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Research Paper

Immunohistochemical Expression of Annexin A5 in Invasive Ductal Carcinoma and Fibroadenoma of the Breast

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ABSTRACT:

BACKGROUND:

Breast cancer is the most common cancer. The commonest type of breast cancer is invasive ductal carcinoma. Fibroadenoma a common benign lesion occur in about one in four women. Annexin-A5 is a tumor marker which is a member of the calcium phospholipid binding protein family that could promote tumorigenesis and progression in certain types of cancers.

OBJECTIVE:

Study the expression of AnnexinA5 in breast cancer and fibroadenoma and correlate the result of study with the clinico-pathological parameters.

PATIENTS AND METHODS:

Comparative case - control study included two groups, group A 30 patients with breast cancer and group B 30 patients with fibroadenoma. Each block of all groups of cases was sliced into two slides (one for Hematoxylin and eosin and the other for immunohistochemistry study).

RESULTS:

AnnexinA5 expression in fibroadenoma was found in 100% of cases which is greater than its expression in invasive ductal carcinoma (70% of cases). There was no significant correlation between A5 expression & age of patients, histologic grade, perineural invasion, vascular invasion and necrosis in invasive ductal carcinoma.

CONCLUSION:

According to the results of present study it was concluded that there is a significant expression of AnnexinA5 in fibroadenoma correlated with age, and non-significant expression of AnnexinA5 in invasive ductal carcinoma in correlation with age, grade, necrosis, vascular and perineural invasion.

KEYWORDS: AnnexinA5, breast cancer, Invasive ductal carcinoma, fibroadenoma.

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Iraqi Postgraduate Medical Journal, 2024; Vol. 23(2): 157-162

DOI: 10.52573/Ipmj.2024.183509, Received: November 15, 2022, Accepted: November 27, 2022

INTRODUCTION:

Breast cancer is the most common cancer worldwide and the most common cause of cancer related death among women[(1)]. The commonest type of breast cancer is the invasive ductal carcinomas not otherwise specified which account for up to 25% of all breast cancers among more than 16 distinct histological special types [(2)]. It is divided into 3 types, Hormone receptor–positive breast cancer makes up 70% of breast cancer cases and has either estrogen receptor (ER) or progesterone receptor (PR) protein in the cancer cells; ERBB2-positive (formerly known as HER2-positive) breast cancer makes up 15% to 20% of breast cancer cases and triple-negative breast cancer which makes up 15% of breast cancer cases and does not have ER, PR, or ERBB2 protein in the cancer cells [(3)].

Fibroadenoma A common benign lesion characterized by a nodule of fibrous tissue with epithelial elements occur in about one in four women and can account for more than two-thirds of all benign lesions in young women [(4)]. It occurs most commonly in women between the ages of 14 to 35 years but can be found at any age [(5)].

AnnexinA5 is the smallest member of the annexin family, has properties of membrane binding on membrane surfaces[(6)]. AnnexinA5 promotes pancreatic adenocarcinoma, tumorigenesis and progression of breast cancer and prostate cancer stem cells. It is involved with metastasis, invasion and development of squamous cell carcinoma, and facilitates nodal progression of bladder cancer and angiogenesis and progression of glioma.

AnnexinA5 de-regulation is associated with drug resistance in nasopharyngeal carcinoma and gastric cancer. AnnexinA5 is negatively correlated with thyroid cancer malignancy [7].

MATERIALS AND METHODS:

1. Patients and methods:

A comparative case control study, patients were divided into two groups, group A included 30 patients proved to have breast cancer and group B included 30 patients proved to have fibroadenoma. The samples were collected from the archives of the teaching lab of Al-Yarmuk general hospital and a private lab in Baghdad for a period from November 2019 to July 2020. Regarding the selection of cases, the types of specimens taken in this study were 52 excisional biopsy specimens and 8 mastectomy specimens, clinico-pathological data including age of patients, histological grade, perineural invasion, vascular invasion and necrosis were collected from the archives reports and prepared for immunohistochemical staining, cases were reviewed for diagnosis by a consultant histopathologist.

2. Quality control

Positive control: Normal human Spleen tissue specimens were considered as a positive control.

