



UTILITY OF PROLINE, GLUTAMIC ACID, AND LEUCINE-RICH PROTEIN 1 AND GATA BINDING PROTEIN 3 EXPRESSION IN BREAST CANCER: AN OBSERVATIONAL STUDY

Breast Surgery

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ABSTRACT

Introduction: Breast cancer (BC) is one of the most common malignancies among women worldwide. Novel diagnostic immuno-histochemical markers with considerably increased sensitivity for primary and metastatic breast cancer (MBC), as well as the ability to predict therapy response and prospective target treatments, are urgently needed. With this background, we aimed the present study to understand the proportion of BC cases expressing PELP 1 and GATA 3 based on IHC and their association with various clinicopathological parameters. **Materials and methods:** An observational study was conducted on the specimens of breast cancer patients sent to the department of pathology in a tertiary care hospital of Maharashtra. After necessary permission from the ethics committee of our institution, we collected the relevant data of BC patients who fitted the criteria. Majority of the specimens were post surgery and a few were cell blocks from pleural effusion and needle biopsies taken from the breast swellings, lymph node or other distant metastasis. The data on age, lymph node involvement, lymphovascular invasion, necrosis, fibrosis, ER, PR, HER-2 neu status, skin invasion etc. were obtained from the pathology reports. Only in-hospital BC cases were included in the study. Histopathology and IHC of the specimens was done on the basis of standard procedure guidelines. Based on ER, PR and Her 2 neu status of the specimens, the final molecular type was categorized into Luminal A, Luminal B, triple negative and HER-2 neu enriched cases. GATA 3 and PELP 1 expression was done using standard IHC procedures. **Results:** Our study found 75% of the BC cases to have GATA 3 expression and 90% to have PELP 1 expression. GATA-3 expression was significantly higher in the age group more than 35 years. ($p < 0.05$) The expression increased with the increase in the grade of tumour. Grade 3 disease had highest expression of GATA-3 by IHC. There was significant association between the lymphovascular invasion and the GATA-3 expression in the present study. GATA-3 expression was higher in luminal type A and triple negative when compared to other molecular type of BC. PELP 1 expression was higher among the patients with age more than 35 years when compared to patients with less than 35 years of age. ($p < 0.05$) Expression of PELP1 among infiltrating duct carcinoma was significantly higher when compared to other types of BC. ($p < 0.001$) PELP 1 expression was higher in luminal type A and triple negative breast cancer patients when compared to other molecular type of BC. **Conclusions:** Expression of GATA 3 marker was in $\frac{3}{4}$ of the patients of BC in the present study. About 9 in 10 patients had diffuse response on PELP 1 marker. Age more than 35 years, grade of tumour and molecular sub type of luminal A and TNBC were significant parameters associated with GATA 3. Age more than 35 years, fibrosis and molecular sub type of luminal A and TNBC were significant parameters associated with PELP 1. GATA 3 and PELP 1 are novel promising markers and cues for newer treatment modalities of BC.

KEYWORDS

GATA 3, PELP 1, Breast cancer; Clinicopathological profile

INTRODUCTION

Breast cancer (BC) is one of the most common malignancies among women worldwide. It is the second leading cause of cancer death in women. In the recent developments, immunohistochemistry remains the main modality in assisting treatment of BC. 1 There was an era when estrogen receptor, progesterone receptor and HER-2 neu receptor status revolutionized the management of BC. 2 Novel diagnostic immuno-histochemical markers with considerably increased sensitivity for primary and metastatic breast cancer (MBC), as well as the ability to predict therapy response and prospective target treatments, are urgently needed. 3 GATA3 has been identified as a new marker for detecting primary and metastatic BC, with considerably more sensitivity than the conventional markers. GATA3 is a zinc-binding transcription factor that controls the differentiation of various human tissue types, including the luminal epithelial cells of the breast. Also, understanding the role of GATA3 can be a promising potential target in BC management, as it was demonstrated as a requirement for estradiol stimulation for cell cycle progression of BC. 4-7 PELP1 is a scaffolding protein that functions as a co regulator of several transcription factors and multiple hormonal receptors and exhibits aberrant expression in many hormone-related cancers. PELP1 over expression had been shown to induce the malignant transformation of normal cells, accelerate cell cycle progression, modulate several signaling cascades, control the cell cytoskeleton, promote tumor cell proliferation, and promote the migration and metastases in BC. 8-11 With this background, we aimed the present study to understand the proportion of BC cases expressing PELP 1 and GATA 3 based on IHC and their association with various clinicopathological parameters.

MATERIALS AND METHODS

An observational study was conducted on the specimens of breast cancer patients sent to the department of pathology in a tertiary care hospital of Maharashtra. After necessary permission from the ethics committee of our institution, we collected the relevant data of BC patients who fitted the criteria. Majority of the specimens were post

surgery and a few were cell blocks from pleural effusion and needle biopsies taken from the breast swellings, lymph node or other distant metastasis.

