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RESEARCH ARTICLE

THE ASSOCIATION OF ki67 PROLIFERATION MARKER WITH ESTROGEN, PROGESTERONE AND HER2 MARKERS AMONG SUDANESE FEMALES WITH BREAST CANCER

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ABSTRACT

Background: Breast cancer aggressiveness can be correlated with proliferation status of tumor cells, which can be ascertained with tumor grade and Ki67 indexing.

We therefore aimed to determine associations of the Ki67 index with other prognostic markers like tumor size, grade, ER, PR and HER2 status.This study aimed to determine the association of ki67 tumor marker with ER, PR, and HER2 markers among Sudanese females with breast cancers during January to august 2012.

Methods: This is a descriptive retrospective study, aimed to detection the association of ki67 tumor marker with PR, ER, HER2 as well as clinicopathological data of breast cancer among Sudanese female attending Radiation and isotope center-Khartoum. 50 Paraffin blocks that were previously diagnosed as breast carcinoma were randomly selected and each paraffin block two sections of 3µm thick one for H&E stain and anther to immunohistochemicaln stain was performed by the DAKO envision method.

Results: out of 50 Paraffin blocks that were previously diagnosed as breast carcinoma, 47(94%) show invasive ductal carcinoma, the majority of the samples 31 (62%) were found to be grade III, the age of the involved patients range between 20 to 80 years old; the majority of the patients (more than 80%) were between the age group 41 to 50 years old, Twenty nine out of 50 sample (58%) were positive for ki67and the remaining 21(42%) were not expressed the marker.

Conclusion: the study present the ductal carcinoma is the most common 47(94%) among 50 Sudanese patients suffering from breast cancer, most of the cases diagnosed with grade III.

Keywords: breast cancer,ki67**,** progesterone receptor**,** estrogen receptor**,** HER2-neu, Sudanese females, haematoxylin and eosin, Immunohistochemical staining.

INTRODUCTION:

Breast cancer (BC) is a common malignant neoplasm comprising a large heterogeneous group of cancers with variable histological types, biological and clinical characteristics[(1)].

Breast cancers is the most commonly diagnosed cancer in women worldwide , and is second only to lung cancer as a leading cause of mortality. It is found in high rates in developing countries as well as low-middle economic countries[(2)] .

In Sudan it is more common in younger age contrary to the West where it is more common in old age, after 60 years[(3)].

Breast cancer refer to cancers originate from breast tissues, most likely from inner lining of milk ducts or the lobules which supply the duct with milk, cancer originate from duct called ductal carcinoma and that originate from lobes called lobular carcinoma[(3)]. some breast cancer requires hormones for its growth estrogen and progesterone receptor, HER2-neu has a receptor also in breast, over expression of HER2-neu leads to developing breast cancer[(4)].

Recently, breast cancer incidence and mortality has been rising. Together with cervical cancer, breast cancer comprises 50% of women's cancer in the Sudan . Breast cancer in Sudanese women is characterized by a younger age at onset, and about 78% present with advanced stages of disease at diagnosis (stages III, IV) [(5)].

Immunohistochemical studies in sporadic cancers have led to identification of novel targets with roles in diagnosis, prognostic and therapeutics. Examples include hormones (e.g. estrogen receptor) and growth factor

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receptors (e.g. epidermal growth factor receptor), tumor-specific oncogene products e.g (HER-2) [6].

Another molecular marker that we can measure (and there are many) is the HER2/neu receptor. When HER2/neu is present in large amounts on the cancer cells, it confers a more aggressive biologic behavior on the tumor cell. We have very effective targeted therapy against HER2/neu, and the name of that treatment is Herceptin (chemical name: trastuzumab). Herceptin is a biologic treatment, not a chemotherapy drug; rather an antibody that blocks the HER2/neu receptor on the cancer cells. And Herceptin shuts down the signal from HER2/neu. In the so-called triple-negative breast cancer, we do not have ER, PR, or HER2/neu as biologic targets. Therefore, we cannot use hormonal therapy or Herceptin in treating this form of breast cancer.

At the present time the standard therapy for triple negative breast cancer is chemotherapy Ki67 is a protein that is present during all active phases of cell division. Ki67 analysis is typically used to evaluate tumor cells that are positive for the protein in order to determine the aggressiveness of the Cancer.

Prostate and Breast Cancer are the two most common Cancers for which the Ki67 protein is evaluated. In evaluating Breast Cancer tumor cells, a high percentage of Ki67-positive tumor cells are associated with aggressive Cancer and predictive of poor prognosis[7].

Our study done to determine the association of ki67 tumor marker with ER, PR, and HER2 tumor markers among Sudanese females with breast cancers (correlate the ki67 intensity with breast tumor type and grade).

