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A Study of Expression of Mammoglobin and Gross Cystic Disease Fluid Protein-15 In Breast Cancer

Neha Suman[1], Manjula Jain[2], Anita Nangia[3], Shaji Thomas[4]

[1]Assistant Professor, Department of Pathology, Lady Hardinge Medical College, New Delhi,
[2]Ex-Director Professor & HOD, Department of Pathology, Lady Hardinge Medical College, New Delhi
[3]Ex-Director Professor, Department of Pathology, Lady Hardinge Medical College, New Delhi
[4]Ex-Director Professor & HOD, Department of Surgery, Lady Hardinge Medical College, New Delhi

Corresponding Author: Dr Neha Suman

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ABSTRACT

Background: Breast cancer is the most common cancer in women worldwide. There is a lack of tissue markers with sufficient sensitivity and/or specificity for detection, and disease evolution in breast cancer. Clinically, ER, PR and HER-2 are the most useful immunomarkers for prognosis and therapy but these are not breast specific. Mammoglobin (MGB) and Gross Cystic Disease Fluid Protein (GCDFP-15) have been suggested as highly specific for mammary differentiation. As there are few studies which have studied the relation of expression of mammoglobin and GCDFP-15 to molecular classification of breast cancer hence there is the need to study these markers.
Aims: To determine expression of mammoglobin and GCDFP-15 in breast carcinoma on tru-cut biopsy specimens and the association of expression of mammoglobin and GCDFP with molecular classification of breast carcinoma using surrogate panel of immunohistochemical markers.
Settings and design: Hospital based descriptive observational cross-sectional study.
Material and methods: Immunohistochemical analysis was conducted on 35 cases of breast carcinoma proven on biopsy/trucut biopsy. The immunohistochemical expression of mammoglobin and GCDFP-15 were analysed and further staining scores were done. Correlation with clinicopathologic parameters and molecular classification of breast carcinoma was done.
Results: 11/35 (31.4%) cases were positive for mammoglobin expression and 9/35 (26.5%) cases were positive for GCDFP. There was overexpression of mammoglobin and GCDFP-15 in Luminal A and Her2 classic cases. Higher mammoglobin score was observed in Luminal A tumors. Most triple negative tumors were negative for mammoglobin and GCDFP-15.
Conclusion: There is overexpression of mammoglobin and GCDFP-15 in breast carcinoma and these markers can serve as a novel and clinically useful immunomarker for breast carcinoma.
Keywords: Breast carcinoma, GCDFP-15, Mammoglobin

INTRODUCTION

Breast cancer is the most common diagnosed malignancy in women worldwide [1]. The development of breast cancer involves a progression through intermediate stages until the invasive carcinoma and finally into metastatic disease. The determination of tumor markers is a useful

tool for clinical management in cancer patients, assisting in diagnostic, staging, and evaluation of therapeutic response, detection of recurrence and metastasis, and development of new treatment modalities [2] especially in the era of personalised medicine. In addition to the known markers used for molecular classification of breast carcinoma, mammoglobin (MGB) and Gross Cystic Disease Fluid Protein (GCDFP-15) has been suggested as highly specific for mammary differentiation [3].

Mammoglobin has been described as one of the 23 members of the uteroglobin/Clara cell protein family of small epithelial secretory proteins, the secretoglobins [4,5]. It is a 93 amino acid glycoprotein and its expression is restricted to normal breast epithelium and frequently upregulated in breast carcinomas by about 10 folds compared to normal breast [6]. It can be used as molecular marker for early detection, staging, prognosis and relapse monitoring for breast cancer. Due to its high specificity and over expression 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 [7]. The reported figures of mammoglobin expression in breast cancer tissues have ranged from 20 to 95% [8]. In addition to breast carcinoma, mammoglobin also reportedly stains in skin sweat gland and salivary gland tumors [9].

