Immunohistochemical Correlation of Her2/Neu Over- expression with Differentiation Grades of Invasive Ductal Carcinoma of Breast in Sudanese Females

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ABSTRACT

Background: HER2 is an intracellular membrane protein detected in the cellular membrane, it is closely related to EGFR and, like EGFR, has tyrosine kinase activity, gene amplification and the corresponding overexpression of c-erbB-2 has been found in a variety of tumors, including breast carcinomas , Amplification or overexpression of this oncogene has been shown to play an important role in the development and progression of certain aggressive types of breast cancer.

Objectives: the objective of this study was to determine the Correlation between degree of differentiation grade of invasive ductal carcinoma and over expression of human epidermal growth factor 2(Her-2/neu) in Sudanese breast cancer patients.

 Materials and methods: Formalin fixed paraffin embedded (F F P E) blocks from 30 Sudanese female with invasive ductal carcinoma were collected from TOTAL LABCare group and different hospitals in Khartoum state and all samples were verified by consultant Histopathologist for hematoxylin and Eosin staining followed by   immunohistochemical studies and processed as per standard guide lines, all data and detailed history and clinical examination of investigation were record.

Results: Among the 30 case of patient with invasive ductal carcinoma, Over expression of Her-2/neu was seen in 4(13.3%) of cases. Overexpression was seen in 2 out of17 grade III tumors (6.7%) and 2out of 10 grade II tumors (6.7%).

 Overexpression was not detected in grade I tumors, and there was no statistically significant correlation between Her-2/ neu over expression and degree of differentiation grade of invasive ductal carcinoma with P value(0.643).

Conclusion: We conclude that there is no statistically significant correlation between Her-2/ neu over expression and degree of differentiation grade of invasive ductal carcinoma in Sudanese patients.

Key words: Breast Cancer, her2/neu, Sudan

INTRODUCTION

Breast cancer continues to be one of the leading causes of cancer death among women (1) Among female one in nine women develop breast cancer at some stage in their life, Mostly in women over the age of 40- 50 years (2) but younger women are sometimes affected. Invasive ductal carcinoma is abnormal cancer cells that began forming in the milk ducts. have spread beyond the ducts into other parts of the breast tissue can also spread to other parts of the body through bloodstream or lymphatic system, it's also called Carcinoma of no special type or infiltrating ductal carcinoma (3). HER2 is an intracellular membrane protein detected in the cellular membrane (4). HER-2 gene encodes a 185 kD Trans membrane phosphoglycoprotein with tyrosine kinase activity and is a member of the human epidermal growth factor receptor gene family (5.6). Her-2/neu (c-erbB-2) gene amplification, which usually results in overexpression of the encoded Trans membrane protein, occurs in approximately 15 to 30% of invasive breast cancers (7-12). There are several possible uses of HER2 status. This protein plays an important role in regulating cell growth, and overexpression induce High mitotic activity and promote tumor genesis, facilitate invasion and metastasis, but also stimulates apoptosis (13.14). Large studies have reported that histologically high grade tumors are associated with an increased rate of her-2/neu positivity (15-21). Other studies reported that the relationship between Her-2/neu amplification and grade of tumor was found to be not significant (24). The current study aimed to correlate between degree of

differentiation grade of invasive ductal carcinoma and over expression of Her- 2/ neu in Sudanese breast cancer females.

MATERIALS and METHODS

All patients in this study were diagnosed with invasive ductal carcinoma between January 1, 2012 to December 31, 2014 at TOTAL LABCare group and different hospitals in Khartoum state, Sudan. Formalin fixed paraffin embedded (F F P E) blocks were collected, all samples were reviewed by two consultant Histopathologists to evaluate histological grading of tumors using Nottingham grading system of Elston and Ellis (22).followed by immunohistochemistry to evaluate Her-2/neu over expression on formalin fixed paraffin embedded sections of invasive ductal carcinoma.

