

The Role of HER2/Neu and BRCA1 Genes in the Diagnosis of Breast Cancer among Sudanese Women

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Abstract

Background: Knowledge of HER2/Neu and BRCA1 Genes might be helpful for development of strategies for decreasing the burden of risk of breast cancer. Therefore, the aim of this study to detect the role of HER2/Neu and BRCA1 Genes expression in diagnosis of breast cancer in Sudanese women.

Methodology: A total of 100 tissue samples obtained from patients with breast cancer in addition to 50 tissue samples obtained from patients with benign breast lesions, were detected the expression of HER2/Neu and BRCA1 Genes by Polymerase Chain Reaction (PCR).

Results: The prevalence of HER2/Neu and BRCA1 Genes, among cases was 6%, and 10% respectively.

Conclusion: HER2/Neu and BRCA1 Genes have a considerable contribution to etiology of breast cancer in Sudan that requires further consideration.

Keywords

HER2/Neu and BRCA1 Genes, Breast Cancer, Sudan

1. Introduction

Breast cancer is the most common cancer in women worldwide, leading to about 300,000 deaths each year. It is considered a biologically heterogeneous disease that is influenced by complex and still incompletely understood interactions

between multiple genetic and environmental risk factors. These interactions may play an important role in the significant geographical differences in breast cancer incidence [1]. The incidence in developed countries is higher than in developing countries, as well as in urban and rural areas [2]. In Sudan, it was reported that about 70% of the women diagnosed with breast cancer were younger than 50 years old and invasive ductal carcinoma was the most common type (71.5%) [3]. The BRCA1 tumor suppressor gene account for approximately 45% of families with a significantly high breast cancer incidence and the HER2/Neu oncogene is in proximity on the long arm of chromosome 17 (17q11-21) [4] [5]. Absence of BRCA1 or functional overexpression of the HER2/Neu gene may be characteristic of the somatic phenotype of breast cancer in premenopausal women, characterized by a poor prognosis, such as a high tumor grade, negative hormone receptors, and a high proliferation rate [6].

2. Materials and Methods

In this study 100 women with breast lesions were retrospectively investigated for the presence of HER2/Neu and BRCA-1 expression by Polymerase Chain Reaction (PCR). Of the 100, Formalin fixed paraffin embedded tissue samples, 50 tissue samples were obtained from women with breast cancer and the remaining 50 tissue samples were from women with benign breast lesions.

DNA extraction

DNA was extracted according to the steps described in DNA extraction kit purchased from Sacace biotechnologies—Casera—Italy. The pellet obtained from previous steps was treated with 300 µl of Reagent 2 (lysis buffer) in addition 100 µl of sample, vortexed, incubated at 65°C for 5 min and centrifuged at (12,000 - 16,000 g) for 10 min at 25°C and transfer the supernatant into new tube (sterile 1.5 ml Eppendorf tube) for DNA extraction. Vortexed vigorously sorbent and added 20 µl to each tube, Vortexed for 5 - 7 sec and incubated all tubes for 3 min at room temperature, then this step was repeated. Then all tubes were centrifugated for 30 sec at 5000 g and used a micropipette with a plugged aerosol barrier tip, carefully removed and discarded supernatant from each tube without disturbing the pellet. Tips were changed between the tubes. 500 µl of Washing Solution was added to each tube. Vortexed vigorously and centrifuged for 30 sec at 10,000 g. Supernatant was removed and discarded from each tube. This step was repeated and incubated all tubes with open cap for 5 - 10 min at 65°C. The pellet was resuspended in 100 µl of DNA-eluent. Incubate for 5 min at 65°C and vortex periodically. The tubes were centrifuged for 1 min at 12,000 ×g. The supernatant was containing DNA ready for amplification stored at -20°C until used.

PCR amplification of *HER2/Neu* and *BRCA1* Genes

The PCR was carried out in a total reaction volume of 40 µl containing between 20 µl mix-1 (contained in PCR tubes), 10 µl of mix-2 and 10 µl of extracted DNA (sample). The PCR program was described in **Table 1**.

Table 1. Showing PCR program used for amplification of HER2/Neu and BRCA1 Genes.

