Int. J. Pure Appl. Sci. Technol., 20(1) (2014), pp. 81-87

International Journal of Pure and Applied Sciences and Technology

ISSN 2229 - 6107

Available online at ***www.ijopaasat.in***

Research Paper

Expression of Epidermal Growth Factor Receptor in Sudanese Patients with Triple Negative Breast Cancer

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(Received: 15-10-13; Accepted: 30-11-13)

Abstract: *Epidermal Growth Factor Receptor (EGFR) mutations have an important role in the pathogenesis of breast carcinoma. This retrospective study aimed to identify EGFR expression in Sudanese female patients with Triple Negative Breast Carcinoma (TNBC) and its relationship with age, histological diagnosis, and tumor grade. Fifty formalin- fixed paraffin-embedded tissue blocks of TNBC were obtained from the archives of the Radiation and Isotopes Center Khartoum (RICK) during the period between June and November 2012. Immunohistochemical staining was carried out using monoclonal antibody for EGFR. Results showed that EGFR was positive in 60% of cases, mostly in middle-aged women, with a significant correlation with tumor grade, estrogen receptor (ER) expression, and progesterone receptor (PR) expression. In conclusion, this study confirmed the increased frequency of EGFR expression with TNBC in Sudanese women as the case in other populations.*

Keywords: Triple Negative Breast Cancer, EGFR, Tumor Grade.

1. Introduction

Breast cancer is one of the most frequently diagnosed malignant tumors and the main cause of cancer related death in women worldwide. About 1.4 million breast cancer cases and 0.5 million breast cancer deaths were diagnosed in the year 2008 **[1]**. Breast cancer is now recognized as a heterogeneous disease with different biological behaviors that requiring distinct therapeutic strategies **[2]**. Numbers of breast cancer in Sudan show annual increasing rate **[3]**.

Triple negative breast cancer (TNBC) is defined by the lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2 neu) expressions **[4, 5]**. It constitutes about 10%–20% of all breast cancers; more frequently affects younger patients, and is more prevalent in African-American women **[6]**. TNBC tumors are generally larger in size, higher in grade, have lymph node involvement at diagnosis, and are biologically more aggressive than other types of breast cancer **[7]**.

The EGFR is a member of the ErbB family of receptors, which includes four receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her3 (ErbB-3) and Her 4 (ErbB-4). EGFR mutations have an important role in pathogenesis of breast carcinoma **[8, 9]**. EGFR-mediated signal transduction pathways are very extensive and important, and they are involved in growth and differentiation of tumor cells **[10]**.

Epidermal growth factor receptor is more frequently expressed in TNBCs than in non-TNBCs **[11, 12]**. Some retrospective studies indicated that EGFR over expression in primary tumors could predict poor prognosis **[13, 14, 15]**, while other studies did not establish such a relationship **[16, 17]**.

Therefore, the EGFR is considered a candidate treatment target for TNBCs **[13]**. However, molecular targeted therapy has been suggested in the treatment of metastatic breast cancer **[14, 15]**

The purpose of this study was to detect the EGFR expression in TNBCs and its correlation with the clinicopathological parameters.

2. Material and Methods

This was a retrospective study of fifty formalin-fixed paraffin-embedded tissue blocks of triple negative breast carcinoma (TNBC) obtained from the archives of the Radiation and Isotopes Center Khartoum (RICK) between June and November 2012. Clinical data of patients was obtained from the hospital medical records; these data included age, histological diagnosis, grading, ER expression, PR expression, and HER-2 neu expression. Using a rotary microtome, two 3-5µm- thick sections were cut from each block and put on slides, one for EGFR immunostaining and the other for H&E staining to confirm the histopathological diagnosis obtained from the records.

Immunohistochemical staining was carried out using monoclonal antibody for EGFR. The tissue sections were mounted on to adhesive slides. Following deparaffinization in xylene, slides were rehydrated through graded series of alcohol and placed in running water. Slides were placed into a tank containing enough sodium Tris buffer (pH 9.0) to cover the sections, boiled at high temperature for 20 minutes, and then were allowed to cool at room temperature (RT). Endogenous Peroxidase activity was blocked with 3% hydrogen Peroxidase and methanol for 10 minutes, and then slides were incubated with 100-200 µl of primary antibody for 20 minutes at RT in a moisture chamber, and then rinsed in Phosphate buffer saline (PBS) for 3 minutes. The binding of the primary antibody for EGFR (EGFR.25, 1:50, Novocastra, UK) was detected by incubating for 20 minutes with dextran labeled polymer (Dako- EnVision TM Flex kit). Finally, the sections were washed in three changes of PBS, followed by adding 3, 3' Diaminobenzedine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides were counter- stained with hematoxylin. For each run of staining, positive and negative control slides were also prepared. The positive control slides contained the antigen under investigation. The negative control slides were prepared from the same tissue blocks but incubated with PBS instead of the primary antibody.

The slides were evaluated by a light microscope and scored. All tissue sections showed fair staining quality and all quality control measures were considered throughout study procedures. Scoring for EGFR expression was based on staining pattern and intensity of cell membrane (positive or negative). EGFR staining was scored as follows: 0 for no membrane staining (negative); 1 for faint, partial

membrane staining (negative); 2 for weak, complete membrane staining in more than 10% of tumor cells (positive); and 3 for intense complete membrane staining in more than 10% of tumor cells (positive). Positive tumors (with a score of 2 or 3) were identified by the dark brown cell membrane staining. Statistical analysis was performed using the SPSS program. The expression of EGFR was correlated with tumor grade, tumor type, age group, ER expression, PR expression, and HER2neu expression.