Immunohistochemistry

IHC was performed with the FDA-approved Ventana PATHWAY rabbit monoclonal antibody 4B5 clone and with the ultra-VIEW DAB Detection Kit (Ventana) on a Benchmark XT automated strainer (Ventana, Tucson, AZ). Briefly, the tissue sections were deparaffinized with EZ Prep at 75°C and 76°C, heat pretreated in Cell Conditioning 1 (CC1) for antigen retrieval at 76°C – 100°C and then incubated with the anti-HER2 primary antibody for 16 min at 37°C after inactivation of the Endogenous peroxidase using UV-inhibitor for 4 min at 37°C. The slides were incubated with a secondary Antibody followed by the application of HRP Universal Multimer for 8 min. Antibodies were detected using chromogen (for 38 min). Before mounting, slides were counterstained with hematoxylin II for 4 min and bluing reagent for 4 min. To support the validity of staining and identify experimental artifact, Positive controls were included in each staining run and consisted of freshly cut breast cancer specimens known to express HER-2/neu.
Negative controls consisted of substituting normal rabbit serum for HER-2/neu antibody. HER-2/neu staining was considered positive when the tumor cells showed intense circumferential cell membrane staining, easily identified with a 10 objective lens.

Statistical analysis

Gross tabulation was used during the statistical analysis using SPSS package version 18 and chi-square test was assessed to correlate between expression of Her-2/ neu and tumor grade, we found no statistically significant relation with p value (0,643).

Ethical clearance for this study is provided by ethical committee of Faculty of Medical Laboratory Sciences –AL-Neelain University, Khartoum.

RESULTS

Between January 2012 and December 2014 30 biopsies were collected from Sudanese female patients with invasive ductal carcinoma from total lab care group and different hospitals in Khartoum state,   average age of the invasive ductal carcinoma patients was 60 year (range 30-90years), 3 cases carcinoma were grade I, 10 cases carcinoma were grade II, and 17 cases carcinoma were grade III, this is depicted in figure 1. The result showed no statistically significant Correlation between Her -2/neu over expression and degree of differentiation grade of invasive ductal carcinoma in Sudanese female P value (0.643). Overexpression of Her- 2/ neu was evaluated as normal expression or loss (negative) or over expression (positive) of immunoreactions. Over expression of her- 2 /neu in invasive ductal carcinoma were detected in 13.3 %(4/30) of the cases.

86.7 %(26/30) of the cases showed normal expression or loss of Her- 2/ neu , correlation between over expression of Her- 2/ neu and histological grade of invasive ductal carcinoma shown in table (2)

And we have noticed over expression of Her- 2/ neu increase with high tumor grade, which detected in grade III tumors 6.7% (2/30) and   grade II tumors 6.7% (2/30), but overexpression was not detected in grade I tumors, p value in statistical chi-squire analysis was   insignificant P value (0.643).
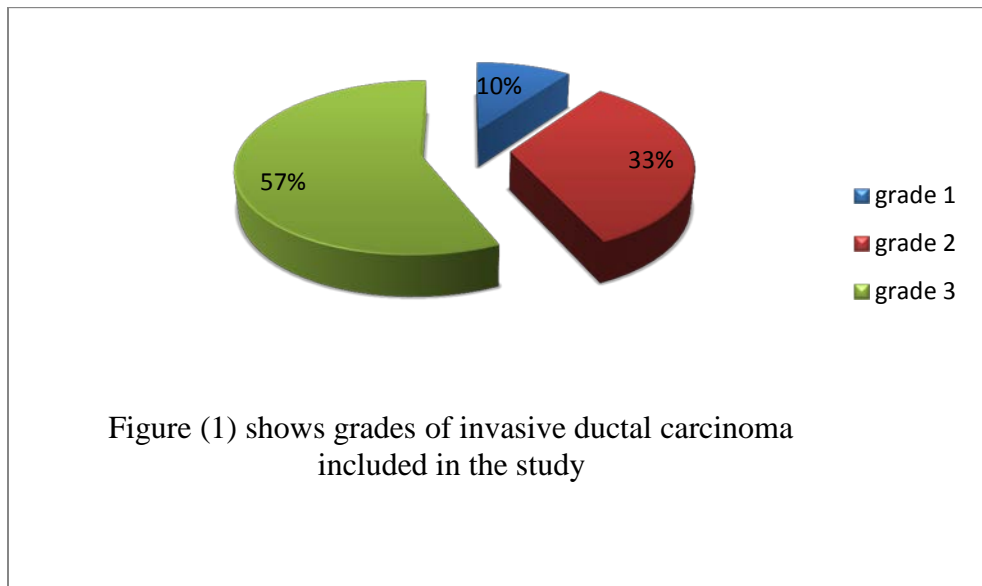
Figure (1) shows grades of invasive ductal carcinoma included in the study

