



Original Research Article

## Expression of Beta Catenin in Sudanese Women with Breast Cancer

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### ABSTRACT

**Study Design and Objective:** this was a retrospective descriptive study aimed to identify expression of beta catenin in breast tissue obtained from Sudanese women with breast cancer.

**Material and Methods:** hundred formalin-fixed paraffin-embedded tissue blocks of breast cancer were included in this study. Tissue sections were stained using monoclonal antibody for Beta catenin, interpreted by a light microscope, and results analyzed by SPSS program and correlated with the available clinicopathological data.

**Results:** The beta catenin expression was positive in 68% of cases and negative in 32% of cases. The expression of beta catenin was positive in 82% of Triple Negative Breast Cancers (TNBCs), 29% of Triple Positive Breast Cancers (TPBCs), 96% of Her2 tumors, 56% of luminal- A tumors, and 29% of luminal- B tumors.

**Conclusion:** Increased expression of beta catenin in triple negative cancer, Her2 positive cancer, and high grade tumors confirms its importance as a prognostic marker for breast cancer.

**Key Words:** beta catenin; breast cancer.

### INTRODUCTION

Breast cancer is mostly found in women and less commonly in men. <sup>[1]</sup> In U.S.A alone, one out of every eight women has the disease. <sup>[2]</sup> The incidence increases with age from the third to the fifth decade and reaches a second peak at age 65. <sup>[3, 4]</sup> In the year 2010, the American Cancer Society estimated approximately 209,060 new cases of breast cancer would be diagnosed and

40,230 deaths due to breast cancer would occur in the United States. <sup>[5]</sup>

According to the records of the Radiation and Isotope Center Khartoum (RICK), about 6622 cases of breast cancer in Sudanese patients were diagnosed during the period between the years 2000 and 2009; most of them were females (95%). In addition, it is noticed that there was an annual increase in numbers of breast cancer cases in Sudan between 2008 and 2010; in

2008 there were 882 cases, in 2009 there were 938 cases and 1068 cases in 2010. [6] Triple Negative breast cancer (TNBC) refers to any breast cancer that does not express the genes for estrogen receptor (ER), progesterone receptor (PR) and Human Epidermal Growth Factor Receptor 2 (Her2/neu). [7] In Triple Positive breast cancer (TPBC), these genes are positively expressed. About 75% of all breast cancers are ER positive, 65% PR positive, and 20 - 25% HER2/neu- positive. [8, 9] Luminal A breast cancer is either estrogen- and/or progesterone-positive and HER2-negative breast cancer. [10] Luminal B breast cancer is either estrogen- and/or progesterone-positive and HER2-positive breast cancer. [10, 11] HER2 over-expressing breast cancer is either estrogen- and/or progesterone-negative and HER2-positive breast cancer. [12-14]

Beta catenin is part of a complex of proteins that constitute adherens junctions. [15, 16] B-catenin plays a role in cell-cell adhesion by controlling cadherin-mediated cell adhesion at the plasma membrane and by mediating the interplay of adherens junction molecules with the actin cytoskeleton. [17-19] Adherens junctions are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. [20, 21] B-Catenin also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete. [22, 23] B-catenin also serves as a pivot between the roles of cell adhesion and gene transcription. The switch between these two cellular functions is controlled by several factors, including the tyrosine phosphorylation/de-phosphorylation of  $\beta$ -catenin. [24] The proper regulation of these two functions has been shown to be crucial for the stages of normal development, with loss of regulation being linked to many

malignancies. Increased  $\beta$ -catenin levels have been noted in several cancers, including breast carcinoma. [25, 26]

This study aimed to identify expression of beta catenin in breast cancer lesions in Sudanese patients by using immunohistochemical staining and then correlation with the clinical and pathological findings.

## MATERIALS AND METHODS

This was a retrospective descriptive study of 100 formalin-fixed paraffin-embedded tissue blocks of breast cancer that obtained from the histopathology department of the Radiation and Isotopes Center-Khartoum (RICK) during the period between July and December 2012; the clinical data of patients was obtained from the hospital medical records.

From each tissue block, 3 $\mu$ m- thick sections were cut and put on special-purpose slides (Dako) for immunohistochemical staining. Procedure was carried out using monoclonal antibody for Beta catenin. Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval PT link. Briefly, slides were placed in a tank containing enough sodium Tris buffer (pH 9.0) to cover the sections, boiled at high Temp for 20 minutes, and then sections were allowed to cool at RT. Endogenous Peroxidase activity was blocked with 3% hydrogen Peroxidase and methanol for 10 min, then slides were incubated with 100-200  $\mu$ l of the primary antibody for Beta catenin (Dako, Carpintera) for 20 minutes at room temperature in a moisture chamber, and then rinsed in Phosphate buffer saline. After washing with PBS for 3 min, binding of antibodies was detected by incubating for 20 minutes with dextrin labeled polymer (Dako- Envision TM Flex kit). Finally, the sections were washed in three changes of

PBS, followed by adding 3, 3 Diaminobenzidine tetra hydrochloride (DAB) (Dako) as a chromogene to produce the characteristic brown color for visualization of the antibody/enzyme complex for up to 5 minutes. Slides were counterstained with hematoxylin.

