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# Expression of Brca1 in a Group of Iraqi Patients with Breast Cancer

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***Background:*** *Breast cancer is the most common malignancy among women worldwide.BRCA1 (breast cancer type 1) is a human tumor suppressor gene(2,3). It is found in all humans; it's a protein, responsible for repairing DNA.*
*Objective: To evaluate the expression of BRCA1 protein in breast cancer and assess associations with histological stages, and grades.*
*Materials and Methods: Seventy cases of breast cancer were subjected to immunohistochemistry for expression of BRCA protein.*
*Results:Out of 70 cases of breast cancer; 46 cases (65.7%) were positive in BRCA1 (over expression). There is asignificant correlation between the results of IHC staining of BRCA1 with age group.*
***Key words:*** *Breast Cancer, BreastCancer Antigen1.*

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## I. Introduction

Breast cancer is the most commonly diagnosed cancer in women ([1]). According to Iraqi Cancer Registry 2012, Ministry of health, Iraq (vsl),2016: breast cancer is amongst the first ten types of cancers in Iraq, where it comes first with an incidence of 34,5% **.**

*BRCA1*( breast cancer type 1)is a human tumor suppressor gene([2,3])**.** It is found in all humans; it's aprotein, responsible for repairing DNA.([4])

If BRCA1 or BRCA2 itself is damaged due to BRCA mutation, damaged DNA is not repaired properly, and this increases the risk for breast cancer.([5,6]) Thus, although the terms "breast cancer susceptibility gene" and "breast cancer susceptibility protein" describe a proto-oncogene (a normal gene that could become an oncogene due to mutations or increased expression), *BRCA1* and *BRCA2* are normal; it is their mutation that is abnormal.([7])

Certain variations of the *BRCA1* gene lead to an increased risk for breast cancer as part of a hereditary breast-ovarian cancer syndrome. Researchers have identified hundreds of mutations in the *BRCA1* gene, many of which are associated with an increased risk of cancer. Women with an abnormal BRCA1 or BRCA2 gene have up to an 80% risk of developing breast cancer by age 90; increased risk of developing ovarian cancer is about 55% for women with BRCA1 mutations and about 25% for women with BRCA2 mutations.([8])

Women having inherited a defective BRCA1 or BRCA2 gene have risks for breast and ovarian cancers that are so high and seem so selective that many mutation carriers choose to have prophylactic surgery.([9])

Approximately 5 to 10% of all breast and ovarian cancers can be attributed to highly penetrates, dominantly inherited mutations in specific genes ([10,11]). Mutations in the *BRCA1* gene are responsible for 45% of familial breast cancer and 80% of families predisposed to both breast and ovarian cancer ([12,13]).

BRCA1 expression is reduced or undetectable in the majority of high grade, ductal breast cancers.([14])

It has long been noted that loss of BRCA1 activity, either by germ-line mutations or by down-regulation of gene expression, leads to tumor formation in specific target tissues. In particular, decreased BRCA1 expression contributes to both sporadic and inherited breast tumor progression**.([15])**

Only about 3% - 8% of all women with breast cancer carry a mutation in BRCA1 or BRCA2([16])

**Aim**:

We set out to study the immunohistochemical expression of BRCA1 protein in breast cancer and assess associations with histologicalstages, and grades.

## II. Materials and methods

**1-Tissue samples:**

70 paraffin – embedded tissue samples belong to Iraqi women diagnosed with breast cancer were collected from the archive for paraffin blocks for histopathological diagnostic service laboratory of both the Central Health Laboratory and the National Center for Breast Cancer Research, University of Baghdad, since November 2014 till June 2016.

Information about the patient's clinical history was obtained from their medical records. The cases were identified by specific numbers and by personal details.

This study was approved by the National Center for Breast Cancer Research.

