Study of BRCA1 gene expression in breast cancer in relation to some clinicopathological parameters in Al-Diwanyia city by immunohistochemistry

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الخلاصة

البحث عن التعبير المناعي النسيجي الكيميائي لجين أل BRCA1 في خلايا سرطان الثدي و معرفة ترابط هذا التغير مع بعض المتغيرات السريرية - المرضية . تمت دراسة(45) عينة مطمورة بالفورمالين من سرطان الثدي و (10) عينات من نسيج الثدي الطبيعي في مختبرات مستشفى الديوانية التعليمي والتي تم استئصالها للفترة منذ بداية تشرين الثاني 2005 وحتى آب 2011 ، تراوحت أعمارهم بين 35 و 75 سنه مع معدل عمر(55) . مع وجود تاريخ عائلي من الدرجة الأولى للمرض في 25 عينة من العينات ، صنفت العينات حسب درجة التمايز إلى(13) عينات من الدرجة الأولى و(32) عينة من الدرجة الثالثة، وحسب النوع إلى 30 عينة سرطان الثدي القنوي و15 عينة من سرطان الثدي الفصيصي . أظهرت الدراسة المناعية النسيجية أن تعبير أل BRCA1 كان موجبا في (9) عينة (20 %) من سرطان الثدي، وكان أكثر في العينات ضمن مجموعة العمر الأصغر من 50 سنة والعينات ذات التاريخ العائلي المرضي والعينات ذات التمايز الضعيف (الدرجة الثالثة) عنه في العينات ذات التمايز القوي (الدرجة الأولى) وفي سرطان الثدي الفصي عنه في سرطان الثدي القنوي كما انه توجد علاقة واضحة لتعبير ا لBRCA1 مع التاريخ العائلي المرضي للمرضى و عمر المرضى و درجة التمايز (قيمة ألفا < 0.05).

Abstract

Background: Breast cancer causes major part of cancer deaths in women and is increasing in incidence. The tumor suppressor gene, BRCA1 has been conferred to increase the susceptibility to breast cancer. We aimed to determine the significance of BRCA1 gene expression in relation to other prognostic factors.

Materials and methods: 45 patients with positive family history of breast cancer were selected from Al-Diwaniya Teaching Hospital / department of pathology for the study. A control group of 10 healthy subjects were also included. BRCA1 expression was assessed and correlated with age, family history , histological type and grade of breast cancer.

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Results: BRCA1 was found in 9 patients 'samples (20% of the breast cancer tissues) while remaining patients (80%) were negative as well as the control group. A positive significant relationship was demonstrated between BRCA1 expression and high histological grade, age of the patient and family history and A significant negative correlation was found between BRCA1 expression and type of the tumor.

Conclusion: The study demonstrated the lack of BRCA1 gene expression in the majority of breast cancer cases and confirmed the relationship between BRCA1 expression and parameters that determine the poor prognosis in breast cancer .

Keywords: Breast cancer, immunohistochemistry, BRCA1

Introduction

Breast cancer is the most frequent neoplasia in female, and the morbidity and specific mortality continue to increase, in spite of remarkable progresses in the field of early diagnosis and adjuvant therapy[1]. Also breast cancer is a major cause of cancer related mortality in women[2]. To some extent, the high proportion of cases in young women reflects the high prevalence of hereditary breast cancers in women[3]. About 90% of breast cancers are sporadic and are diagnosed in women without germline mutations in known susceptibility loci[4]. Approximately 10 % of cases exhibit a familial pattern of inheritance[5].BRCA1 and BRCA2 Germline mutations of these two genes are responsible for approximately two-thirds of all familial breast cancers; the loci for the remaining cases remain unknown[4]. Mutations in BRCA1 have also been found and confer a high risk of approximately 90% of familial breast and ovarian cancers[6-10]. Breast cancer susceptibility gene 1 (BRCA1) associated breast cancers often occur in younger women, and such tumors are high grade and lack estrogen receptor[11,12]. All these features are associated with a poor prognosis. However, the evidence concerning the effect of a BRCA1 or BRCA2 mutation on the prognosis is inconsistent[13,14]. BRCA1 consists of 23 exons over approximately 80 Kb with a coding region of 5.5 Kb. Two

