

Overexpression of mammaglobin in tissue breast carcinoma by immunohistochemistry

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الخلاصة

البيج نقص ن التعبير ر المذ اعى النس يجي الكيمى ائى ل ج ين أل mammaglobin في خلايا سرطان الثدي المعروفة ترابط ه ذا التغير مع درجة تم ايز ال ورم يتم ت دراسة 60 عينة من سرطان الثدي مختبرات مستش فى الديوانية التعليمى للفتره منذ ذ بدايه تشدرين الثاني 2005 وحتى أب 2011 لودت أعم ارهم ب بين 45 و 60 نه ص د نلفغين ذات حسب درجة التمايز إلى 20 عينات من الدرجة الأولى إلى 20 عينة من الدرجة الثانية و 20 عينة من الدرجة الثالثة على التوالي. أظهرت الدراسة المناعية النس يجية أن تعبير ر أل- mammaglobin كان موجبا في 45 عينة (75%) من سرطان الثدي، وكان أكثر في العينات ذات التمايز من (الدرجة الأولى والثانية) عنه في العينات ذات التمايز من (الدرجة الثالثة) كما انه توجد علاقة واضحة لتعبير ال mammaglobin مع درجة التمايز (قيمة ألفا > 0.05) ما يدل على أن ج ين أل- mammaglobin يلعب دور أساسى في تقييم حالة سرطان الثدي المستقبلية.

Abstract

Our purpose was to investigate the mammaglobin overexpression in tissue breast cancer and to determine its correlations with conventional prognostic parameters. There were investigated 60 patients with breast carcinoma, and slides from paraffin blocks were stained with an antibody against mammaglobin. The immunohistochemical reaction was scored based on the percentage of positive tumor cells in tumors. Positive reaction for mammaglobin was diffused cytoplasmic staining found in 75% of tissue breast carcinoma. A significant correlation was found between the mammaglobin expression in the breast cancer and grades of the tumor. Our results suggest that mammaglobin is a good prognostic marker for breast carcinoma

Aim of study:- this study was to investigate the mammaglobin overexpression in tissue breast cancer and to determine its correlations with prognostic significant.

Keywords: breast cancer, immunohistochemistry, mammaglobin

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Introduction

Breast cancer:- is cancer originating from breast tissue, most commonly from the inner lining of milkducts or the lobules that supply the ducts with milk.^[1] Cancers originating from ducts are known as ductal carcinomas; those originating from lobules are known as lobular carcinomas. Breast cancer is a disease of humans and other mammals; while the overwhelming majority of cases in humans are women, men can also develop breast cancer.^[2] Worldwide, breast cancer comprises 22.9% of all cancers (excluding non-melanomaskin cancers) in women.^[3] In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women).^[3] Breast cancer is more than 100 times more common in women than breast cancer in men, although males tend to have poorer outcomes due to delays in diagnosis^[4]. Prognosis and survival rate varies greatly depending on cancer type, staging and treatment, 5-year relative survival varies from 98% to 23%, with an overall survival rate of 85%^[3].

Mammaglobin:-Themammaglobin gene also known as human mammaglobin (hMAM) which is located on chromosome 11q13 and encodes a glycoprotein^[5]. It codifies for a glycoprotein of 23 amino acids^[6]. This gene is often expressed at basal levels in normal breast and its overexpression was assumed to be present in breast cancer^[7]. it is belongs to secretoglobin superfamily . Unlike other secretoglobin family members, mammaglobin expression is breast specific. Mammaglobin, in conjunction with a panel of other markers, may aid in the identification of breast carcinomas presenting at metastatic sites^[8,9]. Although the functional significance of mammaglobin remains unknown, the restricted expression to the mammary tissue led to the evaluation of this molecule as a possible breast cancer marker^[10]. Mammaglobin expression has been reported in 70-80% of primary and metastatic breast tumors and has been associated with unfavorable prognosis^[11].

