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Study of utility of Mammaglobin in diagnosis of breast carcinoma and as a valuable marker for mammary tissue

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Abstract:

Aim: The study was undertaken to establish mammaglobin as a marker to differentiate between benign and malignant breast lesions. **Materials and methods:** The present study was undertaken at Osmania general hospital, Hyderabad from January 2012 to July 2013. A total of 60 samples of various breast lesions were studied. Immunohistochemistry was performed by using mammaglobin antibody. **Results:** Mammaglobin immunoexpression was seen in 75% cases of breast carcinoma. The invasive ductal carcinoma-NOS type showed stronger and diffuse staining pattern than other variants of breast carcinoma. Interestingly high mammaglobin expression observed in low grade tumours compared to high grade. **Conclusion:** Mammaglobin is over expressed in malignant breast lesions compared to non-neoplastic lesions of breast ($P<0.05$). The expression is high in low grade tumours compared to high grade tumours.

Key words: Breast lesions, Carcinoma, Immunohistochemistry, Mammaglobin

Introduction

Cancer is now the second leading cause of death in Indians after cardiovascular disease. Breast cancer is the most commonly occurring female cancer in the world with an age-standardized incidence rate (ASR) of 39.0 per 100,000, which is more than double that of the second ranked cancer (cervical cancer ASR=15.2 per 100,000). Breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents13.7% of all cancer deaths [1]. Breast cancer accounts for 5-8% of all cancers in India and the incidence is on the rise [2]. It is the most common cancer of urban Indian women than in rural women [3].In India, about 60 - 80% of these cases present at a locally advanced stage[4].In 1996 Watson and Fleming identified a novel gene that appeared to be expressed only in a breast tissue, which is named as mammaglobin [5]. It is a novel breast specific and breast cancer associated marker. Expression of this protein is restricted to normal breast epithelium and frequently upregulated in breast carcinomas by about

10 folds compared to normal breast [6]. MG gene is a member of uteroglobin family. This gene was localized on chr11q12-13[7]. It is a 23 amino acid glycoprotein. Frequent amplification of this gene is seen in breast neoplasia [8].

Material and Methods

The present study was undertaken at the Upgraded Department of Pathology at Osmania General Hospital, Hyderabad from January 2012 to July 2013. Clinical data was retrieved from the records. The specimens were fixed in 10% buffered formalin. Specimens were grossed and sections were taken from representative sites. The sections were then processed in automated tissue processor (leica) and embedded in paraffin wax. A total number of 60 cases are studied.

Inclusion criteria for Selection of cases:

- This study includes both benign and malignant breast lesions.
- Clinico-pathological data like age, sex, histopathological diagnosis of the cases.
- No prior treatment history.
- Cases of breast carcinoma with lymphnodal metastasis are also included.

Exclusion criteria

- Congenital breast diseases.
- Inflammatory breast lesions.
- Benign and malignant soft tissue diseases of breast.
- Metastatic deposits to the breast.

For each case 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 immuno-histochemical staining. Histological typing of tumour was done. Combined histological grading (1, 2 and 3) of invasive carcinomas was given according to Elston et al. 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 -antiperoxidase method. The kits for Mammaglobin immunohistochemical staining were obtained from DAKO (clone 304-1A5, denmark). Staining was done according to the manufacturer's protocol. The slides are then examined under microscope for Mammaglobin positivity. Mammaglobin shows diffuse cytoplasmic positivity in tumour cells. The present study uses the following scoring system [9].

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

Statistical analysis was done using Fishers exact test. P value < 0.05 was considered statistically significant.

Results:

The present study was under taken at Osmania General Hospital, Hyderabad from January 2012 - July 2013. A total 60 breast lesions are included in the study, of which 15 are benign (25%), 45 are malignant (75%). Majority of cases were seen in 3rd - 5th decade. The median patient age at presentation was 48 yrs (16 yrs - 80 yrs). Out of 60 cases, 45 are malignant, of which 30 cases (66.6%) are invasive ductal carcinoma NOS type and the remaining 15 are special type (33.3%). Of 15 benign cases majority are fibroadenomas. All the 60 cases presented with lump in the breast.

Table1: Histopathological distribution of benign lesions

Diagnosis	**Number of cases**	**Percentage**
Fibroadenoma	8	53.3%
Phyllodes	3	20%
Intraductal Papilloma	1	6.6%
Fibrocystic change with ID neoplasia	1	6.6%
ADH	1	6.6%
Apocrine metaplasia with Adenoma	1	6.6%

Table 1 shows histopathological distribution of benign cases. In the present study, we have 15 benign breast lesions, of the 15 lesions majority are fibroadenomas (53.3%).

