Immunohistochemical study of breast carcinoma in old age Iraqi women by application of BRCA1 and P53

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الأهداف: تقييم التعبير المناعي ل (BRCA1 وp53) في مختلف الانواع النسيجية لسرطان الثدي وعلاقتهما ببعضهما وتعبيرهما المترافق في النساء العراقياتالمصابات بسرطان الثدي وتعيين علاقة هذين المعلمين الحيويين مع مختلف المعايير المرضية والسريرية الشائعة مثل: درجة الورم، حجمه، حالة العقد اللمفاوية، وعودة الورمللاعمار المتاخرة٠

الطريقة: طبقت هذه الدراسة على ثلاثين حالة مرضية لسرطان الثدي من الاعمارالمتاخرة تتراوح اعمارهمبين ٣٨-٦٠ سنة وكان المعدل العمري٤٩ سنة في فرع الأمراض كلية الطب جامعة الكوفة للفترة من تشرين الثاني٢٠١٠- ايلول٢٠١١، استخدمت طريقة $LSAB^{+}$ لتحديد التعبير المناعي النسيجي ل (BRCA1 وp53).

النتائج: ٢٨ (٩٣٠٤%) من الحالات كانت من السرطان القناتي، بينما٢ (٦٠٦%) حالات من السرطان الحويصلي وكانت نسبة التعبير المناعي لـ(BRCA1 وp53) كانت ٩٢٠٩% و ٥٠% على التوالي.

خاتمة: أثبتت الدراسة أنه كان هناك فرق ذا مغزى بين التعبير الزائد لـ(BRCA1) و بين النوعين المذكورين آنفا،بينما لم يكن هناك فرق ذا مغزى بين التعبير الزائد ل (BRCA1) مع الأحجام المختلفة للورم، حالة العقد اللمفاوية، عمر المريض وعودة الورم. وأثبتت الدراسة انه لم يكن هناك فرق ذا مغزى بين التعبير المناعي ل (p53) مع الاحجام المختلفة للورم ، بينما كان هناك فرق ذا مغزى بين التعبير الزائد ل (p53) مع حالة العقد اللمفاوية ، وعودة الورم,درجة الورم كما إنه يرتبط بعلاقة إيجابيه مع درجة الورم.كلا المعلمين الحيويين يرتبطان مع بعضهما بعلاقة إيجابيه وإن الحالات التي تظهر التعبير المتزامن ل (BRCA1 وp53) هي ذات الحالة المرضية الحيوية الأكثر سوءا".

Abstract

Objectives: To estimate the rate of **BRCA1** and **P53** immunoexpression in different histological types of female breast cancer and to assess whether these biomarkers are significantly correlated with each other and to assess the coexpression of these two biomarkers . To show the correlation of these biomarkers to common clinicopathological parameters such as : tumor grade , size , lymph node involvement, and recurrence of breast carcinoma for old age group .

Methods: Thirty patients with breast carcinoma ,their ages ranging between 38-60 years with a mean age of 49 years, were included in this study and conducted in the Department of Pathology, Faculty of Medicine, Kufa University from November 2010 through September 2011. Labeled Streptavidin -Biotin ($LSAB^{+}$) method was employed for immunohistochemical detection of BRCA1 and p53.

Results:28 cases (93.4%) were of ductal carcinoma while2 cases (6.6%) were of lobular type. The detection rate of BRCA1 and p53 was92.9% and50% respectively .

Conclusion: There was significant difference of BRCA1 overexpression between ductal and lobular carcinoma, while therewas no significant difference of BRCA1 overexpression with different tumor sizes, lymph nodal involvement, age of the patients, and recurrence of breast carcinoma.. P53 immunoexpression was not significantly different among tumor sizes ,while there wasa significant difference with axillary lymph node involvement, tumor grade, and recurrence of breast cancer.

However, p53 positively correlated with tumor grade . Moreover, both biomarkers were positively correlated with each other, and cases coexpressing both BRCA1 and p53 have shown the most unfavorable biopathological profile.