**2- Immunohistochemistry staining test of BRCA1** :( DakoREAL™InVision™ Detection System )

Serial dewaxedsections (5Mm-thick) were immersed in xylene, re-hydrated by serial alcohol, heated in water bath at 95°C for 30 minutes for antigen retrieval, and blocked with peroxide solution for 10 minutes at room temperature after washing in TBS. Sections were incubated at 37 °C with the primary antibodies: anti-BRCA1, a mouse –mono clonal antibody. (1:50, Lab Vision,dako). Sections were next incubated at 37°C for 10 minutes with real invasion solution (dako) ,washed ,stained with DAP (the main stain) and stopped in distilled water, counter stain was added(mayershematoxilline ) then washed ,mounted and examined under 40x objective lens of light microscope.

In arecentstudy, the positive BRCA1 reported only in cytoplasm of tumor cells of breast cancer patients.

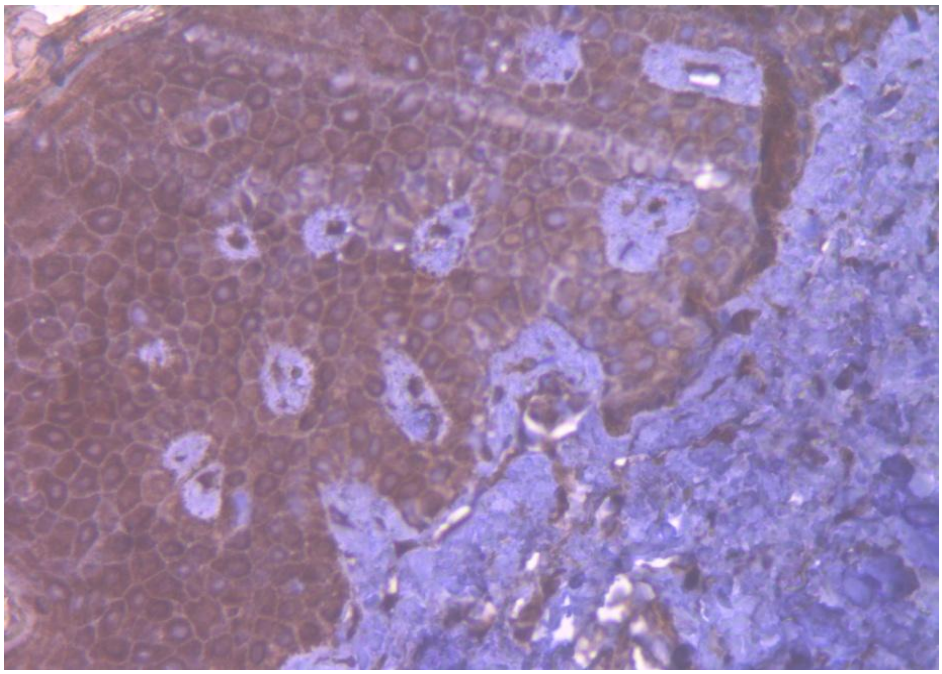
The anti- BRCA1 , a mouse –mono clonal antibody used in this study ,was directed against BRCA1 and seemed to reveal only the cytoplasmic form of this protein ([17])

Tumor cells were given scores depending on the percentage of labeled malignant cells ([18]):