Table 2: Histopathological distribution of Malignant cases

Types	No. of cases	Percentage
IDC NOS	30	66.6%
IDC + DCIS	1	2.2%
Papillary Ca	1	2.2%
Neuro Endocrine Ca	2	4.4%
Mucinous Ca	2	4.4%
Mixed Type	2	4.4%
Cribriform	1	2.2%
Lobular	2	4.4%
Medullary	2	4.4%
Metaplastic	2	4.4%
Total	100%	100%

Table 2 shows histopathological distribution of malignant cases. In 45 malignant breast lesions IDCNOS type compromises 66.6%, majority lesions in the present study sample.

Based on histological features, all the 30 cases of IDCNOS are divided into 3 grades by Bloom Richardson grading system. (Table 3)

Table 3: histological grading of IDCNOS

Grades of IDC	Total Number of cases
Grade 1	12
Grade 2	8
Grade 3	10

The age distribution of the cases in the present study is depicted in Table 4. Accordingly, the commonly affected age group is 4th decade. Most of the malignant lesions are seen between 4th and 5th decades and benign lesions between 2nd and 3rd. The mean age of the sample is 46 years, the minimum and the maximum ages being 16 years and 80 years respectively.

Table 4: Age distribution of cases

Age	Number of Cases	Percentage
<20 yrs	3	5%
21-30	5	8.3%
31-40	16	26%
41-50	18	30%
51-60	9	15%
61-70	5	8.3%
71-80	4	6.6%
Total	60	100%

Even though both breasts can be affected in malignant breast lesions, there is slight preponderance of left breast (55.5%) [Table 5]. Benign lesions of breast also affect both sides but with a slight left preponderance (53.3%). In 13.3% of the cases there is bilateral involvement.

Table 5: Distribution of Lesions According to side affected –Benign &Malignant

	MALIGNANT LESIONS		BENIGN LESIONS	
Laterality of Tumour	Number of Cases	Percentage	Number of Cases	Percentage
Right	20	44.4%	5	33.3%
Left	25	55.5%	8	53.3%
Bilateral	0	0	2	13.3%
Total	45	100%	15	100%

Table 6 shows lymphnode status in the present study. Out of 45 malignant cases, only 40% have shown metastatic deposits and the remaining 60% showed reactive hyperplasia.

Table 6: Histomorphology of enlarged lymphnodes in the study

	Number of Cases	Percentage
Reactive	27	60
Metastatic	18	40
Total	45	100

The expression of mammaglobin in the present study is depicted in the following Table 7. MG shows diffuse cytoplasmic positivity. Score 3 and score 4 are taken as positive. Score 1 & 2 are taken negative.

Table 7: Expression of Mammaglobin in the Present Study

Lesion	Number of cases	Positive	Negative
Benign: Fibroadenoma	8	0	8
Phyllodes	3	0	3
Intraductal Papilloma	1	1	0
Fibrocystic change with ID neoplasia	1	1	0
ADH	1	0	1
Apocrine metaplasia with Adenoma	1	1	0
Malignant: IDCNos	30	22	8
IDC + DCIS	1	1	0
Papillary Ca	1	0	1
Neuro Endocrine Ca	2	2	0
Mucinous Ca	2	2	0
Mixed Type	2	2	0
Cribriform	1	1	0
Lobular	2	2	0
Medullary	2	0	2
Metaplastic	2	0	2

Table 8: Expression of Mammaglobin: Low Grade Vs High Grade IDCC

IDCC	Negative	Score 3	Score 4	P Value
Low Grade	0	2	10	0.0001
High Grade	8	2	0	

Table 9: Expression of Mammaglobin: Benign Vs Malignant lesions

	Total	Negative	Positive	P-Value
Malignant	45	13	32	0.0007
Benign	15	12	3	

Mammaglobin Expression in benign breast lesions:

In this study expression of mammaglobin (MG) is observed in benign breast lesions. All cases of fibroadenomas showed negative expression for MG (0/8). MG is not expressed in stromal cells; because of this property all cases of phyllodes are negative for MG expression (0/3). In the remaining cases of apocrine metaplasia with adenoma, fibrocystic change with ID neoplasia and Intraductal papilloma MG expression was noticed. Out of 15 benign lesions only 3 cases are positive for MG with a score 3(20%).

