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Assessment of HER2/neu among Female with Invasive Ductal Breast Carcinoma, Using Manual Tissue Microarray Technology in Khartoum State, Sudan

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ABSTRACT

The human epidermal growth factor receptor-2 (HER2), which is expressed in large amount in 20%-30% of breast cancer patients, is associated with disease recurrences, shortened disease survival, and bad prognosis. The aim of this study is to evaluate the expression of (Her2/neu) receptor as a diagnostic and prognostic marker in Invasive Ductal Breast cancer in Sudanese female patients. The study was performed on 35 archival paraffin blocks previously diagnosed as invasive ductal breast cancer (IDC), then a second diagnosis was done using IHC staining (Her2/neu). The obtained information is arranged in a standard master sheet, then analyzed using a statistical package for social science (SPSS) version 20 computer program. The age 30-45years was the most frequent age group 48.6% (17/35), followed by 46-60 years 40% (14/35). The majority of samples were grade III 74.3% (26/35) and the samples of grade II were 25.7% (9/35). the majority of TMA results were positive for Her2/neu receptor 65.7% (23/35) and the negative results were 34.3% (12/35) the majority of TMA score were (+) 31.4% (11/35), followed by (++) 17.2% (6/35), then (+++) 11.4% (4/35), then (++++) 5.7% (2/35), and the negative result were, 34.3% (12/35). There was a significant relation between H&E diagnosis with TMA result for Her2/ neu diagnosis that the P-value (0.0002). There was an insignificance relation between H&E diagnosis of grades with TMA results for Her2/ neu diagnosis that the P-value (0.08). In conclusion, HER2 is over-expressed in 65.7% of the patient while internationally it is over-expressed in 15-30%. This provides valuable prognostic and predictive implications in order to choose the suitable treatment for the patients.

Keywords: Breast cancer diagnosis; H&E; HER2/neu; Sudan.

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I. Introduction

Globally breast cancer is the second leading cause of death among women worldwide [1]. Breast cancer (BC) is an important disease that affects thousands of women in developed countries. It is estimated that approximately one woman out of ten is affected by the disease. Breast cancer incidence is low among women less than 25 years. After this age, the incidence rate starts to increase. One quarter of the breast cancer occurs in the age group of 25 to 49 years, also one quarter in women of 50 to 59 years of age, and the peak incidence in women over 60 years [2]. In Sudan data collected from cancer care health centers reflect that breast cancer incidence rate was 14 in every 100 individuals [3].

HER2 gene is normally found but produced in large amount in about 15–30% of invasive breast cancers, and therefore has both prognostic and predictive value. Breast cancers can have up to 25–50 copies of the HER2 gene, and up to 40–100-fold increase in HER2 protein resulting in 2 million receptors expressed at the tumor cell surface. Also, the estrogen hormone has a role in the activation of, HER2 signaling. HER2 amplification is an early event in human breast tumorigenesis, and is seen in half of in situ ductal carcinomas, during progression to invasive disease and metastasis, nodal metastasis, and distant metastasis. HER2 sensitive to certain cytotoxic chemotherapeutic agents and resistance to certain hormonal agents [4]. Tissue microarray (TMA) began with the completion of the human genome sequencing project in early 2001 [5]. TMA technique assists in making large amounts of immunohistological samples of Paraffin tissue blocks, and their subsequent screening for proteins of interest. TMA technology uses novel sampling devices in order to construct an array of up to hundreds of tissue samples onto a single microscope slide, which can then be stained in a similar manner as a standard immune histological sample. Thus, allowing researchers to speed up the diagnosis, also reducing the reagent consuming and preserving most of the sample material for future use [6], [7].

II. The Study Design

This was a cross-sectional retrospective laboratory study, to evaluate the expression of (Her2/neu) receptor as prognostic marker in Invasive ductal breast carcinoma

samples, the study was conducted during the period from June to October 2018. Study samples were selected from Al-Rahma Center at Khartoum state.

The study was performed on 35 archival paraffin blocks previously diagnosed as invasive ductal breast cancer (IDC), then a second diagnosis was done using IHC staining (Her2/neu). The obtained information is arranged in a standard master sheet, then analyzed using a statistical package for social science (SPSS) version 20 computer program.

