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RESEARCH ARTICLE

KI-67(LI) EXPRESSION AMONG SUDANESE WOMEN WITH TRIPLE NEGATIVE AND TRIPLE POSITIVE BREAST CANCER

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ABSTRACT

Aims: The aim of this study was to evaluate the expression of Ki-67(LI) in breast cancer lesions among Sudanese females.
Study design: Cross-sectional descriptive study.
Place and Duration of Study: Radiation and isotope center at Khartoum (RICK), Sudan, during the period between October 2012 and September 2013.
Methodology: Tissue sections obtained from 200 formalin-fixed paraffin-embedded tissue blocks of breast cancer lesions were immunohistochemically stained using monoclonal antibody (MIBI) for Ki-67.
Results: The study showed significant differences in expression of Ki-67 in different grades of triple positive and triple negative breast cancer lesions.
Conclusion: Expression of Ki-67 in breast cancer is an important prognostic factor as it significantly associated with aggressive tumors.

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INTRODUCTION

Breast cancer is now recognized as a heterogeneous disease with different biological behaviors that requiring distinct therapeutic strategies (Weigelt *et al*., 2005). It is a worldwide health problem with the number of cases in sub-Saharan Africa, including Sudan, is rising (Elgaili *et al*., 2010). The highest frequencies were recorded in 1998 (38.4% of all female cancers), followed by the years, 2000, 1999 and 2001, which attended 36.03%, 35.2% and 32.4% respectively (Ahmed and Hussein, 2009). Number of breast cancer cases in Sudan during the period between 2000 and 2009 was 6622 cases (17.2% of all cancers in Sudan) (Records of the Radiation and Isotopes Center Khartoum (RICK), Sudan, 2010). The first Sudanese National Cancer Registry (NCR) was established on 2009; the first data showed that breast cancer was the most common type of cancer in Sudan with an incidence rate of (25.1/100,000) (Saeed *et al*., 2014). Triple negative breast cancer (TNBC) is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2 neu) (Lund *et al*., 2009; Bauer *et al*., 2007). 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 (Haffty *et al*., 2006). The antigen KI-67 is a nuclear protein that is strictly associated with cell proliferation (Bullwinkel *et al*., 2006). During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes (Scholzen and Gerdes, 2000). Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index LI) is often correlated with the clinical course of cancer. The best-studied examples in this context are carcinomas of the prostate, brain, breast and nephroblastoma (Rahmanzadeh *et al*., 2007). Ki-67 positivity is associated with higher probability of relapse in breast cancer patients (De Azambuja *et al*., 2007; Jung *et al*., 2009).

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Table 1. Results of the study

Parameters → → → / Type of ↓ cancer ↓	Age less than 40	Age more than 40	LN +ve	LN -ve	G 1	G 2	G 3	Score 1 Ki-67	Score 2 Ki-67	Score 3 Ki-67
TNBC	83	17	95	5	8	24	68	6	25	69
TPBC	39	61	23	77	39	52	9	72	24	4

$P < 0.05$

The present study aimed to evaluate the expression of KI-67 LI in triple negative (TN) and triple positive (TP) breast cancer in Sudanese female patients.

MATERIALS AND METHODS

A total of 200 formalin-fixed paraffin- embedded tissue blocks from breast cancer lesions were included in this study. They were obtained from the histopathology archives of the Radiation and Isotopes Center at Khartoum (RICK) during the period between October 2012 and September 2013. Clinical data of patients was obtained from the hospital medical records. Using a rotary microtome, two 3µm- thick tissue sections were cut from each block and put on two slides, one was stained by Mayer's hematoxylin and eosin stain for confirmation of the diagnosis obtained from the records, and the other salinized slide (Dako) was stained using monoclonal antibody for Ki-67 LI. Immunohistochemistry staining procedure was as follows:following deparaffinization in xylene, slides were rehydrated through graded series of alcohol, placed in running water, steamed for antigens retrievals (PT link),placed in a tank containing enough sodium Tris buffer (pH 9.0) to cover the section, and then boiled at high temperature for 20 minutes and allowed to cool at room temperature (RT). Endogenous Peroxidase activity was blocked using 3%hydrogen peroxidase and methanol for 10 minutes, and then slides were incubated with 100-200 µl of primary antibody for Ki-67 (Dako, Carpintera). After washing with PBS for 3 minutes, binding of antibodies was detected by incubating for 20minutes with dextran labeled polymer (Dako En Vision TM Flex kit). Finally, sections were washed in three changes by PBS, and followed by adding 3,3Diaminobenzedine tetra hydrochloride (DAB) as a chromogne to produce the characteristic brown color for visualization of the antibody/ enzyme complex for up to 5 minutes. Slides were then counterstained with heamatoxylin.
For each run of staining, positive and negative control slides were also prepared; positive control slides contained the antigen under investigation and negative control slides were prepared from the same tissue blocks but incubated with PBS instead of the primary antibody. Slides were evaluated by light microscope and scored. All tissue sections showed fair staining quality and all quality control measures were considered throughout study procedures. Positive Ki-67 expression was identified as dark brown nuclear staining. Scoring of Ki-67 had been based on Ki-67 labeling index. Statistical Package for Social Science (SPSS) version 20 computer software was used for data storage and analysis; frequencies were counted and data was compared and correlated using t. test and confirmed by analysis of variance (ANOVA).Parameters analyzed were age, tumor grade (G), lymph node involvement (LN), and Ki-67 labeling index (LI) expression.