Introduction:

Breast cancer constitutes around one quarter of all cancers ,making it the most common cancer in females[(1)].It is heterogeneous disease with high individual variability as far as response to treatment is concerned[(2)]. Despite the increasing incidence rates of breast cancer, the morbidity and mortality rates are beginning to fall. This reflect improvement in the methods of diagnosis and treatment[(3)]. Several molecular markers that are important in the clinical aspect of malignancies especially in breast cancer have been detected[(4)]. BRCA1 and P53(both genes map to chromosome 17) are known biomarkers of breast cancer.Although this tumor generally appears in sporadic form, ranging from 5% and 10% of all cases, it is consideredas hereditary disease due to inherited autosomal dominant mutationsin several susceptibility genes [(5)].Indeed About 40% to 50% of hereditarybreast cancers and most hereditary breast and ovarian syndromes are thought to be caused by mutations in breast cancer susceptibilitygene 1 (*BRCA1*) [(6)]. The*BRCA1* gene, identified by positional cloning in 1994, consistsof 24 exons, 22 of which encode for a protein of 1863 aminoacids [(7)].

BRCA1 mRNA is induced at late G_1/earlyS phase before DNA synthesis [(6)], and the expressionof the BRCA1 protein closely follows that of its mRNA [(5)]. Although *BRCA1* mutation has rarely been detectedin sporadic breast cancers [(8)], loss of heterozygocity [(9)], methylation of *BRCA1* promoter region [(7)], and loss or reductionof BRCA1 mRNA and protein expression have been demonstratedin sporadic breast cancers [(5)].

Mutations of P53 genehave been reported in human breast carcinoma, especially in more advanced and/or more aggressive tumors [(10)].

P53 mutation is a strong independent marker for survival in breast cancer with some heterogeneity in the clinical phenotype of various types of mutations [(11)].

P53 immunohistochemical staining has been advanced and introduced in clinical and histological assessment of breast cancer aggressiveness and prognosis, in addition to other several established prognostic factors routinely used in the clinical management of breast cancer, including the extent of axillary lymph node involvement, tumor size, histological differentiation, and estrogen and progesterone receptor status [(12)]. In Iraq, immunohistochemical studies to detect P53 overexpression in breast cancer have been conducted [(13)].

Methods:

After Institutional Ethics Committee approval , thirty specimens of formalin-fixed, paraffin embedded breast cancer tissue, collected from breast cancer patients over a period fromNovember 2010 toSeptember 2011 were included in this study. All cases were referred toKufa University of Medicine Teaching Hospital for histopathological evaluation from different parts of the middle region of Iraq. The age range of patients was 38-60 years with a mean age of 49 years. A group of 10 patients with benign breast lesions(fibroadennoma) was included as comparative group and 10 normal breast tissue sections were included as controls. Confirmation of histopathological diagnosis and grading of tumors were carried out after reviewing all slides before proceeding further to the immunohistochemical analysis. Tissue sections of 4 µm were taken from the formalin-fixed, paraffin embedded blocks for immunohistochemistry.Labeled Streptavidin -Biotin ($LSAB^+$) method was employed for immunohistochemical detection of BRCA1 and p53 using Monoclonal Mouse Anti-Human P53 Protein, 1 ml

DAKO, Clone DO-7, Code N7001,andMonoclonal mouse anti-BRCA1 1 ml, (DAKO, Clone GLK-2, Code NO. M3606**.** Theintensity of BRCA1 cytoplasmic stain and P53 nuclear stain was classified into [14]:

Score 0: Negative, none or <5% of the cells revealed positivity for the marker.
Score +1: Weak or mild staining, (5-10%) positive of tumor cells.
Score +2: Moderate staining, less than 25% of tumor cells are stained positive.
Score +3: Strong staining, (25-50%) of tumor cells are stained positive.
Score +4: Highly strong staining, over 50% of tumor cells are stained positive.

All biopsies were classified into three grades: GradeI,Grade II and Grade III,according to the modified Bloom Richardson Grading System[15]**.** The results were statistically evaluated with Chi square test(at level of significance alpha < 0.05) andcorrelation-regression test (R at a significant level of 0.3) using SSPS software.