carboxyl- terminal BRCT domains are shared with several cell cycle checkpoint proteins, such as p53-binding protein 1[15]. Although BRCA1 is located predominantly in the nucleus, it contains an NH2- terminal nuclear export signal (NES) and can undergo dynamic shuttling between nucleus and cytoplasm[16].The BRCA1 gene, identified by positional cloning in 1994, consists of 24 exons, 22 of which encode for a protein of 220 kDa consisting of 1863 amino acids[17]. BRCA1 mRNA is induced at late G1/early S phase before DNA synthesis[18], and the expression of the BRCA1 protein closely follows that of its mRNA[19].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 a 60% 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[20].

Material and method

Patient group:- This study was conducted on (45) breast cancer female patients. Their ages ranged from 35 to 75 years old. They were referred to the Al-Diwaniya Teaching Hospital in a period from November 2005 – August 2010 .

The diagnosis of breast cancer was based on radiology, mammography, tissue biopsy for histopathological examination and ultrasonography. The cases was grouped according to the age in to tow groups ; (35year -50year) in 28(62.2%) and (55year -75 year) in 17(36.8%) and according to the family history into tow main group ; 25(55.5%) cases with positive family history and 20(44.5%) with negative history . also the cases histopathologically classified according to the system of Bloom and Richardson[21] which was recommended by WHO[22] into:

1- Tow types of tumor :- infiltrative ductal carcinoma in30(66.6%) cases and infiltrativr lobular carcinoma in 15(33.4%) cases
2- Grades in to :- grade I (Well differentiated) in 13(28.8%) cases and grade III (Poorly differentiated) in 32 (71.2%) patients.

Control group:- This group included 10 cases of normal breast tissue .

Immunohistochemical study:- Immunohistochemistry was performed on formalin fixed, paraffin wax embedded samples. Routine sections of 3 μm thick were cut onto DAKO Capillary Gap slides (S2024) (DAKO Corp, Carpinteria, CA), fixed in 10 per cent buffered formalin and dried at 60°C overnight. To study BRCA1 protein expression, deparaffinized sections were rehydrated, and treated with 3% H2O2 in methanol for 30 min to block endogenous peroxidase. Epitope retrieval was carried out in 0.01 M citrate buffer at 95°C in water bath for 20 min. Slide sections dewaxed in xylene transferred to absolute alcohol, and incubated in 3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase. The slides were then transferred to running tap water before being transferred to 3 L of boiling citrate buffer pH 6.0 in a 15-lb pressure cooker. Slides were then rinsed in Trisbuffered saline (TBS) pH 7.4 and incubated in normal goat serum (1:10) for 10 minutes. The sections incubated in primary antibody over the night at the appropriate dilution. monoclonal antibody was used, anti- BRCA1 (GLK-2). After overnight incubation at 4°C with the primary anti-body in a humidified chamber, biotinylated antibody (link antibody) was added, After rinsing in TBS, the slides were then incubated in DAKO Duet (K0492) streptavidin- biotin–horseradish peroxidase complex for 35 minutes, rinsed in TBS, and treated with DAB (3,3' diaminobenzidine chromogen: 896102, Kem-En-Tec, Copenhagen, Denmark) for 10 minutes. The slides were then rinsed in tap water, counterstained in Mayer's hematoxylin and mounted. Nuclear with or without cytoplasmic staining of 10% or more cancer cells was considered positive.

Statistic analysis

Statistical analysis was performed using SPSS ver14,Asignificant correlation was reported for a value of $p<0.05$.

Results

Out of the 45 breast cancer tissues studied, only 9 (20%) showed detectable BRCA1 expression (Table 1). No demonstrable BRCA1 expression was found in 80% of the breast cancer tissues as well as the control group, the expression of BRCA1 was nuclear in all the positive cases (Figure 2and 3).