Table1: shows percentage of invasive ductal carcinoma with over expression. Of her-2/neu

Grading * immuneCross tabulation					
			immuno		
			positive	negative	Total
Grading	grade 1	Count	0	3	3
		% of Total	.0%	10.0%	10.0%
	grade 2	Count	2	8	10
		% of Total	6.7%	26.7%	33.3%
	grade 3	Count	2	15	17
		% of Total	6.7%	50.0%	56.7%
Total		Count	4	26	30

Grading * immuneCross tabulation			immuno		Total
			positive	negative	
Grading	grade 1	Count	0	3	3
		% of Total	.0%	10.0%	10.0%
	grade 2	Count	2	8	10
		% of Total	6.7%	26.7%	33.3%
	grade 3	Count	2	15	17
		% of Total	6.7%	50.0%	56.7%
Total		Count	4	26	30
		% of Total	13.3%	86.7%	100.0%

Table2: shows the correlation between over expression Her-2/neu and histological grade in invasive ductal carcinoma among the study population

Chi-Square Tests			
	Value	df	Asymp. Sig. (2 sided)
Pearson Chi-Square	.882[a]	2	.643

DISCUSSION

One important marker in breast cancer today is c-erbB-2 oncoprotein (HER2). HER-2/neu overexpression and/or amplification occur in approximately 15–30% of invasive breast cancer (7-12). Immunohistochemistry is the most widely used method for c-erbB-2 protein assay, with a variety of polyclonal and monoclonal antibodies Her-2/neu amplification/or overexpression in different Histological grades of female breast cancer has traditionally been a subject of interest. In a study of Hoff et al. (23), infiltrating ductal carcinomas were significantly more likely to show Her-2/neu amplification than infiltrating lobular carcinomas (p<0.005), and higher grade tumors were more likely to demonstrate Her-2/neu amplification than lower grade ductal carcinomas (p<0.001). Similarly other large studies have confirmed that Her-2/ neu over expression correlated with high histological grading (15-21), but our study failed to support these studies that we have mentioned previously Where we did not find statistically significant relationship between over expression of Her-2/ neu and degree of high grade of tumors in invasive ductal carcinoma in Sudanese females P Value (.0643). but this is can be explained by the weakness of the number of samples that target   in our study and heterogeneous Sudanese population But interestingly our study in line with study done in Saudi Arabia the closed by population in which they reported the relationship between Her-2/neu amplification and grade of tumor was found to be not significant And vast majority of Her -2/neu negative tumor were grade III (24).

CONCLUSION

In this study of 30 cases of invasive ductal carcinomas in which Her-2/neu by IHC was performed, Her-2neu Protein over expression was seen in 4 cases (13, 3%),
There was no statistically significant correlation between her neu2 over expression and degree of grade of differentiation. We conclude there is no relation between degree of differentiation grade and over expression of her- 2/ neu of invasive ductal carcinomas in our study.

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