Material and method

This study was conducted on 60 breast cancer female patients. Their ages ranged from 45 to 60 years. They were referred to the Al-Diwaniya Teaching Hospital in a period from November 2005 – August 2011.

The diagnosis of breast cancer was based on tissue biopsy for histopathological examination, all breast cancer patients were graded according to the system of Bloom and Richardson ^[12] which was recommended by WHO ^[13] into:

- Grade I (Well differentiated): It included 20(33.3%) patients.
- Grade II (Moderately differentiated): It included 20(33.3%) patients.
- Grade III (Poorly differentiated): It included 20(33.3%) patients.

Control group

This group included 18 of normal breast tissue adjacent to tumor tissue.

Immunohistochemical study

Streptavidin-biotin technique was used to investigate mammaglobin (MAG). Slide from each case were deparaffinized, hydrated and incubated in 3% hydrogen peroxide for 30 minutes to block the internal peroxidase activity. Antigen retrieval was done by microwave pretreatment for 10 minutes in 0.01 citrate buffer. Slide was incubated with anti-mammaglobin monoclonal antibody (Dako Corporation) at a dilution 1:100, at 4 °C overnight. Sections were then washed twice for 5 minutes with PBS and incubated for 10 minutes with biotinylated secondary antibody (Dako Cytomation). The slides were washed twice for 5 minutes in PBS and incubated for 10 minutes in performed avidin-biotin-peroxidase complex (Dako Cytomation). Chromogen development was accomplished by immersion of the sections in 2, 3-Diaminobenzidine tetrahydrochloride (BAB) (Dako Cytomatin) for 5 minutes. The nuclei were counterstained with hematoxylin, dehydrated, cleared and mounted. For negative controls, the primary antibody was omitted and replaced with PBS ^[14,15].

Mammaglobin gives cytoplasmic staining

Mammaglobin scoring:-According to Han et al ^[16,17], the intensity of mammaglobin expression were scored as no staining, weak, moderate and strong staining based on the number of positive epithelial cells, as follows: negative (0), weak positive with less than 10% positive cells (+1), moderate positive with 11 to 50% stained cells (+2), and strong with over 50% stained cells (+3). The intensity of the final product of reaction was not taken into account, because all stained cells showed a strong reaction.

Statistical analysis:-Statistical analysis was performed using SPSS ver14,A significant correlation was reported for a value of $p < 0.05$.

Results

Mammaglobin overexpression in 45(75%) of 60 specimens with breast cancer and where diffuse cytoplasmic staining was seen and only 15(25%) of breast cancer give negative staining for mammaglobin as show in (table 1).While in normal control breast tissue was less mammaglobin expression and scattered in epithelial cells less than 10% of positive cells.

According to grades of tumors ,mammaglobin was positive in 20(100%) in grade I,17(85%) in grade II , 8(40%) in grade III and 12(60%)was completetly negative cells in grade III, so mammaglobin overexpression was highly significant correlated with tumor grading when comparing high grade tumors (GIII) with low grade tumors (GI, GII) where P value was 0.0000 as show in (table 2). In our study , majority cases of breast cancer 28(60.2%) give strong positive cells score (+3) , 11(24.5%)cases give moderate staining score (+2) and only 6(13.3%) cases give weak staining score (+1) as show in (table 3).According to scoring system in comparison with grading of tumors ,we detect strong staining of mammaglobin score (+3) in 14(70%)of grade I cases,(figure 1) ,13(76.5%) in grade II,(figure 2) and only 1(12.5%) in grade III ,while weak staining was not detected in grade I in comparison with grade IIIwhich was 5(60.5%) cases with weak staining pattern ,(figure 3), so strong staining of mammaglobin was highly significant correlated with grades of tumors where P value was (0.0000) as show in (table 4).

Table 1:- Mammaglobin expression in breast cancer tissue.

Mammaglobin expression	-ve	+ve	Total
	15(25%)	45(75%)	60(100%)

Table 2:- Mammaglobin expression in breast cancer tissue accords to grades of the tumor.