Age distribution of breast cancer cases studied

The patients' ages ranged between 35 and 75 years with a mean of 54 years . 9 cases (20%) showed BRCA1 expression with age group ranged from 35 to 50 years while the other patients without BRCA1 expression their ages ranged from 50 to 75 years. A significant correlation was found between BRCA1 expression and age ($p<0.05$) (Table 2)

Family history of breast cancer cases studied

25(55.5%) cases with positive family history and 20(44.5%) with negative history; 9 cases (20%) showed BRCA1 expression with positive family history while the other patients without BRCA1 expression were with negative family history. A significant correlation was found between BRCA1 expression and family history ($p<0.05$) (Table 3)

Histological types of breast cancer cases studied

tow main histological types were present in this study. The infiltrating ductal carcinoma in (30 cases), which accounted for 66.6% of cases. The infiltrating lobular carcinoma (15 cases), which accounted for 33.4% of cases. 8 cases with BRCA1 expression was infiltrating ductal carcinoma and one case only with BRCA1 expression was There was infiltrating lobular carcinoma no significant association between BRCA1 expression and histological types of breast cancer($p>0.05$)(Table 4).

Histological grades of breast cancer cases studied

In this study of the 45 breast cancer cases the majority (71.2%) were of high histological grade while (28.8%) with low histological grade. There was a significant association between BRCA1 expression and histological grade of breast cancer as the nine cases showing BRCA1 gene expression were of high histological grades ($p<0.05$) (Table 5).

Table 1:- BRCA1 expression in breast cancer tissue.

BRCA1 expression	-Ve	+Ve	Total
	36(80%)	9(20%)	45(100%)

Table 2:- BRCA1 expression in breast cancer tissue according to age of the patient.

Age of the patient/years	BRCA1 expression		Total	P value	χ
	-Ve	+Ve			
35-50	19(67.9%)	9(32.1%)	28(62.2%)	0.0008	6.8304
50-75	17(100%)	0	17(37.8%)		
Total	36(80%)	9(20%)	45(100%)		

Table 3:- BRCA1 expression in breast cancer tissue according to family history of the patient.

Family history	BRCA1 expression		Total	P value	χ
	-Ve	+Ve			
+Ve F.H	16(64%)	9(56%)	25(55.5%)	0.002	9.000
-Ve F.H	20(100%)	0	20(44.5%)		
Total	36(80%)	9(20%)	45(100%)		

Table 4:- BRCA1 expression in breast cancer tissue according to types of the tumor.

Types of the tumor	BRCA1 expression		Total	P value	χ
	-Ve	+Ve			
IDC	22(73.3%)	8(26.7%)	30(66.6%)	0.11	2.500
ILC	14(93.3%)	1(6.7%)	15(33.4%)		
Total	36(80%)	9(20%)	45(100%)		

Table 5:- BRCA1 expression in breast cancer tissue according to grade of the tumor.

Grades of the tumor	BRCA1 expression		Total	P value	χ
	-Ve	+Ve			
I(W.D.C)	13(100%)	0	13(28.8%)	0.03	4.5703
III(P.D.C)	23(71.8%)	9(28.2%)	32(71.2%)		
Total	36(80%)	9(20%)	45(100%)		

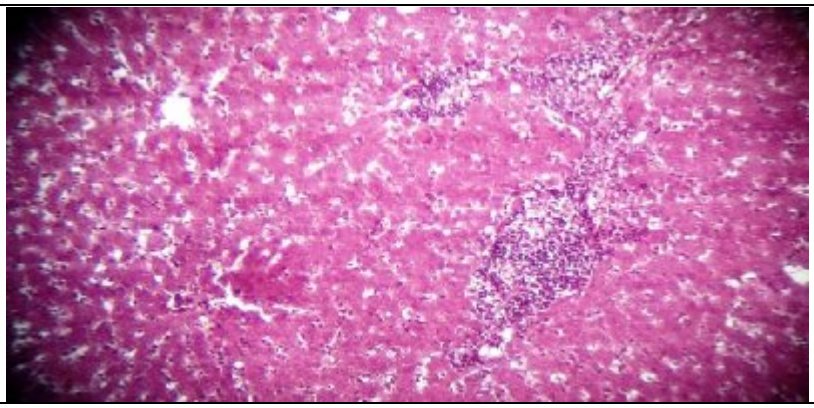

Figure 1:- Normal breast tissue showing negative BRCA1 immunostaining (x40).