III. The Manual Tissue Microarray Technique (TMA)

The tissue microarray technique included a hollow needle used to remove tissue cores as small as 0.6 mm in diameter from regions of interest in paraffin-embedded. These tissue cores were then inserted in the recipient paraffin block in précised spaced, array pattern. Sections from this block were cut using a microtome, mounted on a microscope slide, and then analyzed by any method of standard histological analysis. Each microarray block could be cut into 100–500 sections which could be used for other tests [5].

IV. Immunohistochemistry Techniques (IHC)

One section (3 μm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto salinized slides (Thermo - USA). De paraffinization in xylene, slides were rehydrated through descending grades of ethyl alcohol; 100% (4 min), 90% (2 min), 70% (2 min), and water (2 min), and then placed in distilled water. Antigen retrieval by buffer (PH 0.9) using water bath (histoline- TEC 2601-Italy) at 95 ºC for 40 min. Washing in PBS for 3 min, then blocking of Endogenous peroxides activity with 3% hydrogen peroxide and methanol for 10 min (Dako – USA), and washing in PBS for 3min, then Slides were incubated with (100 μL) of mouse monoclonal antibody (Her2-Thermo) for 30 min at room temperature in a moisture chamber. Washing in PBS for 3 min, binding of antibodies to antigen will be detected by incubating for 20 min in dextrin labeled polymer (Dako – USA). The sections were washed in three changes of PBS, followed by adding 3, 3 di amino benzidine tetra hydrochloride (DAB) a chromogen for up to 5min to produce the characteristic brown colour for the visualization of the antibody/enzyme complex. Washing with distilled water for 3 min Slides were counterstained with Mayer ’s haematoxylin (RAL-faranca) for one min, were washed in running tap water for minutes 7–10 (bluing), then dehydrated in ascending grades of alcohol (70%, 90%, and 100%), cleared in xylene and mount in DPX [8].

A. Result Interpretation

The IHC staining used in this study, showed paled pink color in negative results. Otherwise, the positive result showed intensity and general cell morphology, and the receptor expression, which is localized at the plasma membrane takes brown color [8].

The diagnosis of Breast cancer was established depending on clinical examination and histopathological features of the biopsy. Then the results were confirmed by an experienced histopathologist.

V. Results

A. The Age of the Study Population

The patients' age ranged from 30 to 75 years. The age 30-45years was the most frequent age group 48.6% (17/35), followed by 46-60 years 40% (14/35), and 61-75 years 11,4% (4 /35) (Table I).

TABLE I: Shows the Age of the Study Population

Age	Frequency	Percent
30-45	17	48.6%
46-60	14	40%
61-75	4	11.4%
Total	35	100%

B. Histological Diagnosis Using H&E Stained Sections

The study showed the majority of samples were grade III 74.3% (26/35) and the samples of grade II were 25.7% (9/35) (Table II).

TABLE II: The histological Diagnosis Using H&E Stained Sections

Tumor Grade	Frequency	Percent
Grade II	9	25.7%
Grade III	26	74.3%
Total	35	100%

C. The Histological Diagnosis of IHC Using TMA for Her2/ neu

The study showed the majority of TMA results were positive 65.7% (23/35) and the negative results were 34.3% (12/35) (Table III).

TABLE III: The histological diagnosis of IHC using TMA for Her2/neu

Her2/ neu	Frequency	Percent
Her2/Positive	23	65.7%
Her2/Negative	12	34.3%
Total	35	100%

D. The Score of Immunostaining (Intensity)

The study showed the majority of TMA score were (+) 31.4% (11/35), followed by (++) 17.2% (6/35), then (+++) 11.4% (4/35), then (++++) 5.7% (2/35), and the negative result were, 34.3% (12/35) (Table IV).

TABLE IV: The Score of Immunostaining

Score	Frequency	Percent
+	11	31.4%
++	6	17.2%
+++	4	11.4%
++++	2	5.7%
Negative	12	34.3%
Total	35	100%

E. The Correlation between H&E Diagnosis with TMA for Her2/ neu Diagnosis

There was a significance relation between H&E diagnosis with TMA result for Her2/ neu diagnosis that the P-value<0.05(Table V).

