

Expression of HER2 and BRCA1 in Breast Tumors among Sudanese Women, Immunohistochemical Study

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Abstract: ***Objective:** The aim of this study was to detect HER2/ Neu and BRCA1 expression in breast cancer using immunohistochemical methods. **Methodology:** A retrospective descriptive study was performed on 50 samples from patients diagnosed with breast cancer as well as 50 from benign breast tumors. HER/neu and BRCA1 expression was assessed using immunohistochemistry (IHC). **Result:** HER/neu was expressed in 4/50 (8%) of breast cancer and 11/50 (22%) of benign breast tumors whereas BRCA1 was expressed in 9/50 (18%) of breast cancers and 7/50 (14%) of benign breast tumors, with significance at p values of 0.045 and 0.393 respectively. **Conclusions:** There is insignificant association between HER/neu and BRCA1 expression and breast cancers among Sudanese patients.*

Keywords: Breast cancers- Sudanese- HER/neu- BRCA

1. Introduction

Breast cancer is the most common invasive cancer in women; according to GLOBOCAN 2012, it is the second most common cancer and the fifth most common cause of cancer deaths worldwide, with an age standardized incidence rate (ASR) of 43.3 per 100,000 women-years and a worldwide mortality rate of 12.9% in 2012 (1). Its incidence varies widely among different regions. In the United States breast cancer is accounting for more than 40,000 deaths per year (2). It is lower in Eastern Asia than in Northern America and Europe (ASR: 27.0, 91.6, and 71.1 per 100,000 women-years, respectively). In Asia, reports have indicated that the annual incidence of breast cancer has doubled or tripled over the past two decades (3). In Africa overall, the estimated number of new cases was 92,600 in 2008 and 133,900 in 2012 (4, 5).

Breast cancer continues to be the most common cancer among women in Sudan. Out of 521 patients who visited Khartoum Teaching Hospital during a 5-year period from 1994 to 1999, invasive ductal carcinoma was the most common type (71.5%) and most patients (17.2%) had an advanced stage III and IV disease (6). Furthermore, among breast cancer patients 1255 attending NCI-UG from 1999 to 2006, invasive ductal carcinoma was the most common type (82%) (7), and about 74% of those patients were less than 50 years old and presented with stage III and higher tumors expressing no estrogen or progesterone receptors that had already metastasized (7).

Breast cancer risk factors among Sudanese patients included past history of benign breast disease, previous breast biopsies, pesticide and plasticizer exposure, periods of being overweight, physical inactivity, being unmarried and decreased number of children (8). Recently, genetic and genomic risk factors associated with the development of breast cancer in Sudanese women were examined. BRCA2 exon 11, breast cancer susceptibility genes, and the conserved p53 regions were studied in breast cancer samples from 20 patients. One somatic mutation and one polymorphism in BRCA2 exon 11 and no mutation for all p53 sequences were found indicating a limited role of these regions in the pathogenesis of breast cancer in those patients (9). However, a study that screened for germline BRCA1/2 mutations in patients (N = 35) from Central Sudan detected a total of 60 sequence variants (32 in BRCA1, 28 in BRCA2) in 94% of the cases and five truncating mutations (2 in BRCA1, 3 in BRCA2) in 14% of the patients (10). Furthermore, BRCA1 and BRCA2 mutations were tested in female teenage students (n = 47) attending Marawi Secondary School in Northern State. About 51% of the students with a family history of breast cancer and 20% with no family history of breast cancer had BRCA1 and BRCA2 mutations. Most of the BRCA1 mutations located to exon 11 fragments 11.9 and 11.1 29. Though, in a study that examined methylation status of six tumor suppressor genes that included BRCA1, BRCA2, p14, p16, hMLH, and MGMT in breast (n = 23), other tumor (n = 10), and control tissues (n = 4) suggested that BRCA1, BRCA2, and p14 appeared to be under strong epigenetic silencing. BRCA1, BRCA2, and p14 were strongly hypermethylated in 84%, 84%, and 81% of cancer tissues, respectively (11). Genetic