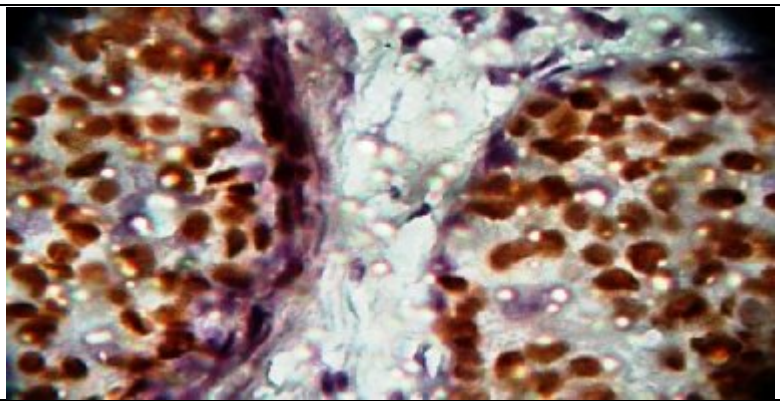

Figure.2: IDC of breast with positive BRCA1 nuclear immunostaining (x 40).

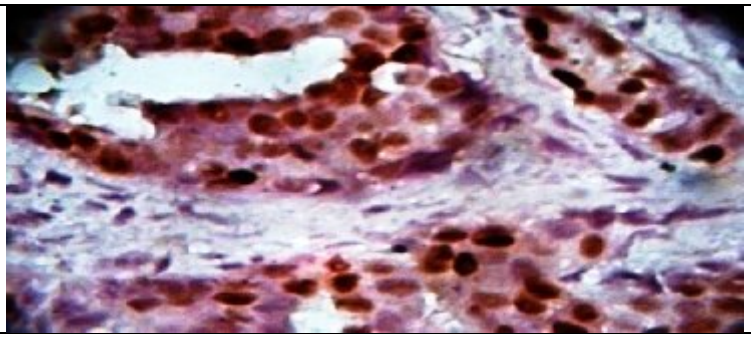

Figure.3: IDC of breast with positive BRCA1 nuclear immunostaining (x 40).

Discussion

The evidence concerning the effect of a BRCA1 or BRCA2 mutation on the prognosis of breast cancer is inconsistent[23-28]. Women who had inherit a mutation in *BRCA1* or *BRCA2* have a greatly increased risk for breast cancer, and the vast majority of breast cancers occurring in such women are attributable to the underlying germline mutation[29]. Over the past decade, much progress has been made in understanding the molecular biology of breast cancer. The use of molecular and immunohistochemical techniques is providing insights that will allow us to tailor the management of patients with breast cancer. BRCA1 is becoming an important prognostic factor for breast cancer. The morphological features of tumors from patients with breast cancers associated with BRCA1 mutation include higher grade, with an excess of medullary/atypical medullary carcinoma[30].The present study showed the lack of BRCA1 expression, in the majority of breast cancer tissues studied, this is in agreement with one of the major studies in Egypt by Manal Kamal et al[31] who are study BRCA1 expression as prognostic marker in Egyptian women.

Although BRCA1 is located predominantly in the nucleus, it contains an NH2- terminal nuclear export signal (NES) and can undergo dynamic shuttling between nucleus and cytoplasm[32] , the expression of BRCA1 was completely nuclear in all positive cases , this is in agreement with Fahad Al-Mulla et al[33] who is confirmed that BRCA1 is expressed as a nuclear and cytoplasmic antigen in

breast cancer tissues while Kashima et al[33] as well as Troudi. W et al[35] detect they BRCA1 expression in cytoplasm of malignant cells only because they used monoclonal Ab that has been shown the mutation in exon 11 only . These contradictory results about the location of the BRCA1 protein probably result from the quality of the archival paraffin embedded breast cancer tissues affecting protein detection and the quality of the antibodies.

