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CORRELATION BETWEEN HER2/NEU AND HISTOPATHOLOICAL FINDING AMONG SUDANESE FEMALE BREAST CANCER PATIENTS

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ABSTRACT

Background: This study is one of very few studies which have investigated HER-2/neu expression and its correlation with histopathological finding. A total of 328 breast cancer tissue samples had been analyzed using immunohistochemical techniques. Objective: To compare the expression of tumor markers (HER-2/neu) with other prognostic parameters for mammary carcinomas. Results: Stage I- III B breast cancer HER-2/neu negative are (62%), whereas positive are (22%). Stage IV breast cancer HER-/neu negative are (4%), whereas positive are (3%). Recurrent breast cancer HER-2/neu negative are (3%) while positive are (5%).Stage I breast cancer HER-2/neu negative are (10%), whereas positive are (1%). Referring to stage II A, II B breast cancer HER-/neu negative are (59%), whereas positive are (16%). Stage III A, III B breast cancer HER-2/neu negative are (10%), while positive are (4%). HER-2/neu negative breast cancer Tumor size ⌈le⌉ 2 cm are (11%), while positive are (2%). HER-2/neu negative breast cancer Tumor size2-5 cm are (23%), whereas positive are (9%).HER-2/neu negative, Tumor size >5 cm are (26%), while positive are (29%). HER-2/neu negative breast cancer Nodal metastasis 0 are (5%), while positive are (2%). HER-2/neu negative Nodal metastasis1-9 are (16%), whereas positive are (19%). HER-2/neu negative breast cancer Nodal metastasis ⌈ge⌉ 10 are (28%), while positive are (30%). HER-2/neu negative histological subtype NIDC are (2%), whereas positive are (1%). HER-2/neu negative histological subtype IDC are (67%), while positive are (25%). HER-2/neu negative histological subtype ILC are (1%), whereas positive are (1%). Lastly, HER-2/neu negative with others histological subtype are (3%), whereas positive are (0%). HER-2/neu negative histological grade I are (6%), whereas positive are (2%). HER-2/neu negative histological grade II are (21%), while positive are (7%). Lastly, HER-2/neu negative histological grade II are (40%), whereas positive are (24%). HER-2/neu negative lympho-

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vascular invasion- are (71%), positive are (29%). HER-2/neu negative with lympho positive or vascular positive are (80%), While among positive are (16%). Conclusion: In this study population, the HER-2/neu expression its relation with histopathological finding determined.

Keywords: (HER2/neu), Histological grade, Tumor size, Histological subtype, Number of lymph node metastases, Histopathological finding, Breast cancer.

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Contribution/ Originality

This study contributes in the existing literature in Radiation Isotopes Centre Khartoum (RICK) among Sudanese female breast cancer patients. This study uses new estimation methodology of immunohistochemical techniques to understand HER-2/neu expression and its correlation with histopathological finding.

1. INTRODUCTION

The International Agency for Research on Cancer (IARC), the specialized cancer agency of the World Health Organization, in its World Cancer Report 2014 observed that as a consequence of growing and ageing populations, developing countries are disproportionately affected by the increasing numbers of cancers. Therefore more commitment to prevention and early detection is desperately needed in order to complement improved treatments and address the alarming rise in cancer burden globally [1].

The incidence of cancer in Sudan has nearly doubled from 2001 to 2007(Cancer registry record). As opined by several experts a potential surge in cancer cases in Sudan is likely to occur in immediate future [2]. Cancer of the breast cancer is the fifth most common neoplasm in the industrialized countries and in the Unites States, with an estimated increasing new cases of breast cancer diagnosed in 2010 [3].

With regard to Sudan population, there is a lag in studies related to major prognostic tumor markers and their correlation in the breast cancer patients in the Sudan to support therapeutic decisions. The present study thus aims to establish a correlation between tumor marker HER-2 and other prognostic factors prevalent in the population thereby providing a footprint of the disease. Such a tumor marker based correlation would be useful in newly diagnosed patients and also serve as predictive markers for disease progression and response to therapy.