Gross cystic disease fluid protein (GCDFP-15) has been identified as one of the glycoproteins present in breast gross cystic disease fluid [10]. Gross cystic disease fluid is a pathologic secretion from breast composed of several glycoproteins, including a unique 15,000-dalton monomer, GCDFP-15 [11]. GCDFP-15 is uniformly expressed in cells of apocrine differentiation. It inhibits T-cell apoptosis induced by CD4 cross-linking and subsequent T-cell receptor activation which may have role in breast tumor progression [12]. Normal breast ducts and lobules do not express GCDFP-15, however, apocrine metaplastic epithelium expresses GCDFP-15[13]. GCDFP-15 is a diagnostic marker for mammary differentiation in histopathology. Salivary gland and skin adnexal tumors (with apocrine differentiation) are the only two major sources with significant immunoreactivity for GCDFP-15. The reported sensitivity of GCDFP- 15 for breast cancer in the literature ranges from 14% to 75% [13,14].

There are few studies which have studied the relation of expression of mammoglobin and GCDFP-15 to molecular classification of breast carcinoma. The aims of this study were, (1) to determine expression of mammoglobin and GCDFP-15 in breast carcinomas and (2) to study the association of expression of mammoglobin and GCDFP-15 with molecular classification of breast carcinoma.

MATERIALS & METHODS

The present study was conducted in the Department of Pathology and Surgery, Lady Hardinge Medical College, New Delhi. Cases of breast carcinoma proven on biopsy/tru-cut biopsy sent from surgical OPD were included in the study after taking patient consent for further processing. Out of 68 cases, a total of 35 cases were included in the study. The exclusion criteria were chemotherapy treated patients, patients whose tissue blocks had inadequate materials and patients not willing to give consent. The study was approved by Institutional Ethics committee (certificate number: LHMC/ECHR/2015/58).

Routine staining was done by H&E following which the stained sections were examined and histopathological diagnosis was made. Immunohistochemistry (IHC) was applied for ER, PR, Her2neu, Ki67, basal markers (CK5/6 and EGFR whenever required) was performed using standard techniques and categorisation for molecular classification was as per classification given by Zhang et al. [15]. Mammoglobin and GCDFP-15 IHC was put up in all cases.

Mammoglobin and GCDFP-15 staining was performed using a primary antibody cocktail of mouse and rabbit antibodies (clone: 31A5 (Mammaglobin) and D6 (GCDFP-15), ready to use, Biocare). Immunohistochemical staining was done manually, according to the manufacturer's protocol.

Mammaglobin shows diffuse cytoplasmic positivity in tumour cells. The present study used the following scoring system [16] (Figure 1):

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

Immunohistochemical staining for GCDFP was evaluated for the intensity and extent of staining (Figure 2).[13] The intensity of IHC staining was recorded on an ordinal scale and since it does not have further benefit, the positive and negative classification of staining is used. The extent of staining (percentage of carcinoma cells staining) was recorded on an ordinary scale:

= ABSENT
= <25%
= 25% TO 49%
= 50% TO 74%
= >75%

STATISTICAL ANALYSIS

Chi square test was performed using SPSS software for statistical analysis. For quantitative data mean, range and standard deviation was calculated. A p value of <0.05 was considered as significant.

RESULT

35 cases of breast carcinoma were included in the present study. The age of patients ranged from 30 years to 70 years with the mean age of 48.64 years. All the patients were females, except for one male patient. 79.6% (27/34) of females with breast cancer were postmenopausal and 20.6% (7/34) were premenopausal.

