.05 level of significance

RESULTS

25 cases of fibroadenoma were placed in two age groups. There were 6 (24%) cases in age group A (0-19 years) and 19 (76%) cases in age group B (20-39 years). The youngest patient whose specimen was taken in this case was 15 years old and the eldest patient was 38 years old. The mean age in this group was 24.40+ 6.59 years (Table 1, Fig. 1) Distribution of various Her-2 staining levels within fibroadenoma cases with reference to different age groups showed that 14 (56%) cases took no immunostaining, 7(28%) cases showed faint level of immunostaining while 4(16%) cases exhibited moderate level of staining. Statistically, for Her-2 at negative level ("0") the distribution of patients in age group A is significantly lower ($p<0.05$) from that of in age group B whereas at other levels of Her-2 (1+ and 2+) the distribution of patients in age group A and B are non significant (Table 1, Fig. 1)

Table 1: Distribution of cases in different age groups of fibroadenoma (n=25)

Age group (in years)	n	%age
10-19	6	24
20-39	19	76
Total	25	100

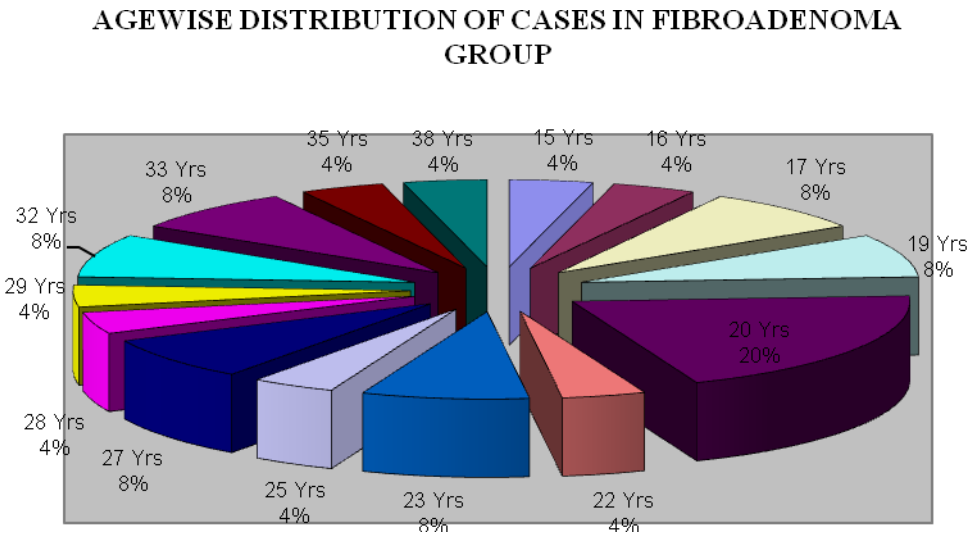
A vs B…….. S S= Significant ($p<0.05$)

Table 2: Distribution of different patterns of HER-2 staining in different age groups of firboadenoma (n=25)

Age group(Yrs)	HER-2			Total
	0	1^+	2^+	
0-19	3(12%)	3(12%)	-	6(24%)
20-39	11(44%)	4(16%)	4(16%)	19(76%)
Total	14(56%)	7(28%)	4(16%)	25(100%)
AvsB	S	NS	NS	

HER-1=Herceptin receptors
0=No membrane staiing observed
1+=Faint staining of the membrane
2+=Moderate, weak complete membrane staining
S=Significant ($p<0.05$) NS=Non significant

Fig. 1:



DISCUSSION

Benign Breast Diseases (BBDs) is a group of breast diseases which is not cancer. It is the most common cause of breast problems in females and it is more frequent than the malignant ones. In our study the age range was 15-38 years with mean age of 24.4+ 6.59 years. Our findings are consistent with the findings of *Goldenberg et al (1968), Haagensen (1971), Matar et al (1998)* and *Mima et al (2013)* who also noted that it is a most common cause of palpable breast mass in women younger than 30 years of age. The peak incidence of fibroadenoma ranged from the 2nd to the 3rd decade of life, which was consistent with the findings of other studies. However, *Levi et al (1994) and Iua et al (1998),* in their studies observed that the median age was 37 years. This discrepancy in the median age between different studies might have risen because of geographical variations, hormonal factors, variable reproductive rates and socio-economic reasons.

In this current study, testing for over-expression of Her-2 receptors on 25 cases of fibroadenoma exhibited 4 (16%) moderately stained cases in age group B (20-39 years). Kalogeraki et al observed that 6 (24%) out of 25 fibroadenomas cases were found to display moderate Her-2 / c-erb B2 over expression in the similar age range. However *Zubor et al (2008)* are of the view that their results indicate that HER-2 over expression in their study might play some role in the etiology of breast fibroadenoma formation. Little discrepancies in the results of these studies could stem from the sources of tissue fixation, antigen retrieval and variable antigenicity of oncoprotein receptors in breast tissue employing the present immunohistochemistry methods are quite sensitive and detect very low levels of protein expression. The significance of this susceptibility, however, will have to be verified by larger studies. *Gown and Yaziji (2000)*, are of the similar view and stated that Her-2 expression on benign breast epithelium never implies true over-expression of the protein but as existing immunohistochemistry methods are sensitive enough that they detect very low levels of protein expression.

CONCLUSIONS

This study was conducted to determine the cell membrane Herceptin receptors (Her-2/neu/c-erb B2) expression in patients of fibroadenoma to find out any association in different age groups and cell membrane Herceptin receptors in above noted patients. Following conclusions were drawn as a result of the above study.

- The age range in 25 patients of fibroadenoma was 15-38 years with mean age 24.4 +/- 6.59 years.
- In fibroadenoma group, out of 25 cases, 4 cases (16%) showed a moderate (2+) level of Her-2 over-expression.
- The clinical significance of Her-2/neu expression in benign breast lesion remains unclear.

SUGGESTIONS

- Her-2 has a promise as a vaccine for active specific immunotherapy. So progress on these modalities of therapy may result in lesser emergence of fibroadenomas. Therefore further research is needed to put this fascinating protein in therapeutic use.
- Further research is also needed in Pakistan to determine the prognostic and predictive roles of

various associations / correlations between HER receptors, their ligands and signal transduction molecules in patients with breast tumours.

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