Negative control: it was accomplished by removing the primary antibody and putting PBS which is antibody diluent in the same slide solely and performing the phases used in immunohistostaining.

3. Microscopical study

A digital light microscope (Motic BA210) was used in the examination of slides, each field was obtained from the region of 5 zones of the slide (corners and the center) which were randomly selected, then the image captured in high definition (HD) using the same device built in camera that displays the image on the LCD screen.

4. Scoring of Annexin V

Depending on the appearance of different brown (cytoplasmic and/or membranous staining), the cells were scored as (positive/ negative), the slides were evaluated with low-power microscopy (10x) to establish the largest staining areas, if no staining seen at low power, re-evaluation performed with high power (40x) to assess weak staining region, all areas of every slide were observed and scored semi quantitatively by measuring the percentage of cells that are positively stained over the entire amount of malignant cells “percent”.

The percentage of cells staining were evaluated using 10% as cut-of value as follow: 10% _ 0.1, 20% _ 0.2, 30% _ 0.3 and so on...

To measure the expression of Annexin V in tissues, the extent and intensity of staining were assessed in each section. All tissues were analyzed and scored independently by experienced pathologist.

Annexin A5 staining intensity was scored as: 0(negative, -), 1(positive, weak, +), 2(positive, moderate, ++), 3(positive, strong, +++).

The extent of staining was scored as 0-1.0 (0-100%)

The final staining score (0-3) was calculated as the multiplication of the intensity score and extent score [8].

5. Statistical analysis

The experiment was designed using completely randomized design. The relationship between the pattern of Annexin A5 expression and clinico-pathological variables was analyzed by the statistical package for the social science (SPSS) version 24. The results were considered statistically significant if the probability p value was <0.05 and highly significant <0.00

RESULTS:

The present study is a comparative case control study divided into two groups the first group included 30 cases of FA and the second group were 30 cases of IDC. These samples were studied concerning the pattern of expression of Annexin A5 and its relation to certain clinico-pathological parameters.

This study focused on the expression of Annexin A5 and its potential clinical value in comparison between fibroadenoma and IDC of the breast. In this study the expression of AnnexinA5 assessed on 30 cases of IDC and 30 cases of fibroadenoma. Result indicated that there was Annexin A5 expression in fibroadenoma and IDC. It has been found in this study that Annexin A5 expression in IDC is (70%), and in fibroadenoma is (100%), In 30 cases of invasive ductal carcinoma 21 out of 30 cases (70%) showed Annexin A5 expression, of them 40% were moderate intensity and 30% were strong intensity staining. while all 30 cases of fibroadenoma showed expression (100%), 70% of them were strong intensity stained and 30% with moderate intensity.

Regarding the selection of cases, the types of specimens taken in this study were 52 excisional biopsy specimens and 8 mastectomy specimens.

Percentage of cases positive for annexinA5 expression in fibroadenoma were 100% meaning all cases showed expression, from them 70% of cases showed strong intensity staining and 30% of cases showed moderate intensity as illustrated in figure 1. B and 1. C respectively. .

Percentage of cases positive for Annexin A5 expression in invasive ductal carcinoma were 70% and percentages of cases showed negative stain were 30% as in figure 1.F From the positive expressed cases 40% of cases showed moderate intensity as in figure 1.D and 30% showed strong intensity. As shown in figure 1. E.