In our study the BRCA1 expression was significantly correlated with the grades of tumor , this is in agreement with Manal Kamal et al[31], Fahad Al-Mulla et al[33] who are study the expression of BRCA1 by immunohistochemistry and real time PCR procedures while our result is in disagreement with Lee et al[35] who is use the immunehistochemistry alone.

Several reports have indicated that a specific histological phenotype can be recognized in breast carcinoma occurring in women with germ line mutations in BRCA1[36,37]. One of these studies reported an excess of ductal carcinoma in breast cancers associated with BRCA1 mutations[36]. An excess of tumors with medullary and atypical medullary pattern within the group of ductal carcinomas was also reported in association with BRCA1 mutations[30,37]. The findings of the present study do not support these findings in our study group, Although 73.3% of breast cancers with negative BRCA1 expression in this study were infiltrative ductal carcinoma, the relationship was not statistically significant and this may be attributed to the fact that the numbers of studied cases were rather small.

Currently, predictions about the likelihood of an individual harboring a germline mutation in *BRCA1* or *BRCA2* are based on clinical data, including family history, age of onset of breast cancer, and ethnicity. Family history, especially where there are clusters of breast and ovarian cancer, has the best positive predictive value of these three. However, family history data is often problematic as it relies on accurate recall of cancer events within the family and the ability to obtain pathology confirmation of those cancers. In our result there is a significant relation between the expression of BRCA1 and the positive family history in first

degree relative and this go with the idea that about 40-50% of hereditary breast cancers and the most of the hereditary breast-ovarian syndromes are thought to be due to mutations in BRCA1 gene[38], also our result is in agreement with Lidereau R. et al[39] and Cortesi L. et al[40] that confirm the BRCA1 over expression in women with family history of breast cancer.

Women carrying a BRCA1 mutation are known to have an increased risk of developing contra lateral primary breast cancer which is even more apparent among women who are younger when diagnosed with a primary breast carcinoma (age <50 years)[18 , 41].our result confirm that BRCA1 expression was higher in the younger age group (35-50 years old) than the older one with significant relationship and this agree with Manal Kamal et al [31] who is find that all the BRCA1 expressed cases was in the younger age group.

Also, it has recently been reported that *BRCA1* expression influences the choice of chemotherapeutic agents used in the treatment of breast cancer[42,43]. Therefore, it is now becoming clear that the knowledge of *BRCA1* expression in breast cancer has important clinical ramifications.

Conclusion

The study demonstrated the lack of BRCA1 gene expression in the majority of breast cancer cases and confirmed the relationship between BRCA1 expression and parameters that determine the poor prognosis in breast cancer in. Our results confirmed the role of BRCA1 in our study population.

References

1- Sotriou. C., Neo. S. Y., Mcshneu L. M., Korne. L., Long. P. M., Jazaeri A., Martai.T P., *Breast cancer classification and prognosis based on gene expression profiles from a population-based study*, Proc Natl Acad Sci USA, 2003, 100(18):10393–10398.

2- Hill AD, Doyle JM, McDermott EW, and O'Higgins NJ. *Hereditary breast cancer. Br J Surg* 1997; 84(10): 1334-9.