Results:

BRCA1 and P53 immunoexpression were positive in 50% and 33.3% of breast cancer cases respectively ,and negative in all sections of the normal and benign breast tissues. The differences between groups were statistically significant($p < 0.05$)(Table 1)**.** BRCA1 overexpression was detected in 92.9% of ductal carcinoma cases and in 50% of invasive lobular carcinoma, the difference was statistically significant ($p <0.05$)**.** As grouped in Table 1, overexpression of BRCA1 was detected in in only 50% of those with pure invasive ductal carcinoma (Figure 1**),** in comparison with 42.9% of pure ductal carcinoma in situ, and 100% in those with invasive ductal carcinoma with Paget's disease(Figure 2**),** the difference was statistically significant ($p <0.05$). BRCA1 overexpression was detected in 40% of gradeI(well differentiated breast cancer), 62.5% of grade II (moderatelydifferentiated breast cancer) ,as compared to 47.1% of grade III(poorlydifferentiated breast cancer)**.**Statistically no significant difference exists ($p > 0.05$). Furthermore ,apositive BRCA1 overexpression was detected in 42.9% of Tis size breast cancer, in 66.7% of T1,60% of bothT2 and T3,and in33.3% of T4 .The differences between sizes were statistically not significant($p > 0.05$)**.** Table 1 shows that there is a high detection rate of BRCA1 overexpression inprimary breast cancer in comparison withrecurrent lesions(52.2% versus 42.9%)($p >0.05$), and in theabsence (negative) orpresence (positive) of axillary lymph nodes (66.7% versus 42.9%) ($p <0.05$)**.** In the present study , p53 immunoexpression was detected in 60.7% of ductal carcinoma cases and 50% of invasive lobular carcinomas (Table 1), p53 immunodetection was detected in 35.7% of pure ductal carcinoma in situ (Figure 3), and 25% of invasive ductal carcinoma (Figure 4)and 50% in those with invasive ductal carcinoma with Paget's disease**.** In all these types the incidence is statistically not different from that found in the control ($p >0.05$)**.** On the other hand ,p53 immunoexpression was detected in 40% ofgradeI(well differentiated breast cancer), 12.5% of grade II (moderatelydifferentiated breast cancer) ,as compared to 41.2% of grade III(poorlydifferentiated breast cancer)**.**Statistically significant difference exists ($p < 0.05$),it is obvious that p53 immunoexpression was highly correlated with tumor grade ($R = 0.13$). Apositive p53 immunexpression was detected in 35.7% of Tis tumor size, in 66.7% of T1, 20% of both T2 andT3 , and 33.3% of T4**.**The differences between sizes were statistically not significant($p > 0.05$). A higher detection rate of p53 overexpression inrecurrentbreast cancer in comparison withprimarylesions(42.9% versus 30.4%)($p <0.05$), and in theabsence (negative) orpresence (positive) of axillary lymph nodes (44.4% versus 28.6%) ($p <0.05$)**.** Both biomarkers are positively correlated with each others with respect to most clinicopathological parameters ($R > 0.3$)**.**The cases that co-expressed both biomarkers were found in 100% of ductal carcinoma, in66.6% ofgrade III(poorlydifferentiated breast cancer), in 66.6% of Tis

tumor size, in 50% of those with positive axillary lymph node and in 62.5% of those that showed recurrent tumors **.**Furthermore , the co- expression of both biomarkers was significantly correlated with histopathological type , tumor grade, axillary lymph node involvement, and tumor recurrence (R > 0.3) (Table 2) .

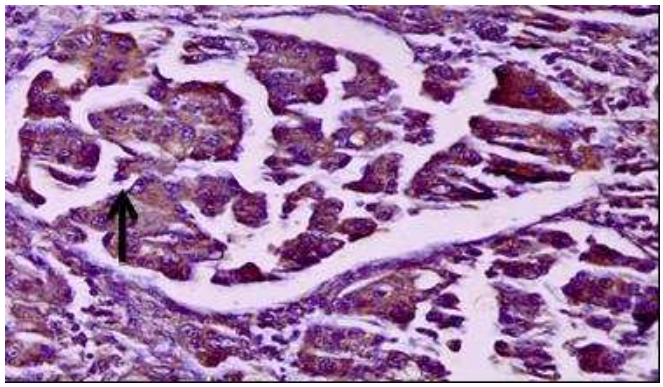

Figure 1 :Invasive ductal carcinoma, (arrowed) showing strong cytoplasmic staining (score 4+)
[immunohistochemical stain for BRCA1 ,40X].