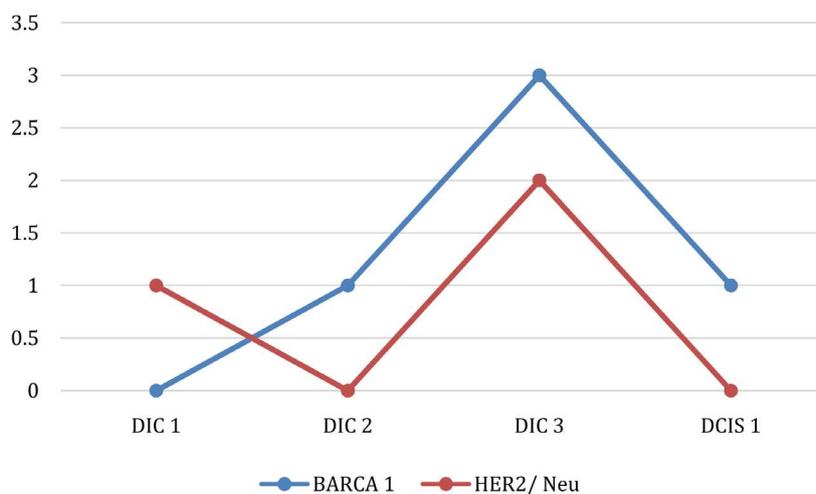
Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5 - 1 minute	25 - 35 cycles
Annealing	42°C - 65°C*	0.5 - 1 minute	25 - 35 cycles
Extension	72°C	1 minutes/kb	25 - 35 cycles
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	indefinite	1 cycle

3. Result

This study investigated 100 patients with breast lesions, their ages ranging from 20 to 80 years with a mean age of 50 years. The expression of HER2/Neu and BRCA1 Genes was investigated among 50 cases (women with breast cancer) and 50 controls (women with benign breast lesions). Positive detections were established among cases in 3/50 (6%) and 5/50 (10%) of HER2/Neu and BRCA1 Genes, respectively. Amongst controls, no expression was identified in case and control. HER2/Neu were found in one case of invasive ductal carcinoma grade 1 (IDC 1) and two cases of IDC 2, BRCA1 were found in two cases of invasive ductal carcinoma grade 1 (IDC 1), one case of IDC 2, one case of IDC 3 and one case of ductal carcinoma in situ (DCIS 1) as indicated in **Table 2, Figure 1.**

Table 2. Distribution of cases by BACA1, HER2/Neu and pathology.

HPV subtype	DIC 1	DIC 2	DIC 3	DCIS 1	Total
BRCA1	0	1	2	1	5
HER2/Neu	1	0	2	0	3
Total	3	3	1	1	8

**Figure 1.** Description of cases by BRCA1 and HER2/Neu in different diagnostic findings.

4. Discussion

Breast cancer is the most common cancer in women in both Sudan and worldwide [7]. It is considered the leading cause of female related mortality in Sudan [8]. The incidence rates are rising all over the world increasing the disease burden and necessity to increase awareness of such devastating disease. The amplification and/or overexpression of the HER2/Neu and BRCA1 oncogenes have been proposed as an important prognostic marker in breast cancer, in our study, HER/neu protein was expressed in 3 (6%) of breast cancers and not expressed in 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 [9]. 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 HER2/Neu Ile655Val variants were suggested to jointly contribute to a higher risk of breast cancer [10]. 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, this finding is supported by study of Lobna *et al.*, who reported that HER-2 over-expression 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 [11]. 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 [12]. 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 [12]. In this study, BRCA1 protein was expressed in 5 (10%) of breast cancers. The expression of BRCA1 in breast cancers is statistically significant, the P value = 0.04. 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 [12]. 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 [12].

In this study we found that BRCA1 protein was expressed in 10% of breast cancer, this study is supported by study of Dokyung *et al.* [13]. 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 finding is inconsistent with the study of [14] 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

In conclusion, the present results suggest there is a weak expression of HER/Neu and BRCA1 proteins in breast cancers among Sudanese women and breast cancers are the most prevalent cancers in Sudanese women. HER/Neu and BRCA1 Genes mutation is not clearly involved in breast cancers among Sudanese women.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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