alterations in estrogen receptor alpha gene (ESR1) such as C325G single nucleotide polymorphism (SNP) are thought to play a role in predisposition to breast cancer. Genotyping C325G in ESR1 in breast cancer patients (n = 100) in comparison to healthy controls (n = 90) revealed a significant association of breast cancer risk in women 50 years and younger who had the C allele (OR: 2.28, 95% CI: 1.104.72) (P = 0.03) suggesting that polymorphism within the low penetrance ESR1 is associated with breast cancer susceptibility in young Sudanese women (12). Similarly, genetic alterations in human epidermal growth factor receptor (HER-2/neu) have been shown to induce breast cancer malignant transformation. The association of HER-2/neu Ile655Val polymorphism and risk of breast cancer in a Sudanese population were examined and found to be borderline significant. Women who are heterozygous Ile/Val carriers have higher risk of breast cancer. Both ESR1325C and HER-2/neu Ile655Val variants were suggested to jointly contribute to a higher risk of breast cancer (13). The HER2-positive subtype comprises ~20% of all breast cancers and is defined as displaying overexpression of the human epidermal growth factor receptor 2 (HER2) proteins or amplification of the *ERBB2* gene, as assayed by immunohistochemistry or fluorescence *in situ* hybridization, respectively(14). HER2 is a 185 kDa trans membrane protein encoded by the HER2/neu gene (15, 16). HER2 is more closely related to EGFR compared to HER3 and HER4 (17). There is no known ligands that bind to HER2 thus far. However, HER2 has a functional tyrosine kinase domain that can be activated upon interactions with ligand-activated EGFR or HER3 (18–20). HER2 is also believed to form homodimers at high concentrations (21). Activated HER2 (hetero- and homodimer) proteins initiate phosphorylation events, similar to EGFR, and leads to activation of several signaling pathways, all of which are implicated in breast cancer progression. These pathways include STAT3, RAS-MAPK, and PI-3K which leads to inactivation of proteins triggering apoptosis and upregulation of genes for cell growth allowing the proliferation of tumor cells (22, 23–25). Overexpression of HER2 is found in 15%-35% of invasive breast cancers (26-29).

2. Material and Methods

In this study, a total of 50 patients (female) aged between 20 and 80 years mean age 50 years, were diagnosed as having breast cancer, as well as 50 samples of benign breast tumor were assessed for HER-2/NEU and BRCA-1 expression using immunohistochemistry. The diagnosis was based on clinical examination and histological features of the biopsy. Breast cancer was classified as grade1, grade2, grade3 and high grade. The diagnosis was verified based on Royal College of Pathologists criteria (30). The sample included full coverage of patients with breast lesions referred to our hospital within Two-year time. Ethical consent was obtained from ethical committee of the Faculty Research Board and Hospital. HER-2/NEU and BRCA-1 immunohistochemistry (IHC) was performed on formalin-fixed paraffin embedded (FFPE) tissue sections using kits from Dako (Real Envision Detection Kit, China.). Paraffin embedded blocks of breast cancer tissues as well as benign breast tumors were retrieved from histopathology laboratories then cut into (3 µm in

thickness) sections using rotary microtome. The sections were mounted on poly-L-lysine-coated slides and dried in hot air oven at 60°C for 1 hour. The sections were dewaxed in xylene 5 minutes, three times, and rehydrated through descending grades of ethyl alcohol beginning with 100% ethyl alcohol, then 90% ethanol, 70% ethanol and finally placed in distilled water, 4 minutes for each change, and then the sections were washed 3 times with PBS, three minutes for each. The sections were boiled in the Target Retrieval Solution of Dako (Real Envision Detection Kit, China) in a water bath at 95°C for 30 min, then left to cool at room temperature and washed three times with PBS. 0.3% hydrogen peroxide in methanol were added to each section for 15 min to block endogenous peroxidase activity, and then washed three times with PBS. The following antibodies (Abs) were used: primary mouse monoclonal mutant HER2 antibody, primary mouse monoclonal mutant BRCA1 antibody. (Gene tech company limited, Shanghai, China) at a working dilution of 1/100, at 37°C for 30 min ; After two washes in PBS, sections were incubated with Chem Mate TME nVision of +/HRP (Gene tech company limited, Shanghai, China), a secondary antibody at room temperature for 30 min, then washed three times in PBS. The immunoreactivity was detected using diaminobenzidine (DAB) (Gene Tech Company limited, Shanghai, China) in a dilution 1/100 as the final chromogenic for 10 min, and then washed in distilled water for 3 min. Finally, sections were counterstained with Mayer's Haematoxyline for 3 min, and washed in running tap water 5min, then dehydrated through a sequence of increasing concentrations of alcoholic solutions and cleared in xylene then mounted with DPX. During each IHC assay, proof slides were coupled with negative and positive controls provided by the manufacturer for each marker, and reactions were observed appropriately. IHC stained sections were examined under the light microscope (Olympus CHT, Optical.Co.Ltd, Japan) using 4×, 10×, 40× 100×, objective and eyepieces of 10× giving a maximum magnification of 1000. Mutated her2 and BRCA1 were observed only as a nuclear staining of epithelial cells, and the nuclei with clear brown color were scored as positive. The intensity of immunohistochemical staining for each marker was score by two investigators based on subjective evaluation of color exhibited (brown) by antigen, antibody and chromogenic complex. It was scored as 0 for negative (no color), 1+ for weak (light brown color), 2+ for moderate (dark brown color), and 3+ for strong staining (very dark brown color) with 0 or 1 scores defined as negative and 2 or 3 defined as positive.