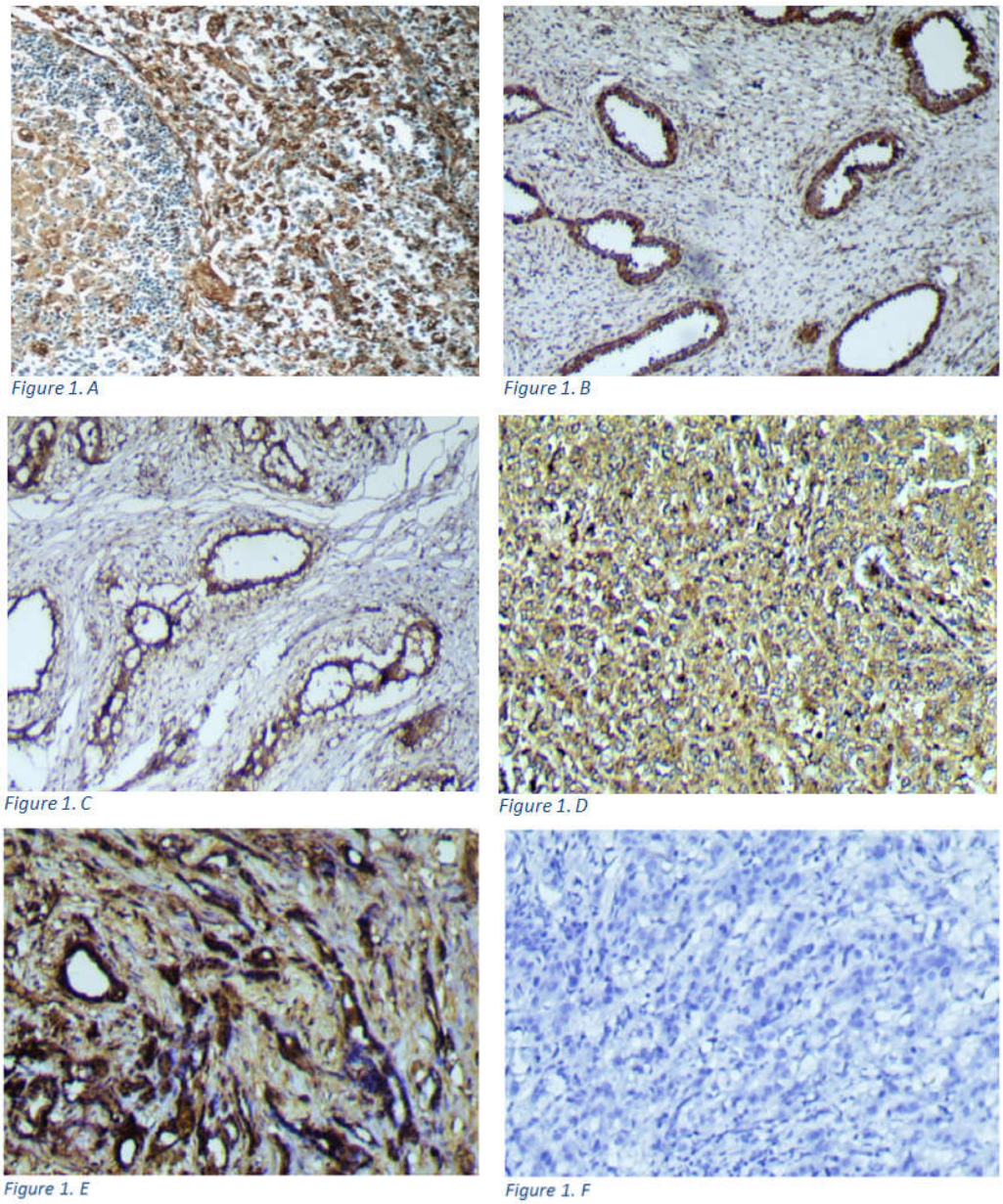

Figure 1: **H&E and Immunohistochemical staining:** Representative H&E of breast cancer and fibroadenoma and immunohistochemical staining of AnnexinA5 A: AnnexinA5 expression in positive control spleen tissue at 200 magnification. B, C: AnnexinA5 expression in fibroadenoma strongly stained at 200 magnification (B), and moderately stained at 200 magnification (C). D, E: AnnexinA5 expression in invasive ductal carcinoma of breast grade III moderatley stained at 200 mgnification (D), and strongly stained at 200 magnification (E). F: negative expression of AnnexinA5 in invasive ductal carcinoma grade III at 200 magnification

Table 1: The relationship of AnnexinA5 expression between Invasive ductal carcinoma and Fibroadenoma in 30 studied cases

Type	No. of cases	mean/SD
Invasive ductal carcinoma	30	1.033/0.887
Fibradenoma	30	2.303/0.607**

Table 2: The Relationship between age and AnnexinA5 expression in invasive ductal carcinoma and fibroadenoma in 30 studied cases