All quality control measures were considered throughout study procedures. 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 were incubated with PBS instead of the primary antibody. All slides were evaluated under a light microscope and scored.

All histological sections showed fair staining quality; positive Beta Catenin staining was identified in form of brown membranous staining. The obtained results and variables were arranged in standard

master sheet, and then were entered into a computer program SPSS and analyzed.

## RESULTS

All the patients included in this study were females, ranged in age between 31 – 55 years. The histological diagnosis of cases included invasive ductal carcinoma (78%), mucinous carcinoma (10%) and medullary carcinoma (12%). About 32% of cases were within grade II, 43% within grade III, and 25% of cases were ungraded.

The molecular classification of breast cancer lesions in this study included 39 cases TNBC, 21 cases TPBC, 24 cases HER2-positive breast cancer, 9 cases luminal- A breast cancer, and 7 cases luminal- B breast cancer.

The beta catenin expression was positive in 68% of cases and negative in 32% of cases. In correlation with age, expression of beta catenin was prominent in the age groups (36-40) and (51-55). (Table 1)

Table (1): Expression of beta catenin according to age groups.

	31-35 y	36-40 y	41-45 y	46-50 y	51-55 y	Total
Negative Beta Catenin	9	11	0	3	9	32
Positive Beta Catenin	16	20	6	5	21	68
Total	25	31	6	8	30	100

Table (2): Expression of beta catenin according to classification of cases.

	IDC	Mucinous Ca	Medullary Ca	Total
Negative Beta Catenin	32	0	0	32
Positive Beta Catenin	47	10	11	68
Total	79	10	11	100

Table (3): Expression of beta catenin according to grade of tumors.

	Grade II	Grade III	Ungraded	Total
Negative Beta Catenin	13	17	2	32
Positive Beta Catenin	19	26	23	68
Total	32	43	25	100

Table (4): Expression of beta catenin according to molecular classification of breast cancer lesions.

	TNBC	TPBC	HER2	LA	LB	Total
Negative Beta Catenin	7	15	1	4	5	32
Positive Beta Catenin	32	6	23	5	2	68
Total	39	21	24	9	7	100

In correlation with histological diagnosis, the expression of beta catenin was positive in all cases of mucinous and medullary

carcinoma and it was positive in about 60% of invasive ductal carcinomas. (Table 2)

In correlation with tumor grade, the expression of beta catenin was positive in about 59% of grade II tumors, 60% of grade III tumors, and 92% of the ungraded tumors. (Table 3)

In correlation with the molecular classification, the expression of beta catenin

was positive in 82% of TNBCs, 29% of TPBCs, 96% of Her2 tumors, 56% of luminal- A tumors, and 29% of luminal- B tumors. (Table 4)

## DISCUSSION

In this study, beta catenin expression was positive in 68% of cases, mainly in high grade tumors, and mostly in TNBCs and HER2 breast cancers. These findings are almost similar to findings in several other studies.

Karayiannakis AJ et al evaluated expression of beta-catenin in 121 breast cancer specimens by immunohistochemistry and reported that altered beta-catenin expression was found in 68% of tumors, most of them were of high grade. [27]

Lin SY et al studied 123 breast cancer specimens and found that 53% of cases were positive for beta catenin expression and that high b-catenin activity significantly correlated with poor prognosis. [28] Dalia M. and Moatasem M. studied 65 formalin-fixed paraffin-embedded primary invasive ductal breast carcinomas and found that positive staining for  $\beta$ -catenin was observed in 63% of cases, significantly associated with large tumor size, high grade, advanced stage, lymph node metastasis, and TNBC. [29]

López-Knowles et al undertook an immunohistochemical study measuring the levels and localization of  $\beta$ - catenin in 276 invasive ductal breast cancers. High beta catenin expression was found to be associated with high tumor grade, large tumor size, lymph node metastasis; negative estrogen receptor (ER), negative progesterone receptor (PR), positive HER2, and luminal- A breast tumors. [30] Khramtsov et al analyzed 190 breast cancer tissues containing luminal- A, luminal- B, HER2-positive, and ER-negative breast cancers and reported high expression of  $\beta$ - catenin in all these subtypes. [31]

FC Geyer et al performed immunohistochemistry on 245 invasive breast carcinoma tissues and found that beta catenin expression was high in about 69% of cases; they were significantly associated with estrogen receptor negativity and tumors of triple-negative phenotype with poor clinical outcome. [32] Wen-Huan Xu et al investigated the prognostic importance of dickkopf-1(DKK1) and beta-catenin expression in 85 cases of triple negative breast cancer. Staining of beta-catenin was observed in 65% of the patients. [33]

## CONCLUSION

High expression of beta catenin in triple negative cancer, Her2 positive cancer, and high grade tumors confirms its importance as a prognostic marker for breast cancer in Sudanese patients, as the case in other populations.

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