Mammaglobin Expression in Invasive Ductal Carcinoma:

Expression of MG was studied in 30 cases of IDC NOS type that were grouped according to the histological grade. All grade 1 tumours (12/12) expressed strong diffuse cytoplasmic positivity with a score of 4 in10 cases (83.3%) and with a score of 3 in 2 cases (20%). All grade 2 (8/8) tumours expressed MG with a score of 3 in 3 cases (3/8) and score 4 in 5 cases (5/8). Most of Grade 3 tumours showed negative MG expression (8/10), only 2 cases positive with a score of 3 (2/10). In this study positive MG expression was observed in 22 cases of IDC NOS type (73.3%) and negative expression observed in 8 cases (26.6%). In the present study, we also observed mammaglobin expression in metastatic lymphnode of IDC NOS type they exhibited positive immunoreactivity for MG.

Mammaglobin Expression in other Specifc Variants of breast carcinomas:

Invasive lobular carcinomas are positive for MG expression with a score of 3 (2/2). The positivity score was less compared to invasive duct cell carcinoma. Invasive carcinoma with cribriform pattern and mixed type are positive for MG expression (3/3), papillary carcinoma showed negative MG expression (0/1). Mucinous carcinoma of breast and neuroendocrine carcinomas also

showed positive MG expression (4/4). Medullary and metaplastic carcinoma showed negative MG expression (0/4).

Discussion

The evolution of breast neoplasia is accompanied by a number of quantitative changes in gene expression [10-12]. Mark A Watson first identified quantitative changes in gene expression that occur in the malignant mammary gland. Watson et al studied 10 fold increased mammaglobin mRNA level in malignant breast lesions compared to normal breast tissue in an analysis of 35 breast tumour biopsies [13]. Stampfer et al observed the absence of mammaglobin expression in primary breast myoepithelial cells and stromal cells [14]. The present study concurs with the above observation that malignant breast lesions show diffuse and intense mammaglobin expression, compared to benign breast diseases. Mark A Watson studied 100 primary breast tumours of which 81% were strongly immune positive for mammaglobin protein. They also studied 11 cases of breast cancers with lymphnodal metastasis, in which 10 out of 11 cases showed positive MG mRNA levels [9]. This over expression did not appear to correlate with histology, tumour grade, stage and receptor status.

Han observed the MG positivity in 59 out of 70 (84.2%) breast cancer cases [15]. Sasaki observed that only 48% breast cancers were immune histochemically positive for MG expression. They also studied mRNA levels of mammaglobin by RT-PCR in breast cancer tissue, they observed that some of the mRNA positive cancers were negative for MG in immunohistochemistry [16]. Gargano detected circulating MG m-RNA positive cells in 52% of patients with localized breast cancer, 75% of patient with regional lymph node metastasis and 86% of patient with distant metastasis [17; 18].

In the present study, we observed high mammaglobin expression in 34 (75%) out of 45 malignant breast lesions. The present study also included breast carcinoma cases with nodal metastasis, these cases showed high mammaglobin expression in metastatic tumour tissue. This observation is comparable with previous studies, so MG can be used as a marker for metastatic deposits suspecting of breast origin. Sonia observed that 79% of breast cancer tumours showed diffuse and intense cytoplasmic mammaglobin expression [19]. They also observed high intensity in low grade tumours compare to high grade tumours and the metastatic ones. It could be due to MG gene expression is highly restricted to low grade tumours. Paul N Span studied mammaglobin expression in various grades of breast carcinoma [20]. They observed that low and intermediate grade tumours expressed significantly higher compared to high grade tumours.

In comparison to the previous results, we also observed a high mammaglobin expression in low and intermediate grade tumours compared to high grade tumours and benign breast lesions. The present study shows that ductal carcinomas are more diffusely and intensely stained with MG as compared to lobular carcinomas. It shows focal and less intense staining pattern with MG and this result was comparable with previous study [7]. In contrast to the above statement, study conducted by Bhargava observed that infiltrating lobular carcinomas shows strong diffuse immune staining for MG compared to infiltrating duct cell carcinoma [21]. This study did not use E-cadherin. Monica observed high levels of MG in patients with benign breast disease [22]. It is due to the fact that certain histological types of benign disease like atypical hyperplasia, fibrocystic changes in breast etc may have an increased chance of developing breast cancer in certain patients.