A study conducted by Moustafa M et al 12 inferred that the proportion of GATA 3 expression among BC patients in their study was around 75%. Using this with 95% confidence interval and 15% absolute error, we found the minimum sample size of 30 for our study. During the above said tenure of 1 year, we found 40 cases of BC and all the cases were included in the present study.

The data on age, lymph node involvement, lymphovascular invasion, necrosis, fibrosis, ER, PR, HER-2 neu status, skin invasion etc. were obtained from the pathology reports. Only in-hospital BC cases were included in the study. Histopathology and IHC of the specimens was done on the basis of standard procedure guidelines. Based on ER, PR and Her 2 neu status of the specimens, the final molecular type was categorized into Luminal A, Luminal B, triple negative and HER-2 neu enriched cases. GATA 3 and PELP 1 expression was done using standard IHC procedures. GATA3 and PELP1 immunoreactivity were evaluated independently in a blind manner by two pathologists using a binocular microscope.

The intensity of the GATA3 nuclear labeling was scored as negative (0), weak (1+), moderate (2 +), or strong (3 +) for association with clinicopathological parameters.

The percentage of tumor cells was scored based on the extent of nuclear staining with a cutoff of 5%, defining GATA3 positive expression for the diagnosis of BC cases. 13 For the ease of analysis negative and weak positive cases were included in one category and moderate to high positive cases into another category. PELP1 staining was categorized into low (< 5%), focal (5-49%), and diffuse ($\geq 50\%$). A cutoff of 5% was used to define PELP1 positivity for the diagnosis of BC cases. 14 For the ease of statistical analysis and

interpretation, focal and low cases categorized into one group and another group had only diffuse cases in the present study.

Statistical analysis plan:

The data was collected, compiled, and analyzed using EPI info (version 7.2). The qualitative variables were expressed in terms of percentages. The quantitative variables were categorized and expressed in percentages or terms of mean and standard deviations percentages. The difference between the two proportions was analysed using the chi-square or Fisher exact test. All analysis was two-tailed, and the significance level was set at 0.05.

Results:

We have included 40 cases of carcinoma breast in the present study

Table 1: Clinicopathological parameters of the present sample

Clinicopathological parameters	Frequency	Percentage
Age group		
<35 years	6	15.00
>35 years	34	85.00
Pathological type		
IDC	30	75.00
Lobular	6	15.00
Special	4	10.00
Grade		
Grade 1	2	5.00
Grade 2	14	35.00
Grade 3	24	60.00
Lymph node		
Negative	18	45.00
Positive	22	55.00
Skin invasion		
Absent	34	85.00
Present	6	15.00
LVI		
Absent	17	42.50
Present	23	57.50
Necrosis		
Absent	24	60.00
Present	16	40.00
Fibrosis		
Absent	10	25.00
Present	30	75.00
Molecular subtype		
Luminal A	18	45.00
Luminal B	3	7.50
HER enriched	4	10.00
Triple negative	19	47.50

The mean age of the BC cases was 45.56 ± 3.66 years. The most common pathological type was IDC (75%), around 60% of the cases were Grade 3, 45% of the lymph nodes were negative, 15% of the cases had skin invasion, 57.50% had lymphovascular invasion, 40% showed necrosis and majority of them were luminal type A and triple negative breast cancers in the present study.

Chart 1: Distribution of the cases based on the GATA-3 expression

25	Negative or weak positive
75	Moderate to high positive
Around 75% of cases showed GATA-3 expression.	

Chart 2: Distribution of the cases based on the PELP 1 expression

10	Negative or focal
90	Diffuse
Around 90% showed PELP 1 expression in the present study.	

Table 2: Association of GATA-3 expression with various

Clinicopathological parameters	Negative or Focal (n=4)	Diffuse (n=36) P value
Clinicopathological Negative or Moderate to high P value		
parameters weak positive (n=10)		
Positive (n=30)		
Age group	Number %	Number %
<35 years	4 40.00	2 6.67 0.0021
>35 years	6 60.00	28 93.33
Pathological type		
IDC	7 70.00	23 76.67
0.3451 Lobular	2 20.00	4 13.33
Special	1 10.00	3 10.00
Grade		
Grade 1	1 10.00	1 3.33 <0.001
Grade 2	6 60.00	8 26.67
Grade 3	3 30.00	21 70.00
Lymph node		
Negative	4 40.00	14 46.67 0.4562
Positive	6 60.00	16 53.33
Skin invasion		
Absent	7 70.00	27 90.00 0.0653
Present	3 30.00	3 10.00
LVI		
Absent	2 20.00	15 50.00 0.0081
Present	8 80.00	15 50.00
Necrosis		
Absent	6 60.00	18 60.00 1.000
Present	4 40.00	12 40.00
Fibrosis		
Absent	3 30.00	7 23.00 0.5623
Present	7 70.00	23 76.67
Molecular subtype		
Luminal A	2 20.00	16 53.33
<0.001 Luminal B	0 0 3 10.00	
HER enriched	1 10.00	3 10.00
Triple negative	7 70.00	12 40.00

GATA-3 expression was significantly higher in the age group more than 35 years. (p<0.05)

The expression increased with the increase in the grade of tumour. Grade 3 disease had highest expression of GATA-3 by IHC. There was significant association between the lymphovascular invasion and the GATA-3 expression in the present study. GATA-3 expression was higher in luminal type A and triple negative when compared to other molecular type of BC.