	Invasive ductal carcinoma				Fibroadenoma	
Age	ANA5 +ve	ANA5 –ve	Total	P value	ANA5 +ve	Percentage
<20	0	0	0	0	5	16.6%
20-29	1 (4.76%)	0	1	0.512	13	43.3%
30-39	1 (4.76%)	2	3	0.165	8	26.6%
40-49	6 (28%)	7	13 (43%)	0.060	4	13.3%
50-59	8 (38%)	0	8 (26.6%)	0.064		
>60	5 (23%)	0	5	0.143		
	21	9	30			

Table 3: Relationship between perineural invasion and Annexin V expression in IDC in 30 studied cases

Perineural invasion	ANA5 +ve	ANA5 –ve	Total	P value
Negative	15 (71.5%)	9	24 (80%)	0.422
Positive	6 (28.5%)	0	6 (20%)	0.108
	21	9	30	

Table 4: The Relationship between histological grade and Annexin V expression in Invasive ductal carcinoma in 30 studied cases

Grade	ANA5 +ve	ANA5 –ve	Total	P value
1	2 (9.5%)	2	4 (13%)	0.382
2	15 (71%)	5	20 (66%)	0.625
3	4 (19%)	2	6 (20%)	0.858
	21	9	30	

Table 5: Relationship between vascular invasion and Annexin V expression in IDC in 30 studied cases

Vascular invasion	annexin 5 +ve	ANA5 –ve	Total	P value
Negative	17 (81%)	5	22	0.456
Positive	4 (19%)	4	8	0.217
	21	9	30	

Table 6: Relationship between necrosis and Annexin V expression in IDC in 30 cases

necrosis	**ANA5 +ve**	**ANA5 –ve**	**Total**	**P value**
positive	12 (54%)	4	16 (53%)	0.895
negative	10 (46%)	4	14 (47%)	0.866
	22	8	30	

**chi test *p value <0.05*

DISCUSSION:

In this study, Expression of Annexin A5 relationship between Invasive ductal carcinoma and Fibroadenoma, a highly significant result found in which Annexin A5 expression in fibroadenoma was found in all 30 cases (100%) which is greater than its expression in invasive ductal carcinoma (70%). In contrast to the study of *H.A.Hassan et al* of "Comparative expression of caspases and annexin V in benign and malignant ovarian tumors", in which Annexin A5 expressed in malignant group more than benign group [(9)]. While in the study of Aziz et al of IHC expression of Annexin A2 and Annexin A6 in breast cancer, stated that the expression of Annexin A2 was significant in mammary invasive ductal carcinoma in relation to fibroadenoma samples, while the annexin A6 expression in invasive ductal carcinoma was not significant [(10)], differences could due to variability in age group, bias in selection and due to factors like different methods and techniques used such as molecular techniques like SISH and/or FISH.

In this study the expression of Annexin A5 in IDC, the correlation with patient's age, histological grade 2, perineural and vascular invasion and presence of necrosis wasn't significant.

In the study of *Tang J et al* of "High Annexin A5 expression promotes tumor progression and poor prognosis in renal cell carcinoma", stated that Annexin V expression in renal cell carcinoma and age did not significantly correlate which is similar to our results in which age did not significantly correlate either, while it stated that Annexin A5 expression exhibited a significantly positive correlation with histological grade and TNM stage, which is unlike our study in which histological grade didn't significantly correlated, this could be due to small sample size taken [(11)].

In another study *Chong-Bing Sun et al* of " Expression of annexin A5 in serum and tumor tissue of patients with colon cancer and its clinical significance stated that annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade [(12)]. In our study tumor grade did not significantly correlated this is may be related to small size of the sample and bias in selection of cases since most of our cases were grade 2.

CONCLUSION:

From the results of the current study, it is concluded that:

There is highly significant expression of Annexin A5 in fibroadenoma correlated with age of the patients while The expression of Annexin A5 in Invasive ductal carcinoma was not significant in relation to the clinico-pathological parameters mentioned which is age, histological grade, presence or absence of necrosis, perineural invasion and vascular invasion and Most of the invasive ductal carcinoma cases recorded were with histologic grade 2 with no perineural invasion, vascular invasion or necrosis.

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