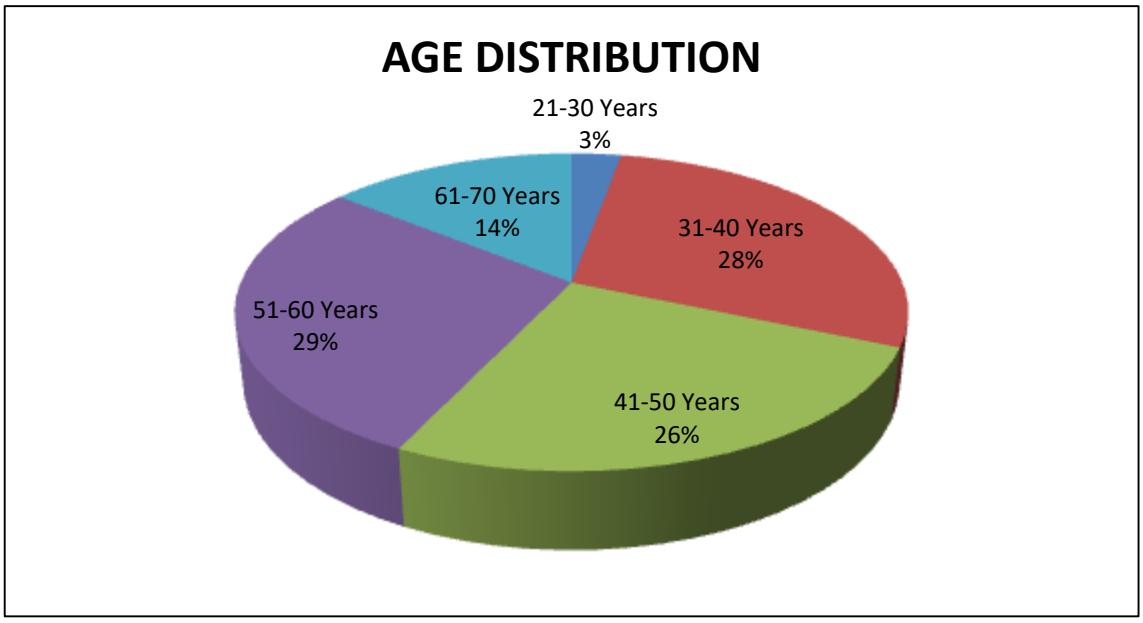

Figure 1 showing age distribution of cases.

Majority of cases belonged to tumor grade T2 category (42.9%) followed by T3 category (31.4%) and T1 category (25.7%). The predominant histological type was IDC

NOS (88.6%). Figure 2 shows histological subtype of cases.

