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A Study of IHC Staining Pattern of Mammaglobin in Various Breast Lesions in a Tertiary Care

Padmaja BVN¹, Devayani N², G Anandam³

1 & 2 - Assistant Professor, Department of Pathology, Prathima Institute of Medical Sciences, Naganoor, Karimnagar, Telangana State

3. Professor & HOD, Department of Pathology, Prathima Institute of Medical Sciences, Naganoor, Karimnagar, Telangana State

Abstract

Aim: The aim of the present study was to study the expression pattern of mammaglobin in various breast lesions. The study was carried out by using mammaglobin as a marker to the difference between benign and malignant lesions. **Methods:** This study was done in the Department of Pathology, at Prathima Institute of Medical Sciences, Karimnagar. Two micro-sections of 4-5 micron thickness were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other on poly-L-lysine coated slide for immunohistochemical staining. Histological typing of the tumour was done. Immunohistochemical staining of mammaglobin protein was done using peroxidase-anti-peroxidase method according to the protocol described by DAKO. The slides were then examined under the microscope and mammaglobin positivity is classified as positive (score 3&4) and negative (score 1&2). **Results:** A total of 40 breast lesions are included in the immunohistochemical study, of which 10(25%) were benign, 30(75%) were malignant. Out of total n=10 benign lesions, mammaglobin expression was found negative in n=9 lesions and the only n=1 lesion was found to be positive for mammaglobin. Out of the 30 malignant lesions, n=22(73.3%) were positive and n=8 (26.67%) were negative for mammaglobin expression. **Conclusion:** It is concluded that Mammaglobin is a useful marker for malignant breast lesions. Mammaglobin is a novel promising marker for neoplastic breast epithelial cells. It can be used as a molecular marker for early detection, prognosis and relapse monitoring for breast cancer cases.

Keywords: Breast Lesions, IHC Staining, Mammaglobin

Address for correspondence: Dr. Devayani N, H. No: 2-7-76, Sanjeeviah Nagar, Near Old Petrol Pump, Sircilla - 505301, Karimnagar District, Telangana State. drdevayani726@gmail.com Mobile: 9885900664.

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Introduction

Worldwide breast cancer is the most frequently diagnosed cancer in women and represents the leading cause of cancer deaths among women. [1] Breast cancer is now the most commonly diagnosed cancer in urban Indian women. [2] There is a significant trend of increase in the incidence of breast cancer in Indian population probably explained by the westernization of lifestyles and changes in reproductive behavior. Breast cancer accounts for 5-8% of all cancers in India and the incidence is on the rise. [3] It is

the most common cancer of urban Indian women and the second most common in rural women. [4] Locally advanced breast cancer accounts for 50% of all breast cancers. [5] Several molecular components related to the development of breast carcinomas and associated with therapeutic and prognostic value, like p53, RB, ER, PR, HER-2/neu; have been studied in great detail. [6, 7] Recent emphasis is on identifying markers related to tumor metastasis, which is the major cause of morbidity and mortality in these patients. [8] The evolution of human breast cancer is

accompanied by multiple changes in gene expression. Identification of these genes and evaluation of the mechanisms controlling their patterns of expression could lead to novel diagnostic and therapeutic targets for clinical management of breast carcinoma. [9] Watson and Fleming in 1996 identified a novel gene that appeared to be expressed only in breast tissue, which is named as mammaglobin. It is a novel breast specific and breast cancer associated marker. [10]

Materials and Methods

The study was done in the Department of Pathology, at Prathima Institute of Medical Sciences, Karimnagar. Institutional Ethical Committee permission was obtained for the study. Clinical data were retrieved from HPE records. The specimens were fixed in 10% buffered formalin, grossed and sections were taken from representative sites. The sections were then processed in automated tissue processor and embedded in paraffin wax. Inclusion criteria: This study includes both benign and malignant breast lesions, No prior treatment history. Cases of breast carcinoma with lymph nodal metastasis were also included. Exclusion criteria: Congenital breast diseases, Inflammatory breast lesions. Benign and malignant soft tissue diseases of the breast, Metastatic deposits to the breast were excluded. Two micro-sections of 4-5 micron thickness were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other on poly-L-lysine coated slide for immunohistochemical staining. Histological typing of the tumour was done. Combined histological grading (1, 2 and 3) of invasive carcinomas was given according to Elston. It includes tubular formation (1 to 3), Nuclear Atypia (1 to 3), and mitoses (1 to 3). Immunohistochemical staining of mammaglobin protein was done using peroxidase-anti-peroxidase method according to the protocol described by DAKO. The slides were then examined under the microscope and mammaglobin positivity is classified as positive (score 3&4) and negative (score 1&2). Mammaglobin shows diffuse cytoplasmic positivity in tumor cells. The present study uses the following scoring system (table 1).