3. Result

In this study we tested 100 samples of confirmed female breast tumor (50 benign and 50 malignant) for expression of HER2 and BRCA1 using immunohistochemistry methods. The samples we retrieved from different histopathology laboratories of Khartoum hospitals. The patients` age ranged from 20 to 80 years with mean age 50 year. The great number of study population 41 (41%) was found in the age group (20-30) whereas the rest numbers were distributed as the following: 21(21%), 20(20%),12(12%), 2(2%), 4(4%) were found in the age group (31-40), (41-50), (51-60), (61-70) and (71-80) year respectively as shown in figure (1) .

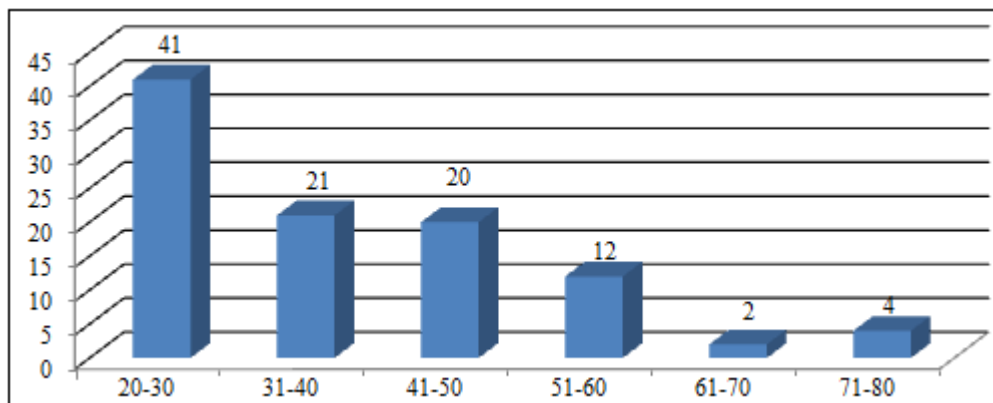


Figure 1: Distribution of study population by age

According to relationship between age group and type of tumor, the highest incidence of benign tumors 35(70%) were found in the age group (20-30) whereas 12(24%), 1(2%), 2(4%) were seen in the age group (31-40), (41-50), (51-60) respectively. On the other hand the highest incidence of malignant tumors 19(38) were found in the middle age group (41-50) with only 6(12%), 9(18%) were found in the lowest age group (20-30) , (31-40) respectively and 10(20%), 2(4%), 4(8%) were seen in age group (51-60), (61-70), (71-80) respectively as shown in table (1)

Table 1: Distribution of age by tumors

Age group	Malignant	Benign	Total
20-30	6(12%)	35(70%)	41(41%)
31-40	9(18%)	12(24%)	21(21%)
41-50	19(38%)	1(2%)	20(20%)
51-60	10(20%)	2(4%)	12(12%)
61-70	2(4%)	0(0%)	2(2%)
71-80	4(8%)	0(0%)	4(4%)
Total	50(100%)	50(100%)	100(100%)

Regarding to histological grades of breast cancers, they were found as: grade one 8(16%), grade two 24(48%), grade three 16(32%) and high grade which is only comprised 2(4%) of the study population as shown in table (2).