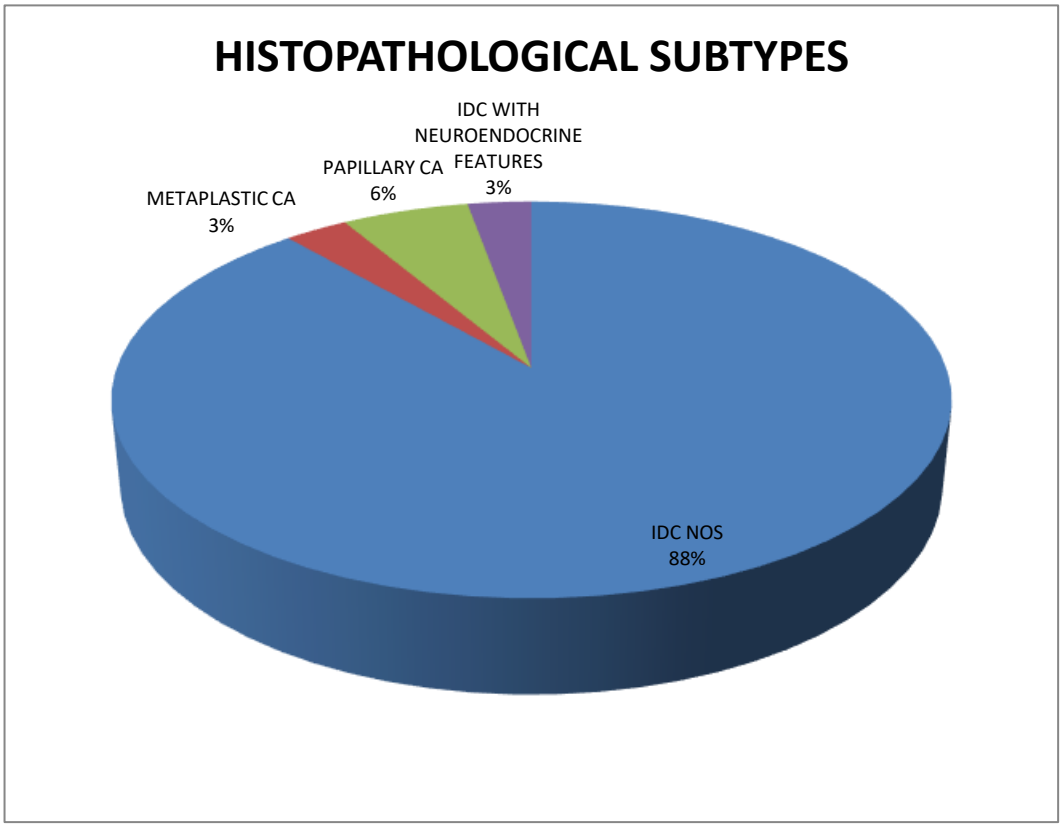

Figure 2 showing histological subtypes of cases.

Positive mammoglobin expression was found in 11/35 (31.4%) cases. 24/35 cases (68.6%) were negative. Figure 3 shows distribution of scoring pattern of mammoglobin expression in breast carcinoma.

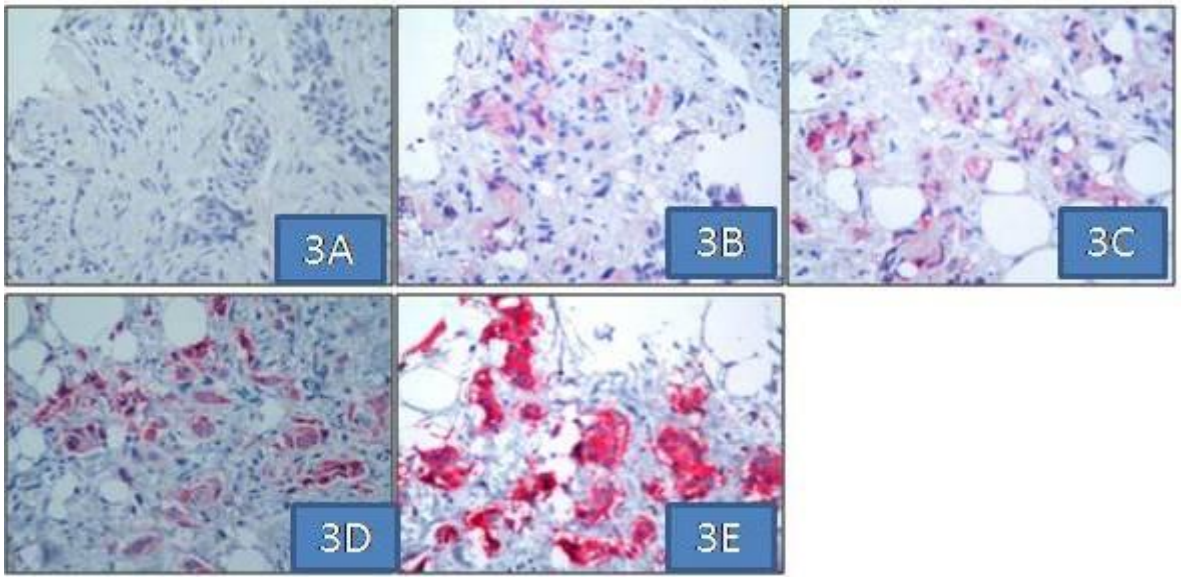

Figure 3: Tumor cells showing mammoglobin IHC staining (A) Score 0 (B) Score 1 (C) Score 2 (D) Score 3 (E) Score 4

The 11 positive cases were further classified according to the scoring system (Table 1).

TABLE 1: DISTRIBUTION OF SCORING PATTERN OF MAMMOGLOBIN POSITIVE EXPRESSION IN BREAST CANCER

Score	Cases (%)
0	24(68.6)
1	1(2.9)
2	1(2.9)
3	1(2.9)
4	8(22.7)

Positive GCDFP expression was found in 9/35 (26.5%) cases. 26/35 cases (73.5%) were negative. Figure 4 shows distribution of scoring pattern of GCDFP-15 expression in breast carcinoma.