TABLE V: THE CORRELATION THE BETWEEN H&E DIAGNOSIS WITH TMA FOR HER2/ NEU DIAGNOSIS

H&E Diagnosis	TMA for Her2/ neu	P-value
Grade II (9)	Positive (23)	significant
Grade III (26)	Negative (12)	0.0002
35	35	Total

F. The Correlation between the Tumor Grade and Score of Her2/ neu

There was insignificant relation between the score of Her2/ neu and the tumor grade the P-value >0.05 (Table VI).

TABLE VI: THE CORRELATION BETWEEN THE TUMOR GRADE AND SCORE OF HER2/ NEU

H&E Diagnosis	TMA for Her2/ neu Score	P-value
Grade II (9)	+(11)	Insignificant
	++(6)	0.08
Grade III (26)	+++(4)	
	++++(2)	
35	23	Total

VI. DISCUSSION

The age 30-45years was the most frequent age group 48.6% (17/35), followed by 46-60 years 40% (14/35). This agrees with Hamad *et al.* [9], Babiker *et al.* [10], Saleh *et al.* [11], Juneja *et al.* [12], who found the majority of breast cancer cases are in this age group.

The study showed the majority of samples were grade III, 74.3% (26/35) and the samples of grade II were 25.7% (9/35). This agrees with Hamad *et al.* [11], Galvez *et al.*, [13] and Sundquist *et al.* [14].

The study showed the majority of TMA results were positive 65.7% (23/35) and the negative results were 34.3% (12/35). This study agrees with Cooke *et al.* [15], who published that 85% of all breast tumor samples expressed HER2 at higher levels. Of these, 23% expressed HER2 at levels between 45 and 480 times greater than normal and had a relation with a bad prognosis.

There was a significance relation between H&E diagnosis of grades with TMA results for Her2/ neu diagnosis that the P.value 0.0002 (<0.05). This finding disagrees with Bushra Sikandar *et al.* [16], who published that no significant relation of grade with HER2/neu. This finding agrees with Rafil Toma Yaqo *et al.* [17]. they found that there was a significant relation between Her2 level and grade of breast cancer.

Maria Teresa Ramieri *et al.* [18] found a relation between HER2 protein expression and gene amplification in breast carcinoma.

The data of Bushra Sikandar *et al.* [16] demonstrate dreadful findings in breast cancer, patients from Pakistan, their results showed that there was a relation between early onset of breast cancer and bad prognosis of the disease. This provides valuable prognostic information in order to decide the treatment options.

The study showed the majority of TMA score were (+) 31.4% (11/35), followed by (++) 17.2% (6/35). There was insignificant relation between the score of Her2/neu and the tumor grade the P.value 0.08 (>0.05). The study agrees with Skaland *et al.* [19]. They published that no relation between Her2 3+ and FISH amplification with the score. This may be due to disadvantages in the FISH scoring protocol. There are several possible reasons for this, including errors in the staining protocol and inaccurate expression scoring.

This result does not agree with Keshgegian and Cnaan (1997), who had shown an association between moderate to strong cytoplasmic staining of HER2 and with bad prognosis [20]. There is a limitation of IHC scoring system, especially in cases with moderate (2+) membrane staining [21].

IHC analysis of HER2 is a simple-to-perform, available and cheap test. It is also affected by several factors including tissue-fixation methods, reagents, assay protocols, antibody sensitivities and specificities, and scoring systems [22]–[24].

HER2 IHC 2+, 3+ accounted for 28.69% and HER2 gene amplification was detected in 31% of cases [25]. HER2 gene amplification was statistically associated with cell proliferation index Ki67, but not significantly associated with another clinicopathological factor in breast cancer in Vietnamese women.

VII. CONCLUSION

The most frequent age group 30–45years, which accounts for 48.6%. Human epidermal growth factor receptor 2 (HER2) is over-expressed in 65.7% of the patient while internationally it is over-expressed in 15-30%. This provides valuable prognostic and predictive implications in order to choose the suitable treatment for the patients.

There was a significance relation between H&E diagnosis of grades with TMA results for Her2/ neu diagnosis, and also there was insignificant relation between the score of Her2/ neu and the tumor grade.

ETHICAL APPROVAL

Tissue blocks were used (the samples from the Bank), numbered samples, no patients' name.

Ethical approval for this study was obtained from the research ethical committee from the Gezira State Ministry of Health. The samples were collected after taking ethical acceptance from Al-Rahma Center at Khartoum State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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