We also found MG expression in apocrine metaplasia with adenoma, ID neoplasia and Intraductal papilloma which are benign breast lesions. From this observation MG is helpful in predicting the potential of malignant transformation in certain benign lesions, thereby reducing the risk of development of obscure malignancy in future. Corradini used MG expression to study occult breast cancer cells in the peripheral blood and bone marrow and this marker is not detected in healthy subjects [23]. So they concluded that MG is a useful marker for RT-PCR detection of minimal residual disease in breast cancer patient who are on post therapeutic follow up. Also studies have been done to know the expression pattern of mammaglobin in medullary and metaplastic carcinomas. One large study done by Reyes et al on breast carcinoma consisting 1079 cases, of which 38 are medullary carcinoma and 36 cases are metaplastic carcinoma, all the 74 (38+36) cases are negative for mammaglobin expression [24]. In another study done by Sasaki it was observed that 1 case of medullary and 2 cases of metaplastic carcinoma showed negative mammaglobin expression [16]. We also observed same result. In the present study we included 2 medullary carcinoma, 2 metaplastic carcinoma and all are negative for MG

expression. In the present study, we compared [Table 10] mammaglobin expression in the malignant lesions of the breast with previous literature. Our results are comparable with previous studies.

Table 10: Comparative analysis

	Number of Cases	**Positive for Mammaglobin**	**Percentage**
Watson et al [1999]	100	81	81%
Han et al [2003]	70	70	84.3%
Bernsteinet et al [2005]	237	190	72%
Sonia et al [2007]	48	38	79%
Bhargava et al	121	67	56%
Present Study	45	34	75%

Conclusion

From this study, we conclude that

- MG is a useful marker for malignant breast lesions.
- Mammaglobin is a novel promising marker for neoplastic breast epithelial cells. It can be used as molecular marker for early detection, staging, prognosis and relapse monitoring for breast cancer.
- MG is one of the first relatively mammary specific and mammary sensitive markers and its over expression associated with low grade, steroid receptor positive tumours.
- Due to its high specificity and over expression of MG in the malignant breast tissue it can be used to confirm a breast origin in a metastatic or undifferentiated tumour with unknown primary.
- It also helps to differentiate benign proliferating breast diseases mimicking malignancy on routine H&E from in situ or invasive carcinoma.
- 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.

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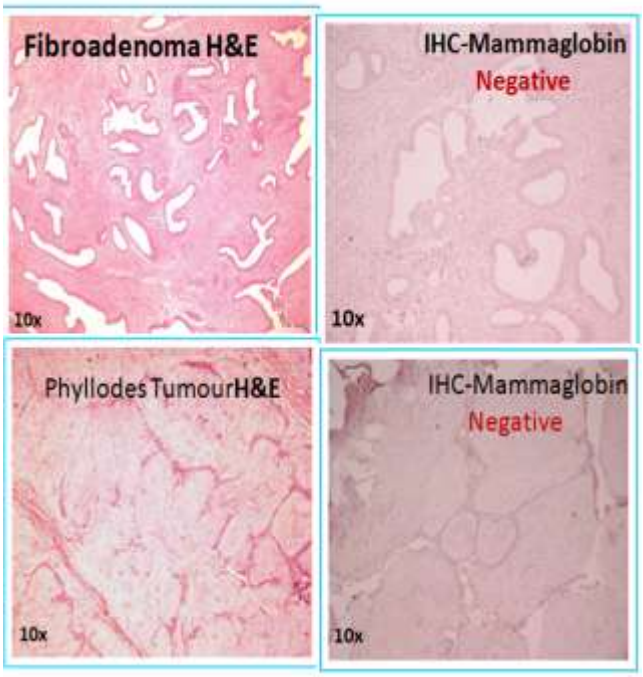

Figure 1: H&E and IHC of fibroadenoma and phyllodes showing negative MG immunostaining