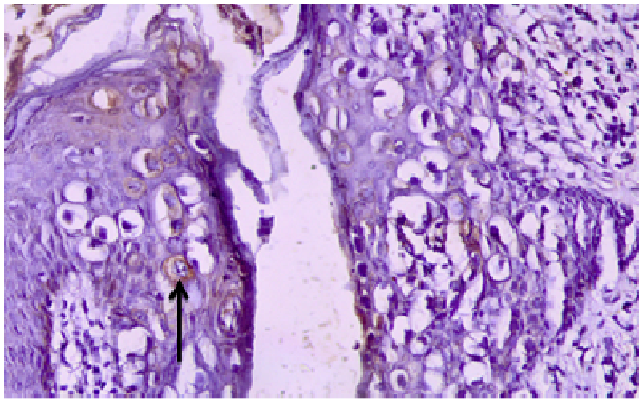

Figure 2:Invasive ductal carcinoma, with Paget's dis. (arrowed)showing weak cytoplasmic staining (score 2+)[immunohistochemical stain for BRCA1, 40X].

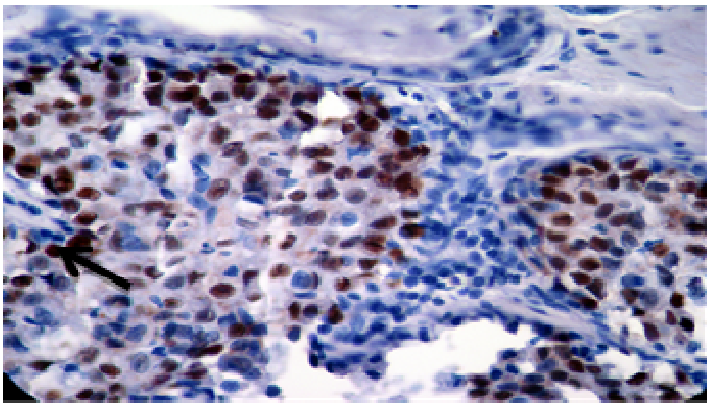

Figure3 :Insitu ductal carcinoma, (arrowed) showing nuclear staining(score 3+) [immunohistochemical stainfor p53, 40X].

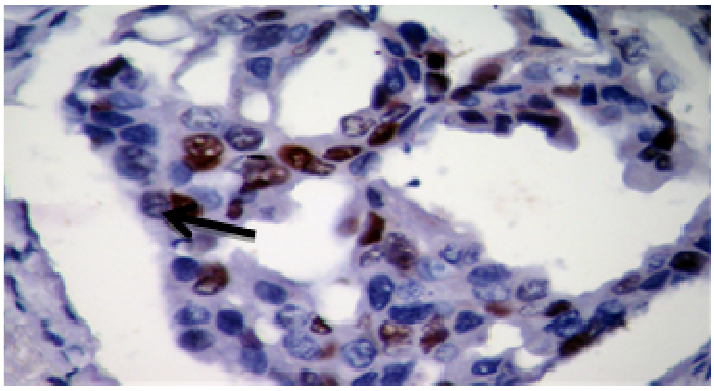

Figure4 :Invasive ductal carcinoma(arrowed) showing strong nuclear staining (score 2+) [immunohistochemical stainfor p53, 40X].

Table 1Immunoexpression of BRCA1 and p53 in relation to clinico pathological parameters of breast carcinomaof old age group.