MATERIALS AND METHOD:

DESIGN :

This is a descriptive retrospective study, aimed to detection the association of ki67 tumor marker with PR, ER, HER2 as well as clinicopathological data of breast cancer among Sudanese female attending Radiation and isotope center-Khartoum ,Fifty paraffin wax blocks were enrolled.

Subject selection:

50 Paraffin blocks that were previously diagnosed as breast carcinoma were randomly selected. Obtained from patient attending Radiation and isotope center-Khartoum (RICK).

EXPERIMENTAL WORK:

COLLECTION OF SPECIMEN & PREPARATION:

From each paraffin block two sections of 3µm thick were cut sections floated into preheated floating water bath at 40˚c, one slides were coated with adhesive salinized glass slide for Immunohistochemistry the other one was placed in clean microscopical slide for H&E, incubated in oven at 58˚c.

STAINING PROCEDURES:

HAEMATOXYLIN AND EOSIN:

as described by Mayer's (1903) will be used in which Sections will be taken to distilled water, stain with haematoxylin and eosin, then slides will mounted in D.P.X and examine primary by investigator and then results will be confirmed with histopathologist to verify that an adequate number of breast cancer cells were present and to classify it morphologically and that the quality of fixation was sufficient for immunohistochemical analysis.

IMMUNOHISTOCHEMICAL STAINING:

Procedure was carried out using monoclonal mouse anti human ki67, cloneMIB-1, code IS62630

The immunohistochemical procedure will be done as follows:

Sections (3µm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto salinized slides (Dako). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and was placed in running water. Section taken for antigen retrieval for KI67 slides were placed in Coplin jars containing enough sodium citrate buffer (pH 9.0) to cover the sections, then were boiled at high Temp for 10minutes then sections were cooled at RT.. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 minute ,then Slides will be incubated with 100-200 µl of primary antibodies for 20 minute at room temperature in a moisture chamber, and then will be rinsed in Phosphate buffer saline. The primary antibody for,KI67 (monoclonal) was ready to use (Dako, Carpintera). After washing with PBS for 3 min, binding of antibodies was detected by incubating for 20 minutes with dextran labelled 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 chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides will be counterstained with haematoxylin. For each run of staining, positive and negative control slides were also prepared. The positive control slides were contain the antigen under investigation and the negative control slides was prepared from the same tissue block, but will be incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results will be confirmed by consultant histopathologist and scored

All histological sections showed fair staining quality and all quality control measures was considered throughout study procedures.

Positive KI67 staining was identified in form of dark nuclear staining. Scoring for KI67 was based on percentage of nuclei stained.(<20% consider negative)

The obtained results and variables were arranged in standard master sheet, then will be entered a computer program SPSS and analyzed.

STATISTICAL ANALYSIS:

The obtaining results, as well as all clinical information data were entered a computer, SPSS (version 11.5) program was used to analyze data.

RESULTS:

In this descriptive retrospective study, the ki67, ER , PR and HER2 markers were investigated in 50 breast paraffin wax embedded blocks, of which 47 (94%) were invasive ductal carcinoma, 2(4%) were mucinous carcinoma, and the remaining one sample (2%) was papillary carcinoma Table (1) .

As shown in Table (2), the age of the involved patients range between 20 to 80 years old; the majority of the patients (more than 80%) were between the age group 41to 50 years old.

Table (3) described the demonstrated samples as follow, one sample was describe as grade –I, ten samples (20%)were describe as grade II, the majority of the samples 31 (62%) were found to be grade III, the remaining 8(16%) were described as not graded tumor.

The expression of the ER, PR and HER2 markers was described by Tables (5, 6 and 7); 27 (54%) of the study samples were positive for ER, 15(30%) were positive for PR, while for HER2 the result was as follow; 28 samples (56%) were expressed the marker, of which seven samples (14%) were +, ten samples (20%) were ++, 11 samples (22%) were +++.

Twenty nine out of 50 sample (58%) were positive for ki67and the remaining 21(42%) were not expressed the marker.

The positivity of ki67marker (>20) among the different pathological conditions was shown in Table (4); 47 (94%) of the samples were invasive ductal carcinoma, 29 (58%) out of them were positive for the marker and 18 (36%) were negative, only one sample was papillary carcinoma and show negative result, the remaining two samples were mucinous carcinoma, both samples were negative for ki67.