Table 1: Scoring of Mammaglobin Expression Score

0	No Staining
1	Weak and sporadic staining in less than 50% of tumor cells
2	Weak staining in greater than 50% of tumor Cells
3	Strong, diffuse cytoplasmic staining in less than 50% of tumor cells
4	Strong, diffuse cytoplasmic staining in more than 50% of tumor cells

Results

A total of 40 breast lesions are included in the immunohistochemical study, of which 10(25%) were benign, 30(75%) were the malignant majority of cases were seen in 3rd - 5th decade. The mean patient age at presentation was 49 yrs (18 yrs - 75 yrs). Out of 30 malignant, 21 cases (70%) were invasive ductal carcinoma NOS type and in the remaining 2 are IDC with DCIS component, ILC(2) and one each of Mucinous, Cribriform, Medullary, Papillary and metaplastic. Of 10(25%) benign cases majority are fibroadenomas followed by phyllodes, intraductal papilloma, fibrocystic change and ADH (Atypical Ductal Hyperplasia). The histological grading of IDC were grade I (n=8) grade II(n=8) and grade III(n=5).

Table 1: Age and Sex distribution

Age	Male	Female	Cases	%
<25	-	3	3	7.5%
26-35	-	5	5	12.5%
36-45	-	6	6	15%
46-55	1	13	14	35%
56-65	-	6	6	15%
66-75	-	5	5	12.5%
Total	1	39	40	100%

Table 2: Histopathological distribution of Benign Lesions

Diagnosis	Cases	Percentage
Fibroadenoma	5	50
Phyllodes	2	20
Intraductal Papilloma	1	10
Fibrocystic Change	1	10
ADH	1	10

Table 3: Histopathological distribution of Malignant Cases

Types	No of cases	Percentage
IDC NOS	21	70
IDC+DCIS	2	6.6
Mucinous	1	3.3
Cribriiform	1	3.3
Medullary	1	3.3
Papillary	1	3.3
Lobular	2	6.6
Metaplastic	1	3.3
Total	30	100

Even though both breasts can be affected in malignant breast lesions, there is a slight preponderance of left breast (56.6%) in the present study. Benign lesions of the breast also affect both sides but with a slight left preponderance (60%) in this study. The majority of tumors were in size range of 2 to 5 cm. The number of cases with positive lymph node positive and n=18(60%) and a number of cases with negative lymph node was n=12(40%).

Table 4: The Expression of mammaglobin in benign breast lesions

Diagnosis	Cases	Positive	Negative
Fibroadenoma	5	0	5
Phyllodes	2	0	2
Intraductal Papilloma	1	1	0
Fibrocystic change	1	0	1
ADH	1	0	1

Out of total n=10 benign lesions, mammaglobin expression was found negative in n=9 lesions and the only n=1 lesion was found to be positive for mammaglobin table 4. Out of the 30 malignant lesions, n=22(73.3%) were positive and n=8 were negative for mammaglobin expression table 5.

Table 5: Expression of mammaglobin in malignant breast lesions

Cases	Cases	Positive	Negative
IDC NOS	21	17(56.66%)	4 (13.3%)
IDC+DCIS	2	1(3.3%)	1 (3.3%)
Mucinous	1	1(3.3%)	0
Cribriiform	1	1(3.3%)	0
Medullary	1	0	1(3.3%)
Papillary	1	0	1(3.3%)
Lobular	2	2(6.6%)	0
Metaplastic	1	0	1(3.3%)
Total	30	22(73.3%)	8 (26.7%)

Table 6: Expression of mammaglobin in Invasive carcinomas

	Grade 1	Grade 2	Grade 3
Positive	10	11	1
Negative	1	1	6

Table 7: Expression of mammaglobin: Low Grade Vs High Grade

IDC NOS	Negative	Score 3	Score 4	P Value
Low Grade	0	2	6	0.0069*
High Grade	4	1	0	