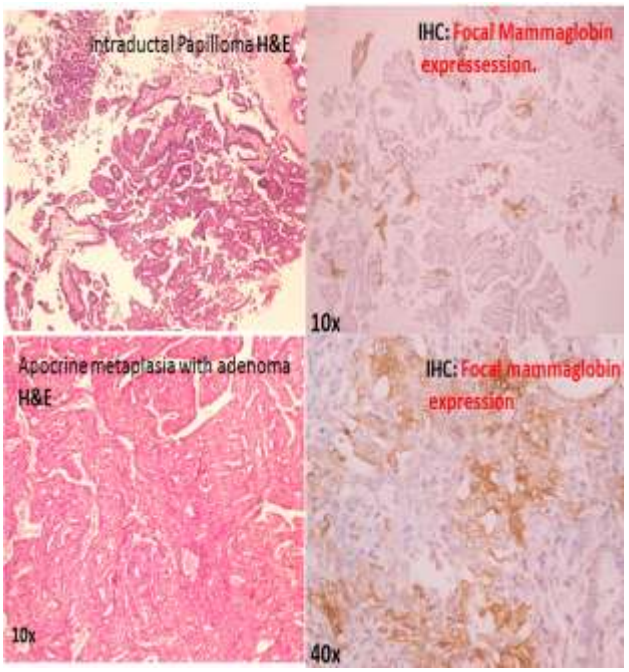

Figure 2: H&E and IHC of intraductal papilloma and apocrine metaplasia with adenoma showing focal mammoglobin positivity

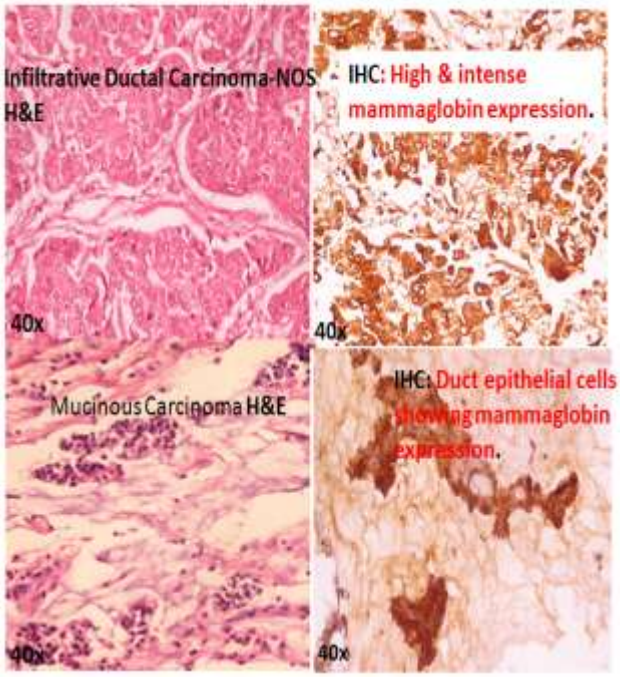

Figure 3: H&E and IHC showing high MG immunopositivity in IDC-NOS and in mucinous carcinoma.

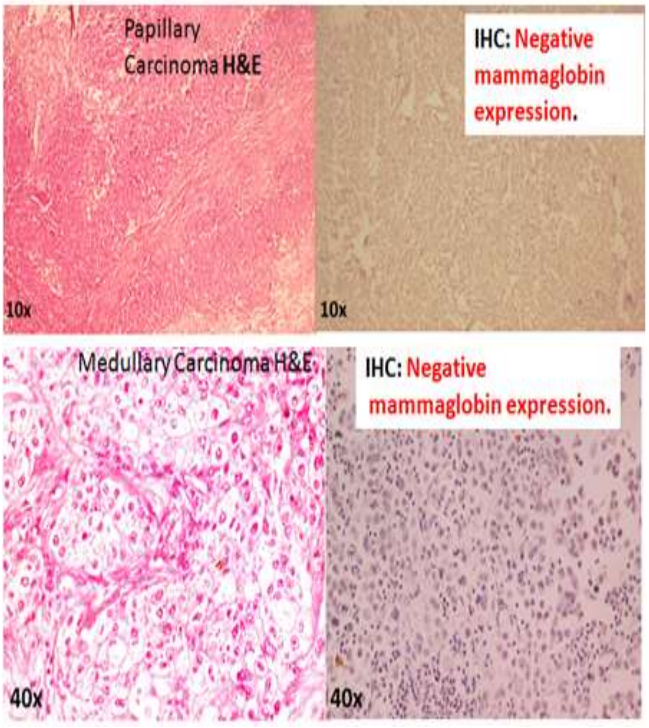

Figure 4: showing H&E of papillary carcinoma and medullary carcinoma and IHC showing negative MG expression.

