

Assessment of immunohistochemical Expression of BRCA1 among Patient with Invasive ductal carcinoma by using manual tissue Microarray, Sudan

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Abstract: BRCA1 gene is tumor suppressor gene, most inherited cases of breast cancer are associated with abnormal genes BRCA1. The function of the BRCA1 genes is to repair cell damage and keep breast cells growing normally. But when these genes contain abnormalities that may pass through generations⁽¹⁾. This is retrospective cross sectional study was aimed to assessment of immunohistochemical expression of BRCA1 among patients with invasive ductal carcinomas using manual tissue microarray at Khartoum state in period from July 2018 to October 2018. Fourty samples of tissue blocks of breast tumor more collected. Using tissue microarray breast sections were cut stained and examined using monoclonal antibodies of BRCA1. Data were analyzed using spss version 16 computer program. Result show the following 41-50 years was the most frequent age group 13(33.5%) and female above 60 years is less frequent age group 6(15.0 %). In patient aged between 30-70 years, with mean age 48.5 years. In immunohistochemical result showed 75% of cases positive with IHC and 25% negative. Also correlation between histological grade and expression of BRCA1 represent invasive ductal carcinomas grade (III) is most common than invasive ductal carcinomas grade (II). The study concluded that 75% of female positive affected with breast cancer and 25% of female was negative. The study recommended that all women undergo breast self examination monthly from the age of 20 and above.

Key words: 1-BRCA1 2-ductal 3- carcinomas 4- immunohistochemical 5- microarray 6- tissue

Introduction:

Breast cancer is the most commonly diagnosed cancer among women, with approximately 182,000 women diagnosed with breast cancer annually in the United States, accounting for approximately 26% of all incident cancers among women. Each year 40,000 women die of breast cancer, making it the second leading cause of cancer death, Among American women after lung cancer. The life time risk of dying of breast cancer is approximately 3.4 %⁽²⁾ (Graham A, 2015).

The international incidence of female breast cancer varies markedly, being highest in the United States and northern Europe, intermediate in southern and Eastern Europe and South American and lowest in Asia.

In recent years, incidence rates have been stable in white women and increasing slightly (by 0.3% per year) African American women. Breast cancer is more common in these women, compared to women of other races/ethnicities.

The incidence of breast cancer slightly higher for whites, but it has increased in blacks by 25%. the highest incidence of breast cancer occurs after age 35, with 83% of the cases occurring after age 50 and only 1.5 under age of 30⁽³⁾ (Rojas, 2016).

Breast cancer starts when cells in the breast begin to grow out of control. These cells usually form a tumor that can often be seen on an x-ray or felt as a lump. The tumor is malignant (cancer) if the cells can grow in to (invade) surrounding tissue or spread (metastasize) to distant areas of the body. Breast cancer occurs almost entirely in women, but men can get breast cancer, too.

Breast cancers can start from different parts of the breast. Most breast cancers begin in the ducts that carry milk to the nipple (ductal cancers). Some start in the glands that make breast milk (lobular cancers). There are also other types of breast cancer that are less common.

Breast cancer can spread when the cancer cells get in to the blood or lymph system and are carried to other parts of the body⁽⁴⁾ (Marchant, 1982).

If cancer cells have spread to lymph nodes, there is a higher chance that the cells could have traveled through the lymph system and spread (metastasized) to other parts of your body.

Change or mutations in DNA can cause normal breast cells become cancer because of changes (mutations) in DNA. Some DNA mutations are inherited or passed to you from your parents. But most DNA changes linked to breast cancer are acquired. This means the change takes place in breast cells during a person's life rather than having been inherited or born with them. Acquired DNA changes take place over time and are only in the breast cancer cells⁽⁶⁾ (Ganesh, 2010).

About 85 percent of breast cancers begin in the mammary ducts, while about 15 percent arise in the lobules. Tumors in the breast tend to grow slowly. By the time a lump is large enough to feel, it may have been growing for as long as 10 years. However, some tumors can be aggressive, and grow much more rapidly. Breast cancer may grow from a tiny tumor to a large one, later traveling to nearby lymph nodes.⁽⁷⁾ (Christopher, 2010).

Material and Method

Study area

Study was conducted in Khartoum state , Sudan .

Study design

Retrospective cross sectional laboratory based study, use tissue microarray technology to assessment of immunohistochemical expression of BRCA1 among patients with invasive ductal carcinomas 2018.

Study population

Women with invasive ductal carcinomas.

Inclusion criteria

Study inclusion women with breast cancer. Type invasive ductal carcinomas

Exclusion criteria

Study exclusion women who do not have breast cancer.

Women have breast cancer. other Type

Sample size

40 samples collected from women with breast cancer. Type invasive ductal carcinomas

Collection of sample

Formalin fixed, paraffin –embedding tissue blocks containing breast cancers Type invasive ductal carcinomas along with their corresponding heamatoxylin and eosin stained slides

Data collection

Personal data obtained from archives of Alrahma center.