Table 2: Distribution of the histological grades of Breast cancer

Histological grades	Frequency
Grade 1	8(16%)
Grade 2	24(48%)
Grade 3	16(32%)
High grade	2(4%)
Total	50(100%)

In the present study HER2/neu was expressed in 4(8%) of breast cancers and 11(22%) of benign breast tumors p. value=0.045. Statistically, the expression of HER/neu is insignificantly associated with breast cancers P. value ≥ 0.005 as shown in table (2). BRCA1 was found to be positive in 9(18%) of breast cancers and 7(14%) of benign breast tumors, p. value=0.393. Statistically, the expression of BRCA1 is insignificantly associated with breast cancers p. value ≥ 0.005 as shown in table (3)

Table 3: Distribution of HER2/neu and BRCA1 by tumors

Marker		Tumor		Total	P value
		Malignant	Benign		
HER2/neu	Positive	4(8%)	11(22%)	15(15%)	0.045
	Negative	46(92%)	39(78%)	85(85%)	
	Total	50(100%)	50(100%)	100(100%)	
BRCA1	Positive	9(18%)	7(14%)	16(16%)	0.393
	Negative	41(82%)	43(86%)	84(84%)	
	Total	50(100%)	50(100%)	100(100%)	

4. Discussion

In our study, HER/neu protein was expressed in 4(8%) of breast cancers and 11(22%) of benign breast lesion. The expression of HER/neu in breast cancers is statistically insignificant, the P value=0.045. This study showed decreased expressions of HER/neu in breast cancers. In contrast to our finding, *Citri and Yarden* reported that the HER2-positive subtype comprises ~20% of all breast cancers and is defined as displaying overexpression of the human epidermal growth factor receptor 2 (HER2) proteins or amplification of the *ERBB2* gene, as assayed by immunohistochemistry or fluorescence *in situ* hybridization, respectively (14). Other studies conducted in Sudan, have also shown to considerable association between HER/neu and breast cancers. *Siddig et al* reported that genetic alterations in human epidermal growth factor receptor (*HER-2/neu*) have been shown to induce breast cancer malignant transformation. The association of *HER-2/neu* Ile655Val polymorphism and risk of breast cancer in a Sudanese population were examined and found to be borderline significant. Women who are heterozygous Ile/Val carriers have higher risk of breast cancer. Both *ESR1325C* and *HER-2/neu Ile655Val* variants were suggested to jointly contribute to a higher risk of breast cancer (13). In the present study we can attribute the reduced expression of HER/neu in breast cancers to the aggressiveness parameters such as high histological grade, because other previous studies linked between the over expression of HER/neu and certain histological grades of breast cancer particularly the high grades. In our study there are only two samples out of the fifty malignant breast cancers were found to be high grade, the remaining samples were grade one 16%, grade two 48% and grade three 32%. This finding is supported by study of *Lobna et al*, who reported that HER-2 overexpression was observed in 18.1% of Tunisian breast carcinoma affecting female patients. This group presents

apparently an aggressive form of breast carcinoma with high histological grade (31).

In this study, BRCA1 protein was expressed in 9(18%) of breast cancers and 7(14%) of benign breast lesion. The expression of BRCA1 in breast cancers is statistically insignificant, the P value=0.393. BRCA1 and BRCA2 are normally expressed in the cells of breast and other tissues, where they help repair damaged DNA, or destroy cells if DNA cannot be repaired. They are involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double-strand breaks (32) If BRCA1 or BRCA2 itself is damaged by a BRCA mutation, damaged DNA is not repaired properly, and this increases the risk for breast cancer(32). In this study we found that BRCA1 protein was expressed in 18% of breast cancer, this study is supported by study of *Dokyung et al.* (33) who reported that BRCA protein was expressed in 24.7% of breast cancers. In the present study we also found the expression of BRCA1 protein in breast cancer is higher than in normal breast lesion, this findings is inconsistent with the study of *Abeer, et al* (34) who reported that a high uniform expression of BRCA1 was observed in normal breast tissue while absent or reduced expression was found only in malignant tissues.

5. Conclusion

Our study disclosed weak association between HER/neu and BRCA1 expression and breast cancers among Sudanese women.

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