Parameters	Total number of patients	BRCA1 overexpression		P53 immunoexpression	
		Positive No. %	Negative No. %	Positive No. %	Negative No. %
Type of breast tissue Normal Benign(fibroadenoma) Malignant	10 10 30	0 (0) 0 (0) 15(50) **P<0.05**	10 (100) 10(100) 15 (50)	0 (0) 0 (0) 10 (33.3) **P< 0.05**	10 (100) 10 (100) 20 (66.6)
Histological type Ductal carcinomas Lobular carcinomas	28 2	14 (50) 1 (50) **P<0.05**	14 (50) 1 (50)	9 (32.2) 1 (50) **P>0.05**	19 (67.8) 1 (50)
Tumor grade I II III	5 8 17	2 (40) 5 (62.5) 8 (47.1) **P>0.05,R<0.3**	3 (60) 3 (37.5) 9 (52.9)	2 (40) 1 (12.5) 7 (41.2) **P<0.05,R>0.3**	3 (60) 7 (87.5) 10 (58.8)
Tumor size Tis T1 (≤ 2 cm) T2 (2>-5 cm) T3 (> 5 cm) T4 (anyT+other)	14 3 5 5 3	6 (46.9) 2 (66.7) 3 (60) 3 (60) 1 (33.3) **P>0.05,R>0.3**	8 (57.1) 1 (33.3) 2 (40) 2 (40) 2 (66.7)	5 (35.7) 2 (66.7) 1 (20) 1 (20) 1 (33.3) **P>0.05,R<0.3**	9(64.3) 1(33.3) 4 (80) 4 (80) 2 (66.7)
Axillary lymph nodes Negative Positive	9 21	6 (66.7) 9 (42.9) **P<0.05,R<0.3**	3 (33.3) 12 (57.1)	4 (44.5) 6 (28.5) **P<0.05,R>0.3**	5 (55.6) 15 (71.9)
Tumor recurrence Primary Recurrent	23 7	12 (52.2) 3 (42.9) **P>0.05,R>0.3**	11 (47.8) 4 (57.1)	7 (30.4) 3 (42.9) **P<0.05,R<0.3**	16(69.6) 4 (57.1)

Table 2Coexpression of BRCA1 and p53 in relation to clinico pathological parameters of breast carcinomaof old age group.

Parameters	Both BRCA1andp53 positive	Only BRCA1 positive	Only p53 positive	Both BRCA1andp53 negative	Total
Histological type					
Ductal carcinomas	6(100%)	8 (88%)	4(80%)	10 (100%)	28 (93.3%)
Lobular carcinomas	0(0%)	1 (11%)	1 (20%)	0 (0%)	2 (6.7%)
	R >0.3				
Tumor grade					
I	1(16.6%)	1(11%)	1(25%)	2 (18%)	5 (16.6%)
II	1(16.6%)	4 (44.4%)	0 (0%)	3 (27.2%)	8 (26.6%)
III	4(66.6%)	4 (44.4%)	3 (75%)	6 (54.5%)	17 (56.8%)
	R >0.3				
Tumor size					
Tis	4(66.4%)	2 (22.2%)	1 (25%)	7 (63.6%)	14(46.8%)
T1 (≤ 2 cm)	1(16.6%)	1 (11%)	1 (25%)	0 (0%)	3(10%)
T2 (2>-5 cm)	0(0%)	3 (33.3%)	1 (25%)	1 (9.1%)	5 (16.6%)
T3 (> 5 cm)	0(0%)	3 (33.3%)	1 (25%)	1 (9.1%)	5 (16.6%)
T4 (anyT+other)	1(16.6%)	0 (0%)	0 (0%)	2 (18.1%)	3 (10%)
	R>0.3				
Axillary lymph nodes					
Negative	3(50%)	3 (33.3%)	1 (25%)	2 (18.1%)	9 (30%)
Positive	3(50%)	6 (66.6%)	3 (75%)	9 (81.9%)	21 (70%)
	R>0.3				
Tumor recurrence					
Primary	3(12.5%)	8 (88.9%)	2 (100%)	10 (91%)	23 (76.6%)
Recurrent	5(62.5%)	1 (11.1%)	0 (0%)	1 (9%)	7 (23.4%)
Total	8(26.6%)	9(30%)	2(6.6%)	11(36.6%)	30(100%)
	R>0.3				

Discussion:

The biology of breast carcinoma remains poorly understood as the knowledge about individual prognostic factors provides limited information[16]. A wide variety of morphology-based and molecular-based prognostic factors and tumor markers have been studied according to their potentials to predict the outcome in breast cancer. Verifying molecular abnormalities in breast cancer is an important strategy for its early detection, assessment of prognosis, and treatment selection[17]..One major goal of this study was to choose an appropriate and reliable prescreening method for BRCA mutation analysis. We performed immunohistochemical analysis targeting BRCA1 protein in tumor cells. This protein is predicted to accumulate in cytoplasm when the mutation is in exon 11 . Our aim was to detect the cytoplasmic form of BRCA1 protein, considering that this abnormal location could be an indicator for BRCA1 mutation (at least in exon 11). [18]Up to our knowledge there was no published paper studying the correlation between BRCA1 and p53 immunoexpressionin different clinicopathological parameters and evaluating the behavior of the tumor in early age patients with breast cancer in our country.

In the present study the percentage of immunoexpression in malignant breast lesionswas 50% for BRCA1 ,33.3% for p53 out of 30 breast cancer cases ,andnegative in all sections of the normal and benign breast tissues,These results might lead to say that BRCA1 overexpression is exclusively reported in malignant breast tissue[19-20],andp53 immunoexpression looks to be characteristic of malignant breast tissue and p53 expression represent early and important event in the molecular pathogenesis of BRCA1

– breast cancer, and does not play a role in normal breast tissues or fibro adenoma. This study has found that BRCA1 was overexpressed in ductal carcinomas was significantly greater than that in infiltrating lobular carcinomas. The current study showed that 50% of pure IDC, 42.9% of DCIS,and 100%of those with invasive ductal carcinoma with Paget's disease, were BRCA1 positive,thus, supporting the view that such tumors represent adefined subtypes of breast carcinoma in relation to the age. While p43 was overexpressed in 60.7% of ductal carcinoma cases and 50% of invasive lobular carcinomas , p53 immunodetection was detected in 35.7% of pure ductal carcinoma in situ ,25% of invasive ductal carcinoma, and50%of those with invasive ductal carcinoma with Paget's disease**.** In all these types the incidence is statistically not different from that found in the control ($p > 0.05$)**.**There was a strong positive correlation between detection of BRCA1 and p53 biomarkers regarding all the three grades ($R > 0.3$) , with significant difference among these grades ($P < 0.05$), a result that is similar to those reported elsewhere[21]**.**Both parameters were associated with worse grades and high proliferation rate.

The degree of the differentiation does not contribute to the increase of the expression of both markers ,though it may reflect the possible role of other pathways by which the tumor is advancing independently from increase in signaling pathways of both BRCA1 and p53 genes.The detection rate of p53 does not increase with size of tumor and there was no significant difference among various tumor sizes ($p > 0.05$) . This result is consistant with previous investigations[22-23]**.** There was significantly higher p53 immunoexpression in recurrent breast cancer patients compared with primary lesions($P < 0.05$), this is comparable with findings in previous studies[24],as the strong correlation between BRCA1 and p53co-expression and grade, lymph node and tumor recurrence is found in this study**.**The present work confirms previous findings that combined alteration in the expression of BRCA1 and p53 are linked to accelerated tumor behavior and a poor prognosis[25-26]**.**

Conclusion: In conclusion, p53 and BRCA1 over-expression play an important role in pathogenesis of breast carcinoma evolution, as their positivity associated with biologically aggressive tumors , so incorporation of these biomarkers with other parameters into a prognostic index will more accurately predict clinical outcome and determine the effects of anti-cancer therapy.However, their results remain controversial, the inconsistency of the results reported may be explained by the small number of cases, inhomogeneity of the methods used to assess the expression of these molecular markers, and short duration of follow-up. Sofurther investigation of larger number of patients with breast cancer in a prospective study with a longer duration of follow-up and studying the survival rates will provide a better insight and validate our findings,considering further studies including DNA/mRNA and protein levels by FISH(Florescent insitu hybridization) or PCR(Polymerase Chain Reaction) to confirm the molecular basis of these genes alteration, and incorporation of these biomarkers with other factors into a prognostic index will more accurately predict clinical outcome and determine the effect of anti-cancer therapy.

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