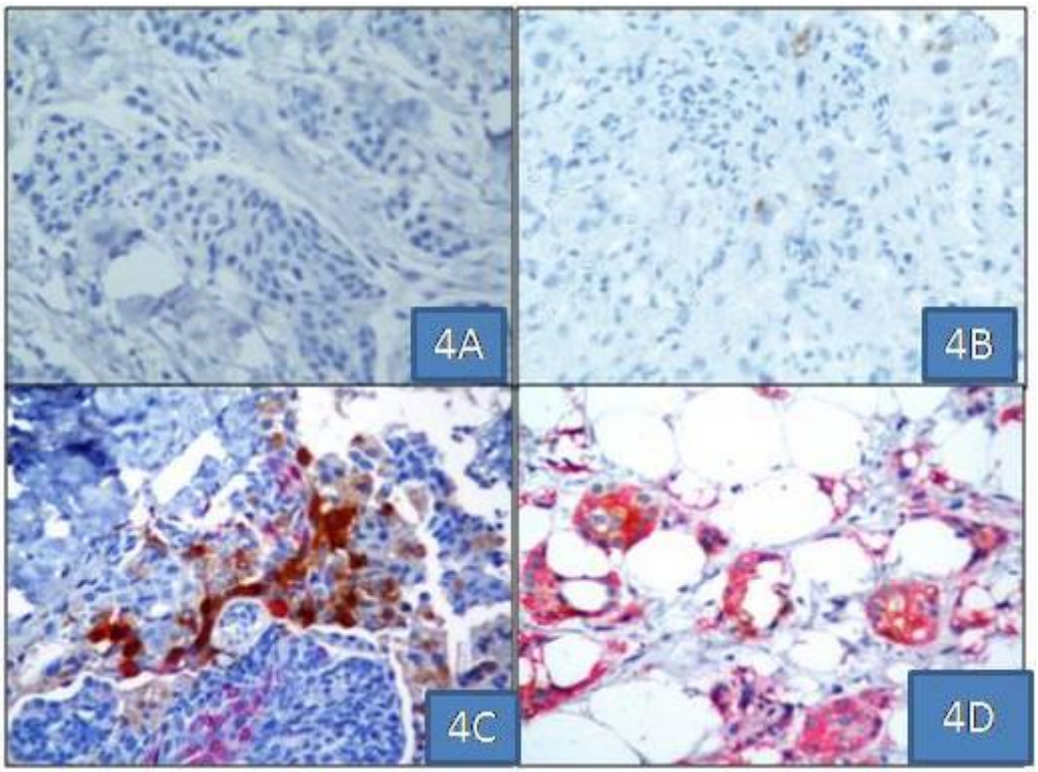

Figure 4: Tumor cells showing GCDFP-15 IHC staining (A) Score 1 (B) Score 2 (C) Score 3 (D) Score 4

The cases were further classified according to the scoring system (Table 2).

TABLE 2: DISTRIBUTION OF SCORING PATTERN OF GCDFP POSITIVE EXPRESSION IN BREAST CANCER

Score	Cases (%)
1	26(74.2)
2	5(14.3)
3	2(5.7)
4	2(5.7)
5	0(0)

Table 3 shows relationship of mammaglobin and GCDFP-15 expression in breast cancer patients with clinicopathological factors and distribution of mammoglobin and GCDFP-15 expression in different morphological types of breast carcinoma.

TABLE 3: RELATIONSHIP OF MAMMAGLOBIN AND GCDFP-15 EXPRESSION IN BREAST CANCER PATIENTS WITH CLINICOPATHOLOGICAL FACTORS

Clinicopathological Factors	Percentage frequency of mammaglobin expression in breast cancer patients (%)	p-Value	Percentage frequency of GCDFP-15 expression in breast cancer patients (%)	p-Value
TUMOR SIZE				
≤ 2 cm	4/9		2/9	
2-5 cm	3/14		5/14	
>5 cm	4/12	0.609	2/12	0.521
LYMPH NODE STATUS				
Metastasis Present	3/6		3/6	
Metastasis Present	8/29	0.282	6/29	0. 135
HISTOLOGICAL TYPE				
Infiltrating ductal carcinoma (IDC NOS)	8/31		8/31	
Papillary carcinoma	50		0	
IDC with neuroendocrine features	100		100 %	
Metaplastic ca	0		0	