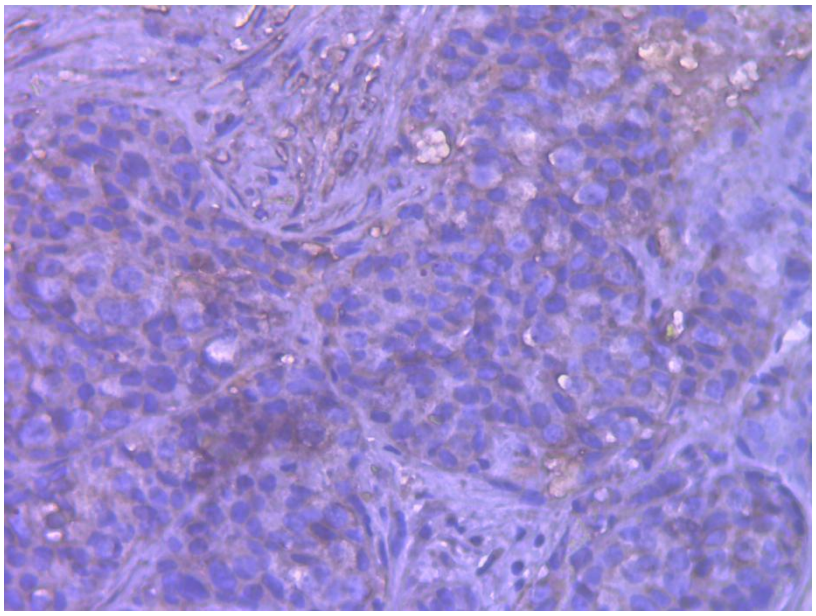
1. Score 0 (-ve) for BRCA1:Tumar cells staining less than 10 %.
2. Score 1 (+)for BRCA1: staining observed in 10%- 40%.
3. Score2 (++)for BRCA1: staining observed in 40%-70%.
4. Score 3(+++)for BRCA1:staining observed in greater than 70%.

Staining was considered positive when greater than 10% 0f the tumors cells showed positive staining ([18]) Statistical analysis was used to compare the clinicopathological data pertaining breast cancer patients with immunohistochemical expression of BRCA1, SPSS V. 22.

(Statistical package) for the social sciences was utilized for data input and analysis [(19)]



**Score** 3



**Score** 1

## III. Results

The results of BRCA1immunostaining tests for all (paraffin – embedded tissue) samples are listed in (table-1).

**Table -1 (BRCA1immunostaining results):**

| BRCA1 | | frequency | Percent. | Cumulative percent |
|---|---|---|---|---|
| Valid | strong | 8 | 11.4 | 11.4 |
| | moderate | 21 | 30.0 | 41.4 |
| | week | 17 | 24.3 | 65.7 |
| | negative | 24 | 34.3 | 100.0 |
| | total | 70 | 100.0 | |

Out of 70 cases of breast cancer; 46 cases (65.7%) were positive in BRCA1 (over expression): ( 41.4% ) were accumulative percentage of positivity in BRCA1 include scoring of strong and moderate as the fallowing : 11.4 % (8 cases) were strong, 30.0 % ( 21cases) moderate while 58.6% were equivocal : 24.3% (17cases) were weak(threshold)which can be considered as loss of BRCA1 (**[20]**) and 34.3 %( 24 cases)were negative .We examined 70 patients aged from 25 to80 years.

**Table -2 (The correlation between the results of immunostaining of BRCA1 with age group):**

| Age group | BRCA1 | | | | Row total | |
|---|---|---|---|---|---|---|
| | strong | Moderate | week | Negative | | % |
| 25-34 | 0 | 2 | 1 | 0 | 3 | 4.3% |
| 35-44 | 1 | 9 | 3 | 15 | 28 | 40% |
| 45-54 | 4 | 5 | 3 | 3 | 15 | 21.4% |
| 55-64 | 0 | 5 | 8 | 5 | 18 | 25.7% |
| 56 &more | 3 | 0 | 2 | 1 | 6 | 8.6% |
| p< .05. The *p*- value is .003531), mean of age (48.30) andstandard deviation 11.34 | | | | | 70 | 100% |

The correlation between the results of IHC staining of BRCA1 with **age group (table -2) was significant** at p< .05. The *p*- value is .003531) according to (social sciences statistics). Approximately ( 40 %) of the breast cancer patients were diagnosed in the age period (35-44). BRCA1 more aggressive in this age than other.

The correlation between the results of IHC staining of BRCA1 with type, grade, and stage of breast cancer, were not significant at p<.05. According to (social sciences statistics) as shown in (table 4, 5,6) respectively.