3. Results and Discussion

A total of 50 women diagnosed with breast cancer were included in this study, ranging in age between 30 to 70 years. The most prevalent type of cancer was invasive ductal carcinoma (90%) followed by invasive papillary carcinoma (4%), medullary carcinoma (4%), and invasive tubule-lobular carcinoma (2%). Most invasive ductal carcinomas were in middle-aged women. Bircan Erbas et al investigated incidence rates for invasive ductal carcinoma of the breast in 474,808 women who attended the Australian Breast Screen Victoria from January 1, 1993 to December 31, 2000. Of these women, 5,301 were diagnosed with invasive ductal carcinoma and 1,127 were diagnosed with ductal carcinoma in situ **[18]**. Christie R. Eheman et al reviewed breast cancer incidence in USA between 1999 and 2004; they found invasive ductal carcinoma was predominant, with a dramatic increase within the age group 40-49 years **[19]**.

The majority of cases in the study were high grade TNBCs. Roohi Ismail-Khan and Marilyn M. Bui reviewed the literature and showed that TNBCs have high incidence rates, poor prognosis, and high frequency in premenopausal women **[20]**. Kanapathy Pillai et al reviewed and analyzed 340 Malaysian patients diagnosed with primary breast cancer between 2002 and 2006; they reported incidence of TNBC was 12.4% (42/340), and was strongly associated with younger ages and higher grade tumors. Further immunohistochemical analysis suggested that TNBC in Malaysian women was strongly associated with EGFR **[21]**.

EGFR was positive in 60% of cases in this study (Table No 1). Teng et al. analyzed seventy samples of triple negative breast tumors from Singapore General Hospital and confirmed the presence of EGFR mutations in these tumors **[22]**.

All EGFR-positive cases in this study were of high grade invasive ductal carcinomas (Tables 2 and 3). G. Viale et al evaluated 284 patients with triple-negative invasive ductal carcinoma of the breast. They reported that EGFR immunoreactivity significantly correlated with worse prognosis in patients with triple-negative invasive ductal carcinoma **[23]**.

S.A. Aziz et al studied 315 tumor specimens of infiltrating ductal carcinoma of breast to assess the prognostic value of epidermal growth factor receptor (EGFR). Mutations of EGFR were observed in 70 (22.00%) tumors. Eleven (16%) were grade I, 43 (61%) grade II and 16 (23%) grade Ill tumors. Significant number of EGFR positive patients developed local recurrence and distant metastases to brain, liver and bone **[24]**.

Of the cases positive for EGFR expression (30 cases), ER expression was positive in only 4 cases (P=0.01), PR expression was positive in only 2 cases (P=0.02), and HER2 neu expression was positive in 11 cases (Tables 4, 5, and 6). Mothaffar F. Rimawi et al studied 475 EGFR- positive tumors and found them more common in younger black women and were larger, more likely to be HER2-positive, but less likely to be ER-positive, or PR-positive **[25]**.

Table No 1: EGFR expression and age groups

Age group	EGFR				Total	
	Positive		Negative			
30-40	12	24%	9	18%	21	42%
41-50	12	24%	4	8%	16	32%
51-60	6	12%	5	10%	11	22%
61-70	0	0%	2	4%	2	4%
Total	30	60%	20	40%	50	100%

Table No 2: EGFR expression and Histological Diagnosis

Diagnosis	EGFR				Total	
	Positive		Negative			
Invasive ductal carcinoma	30	60%	15	30%	45	90%
Invasive papillary carcinoma	0	0%	2	4%	2	4%
Invasive tubulo- lobular carcinoma	0	0%	1	2%	1	2%
Medullary carcinoma	0	0%	2	4%	2	4%
Total	30	60%	20	40%	50	100%

Chi-Square Tests p 0.04

Table No 3: EGFR expression and Tumor Grade

Grade	EGFR				Total	
	Positive		Negative			
Grade I	0	0%	0	0%	0	0%

Grade II	**7**	**14%**	**2**	**4%**	**9**	**18%**
Grade III	**22**	**44%**	**10**	**20%**	**32**	**64%**
Not graded	**1**	**2%**	**8**	**16%**	**9**	**18%**
Total	**30**	**60%**	**20**	**40%**	**50**	**100%**

Table No 4: EGFR expression and ER expression

ER expression	**EGFR**				**Total**	
	Positive		**Negative**			
Positive	**4**	**8%**	**9**	**18%**	**13**	**26%**
Negative	**26**	**52%**	**11**	**22%**	**37**	**74%**
Total	**30**	**60%**	**20**	**40%**	**50**	**100%**

Table No 5: EGFR expression and PR expression

PR expression	**EGFR**				**Total**	
	Positive		**Negative**			
Positive	**2**	**4%**	**6**	**12%**	**8**	**16%**
Negative	**28**	**56%**	**14**	**28%**	**42**	**84%**
Total	**30**	**60%**	**20**	**40%**	**50**	**100%**

Table No 6: EGFR expression and HER2 expression

HER-2 expression	**EGFR**				**Total**	
	Positive		**Negative**			
Positive	**11**	**22%**	**6**	**12%**	**17**	**34%**

Negative	**19**	**38%**	**14**	**28%**	**33**	**66%**
Total	**30**	**60%**	**20**	**40%**	**50**	**100%**

4. Conclusions

From this study, it is concluded that EGFR expression in TNBCs in Sudanese women is almost similar to other populations. In addition, immunodetection of EGFR in paraffin-embedded tissue can be a useful alternative to the standard fresh-tissue assay and can accurately reflect the level of EGFR expression in human breast cancer.

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