On correlating the immunohistochemical expression of mammoglobin with molecular classification of breast carcinoma, it was found that 55.5% of Luminal A cases (Figure 3) and 50% of Her2neu classic cases showed mammoglobin positivity. Higher score was also seen in Luminal A and Her2neu classic cases. Only 16.6% cases of Luminal Her2neu showed positive mammoglobin expression. On correlating GCDFP-15 expression with the molecular classification of breast carcinoma, it was observed that 5/9 (55.5%) of Luminal A subtype (Figure 3), 2/6 (3.3%) of Luminal Her2neu, 1/12 (8.3%) of Her2neu classic and 1/4 (25%) of triple negative non basal phenotype showed positive GCDFP-15 expression. None of the Luminal B and triple negative basal phenotype cases showed positive expression for mammoglobin and GCDFP-15 (Table 4). However, no statistically significant correlation was present.

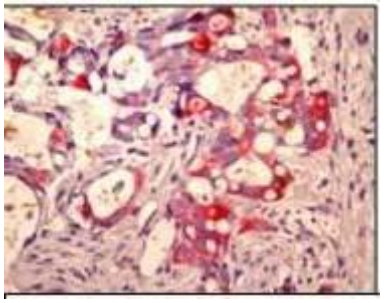

Figure 3: Invasive ductal carcinoma, Luminal A type showing mammoglobin score 4 and GCDFP-15 score 3 (400x)

TABLE 4: DISTRIBUTION OF MAMMOGLOBIN AND GCDFP-15 POSITIVE EXPRESSION IN BREAST CARCINOMA (MOLECULAR CLASSIFICATION)

MOLECULAR CLASSIFICATION OF BREAST CARCINOMA	MAMMOGLOBIN EXPRESSION SEEN IN (%)	GCDFP-15 EXPRESSION SEEN IN (%)
Luminal A (n=9)	5 (55.5%)	5 (55.5%)
Luminal B(n=2)	0 (0%)	0 (0%)
Luminal Her2neu (n=6)	1 (16.6%)	2 (3.3%)
Her2neu classic (n=12)	4 (50%)	1 (8.3%)
Triple negative (Basal Phenotype) (n=2)	0 (0%)	0 (0%)
Triple negative (Non-Basal, Normal Breast like Phenotype) (n=4)	1 (25%)	1 (25%)

DISCUSSION

In the present study, the immunohistochemical expression of mammoglobin and GCDFP-15 was studied in breast carcinoma. Positive mammoglobin expression was found in 11/35 (31.4%)

cases and GCDFP-15 expression in 9/35 (26.5%) cases. Previous studies have reported the expression of mammoglobin from 20 to 95%.[8] The broad range of mammoglobin expression may be attributed to various factors including environmental and genetic factors, preservation methods of tissues (fresh/frozen tissue and paraffin-embedded blocks) or variability in detection techniques (RT-PCR, immunohistochemical staining or in situ hybridization). Also, some of the studies were done in triple negative breast carcinomas where a lower expression was observed in comparison to other studies. Our findings were in concordance with Noriega et al[17]. Some of the previous studies (Fritzsche et al [3], Bhargava et al [9], Sasaki E et al [18] and El-Sharkawy SL et al [19]) have correlated the mammoglobin expression with ER and PR status, and Her2neu expression. Higher percentage of ER (+) and PR (+) cases and lower expression of Her2neu had been reported in their study which might have accounted for higher expression of mammoglobin also. In our study, the expression of Her2neu was higher which could be the reason for lower positivity with mammoglobin.

Richter-Ehrenstein et al [20] and Eugeni Lopez-Bonet et al [21] have reported positive expression of mammoglobin in IDC with neuroendocrine features. Krishnaiah et al [7] and Sasaki E et al [18] have found negative expression of mammoglobin in metaplastic carcinoma. Similar results were obtained in present study.