Cancer is a disease characterized by abnormal growth and development of normal cells beyond their natural boundaries. Despite of global efforts to limit the incident of this disease, cancer has become the leading cause of death in the last 50 years [4]. The burden of cancer is increasing in economically developing countries as a result of population aging and adoption of cancer-associated lifestyle including smoking and physical inactivity. Based on the most recent report available, GLOBOCAN estimated that about 12.7 million cancer cases and 7.6 million cancer deaths have occurred in 2008; of these, 56% of the cases and 64% of the deaths occurred in

the economically developing world [5]. While cancer rates in general are decreasing in the United States and many western countries, they are increasing in less developed and economically transitioning countries [6].

Breast cancer results when cell in the breast begin to grow unregulated and out of control and can then invade near-by tissues or spread throughout the body. Large collections of this out of control dividing tissues are called tumors. However, some tumors are not really cancer because they cannot spread or threaten someone's life. These are called benign tumors. The tumors that can spread throughout the body or invade nearby tissue are considered cancer and are called malignant tumors [7].

Tissue biomolecular markers, aside from being prognostic and predictor factors also play a central role in targeted therapies that are among the emerging directions of cancer therapeutics [8]. Selective biomarkers may be able to define susceptibility risks and assist in tumor detection and diagnosis allowing timely therapeutic interventions for an effective treatment. In most cases, survival rates for patients with the cancers, especially those detected at an advanced stage, remain discouragingly low. Patients with early detection of cancer have better rate of recovery and survival than patients with more advanced cancer. In most cases, detection of stage 1 cancers is associated with a >90% five-year survival rate [9].

Human epidermal growth factor receptor-2 (HER2/erbB-2) belongs to a family of four transmembrane receptors involved in signal transduction pathways that regulate cell growth and differentiation. Overexpression/amplification of HER2 is associated with malignancy and a poor prognosis in breast cancer [10]. HER2 acts as a networking receptor that mediates signaling to cancer cells, causing them to proliferate. If the tumor is found to have the HER-2/neu receptor, special recommendations for targeted biological therapy might be recommended as part of the treatment. HER-2 negative is a favorable prognostic factor; being positive is not favorable [11].

Any uncontrolled growth of breast tissue cells, which has the capacity to spread, is breast cancer." Early" breast cancer is confined to the breast and the tumor is not larger than 5 cm (about 2 inches) across. It may also involve the lymph glands under the armpit, called the "axillary lymph nodes". It has not spread to any distant sites in the body, as far as can be told with today technology. "Locally Advanced" Breast Cancer is noted by a tumor greater than 5 cm across, or a fixed lump in the axilla representing cancer, ulceration of the skin from cancer, or involvement of the deep chest muscles. "Inflammatory Breast Cancer" is a hot, tender breast with skin looking like an orange peel, called "peau de orange" and almost always has spread to the axilla. "Metastasis" breast cancer means the disease has spread to other areas of the body, such as the lung, liver, brain, skin or bone.

Clinical factors of recognized prognostic importance for breast cancer recurrence and survival include lymph node involvement, tumor size, hormone receptor positivity, and several histological factors including tumor grade [12], [13].

Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behavior. Clinical and pathological variables such as tumor size, histologic grade, histologic type, lymph

node metastases, vascular space invasion, tumor cell proliferation, tumor necrosis, extent of ductal carcinoma in situ, age, and pregnancy may help in predicting prognosis and the need for adjuvant therapy [14].

2. MATERIALS AND METHODS

This is a descriptive study to evaluate the tumor marker (HER-2/neu) expression in malignant breast palpable lumps. The study conducted in Radiation Isotopes Centre Khartoum (RICK) during the period from January 2012 to August 2014. 328 females, all originating from the Sudanese, were eligible for analysis. An age-stratified random sample of 100 women was used as a control group, derived from the Breast Unit's database of screened patients who had not developed breast cancer after a median follow up period of 40 months (range 12–92 months).

2.1. Sample Collection

428 sections (328 cancer patients and 100 patients used as control), taken from patients with breast palpable lumps. For histopathology and immunohistochemistry biopsies collected from patients.

2.2. Sample Processing

2.2.1. For Histopathology

Biopsies will be collected form tissues, and stained in hematoxylin & eosin.