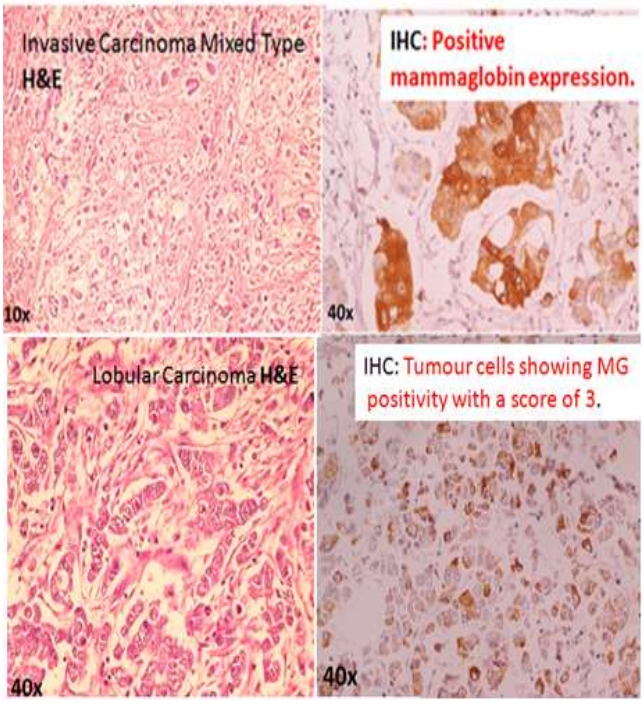

Figure 5: showing invasive carcinoma and lobular carcinoma and IHC showing MG positivity

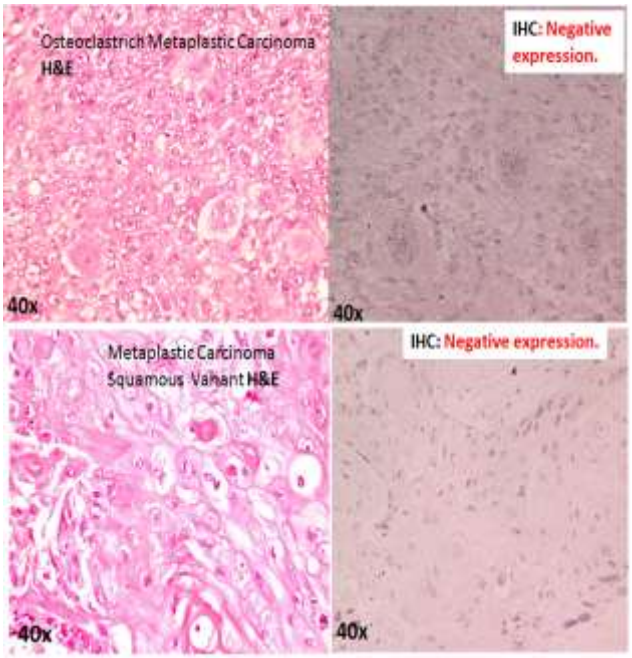

Figure 6: showing osteoclast rich metaplastic carcinoma and metaplasic carcinoma with squamous variant and IHC showing negative MG expression.

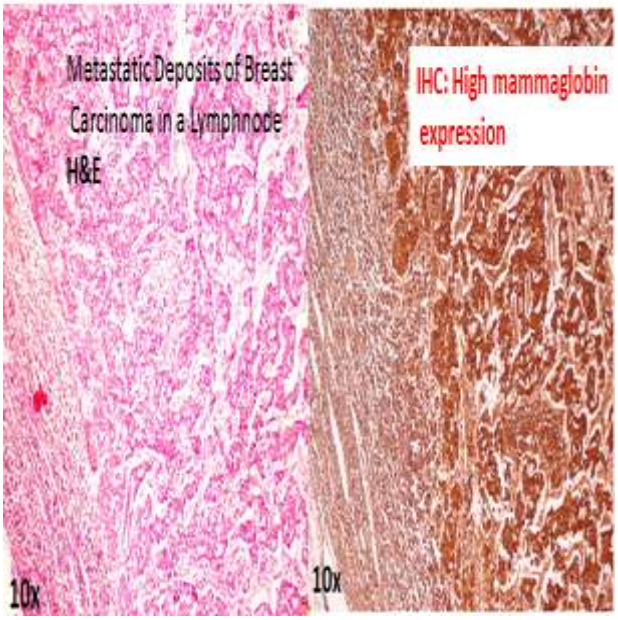

Figure 7: showing metastatic deposits of breast carcinoma in alymphnode and IHC showing high MG expression.

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