Grades of the tumor	Mammaglobin expression		Total	P value
	-ve	+ve		
I	0	20(100%)	20(33.3%)	0.000000
II	3(15%)	17(85%)	20(33.3%)	
III	12(60%)	8(40%)	20(33.3%)	
Total	15(25%)	45(75%)	60(100%)	

Table 3:- scoring system of Mammaglobin positive cells expression.

Mammaglobin expression	Scores of expression			Total
	+1	+2	+3	
	6(13.3%)	11(24.5%)	28(62.2%)	45

Table 4- scoring system ofMammaglobin positive cells expression in relation to grades of tumor.

Grades of tumor	Scores of expression			Total	P value
	+1	+2	+3		
I	0	6(30%)	14(70%)	20	0.00004
II	1(5.9%)	3(17.6%)	13(76.5%)	17	
III	5(62.5%)	2(25%)	1(12.5%)	8	
Total	6	11	28	45	

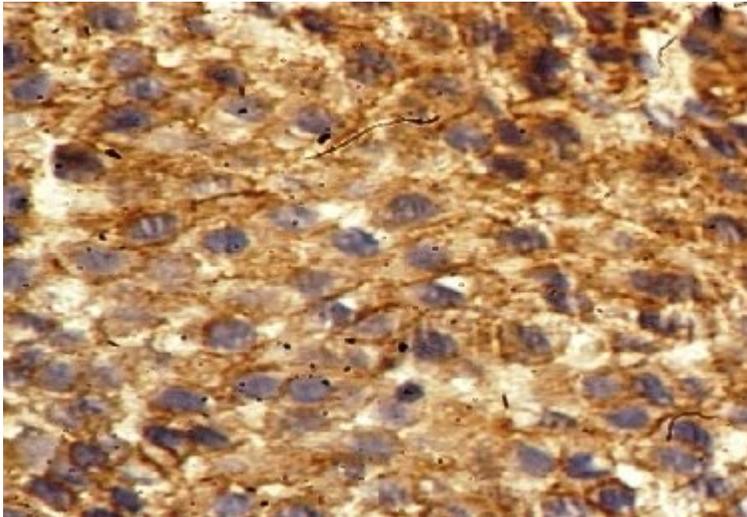


Figure 1:- Mammaglobin over expression in grade I breast carcinoma with diffuse cytoplasmic staining (score 3) X40.

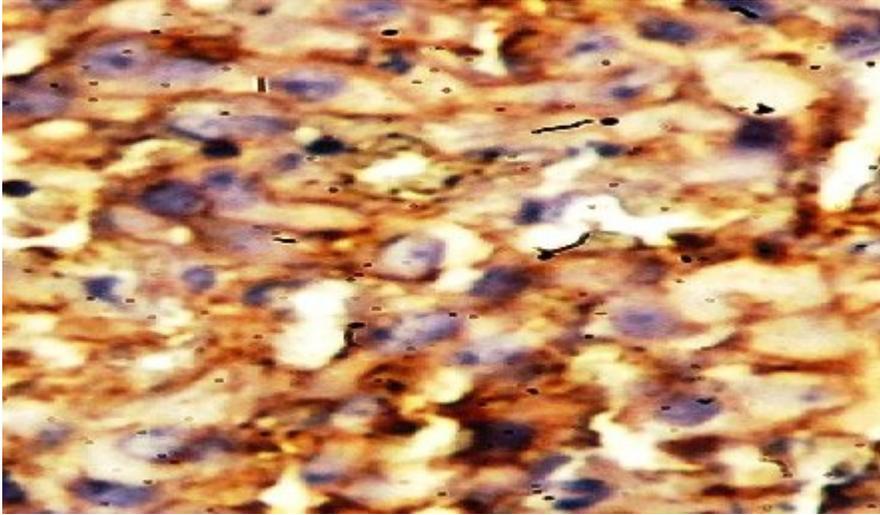


Figure 2:- Mammaglobin over expression in grade II breast carcinoma with diffuse cytoplasmic staining (score 3) X40.