A two-sided P value of < 0.05 was considered to indicate the statistical significance. Overall study was approved by Radiation and isotope center at Khartoum (RICK) and the Council of Ethical Clearance at Neelain University, Khartoum, Sudan.

RESULTS

All lesions in this study were invasive ductal breast carcinoma, 100 of them were triple negative (TN) and 100 were triple positive (TP) breast cancer (BC). Most of the patients with TNBC were under 40 years of age while most of the patients with TPBC were over 40 years of age. More than 90% of patients with TNBC had lymph node (LN) metastases while more than 75% of TPBC patients were without lymph node involvement. Considering tumor grade (G), about two-thirds of TNBC tumors were into grade 3 while only 9% of TPBC tumors were diagnosed as grade 3 tumors.

Ki-67 (LI) expression was categorized into three scores: low (<15 %), moderate (16%- 30%), and high score (> 30 %). Most of TNBC tumors (69%) were of high score while most of TPBC tumors (72%) were of low score.

These findings are shown in Table 1.

All differences between TNBC and TPBC were statistically significant ($P< 0.05$).

DISCUSSION

Clearly, the study showed significant differences between TNBC and TPBC in expression of Ki-67 LI. Findings of this study agree with several world-wide similar studies. In wald *et al.* (2013) analyzed Ki-67 expression in 3,658 patientsw ith invasive breast cancer. Similar to our study, they found the tumors more aggressive with high expression of Ki-67; they found that the 5-year survival rate was 86.7 % in patients with a Ki-67 value less than 15 % compared to 75.8 % in patients with a Ki-67 value more than 45 %. Feng-yan Li *et al*. (2014) retrospectively reviewed the medical records of 1131 Chinese BC patients and reported that Ki-67 expression was associated significantly with histological grade, ER, PR, HER2 status and overall survival of the patient. Saroona Haroon *et al*. (2013) aimed to determine associations of the Ki67 index with other prognostic markers like tumor size, grade, lymphnode metastasis, ER, PR and HER2neu status in 194 cases of newly diagnosed breast cancer; significant association was found between Ki67 and tumor grade, PR, HER2neu positivity and lymph node status. However, they disagreed with our study in that tumors with Ki67 higher than 30% had better prognostic profile compared to tumors with intermediate Ki67 level, as

reflected by slightly lower frequency of lymph node metastasis and HER2neu expression. In a review of the literature between 1990 and 2010, Elisabeth Luporsi *et al*. (2012) screened 314 references focusing on the prognostic and predictive value of Ki-67 and reported that KI-67 could be considered as a prognostic biomarker for therapeutic decision; however, international guidelines are needed for routine clinical use. Munzone *et al*. (2012) investigated the usefulness of Ki-67 labeling index (LI) for the identification of different prognostic subgroups in primary node-negative, triple negative breast cancer (TNBC) in 496patients. They reported that Ki-67LI was increased with increasing tumor size ($P < 0.01$) and grade ($P < 0.01$), and that the risk of death from BC increased steeply with increasing Ki-67LI up to about 35 %.

Conclusion

There are significant differences between TNBC and TPBC in expression of Ki-67 LI. The study confirmed the association of high expression of Ki-67 with aggressiveness of breast cancer.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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