* Significant

Table 8: Expression of mammaglobin

Lesions	Negative	Positive	Total	P Value
Malignant	30	8	22	0.00068*
Benign	10	9	1	

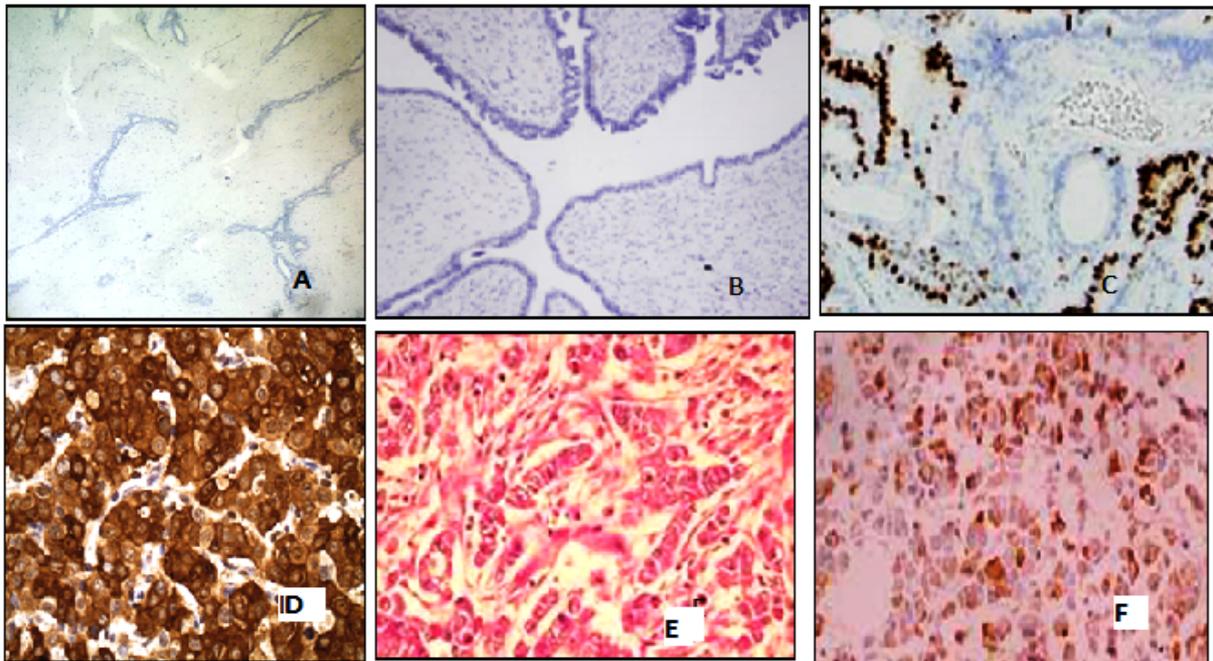
* Significant

Discussion

Mammaglobin has been extensively studied by immune-histochemical analysis in human tissues by the polymerase chain reaction-based methods. Most of the studies have focused on mRNA to detect mammaglobin over expression. [12,13] Our Immunohistochemical study comprised of 40 cases of breast neoplasms out of which 10 were benign tumors and 30 were malignant tumors. In the present study of malignant tumors, the predominant histological subtype was infiltrating ductal carcinoma (NOS) accounting for n=21 cases (70%) followed by n=2(6.67%) cases each of Lobular carcinoma, n=1 case (3.33%) each of Mucinous, Cribriiform, Medullary, Papillary, and Metaplastic carcinoma. In this study the incidence of IDC was 70% correlates with that of A. Krishnaiah et al; who have found the similar incidence of invasive ductal carcinoma. [14] In the present study the incidence of medullary carcinoma (3.33%) correlates with that of Farekha Rehman et al; [15] in the study done by Bane et al; maximum was IDC (NOS) cases were followed by Medullary carcinoma. Whereas in the study done by Mudholkar maximum were IDC (NOS) followed by Mucinous and Metaplastic carcinomas. In the present study, histological grading was done using a modified Scarf Bloom Richardson grading. N=11 cases (36.67%) were grade I, n=12 cases (40%) grade II and n=7

cases (12.12%) were grade III. A similar observation was made by Ayadi L et al; [16] and Azizun-Nisa et al; [17] hence it becomes clear from this study as well as other studies that grade II is more common. Age of the cancer patient is an important factor both for occurrence and management of the cases. In India, breast cancer incidence peaks among women of 45–50 years of age. In the present study also the mean patient age at presentation was 49 yrs. In this study, 73.3% cases had the tumor size between 2-5 cms, 16.67% cases had tumor size <5cm and 10% of cases had tumor size > 5 cm. Dalia Kadry, Amal Fawzy, et al; [18] found 68% of cases had the tumor size between 2-5 cms, 17% of cases had tumor size <2 cms. The present study correlated with observations made by Dalia Kadry, Amal Fawzy et al; [18]