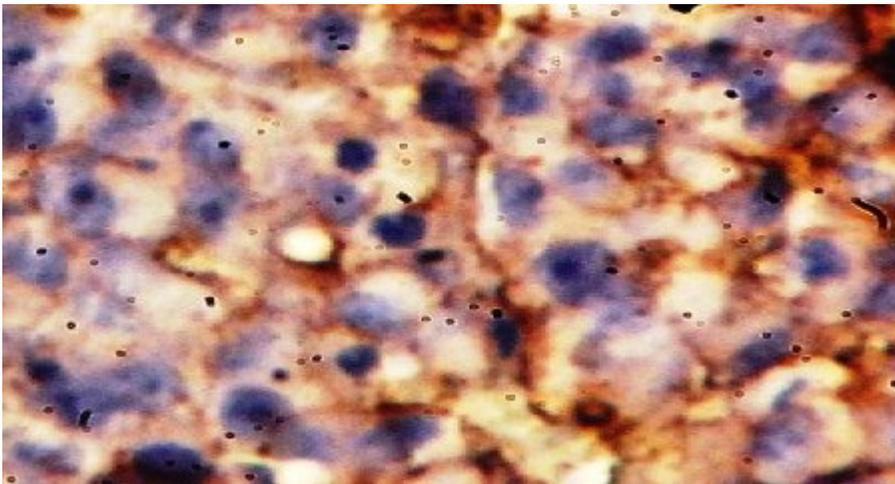


Figure 3:- Mammaglobin over expression in grade III breast carcinoma with diffuse cytoplasmic staining (score 1) X40.

Discussion

Almost all studies showed that mammaglobin expression in tissue breast cancers varies from 20% to 95%^[18, 19]. Such broad range might be due to several factors, such as tumors storage methods (fresh/frozen tissue and paraffin-embedded blocks) and/or the different techniques used for assessing the different expression levels (RT-PCR, immunohistochemical staining or in situ hybridization)^[17,19,20].

In our study, the expression of mammaglobin in tissue breast cancers by immunohistochemistry was 75%, had diffuse cytoplasmic staining, this results similar to Sonia et al^[21] that found mammaglobin overexpression in breast cancer tissues was detected in 79% of the carcinoma cases. Also our results agree with Raica et al^[22] those found mammaglobin overexpression in breast cancer tissues by immunohistochemistry was detected in 78.72% of cases.

On the contrary, results of Gargano et al^[23] and Roncella et al^[24] were showed that 90% and 93% respectively of breast cancers expressed mammaglobin by PCR technique. This controversy may be attributed to PCR method used by those which is more sensitive than immunohistochemical technique but in our work used immunohistochemical technique because this method is easier, practical and give good sensitivity.

On the other hand, we found in our study, all cases of GI and majority of G II give positive cell staining while majority cases of GIII give negative staining for mammaglobin and on other hand, majority of GI and GII give strong staining while majority of GIII give weak staining. So, the mammaglobin expression is significantly association with grade of tumors and highly restricted to low grade tumors and this results similar to many studies by gilbey et al^[25], Gargona et al^[23], Span et al^[26], and Bernstein et al^[27], those found that GI and GII express significantly higher mammaglobin than GIII tumors and all of the breast cancer negative staining belong to GIII thus suggesting that even mammaglobin expression may conflict prognostic factor for breast cancer. The reasons responsible for the absence of mammaglobin expression in a proportion of breast cancer GIII cases are currently unknown.

Conclusion

Mammaglobin is specific tumor marker for breast tissues ,and overexpression in breast cancer tissues is associated with a better prognosis.

Recommendation

Further research about mammaglobin overexpression in blood of patient with breast cancer in comparison with tissue and using of other method for detection of mammaglobin overexpression like PCR is recommended.

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