Data analysis

Data analyzed by using statistical package for social science V. 16

Ethical consideration

The study protocol approved by ethical committee of Algezira university permission of general managers of hospital and permitted center will take and all participants will be known with this study.

Method procedure:

In the tissue microarray technique, a hollow needle is used to remove tissue cores as small as 0.6mm in diameter from regions of interest in paraffin –embedded tissues such as clinical biopsies or tumor samples. These tissue cores are then inserted in recipient paraffin block in précised spaced, array pattern. Sections from this block are cut using a microtome, mounted on a microscope slide and then analyzed by any method of standard histological analysis. Each microarray block can be cut in to 100-500 sections, which can be subjected to independent tests. Tests commonly employed in tissue microarray include immunohistochemistry . Tissue microarrays are particular useful in analysis of cancer samples (Olli P.2001).

Sample processing:

Specimen were collected from Khartoum hospital and preserved in 10% formalin solution. They were examined grossly for signs of inflammation, any swelling hemorrhage or perforation and measurements were recorded. Three cut sections were made at the following sites: base, tip and middle of the breast specimen as well as from any other abnormal region. Selected pieces of tissue have been inserted in a plastic cassette and labeled, then processed in tissue processor according to the following procedure. Sample were dehydrate through 50% ethanol for half an hour, 70% ethanol for one hour, 90% ethanol for one hour, and two changes of absolute ethanol for two and half hour, then were cleared in two changes of xylene for four hours, then impregnated in paraffin wax for six hour, remove paraffin core from donor paraffin block and insert tissue cores in a recipient block , place the block in oven 60c for 30 min, then sectioning in slide by using rotary microtome.

Heamatoxylin and eosin:

The sections were taken to water (dewax in xylene 4 min and rehydrated in ethyl alcohol 100% 4 min, 90% 2 min, 70% 2 min, and water 2 min), then the nucleus was stained by harrisheamatoxylin for 5 min, washing in running tap water until become blue, then

differentiated in 1% acid alcohol(1% acetic acid in 70% ethyl alcohol) for 5-10 second, then were washed in running tap water for several min 7-10(bluing) and the cytoplasm was stained in 1% eosin for 5 min, then wash in running tap water for 1-5 min ,finally dehydrated through alcohols, cleared, and mounted(Bancroft, 2008).

Immunohistochemistry techniques:

The immunohistochemical procedure was done as follows: one section (3 um) from formalin –fixed, paraffin- embedded tumors were cut and mounted on to salinized slides. Following deparaffinization in xylene, slides were rehydrating through a graded series of ethyl alcohol 100% 4 min, 90% 2 min, 70% 2 min, and water 2 min. and were placed in distilled water. Samples were steamed for antigen retrieval using high PH (7.2) by water path at 95°C for 40 min. after wash with PBS for 3 min endogenous peroxidase activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and after washing with PBS for 3 min then slides were incubated with (100 ul) of mouse monoclonal antihuman BRCA1, against LMP-1 for 3 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 min with dextrin labeled polymer (DAKO). Finally, the sections washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex to 5 min. After wash with distil water for 3 min slides were counter staining with mayers heamatoxylin for one min were washed in running tap water for several min 7-10(bluing), then rehydrated ,clear and mount in DPX(Caroline,2012).

Result interpretation:

All quality control measures were adopted positive and negative slides were used during immuohistochemistry staining.

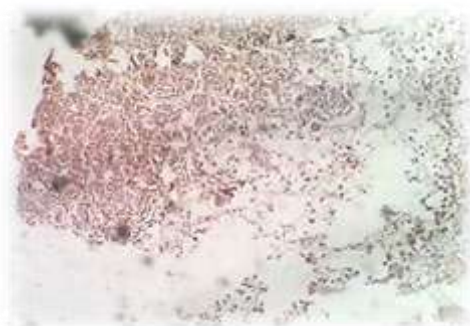


Photo (1): a specimen of breast IDC, stained with IHC stain and using BRCA1 marker show strong positive +++ (10x).



photo (2)A specimen of breast IDC, stained by IHC stain and using BRCA1 marker showing strong positive ++(10x).

showing negative (10X)

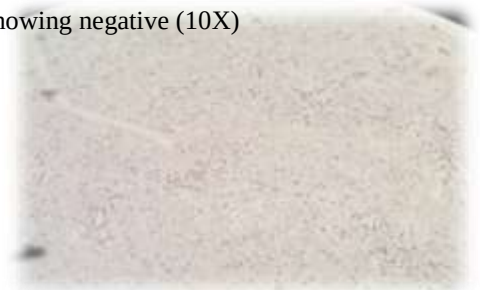


Photo (3) A Specimen of breast IDC stained by IHC and using BRCA1 marker

Results and Discussion

A total of 40 representative samples cores (specimens that contained cancer cells), were analyzed with immunological stains by use BRCA1 tumor marker.

The patients' age ranged between 30-60 years, in 41-50 the most of frequent age group 13 (32.5%), followed by 51-60 years 11 (27.5%), in age 30-40 years frequent 10(25.0%) and 6(15.0%) in age above 60 years(table 4.1).