Similar observation was made by Zafrani B et al; [19] Huang JH et al; [20] and Onitilo et al; [21]. Lymph node involvement is an important prognostic factor. Positive lymph nodes are associated with the worst outcome. The infiltrating ductal carcinomas demonstrated strong cytoplasmic staining for mammaglobin protein in 80% (n = 40). In the present study, 81% of IDC (NOS) breast cancers are positive for mammaglobin. Expression in Medullary and metaplastic types were negative in studies done by Sasaki et al; [22] and Reyes C et al; [23] on breast carcinoma consisting 1079 cases, of which 38% were medullary carcinoma and 36% cases were metaplastic carcinoma. All the (38+36) cases were negative for mammaglobin expression. This is in correlation with the results of the present study.



A: FIBROADENOMA SHOWING MG NEGATIVITY (10X); B: BENIGN PHYLLODES IHC SHOWING NEGATIVE MAMMAGLOBIN; C: INTRA DUCTAL PAPILOMA SHOWING FOCAL MG EXPRESSION(40X); D: INVASIVE DUCTAL CARCINOMA NOS– POSITIVE MG EXPRESSION 40X; E: INVASIVE LOBULAR CARCINOMA– H&E(40X); F: INVASIVE LOBULAR CARCINOMA– SHOWING MG POSITIVITY (40X)

Krishnaiah et al; [14] observed positive MG expression in 22 cases of IDC (NOS) type (73.3%) and negative expression in 8 cases (26.6%). Invasive lobular carcinomas were positive for MG expression with a score of 3 (2/2). The positivity score was less when compared to invasive duct cell carcinoma. Invasive carcinoma with cribriform pattern and mixed type were positive for MG expression

(3/3). Papillary carcinoma showed negative MG expression (0/1). In the present study 21 cases of IDC NOS type that were grouped according to the histological grade. Invasive lobular carcinomas were positive for MG expression with a score of 3. The positivity score was less compared to invasive duct cell carcinoma. Invasive carcinoma with the cribriform pattern was positive for MG expression. Mucinous

carcinoma of breast showed positive MG expression and papillary carcinoma showed negative MG expression. Medullary and metaplastic carcinoma showed negative MG expression. This study showed that ductal carcinomas stained more diffusely and intensely with MG as compared to lobular carcinomas. It also shows focal and less intense staining pattern with MG and this result was comparable with previous studies. In contrast to the above statement, a study conducted by Bhargava et al; [24] observed that infiltrating lobular carcinomas shows strong diffuse immune staining for MG compared to infiltrating duct cell carcinoma. This study did not use E-cadherin. Sasaki et al; [22] observed that only 48% of breast cancers were immunohistochemically positive for MG expression. This is in contrast to the present study which showed mammaglobin expression in 73% of breast cancers. Farekha Rehman et al; [15] observed that 79% of breast cancer tumors showed diffuse and intense cytoplasmic mammaglobin expression. They also observed high intensity in low-grade tumors compared to high-grade tumors and the metastatic ones. It could be due to MG gene expression is highly restricted to low-grade tumors. Paul N Span [25] studied mammaglobin expression in various grades of breast carcinoma. They observed that low and intermediate grade tumors expressed significantly higher compared to high-grade tumors. In comparison to the previous results, the present study shows that ductal carcinomas are more diffusely and intensely stained with MG as compared to lobular carcinomas. Invasive Lobular, Cribriform and Mucinous carcinoma of breast showed positive MG expression. Papillary carcinoma showed negative MG expression. Medullary and Metaplastic carcinoma showed negative MG expression.

Conclusion

Within the limitations of the present study, it is concluded that Mammaglobin is a useful marker for malignant breast lesions. Mammaglobin is a novel promising marker for neoplastic breast epithelial cells. It can be used as a molecular marker for early detection, prognosis and relapse monitoring for breast cancer cases. Circulating MG m-RNA levels may be used as a tumour marker to monitor the efficiency of therapy. MG over expression in breast tissues is

associated with better differentiation, higher hormone dependence and lower proliferation. It means a better prognosis.

Conflict of Interest: None declared

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Ethical Permission: Obtained

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