**Table- 4(The correlation between the results of IHC staining of BRCA1 with type of breast cancer)**

| Type of cancer | BRCA1 | | | | Total | |
|---|---|---|---|---|---|---|
| | strong | moderate | week | negative | | % |
| ductal | 7 | 18 | 14 | 16 | 55 | 85.9% |
| lobular | 1 | 2 | 1 | 5 | 9 | 14% |

| | | | | | | |
|---|---|---|---|---|---|---|
| Column total | 8 | 20 | 15 | 21 | 64 | 100% |
| (The *p* - value is .45415) | | | | | | |

(Note: 6 cases of unknown type of breast cancer is considered missing data)

**Table -5( The correlation between the results of IHC staining of BRCA1 with grade of breast cancer)**

| **Grade** | **BRCA1** | | | | **Row Totals** | |
|---|---|---|---|---|---|---|
| | **Strong** | **moderate** | **week** | **negative** | | **%** |
| grade 1 | 0 | 0 | 0 | 1 | 1 | 2.6% |
| grade2 | 2 | 8 | 6 | 9 | 25 | 64.1% |
| grade3 | 0 | 5 | 4 | 4 | 13 | 33.3% |
| The *p* - value is .780093 | | | | | 39 | 100% |

(Note: 31cases of unknown grade is considered missing data)

**Table- 6( The correlation between the results of IHC staining of BRCA1 with stage of breast cancer)**

| **Stage** | **BRCA1** | | | | **Total** | |
|---|---|---|---|---|---|---|
| | **strong** | **moderate** | **week** | **negative** | | **%** |
| Stage1 | 2 | 6 | 2 | 4 | 14 | 28.5% |
| Stage 2 | 2 | 7 | 8 | 7 | 24 | 48.9% |
| Stage 3 | 2 | 5 | 2 | 2 | 11 | 22.4% |
| ( The *p* - value is .446683) | | | | | 49 | 100% |

(Note: 21cases of unknown stage is considered missing data)

The main histological type was ductal carcinoma (85.9 %) while lobular type was (14%)( table 4), in which pathological changes of **grade II** and **III** were observed in 64.1 % and 33.3 % respectively (table 5) **.**Mostly of these patients presented in **stage I** ( 28.5%) and **stage II** ( 48.9 %) (table 6) .

## IV. Discussion

Globally and According to GLOBOCAN, theprevalence of Breast cancer is 1,461,445 worldwide with theincidence of 1,824,701 and mortality of 1,589,925 ([21]).

We have found out a significant correlation with age group (*p*- value = .003531) table-2 .the majority of the cases located within the age group (35-55) and the peak frequency of the high intensity observed among middle aged women (45-55). These findings coincides with ([22]) . Moreover ([23]) found that The most affected age group was 35-44.(Young age at diagnosis, especially in *BRCA1* mutation carriers, has been associated with an increased risk of contra lateral breast cancer ([24,25,26])

There are other features that justify increasing efforts for breast cancer control including **the tendency for this disease to affect younger women, the obvious rise in incidence rates** ([27,28])

Invasive ductal carcinoma (IDC) not otherwise specified (NOS) is the most common histological type among sporadic breast cancer, comprising 70-80% of all cases ([29]),Which coincide with our study in which the majority of the cases were Ductal carcinoma which comprises about 85% f the total cases (55 out of 64) table -4. Breast carcinomas are routinely graded based on an assessment of tubule formation, nuclear pleomorphism and mitotic counts. This method of tumor graduation consists of scoring 1-3 for each factor.

Although BRCA1 tumors are more frequently high-grade (grade 3) tumors because they show less tubule formation, we found the majority of the patients with grade 2 showed over-expression of BRCA1 (Table-5)this result attributed to the nonhomogeneous sampling of stage cases.

In conclusion, we support adding BRCA1 to the diagnostic panel of breast cancers biomarkers in Iraqi laboratories especially for prediction of cancer recurrence. Further studies of larger number of samples of BRCA1 associated tumors are necessary to clarify and confirm our observations.

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