The scoring system used in our study is same as used by Rehman F et al [15]. They found more number of mammoglobin positive cases with higher score. However, Yadav R et al [22] reported more number of cases with lower score. In present study, mammoglobin positive cases with score 1, 2 and 3 were less and score 4 was more as compared to their study.

In the present study, positive GCDFP-15 expression was found in 9/35 (26.5%) cases. Similar result (23.1% cases) has been observed in study by Bhargava et al [9]. Previous studies have reported the expression of GCDFP-15 from 14 to 75% [3, 14]. The scoring pattern of GCDFP-15 has not been documented to be of any advantage. GCDFP-15 positivity in cases of IDC with neuroendocrine features has been reported by Richter-Ehrenstein et al[20] in 66.4% cases and Eugeni Lopez-Bonet et al [21] in 77.7% cases. Strong staining of GCDFP-15 was observed in similar case in the present study.

Few studies have studied the expression of both the markers (mammoglobin and GCDFP-15) in breast cancer (Table 5).

TABLE 5: COMPARISON OF PERCENTAGE OF MAMMOGLOBIN AND GCDFP-15 EXPRESSION IN VARIOUS STUDIES

Studies	Mammoglobin expression (%)	GCDFP-15 expression (%)
Fritzsche et al [3]	72.1%	73.3%
Bhargava et al [9]	55.4%	23.1%
Noriega et al [17]	34.5%	45%
Chia S Y et al [23]	47.8%	11.3%
Huo et al [24]	25%	30%
Gloyeske N C et al [25]	52%	26 %
Present study	31.4%	25.7%

No significant association was found between expression of mammoglobin and GCDFP-15 with tumor size and lymph node metastasis similar to other studies. However, it was observed that cases negative for lymph node metastasis showed higher score for mammoglobin and more number of cases with lymph node metastasis showed GCDFP positivity as compared to cases negative for lymph node metastasis.

Very few studies have correlated the immunohistochemical expression of mammoglobin and GCDFP-15 with molecular classification of breast

carcinoma. Study done by Gloria H. Lewis et al [14] reported a higher percentage of Luminal A, Luminal B, Her2neu classic cases, basal-like carcinoma cases to be mammoglobin and GCDFP-15 positive as compared to ours. However, they have reported mammoglobin positivity in 17% of non basal normal breast like phenotype and GCDFP positivity in 5% of basal phenotype cases which is lower than our study. Similar results were reported in Luminal subtype and UTNC by E Sasaki et al [18]. However, the mammoglobin positivity was lower in Her2 subtype (53%) and 20% in basal subtype. Lei Huo et al [24] observed positive expression of mammoglobin and GCDFP-15 in 25% and 14% of primary triple negative breast cancer, respectively which is in concordance with present study. However, none of the studies have correlated the scoring pattern and molecular subtypes.

Mammoglobin and GCDFP-15 did not show any statistically significant correlation with tumor size and lymph node metastasis similar to other previous studies (Yadav R et al [22]). However, it was observed that 3 cases with lymph node metastasis were positive for mammoglobin and out of 3, 2 cases showed a higher score (score 4) of mammoglobin positivity. More number of cases with lymph node metastasis (3/6,50%) showed GCDFP positivity as compared to cases negative for lymph node metastasis. No specific trend for GCDFP score was noted with lymph node metastasis. Hence, it can be inferred that mammoglobin and GCDFP-15 may help to establish the mammary differentiation of tumors, especially in cases of metastatic carcinoma.

CONCLUSION

Our results demonstrate that there is overexpression of MGB and GCDFP-15 in breast cancer. Luminal A and Her2 classic cases show higher expression. Higher MGB score was observed in Luminal A tumors. However, GCDFP scoring pattern did not correlate. So, we do recommend scoring of MGB to be useful but GCDFP scoring was not useful. More number of cases needs to be studied to evaluate the significance of mammoglobin and GCDFP in breast carcinoma.

Declaration by Authors

Ethical Approval: The study was approved by Institutional Ethics committee (certificate number: LHMC/ECHR/2015/58).

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Conflict of Interest: The authors declare no conflict of interest.

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