2.2.2. For Immunohischemistry

Sections were cut at 3-5 µm thicknesses, mounted onto salinized slides, and left to dry overnight at 37°C. Sections were then deparaffinized and rehydrated. Antigen retrieval was achieved by heat retrieval using a bench autoclave. Briefly, slides were placed in Coplin jars containing enough 0.01 M sodium citrate solution (pH 6.0) to cover the sections, then autoclaved at 121°C for 10 minutes for Her-2 (waterpath 95°C for 30 min). Slides were incubated with Peroxidase blocking reagent for 10min followed by protein blocking reagent for 10min, then rinsed in PBS Slides were incubated with 100-200 µl of primary antibodies for 30 minutes at room temperature in a moisture chamber, then rinsed in PBS. The dilution of the primary antibodies against Her-2/neu (Dako, Carpintera, Ca, USA) 1:50. After washing, binding of antibodies was detected by incubation for 10 minutes with biotinylated goat anti-mouse antibody ready to use (LSAB2) from Dako; the slides were then rinsed with PBS. Sections were then incubated with streptavidin-horse radish peroxidase for 10 minutes. Finally, the sections were washed in 4 times in 4 minute changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (Biogenex) as a chromogen to produce the characteristic brown stain.

For each run of staining, a positive and negative control slide were also prepared. The positive control slides were prepared from breast carcinoma known to be positive for the antigen

under study. The negative control slides were prepared from the same tissue block, but incubated with PBS instead of the primary antibody.

Her-2/neu was scored on a 0 to 3 scale according to the criteria set by Dako. The staining was scored as: negative (0) when no membrane staining was observed, or when membranous staining was observed in less than 10% of the tumor cells; weak positive (1+) if weak focal membrane staining was seen in more than 10% of the tumor cells; intermediate (2+) if weak to moderate, complete membrane staining was seen in more than 10% of the tumor cells; and strongly positive (3+) if intense membrane staining with weak to moderate cytoplasmic reactivity was seen in more than 10% of the tumor cells. In the final analysis, however, scores 0 and 1 were considered negative; score 2 was considered weakly positive; and score 3 was considered strongly positive. Only score 3 cases were considered as Her-2 overexpressing cases.

3. ASSESSMENTS OF RESULTS

Section will be examined by two different histopathologists for pathological conditions. Then compared with immunohistochemistry result.

3.1. Ethical Considerations

The aims methods of this study are fully explain to the patients and their consent to participate in this study is obtain. Sample will be taken form patient who consent to participate. The questionnaire filled in the presence of patient; the results of breast biopsy of histopathology and immunohistochemistry shown and discussed with the patients.

3.2. Statistical Analysis

Data will be analyzed using SPSS program.

Were calculated to measure the association of the HER-2/neu expression its relation with histological grade, tumor size and number of lymph node metastases.

4. RESULT

Stage I- III B breast cancer cases with HER-2/neu negative expression are 205 (62%), whereas in stage I- III B breast cancer cases with HER-2/neu positive status are 73 (22%). Referring to stage IV breast cancer cases with stage IV having HER-2/neu negative are 14 (4%), whereas in stage IV with HER-2/neu positive status are 10 (3%). Recurrent breast cancer cases with HER-2/neu negative status are 10(3%) while among recurrent breast cancer cases with HER-2/neu positive status are 16 (5%).

Stage I breast cancer cases with HER-2/neu negative expression are 27 (10%), whereas in stage I breast cancer cases with HER-2/neu positive status are 3 (1%). Referring to stage II A, II B breast cancer it shows cases with stage 1IA, 1IB having HER-2/neu negative expression are 164 (59%), whereas in stage IIA, IIB with HER-2/neu positive status are 44 (16%). Stage III A,

III B breast cancer cases with HER-2/neu negative status are 29 (10%) while among stage III A, III B cases with HER-2/neu positive status are 11 (4%).

HER-2/neu negative status cases with breast cancer Tumor size [le] 2 cm are36(11%), while HER-2/neu positive status and breast cancer Tumor size [le] 2 cm are 7 (2%).While HER-2/neu negative with breast cancer Tumor size2-5 cm are 76 (23%), whereas HER-2/neu positive status and breast cancer Tumor size2-5 cm are 22 (9%). While HER-2/neu negative status cases with breast cancer Tumor size > 5 cm are 91 (26%), while HER-2/neu positive status and breast cancer Tumor size>5 cm are 96 (29%).