3- Parkin DM, Whelan JF, Ferlay J, Teppo L, and Thomas DB. eds. *Cancer incidence in five continents Volume VII Texte imprimé*. IARC scientific publications. IARC, Lyon. 1997, Series vol. 143: 155.
4- Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. *A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1*. Science 1994;266:66±71.
5- Nathanson KL, et al. Breast cancer genetics: What we know and what we need. Nat Med. 2001; 7:552-6.
6- Alberg AJ, Helzlsouer KJ. *Epidemiology, prevention, and early detection of breast cancer*. Curr Opin Oncol 1997;9:505±511.
7- Paterson JW. BRCA1: *a review of structure and putative functions*. Dis Markers 1998;13:261±274
8- Arnold N, Peper H, Bandick K, et al. Establishing a control population to screen for the occurrence of nineteen unclassified variants in the BRCA1 gene by denaturing high-performance liquid chromatography. Journal of Chromatography B 2002; 782: 99-104.
9- Brose MS, Rebbeck TR, Calzone KA. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst 2002; 94:1365-72.
10- Thompson D, Easton DF. Cancer incidence in BRCA1 mutation carriers. J Natl Cancer Inst 2002; 94:1358-65.
11- Johannsson OT, Idvall I, Anderson C*, et al.* Tumour biological features of BRCA1-induced breast and ovarian cancer. *Eur J Cancer* 1997; 33(3): 362-71
12- Karp SE, Tonin PN, Begin LR*, et al.* Influence of BRCA1 mutations on nuclear grade and estrogen receptor status of breast carcinoma in Ashkenazi Jewish women. *Cancer* 1997; 80(3): 435-41.
13- Brekelmans CT, Seynaeve C, Menke-Pluymers M*, et al.* Survival and prognostic factors in BRCA1-associated breast cancer. *Ann Oncol* 2006; 17(3): 391-400.
14- El-Tamer M, Russo D, Troxel A*, et al.* Survival and recurrence after breast cancer in BRCA1/2 mutation carriers. *Ann Surg Oncol* 2004; 11(2): 157-64.

15- Koonin EV, Altschul SF and Bork P. BRCA1 protein products. Functional motifs. Nat. Genet. 1996;13:266–268
16- Rodriguez JA, Henderson BR. Identification of a functional nuclear export signal in BRCA1. J. Biol Chem 2000;275:38589-96.
17- Lose F, Duffy DL, Kay GF, Kedda MA, and Spurdle AB. Skewed X chromosome inactivation and breast and ovarian cancer status: evidence for X-linked modifiers of BRCA1. *J Natl Cancer Inst* 2008; 100(21): 1519-29
18- Verhoog LC, Brekelmans CT, Seynaeve C, Meijers-Heijboer EJ, and Klijn JG. Contralateral breast cancer risk is influenced by the age at onset in BRCA1-associated breast cancer. *Br J Cancer* 2000; 83(3): 384-6.
19- Chen CF, Li S, Chen Y, *et al.* The nuclear localization sequences of the BRCA1 protein interact with the importin-alpha subunit of the nuclear transport signal receptor. *J Biol Chem* 1996; 271(51): 32863-8. *Anticancer Res* 1999; 19(4A): 2551-5.
20- Bochar, D.A.; Wang, L.; Beniya, H.; Kinev, A.; Xue, Y.; Lane, W.S.; Wang, W.; Kashanchi, F.; Shiekhattar, R. "BRCA1 Is Associated with a Human SWI/SNF-Related Complex Linking Chromatin Remodeling to Breast Cancer". *Cell* 102 (2): 257–265.2008.
21- Bloom HJG, Richardson WW. Histopathological grading and prognosis in breast cancer. Br J Cancer 1957;11:359-77.
22- Wilson RE. The Breast. In: Sabiston DC, editor. *Textbook of Surgery: The biologic basis of modern surgical practice*. 13th ed. London, Philadelphia, Toronto: W.B. Saunders Co. Ltd, 1986;530-72.
23- Lacroix, M; Leclercq, G. "The "portrait" of hereditary breast cancer". *Breast Cancer Research and Treatment* 89 2005(3): 297–304.
24- Struewing, JP; Abeliovich, D; Peretz, T; Avishai, N; Kaback, MM; Collins, FS; Brody, LC. "Isolation of two human tumor epithelial cell lines from solid breast carcinomas". *Journal of the National Cancer Institute* 61 1978(2): 967–978
25- Tonin, PN; Serova, O; Lenoir, G; Lynch, H; Durocher, F; Simard, J; Morgan, K; Narod, S. "BRCA1 mutations in Ashkenazi