Table 3: Association of PELP 1 IHC expression with various clinicopathological parameters

Clinicopathological parameters	Negative or Focal (n=4)	Diffuse (n=36) P value
Age group	Number %	Number %
<35 years	0 0 6 16.67 0.0451	
>35 years	4 100.00	30 83.33
Pathological type		
IDC	1 25.00	29 80.56 <0.001
Lobular	1 25.00	5 13.89
Special	2 50.00	2 5.56
Grade		
Grade 1	0 0 2 5.56 0.4532	
Grade 2	1 25.00	13 36.11
Grade 3	3 75.00	21 58.33
Lymph node		
Negative	2 50.00	16 44.44 0.5644
Positive	2 50.00	20 55.56
Skin invasion		

Absent 3 75.00 31 86.11 0.3421
 Present 1 25.00 5 13.89
 LVI
 Absent 2 50.00 15 41.67 0.7652
 Present 2 50.00 21 58.33
 Necrosis
 Absent 2 50.00 21 58.33 0.7532
 Present 2 50.00 14 38.89
 Fibrosis
 Absent 1 25.00 9 25.00 1.000
 Present 3 75.00 27 75.00
 Molecular subtype
 Luminal A 1 25.00 17 47.22
 <0.001 Luminal B 0 0 3 8.33
 HER enriched 1 25.00 3 8.33

Triple negative 2 50.00 17 47.22

PELP 1 expression was higher among the patients with age more than 35 years when compared to patients with less than 35 years of age. (p<0.05) Expression of PELP1 among infiltrating duct carcinoma was significantly higher when compared to other types of BC. (p<0.001) PELP 1 expression was higher in luminal type A and triple negative breast cancer patients when compared to other molecular type of BC.

DISCUSSION:

Expression of novel IHC markers like GATA 3 and PELP 1 will assist the medical oncologist in trying newer management techniques for the patients with BC. 2,7 Very few studies have been conducted on these markers and hence we planned this study to understand the various clinical and pathological parameters with these markers. In the present study, the expression of GATA 3 was present in around 75% of the BC cases.

Age group more than 35 years, grade 3 BC cases and presence of lymphovascular invasion were significant factors associated with GATA-3 expression. Similar inferences were reported by Mustafa M et al 12 in their study. The proportion of GATA 3 expression in a study by Mustafa M et al 12 was around 76.67% of BC cases. Of the two studies that have specifically evaluated the expression of GATA3 in TNBC, the reported range of GATA3 expression was 5% and 16%. 15,16 Our study reported higher proportion of GATA 3 expression among TNBC patients. Loss of GATA3 expression has been associated with unfavorable clinical outcomes and worse survival. However, no association with the outcome has been observed in other studies. 17,18 PELP 1 expression was diffuse in 90% of the cases studied in the present study.

The significant higher expression was seen in patients with more than 35 years of age, infiltrating duct carcinoma type, luminal A and triple negative breast cancers. Based on the pathological type, Mustafa M et al 12 reported that IDC was the most common type of BC which had higher expression of PELP 1 marker and was more common in luminal type A BC cases. The immunohistochemical expression of PELP1 in tumor cells was positive in 58 out of 60 cases (96%), and only 2 out of 58 show focal staining in a study by Moustafa M et al. 12 Recent research showed a high expression rate of PELP1 in BC and its superiority over GATA3 in identifying TNBC. In a study conducted by Wang X et al 19 the PELP 1 expression was low in 29.5%, focal in 2% and diffuses in 68.5% of the BC patients. Higher age, molecular subtype of BC and presence of fibrosis were the significant factors associated with PELP 1 expression in a study conducted by Wang X et al. 19 Zhang et al 20 studied the prognostic significance of 129 cases of TNBC on expression of PELP 1. They found presence of positive lymph node was associated with expression of PELP 1 marker. PELP1 revealed a (96.67%) positive expression rate in primary TNBC and a (86.67%) positive in metastatic TNBC. In comparison to GATA3, revealed (53.33%) positive expression rate in primary TNBC and (60%) positive in metastatic TNBC. Furthermore, the majority of the PELP1 positive cases showed diffuse strong staining, making observation of the staining easy and suggested that PELP1 may be a molecular target for TNBC therapy.

CONCLUSIONS

Expression of GATA 3 marker was in ¾ of the patients of BC in the present study. About 9 in 10 patients had diffuse response on PELP 1 marker. Age more than 35 years, grade of tumour and molecular sub

type of luminal A and TNBC were significant parameters associated with GATA 3. Age more than 35 years, fibrosis and molecular sub type of luminal A and TNBC were significant parameters associated with PELP 1. GATA 3 and PELP 1 are novel promising markers and cues for newer treatment modalities of BC. More studies are warranted to understand the patho-biology of these markers in management of primary and metastatic BC.

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