Table (4.1): distribution of study population

	Frequency	Percent
Valid 30 - 40 Years	10	25.0
41 - 50 Years	13	32.5
51 - 60		

Immunohistochemistry Result: 30 samples positive by IHC 75% and 10 samples were negative 25% in cases (Table 4.2).

Table(4.2)Distribution of according to their result

	Frequency	Percent
Valid Negative	10	25.0
Positive	30	75.0
Total	40	100.0

Grading Result: The main type of cancer in the study invasive ductal carcinomas .histological grade II cancers comprised 25% of the cases, while histological grade III cancers comprised 75 %(table4.3).

Table (4.3) Distribution of according to their grade(II,III)

Grade	Frequency	Percent
II	10	25.0
III	30	75.0
Total	40	100.0

Table 4.4 correlation between expression of BRCA1 and patient age by using TMA

Age	Negative	positive	Total
30 - 40 Years	2	8	10
41 - 50 Years	4	9	13
51 - 60 Years	2	9	11
> 60 Years	2	4	6
Total	10	30	40

P= 0.835

Table 4.5correlation between expression of BRCA1 and tumor grade by using TMA

grade	result		Total
	negative	positive	
II	3	7	10
III	7	23	30

Total	10	30	40

P= 0.677

Discussion:

Invasive ductal carcinoma (IDC), also known as infiltrating ductal carcinoma, is cancer that began growing in a milk duct and has invaded the fibrous or fatty tissue of the breast outside of the duct. IDC is most common form of breast cancer, representing 80% percent representing of all breast cancer diagnoses. The study conducted in Khartoum state, in forty people are infected with breast cancer in this study the most frequent age group was (30-70) year and female above 60 years is less frequent. Cytoplasm or nuclear BRCA1 expression was identified is a total of 64 breast cancer patients. BRCA1 expression was associated with higher histologic grade, and majority of BRCA1 positive in patients were below 50 years of age agree with (Burkadze G, et al, 2010). Other study immunohistochemical staining of human breast specimens also revealed, BRCA1 expression was reduced or undetectable in the majority of high grade of invasive ductal carcinomas disagree with (Wilson, et al, 1999). In the present study all patients were females with an average age of (43-71). fourteen out of the 40 tumors were BRCA1 positive the most common histological type in our study was invasive ductal carcinomas. The majority of the tumors were high grade III supporting with (Maria et.al, 2009). In this study, eighty two female's patients in age 45 years diagnosed with breast cancer BRCA1 positive expression was demonstrated in 39.02% out of all patients with breast carcinomas agree with (Wijdan et.al, 2015). In the present study immunohistochemical assessment of BRCA1 expression could provide additional clinically relevant information in routine classification of breast cancer. BRCA1 alteration may play a significant role in the development and progression of breast cancer agree with (Emad A, et.al, 2007).

Conclusions:

75% of female have Type invasive ductal carcinomas in study group positive to BRCA1 and 25% of female was negative.

Most Of positive to BRCA1 in grade III (23/30)

41-50 years was the most frequent age group and female above 60 years less frequent.

Recommendations:

On the bases of this study we recommended that:

The study recommends that all women undergo breast self examination monthly from the age of 20 and above.

Examine the history of genetic family because breast cancer is a multi hereditary disease women have breast cancer if they have a genetic predisposition that makes them susceptible to affect.

Prophylactic surgery for proper breast excision in cases of high risk factors such as the presence of family history or the presence of breast cancer genes such as BRCA1.

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IDC: Age:51 Grade: III	IDC: Age:43 Grade: III	IDC: Age: 40 Grade: III	IDC: Age: 55 Grade: II
IDC Age:33 Grade: III	IDC: Age:52 Grade: II	IDC: Age: 50 Grade:	IDC: Age:61 Grade: III
IDC: Age:37 Grade: III	IDC: Age:41 Grade: III	IDC: Age: 54 Grade: III	IDC: Age: 64 Grade: III
IDC: Age:30 Grade : II	IDC: Age:50 Grade: III	IDC: Age: 63 Grade: III	IDC: Age:57 Grade: II
IDC: Age:43 Grade: II	IDC: Age:56 Grade: II	IDC: Age: 44 Grade: III	IDC: Age:39 Grade: III
IDC: Age:31 Grade: III	IDC: Age:37 Grade: II	IDC: Age: 71 Grade: III	IDC: Age: 51 Grade: III
IDC: Age:60 Grade: III	IDC: Age:44 Grade: II	IDC: Age:60 Grade: III	IDC: Age: 42 Grade: III
IDC: Age:40 Grade: III	IDC: Age: 48 Grade: II	IDC: Age:31 Grade: III	IDC: Age: 36 Grade: III
IDC: Age:44 Grade: III	IDC: Age:53 Grade: III	IDC: Age:55 Grade: III	IDC: Age:50 Grade: III
IDC: Age:47 Grade:II	IDC: Age:70 Grade: III	IDC: Age: 47 Grade: III	IDC: Age:65 Grade: III

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