Jewish women". *American Journal of Human Genetics* 57 1995 (1): 189. PMC 1801236.PMID 7611288.
26- Narod, SA; Foulkes, WD. "BRCA1 and BRCA2. *Nature Reviews on Cancer* 4 2004, (9): 665–676.
27- Brekelmans CT, Seynaeve C, Menke-Pluymers M*, et al.* Survival and prognostic factors in BRCA1-associated breast cancer. *Ann Oncol* 2006; 17(3): 391-400.
28- El-Tamer M, Russo D, Troxel A*, et al.* Survival and recurrence after breast cancer in BRCA1/2 mutation carriers. *Ann Surg Oncol* 2004; 11(2): 157-64.
29- Kelly-Anne Phillips , Immunophenotypic and Pathologic Differences Between *BRCA1* and *BRCA2* Hereditary Breast Cancers, J Clin Oncol 18:107s-112s. 2000 by American Society of Clinical Oncology
30- Marcus JN, Watson P, Page DL, et al: Hereditary breast cancer: Pathobiology, prognosis and BRCA1 and BRCA2 gene linkage. Cancer 77:697-709, 1996
31- Manal Kamal, Khadiga ABU Gabal, Samia EL-Naggar, Yasser Hassan Shaaban, Mohamed Shehata, BRCA1 gene expression in breast cancer in relation to other prognostic markers in Egyptian women, AnationalJournal of obstetric and gynocolog ,ISSN:1308-8254, 2011
32- Rodriguez JA, Henderson BR. Identification of a functional nuclear export signal in BRCA1. J. Biol Chem 2000;275:38589-96.
33- Al-Mulla F, Abdulrahman M,Varadharaj G, et al. BRCA1 gene expression in breast cancer: A correlative study between real time RT-PCR and immunohistochemistry. J Histochem Cytochem 2005; 53:621-9.Kashima K, Oite T, Aoki Y, et al. Screening of BRCA1 mutation using immunohistochemical staining with C-terminal antibodies in familial ovarian cancers. Jpn J Cancer Res 2000; 91:399-409.
34- W. Troudi,1-2 N. Uhrhammer,3 K. Ben Romdhane,2 C. Immunolocalization of BRCA1 protein in tumor breast tissue: prescreening of BRCA1 mutation in Tunisian patients with hereditary breast cancer? European Journal of Histochemistry 2007; vol. 51 issue 3 (July-September):219-226

35- Lee WY. Frequent loss of BRCA1 nuclear expression in young women with breast cancer: an immunohistochemical study from an area of low incidence but early onset. *Appl Immunohistochem Mol Morphol* 2002; 10(4): 310-5.
36- Elston CW and Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991; 19(5): 403-10.
37- Lakhani SR, Van De Vijver MJ, Jacquemier J*, et al.* Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. Breast Cancer Linkage Consortium*. Lancet* 1997; 349(9064): 1505-10
38- Lose F, Duffy DL, Kay GF, Kedda MA, and Spurdle AB. Skewed X chromosome inactivation and breast and ovarian cancer status: evidence for X-linked modifiers of BRCA1. *J Natl Cancer Inst* 2008; 100(21): 1519-29.
39- Lidereau R, Eisinger F, Champeme M-H, et al: Major improvement in the efficacy of BRCA1 mutation screening using morphoclinical features of breast cancer. Cancer Res 60:1206-1210, 2000
40- Cortesi L, Turchetti D, Bertoni C, et al: Comparison between genotype and phenotype identifies a high-risk population carrying BRCA1 mutations. Genes Chromosomes Cancer 27:130-135, 2000
41- Robson M, Gilewski T, Haas B*, et al.* BRCA-associated breast cancer in young women. *J Clin Oncol* 1998; 16(5): 1642-9.
42- Egawa C, Motomura K, Miyoshi Y, Takamura Y, Taguchi T, Tamaki Y, Inaji H, et al. Increased expression of BRCA1 mRNA predicts favorable response to anthracycline-containing chemotherapy in breast cancers. Breast Cancer Res Treat 2003, 78:45–50
43- Zhou C, Smith JL, Liu J .Role of BRCA1 in cellular resistance to paclitaxel and ionizing radiation in an ovarian cancer cell line carrying a defective BRCA1. Oncogene 2003, 22:2396–2404