HER-2/neu negative status cases with breast cancer Nodal metastasis 0 are 18 (5%), while HER-2/neu positive status and breast cancer Nodal metastasis 0 are 8 (2%). HER-2/neu negative with breast cancer Nodal metastasis1-9 **are** 51(16%), whereas HER-2/neu positive status and breast cancer Nodal metastasis1-9 are 62 (19%). HER-2/neu negative status cases with breast cancer Nodal metastasis [ge] 10 are 91 (28%), while HER-2/neu positive status with breast cancer Nodal metastasis [ge] 10 are 98 (30%).

HER-2/neu negative status cases with histological subtype NIDC are 7 (2%), whereas among HER-2/neu positive status with histological subtype NIDC are 4 (1%). HER-2/neu negative status cases with histological subtype IDC are 217 (67%), while HER-2/neu positive status with histological subtype IDC are 81 (25%). HER-2/neu negative status cases with histological subtype ILC are 4 (1%), whereas HER-2/neu positive cases with histological subtype ILC are 3 (1%). Lastly, HER-2/neu negative status cases with others histological subtype are 10 (3%), whereas HER-2/neu positive cases with others histological subtype are 0 (0%).

HER-2/neu negative status cases with histological grade I are 21 (6%), whereas among HER-2/neu positive status with grade I are 7 (2%).While HER-2/neu negative status cases with histological grade II are 71 (21%), in HER-2/neu positive status with grade II are 23 (7%). Lastly, HER-2/neu negative status cases with histological grade II are 127 (40%), whereas in controls HER-2/neu positive cases with grade III are 79 (24%).

HER-2/neu negative status cases with lympho-vascular invasion- are 155 (71%), while among HER-2/neu positive status with lympho negative and vascular negative are 63 (29%). Whereas, HER-2/neu negative cases with lympho positive or vascular positive are 88 (80%), While among HER-2/neu positive status with lympho positive or vascular positive are 22 (16%).

5. DISCUSSION

Overexpression of HER-2 determined was detected in of primary breast cancer and also found it in stages I-IIIB breast cancer cases. However, histological grades 2 and 3 were marginally associated with overexpression of HER-2. Similarly, we were unable to show a significant relationship between Her-2 expression and the histologic grade of breast carcinoma. It should be pointed out, however, that the low number of grade 1 carcinomas (seven cases Her-2 expression) in this study would not allow us to evaluate this variable with any degree of confidence.

The positive rate of HER-2 expression was higher in advanced breast cancer than in operable breast cancer. These results suggest that HER-2 overexpression of breast cancer is associated with aggressive behavior and that targeting therapies to neutralize HER-2 gene product may be more indicated for advanced breast cancer patients than for early breast cancer patients.

Her-2 was expressed in 25% of the infiltrating ductal carcinoma cases compared to only Her-2 was expressed in 1% of the none infiltrating ductal carcinoma and1% of our lobular carcinoma cases. This pattern of low Her-2 expression in lobular carcinoma is in agreement with data reported in the literature.

Our results show a tendency of Her-2 overexpression to be more associated with larger tumor size. Similarly, the fraction of tumors larger than 5 cm tended to have higher rates of Her-2 expression than those 2 to 5 cm in size (29% versus 9%).

Our data reveal that 41% of Her-2 overexpressing tumors had more than three lymph node metastases, as opposed to 39% of Her-2 negative cases, although this difference was not statistically significant.

6. CONCLUSION AND RECOMMENDATION

Their findings will provide us with greater insight into breast cancer an etiology and will help us identify any association that would help discriminate subgroups of HERR-2/neu over expression and histological grade, tumor size, histological subtype and number of lymph node metastases.

Further innovative studies with larger sample sizes are needed to examine how the status of this potentially modifiable HERR-2/neu over expression and histological grade, tumor size, histological subtype and number of lymph node metastases.

Lastly, we recommend further studies in this field with wider scope.

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