The association between ki67 and ER, PR, and HER2 was summarized in Tables (5, 6 and 7); 16(32%) out of 27(54%) of the samples positive for ER were expressed ki67, ten (20%) out of 15(30%) of the samples positive for PR were expressed ki67. For HER2; seven samples(14%) were (+) for HER2, three out of them (6%) were expressed ki67, ten (20%) of the samples were (++) for HER2, of which 8(16%) were positive for ki67, and 11(22%) of the samples their intensity for HER2 was (+++), 8(16%) were positively for ki67, the remaining g 22 samples which were negative for HER2 , 11 out of them were expressed the ki67 and the other 11samples were not.

Table 1: Classification of study population by diagnosis

Diagnosis	Frequency	Percent
Ductal carcinoma	47	94.0
Mucinous carcinoma	02	04.0
Papillary carcinoma	01	02.0
Total	50	100.0

Table 2: Distribution of study population by age (years)

Age group	Frequency	Percent
20-30	02	04.0
31-40	10	20.0
41-50	20	40.0
51-60	13	26.0
61-70	04	08.0
71-80	01	02.0
Total	50	100.0

Table 3: The description of study population by tumor grade

Grade	Frequency	Percent
Grade I	01	02.0
Grade II	10	20.0
Grade III	31	62.0
Not graded	08	16.0
Total	50	100.0

Table 4: Diagnosis and Ki67 correlation

Diagnosis	KI67		Total
	<20%	>20%	
Invasive ductal carcinoma	18 36%	29 58%	47 94%
Invasive papillary carcinoma	01 2%	0.0 0.0%	01 2%
Mucinous carcinoma	02 4%	0.0 0.0%	02 4%
Total	21 42%	29 58%	50 100%

Table 5: ER and KI67

ER expression	**KI67**		**Total**
	<20%	>20%	
Positive	11 22%	16 32%	27 54%
Negative	10 20%	13 26%	23 46%
Total	**21 42%**	**29 58%**	**50 100%**

p. value = 0.11

Table 6: PR and KI67:

PR expression	Ki67		Total
	<20%	>20%	
Positive	5 10%	10 20%	15 30%
Negative	16 32%	19 38%	35 70%
Total	21 42%	29 58%	50 100%

Table 7: HER2 and Ki67

HER-2 expression	KI67		Total
	<20%	>20%	
+1	4 8%	3 6%	7 14%
+2	3 6%	7 14%	10 20%
+3	3 6%	8 16%	11 22%
_ve	11 22%	11 22%	22 44%
Total	**21 42%**	**29 58%**	**50 100%**

DISCUSSION:

Breast cancer (BC) is a common malignant neoplasm comprising a large heterogeneous group of cancers with variable histological types, biological and clinical characteristics[1].

Breast cancers is the most commonly diagnosed cancer in women worldwide[2] , and is second only to lung cancer as a leading cause of mortality. It is found in high rates in developing countries as well as low-middle economic countries.

The management of breast cancer depends on a number of factors. These include tumor type and grade, the invasion of tumor, and the presence or absence of particular tumor markers (e.g. ER, PR[8].

The majority of the investigated samples were invasive ductal carcinoma (47 (94%)) of samples, this is match with international distribution of breast cancer types, worldwide the invasive ductal carcinoma is the most common breast cancer[9].

The present study revealed that; the majority of the diagnosed patients (more than 80%) were between the age group 30 to 60 years old. this finding support by Mahmood,(3) they found that, breast cancer is more common in younger age among Sudanese, which is not match with international finding as breast cancer is more common in older ages[10].

Thirty one out of fifty (62%) of the selected samples were diagnosed as grade –III, which indicate bad prognosis, this may be due to late in detection of the condition.

The expression of the demonstrated was shown as follow; 27 (54%) of the study samples were positive for ER, 15(30%) were positive for PR, while for HER2 the result was as follow; 28 samples (56%) were expressed the marker, of which seven samples (14%) were +, ten samples (20%) were ++, 11 samples (22%) were +++, similar findings published by many authors'[7,11].

Ki67 is a proliferation marker, its positivity and intensity indicate bad prognosis, there fore it can use in follow up for therapy[7].

47 (94%) of the samples were invasive ductal carcinoma, 29 (58%) out of them were positive for the marker and 18 (36%) were negative, only one sample was papillary carcinoma and show negative result, the remaining two samples were mucinous carcinoma, both samples were negative for ki67, this finding indicate that the invasive ductal carcinoma show bad prognosis compared with other type of breast tumors.

Regarding the association of ki67 expression to other markers, our findings show no statistical association.

CONCLUSION:

- At the end of the present study we conclude that:
- The invasive ductal carcinoma is the most common breast cancer among Sudanese, most of the cases diagnosed with grade III.
- Breast cancer occur among younger age among Sudanese patients
- There were no differences between the different between the breast cancer types regarding their expression to ER, HER2 and KI67 markers.
- No statistic association between the ER,PR and HER2 markers and Ki67 expression.

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