Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.202294450-455

Study of GATA-3 Expression in Breast Carcinoma in a Tertiary Care Hospital in Eastern India

Lahari Banik*[a], Mallika Pal[a], Nabanita Mayur[a]

[a]*Department of Pathology, Nil Ratan Sircar Medical College & Hospital (NRSMCH), Kolkata*

ARTICLE INFO

Received:
18 May 2022
Revised:
2 July 2022
Accepted:
19 July 2022

Keywords:
breast carcinoma, GATA-3, immunohistochemical marker

ABSTRACT

Background: Transcription factor GATA-3 is a relatively new and specific biomarker for breast carcinoma that regulates luminal cell differentiation. However, further studies that elucidate its role and diagnostic utility in primary breast cancer are required. Therefore, this study was conducted to assess GATA-3 expression in primary breast carcinoma and determine its association with various known prognostic and predictive factors of breast carcinoma.

Methods: This was a cross-sectional, observational study where 110 breast carcinoma cases were examined histologically on H&E stained slides for routine parameters such as tumor morphological type and histologic grade. ER/PR/Her-2-neu/ki67 and GATA-3 expression was determined using immunohistochemistry. All cases were assessed to determine the association of GATA-3 with different established clinicopathological characteristics in breast cancer such as patient age, axillary lymph node status, tumor grade, lymphovascular invasion, molecular subtype, etc. The results were statistically analyzed and P value < 0.05 was considered to be significant.

Results: Overall, 97 cases (88.1%) expressed GATA-3 which was significantly associated with most of the established clinico-pathological parameters like family history of breast cancer, tumor size and histology grade, lymph node and margin status, presence of lymphovascular invasion and carcinoma in-situ component. GATA-3 expression was also significantly associated with ER/PR/HER2neu/ki67 expression.

Conclusion: GATA-3 expression is significantly associated with the different established prognostic parameters of breast cancer. Therefore, it may be considered as a relevant marker in primary breast carcinoma more routinely. Further studies of GATA-3 in breast cancer may refine prognostic models, predict clinical outcomes, and modify treatment guidelines in future.

INTRODUCTION

Transcription factor GATA-3 binds to the DNA consensus sequence (A/T) GATA (A/G)[1] and plays a crucial role at multiple stages of breast development including luminal cell differentiation.[2] Therefore, GATA-3 dysregulation has been implicated in the pathogenesis of breast cancer. GATA-3 expression has been detected in the luminal breast cancer subtypes[3-5], and somatic mutations of GATA-3 have been identified in ER-positive breast carcinomas.[6,7] GATA-3 shows promise as a sensitive and relatively specific marker for breast cancer as its expression is retained in "estrogen/progesterone receptor loss" metastases of breast carcinoma.[8] Several studies have also shown that higher levels of GATA-3 are associated with better survival in breast cancer[9],

***Address for correspondence:**
Lahari Banik, MD,
DNB Pathology
Department of Pathology, Nil Ratan Sircar Medical College & Hospital (NRSMCH), 7A Kalimohan Banerjee Lane,-700025, Kolkata
Tel: +919007089841/+918276931260
E-mail: laharibanik@gmail.com

making it a biomarker with potential independent prognostic value. Considering the relative paucity of studies aimed at understanding the role of GATA-3 in breast carcinoma in India, this study was conducted to detect GATA-3 expression using immunohistochemistry and determine its association with the different established prognostic parameters of breast carcinoma such as the histologic grade, morphological and molecular subtypes of tumor.

MATERIALS AND METHODS

This cross-sectional, observational study was conducted in the Department of Pathology, NRSMCH, Kolkata on 110 breast cancer patients who underwent surgery between February 2019 and July 2020 and gave consent for the study. Relevant history and clinical findings were noted. Formalin-fixed and paraffin-embedded tissue blocks were cut at 4µm thickness, stained with haematoxylin and eosin and examined for routine microscopic findings such as tumor morphological type[10], histology grade[11], TNM stage, lymphovascular invasion[12], in-situ component, margin involvement, etc. Blocks rich in tumour tissue were selected for IHC with ER, PR, HER-2-NEU, ki-67 and GATA-3. Then, primary antibodies were used - ER: Mouse monoclonal Anti-Human, RTU, 6F11, Novocastra, Leica, PR: Monoclonal Mouse Anti-Human, RTU, Novocastra, Leica, HER2: Polyclonal Rabbit Anti-Human, CB11, Cell Marque, Ki-67: Rabbit Monoclonal Antibody, SP6, Cell Marque, GATA-3: Mouse Monoclonal Antibody, Anti-GATA-3 (L50-823), Cell Marque. Interpretation of ER/PR was done using Allred scoring system[13], and ASCO/CAP guidelines were used for HER2 immunostaining[14]. Each case was classified as either-luminal A [ER and/or PR positive, HER2 negative and low Ki-67 (<14%)] or luminal B [ER and/or PR positive, HER2 variable and intermediate or high Ki-67 (>14%)], HER2 enriched [HER2 positive, ER and PR negative, high ki67] or triple negative [ER, PR and HER2 negative].[15] For GATA-3, only nuclear staining was considered as positive.[16] The immunoreactivity score for GATA-3 expression was calculated by multiplying the percentage of immunoreactive cells (determined as Score 0: No tumor cells stained, Score 1: 1–10%, Score 2: 11–50%, Score 3: 51–80% and Score 4: 81–100% tumor cells stained) with the staining intensity (Staining Score 0: No tumor cells stained; Score 1, 2 and 3: Weak, moderate and strong staining respectively) (Table 1) (Figure 1).

Table 1. Interpretation of GATA-3 Immunohistochemistry

GATA 3 Group	Immunoreactivity score	Interpretation
1	0-1	Negative
2	2-4	Weak Positive
3	5-8	Moderately Positive
4	9-12	Strongly Positive

Statistical Analysis

All statistical evaluation was performed using the software Statistica version 8 [Tulsa, Oklahoma: StatSoft Inc., 2007] and GraphPad Prism 5 [San Diego, California: GraphPad Software Inc., 2007]. Pearson Chi-square test was used to analyse the cross-tabulated categorical data. P-value<0.05 was considered statistically significant.

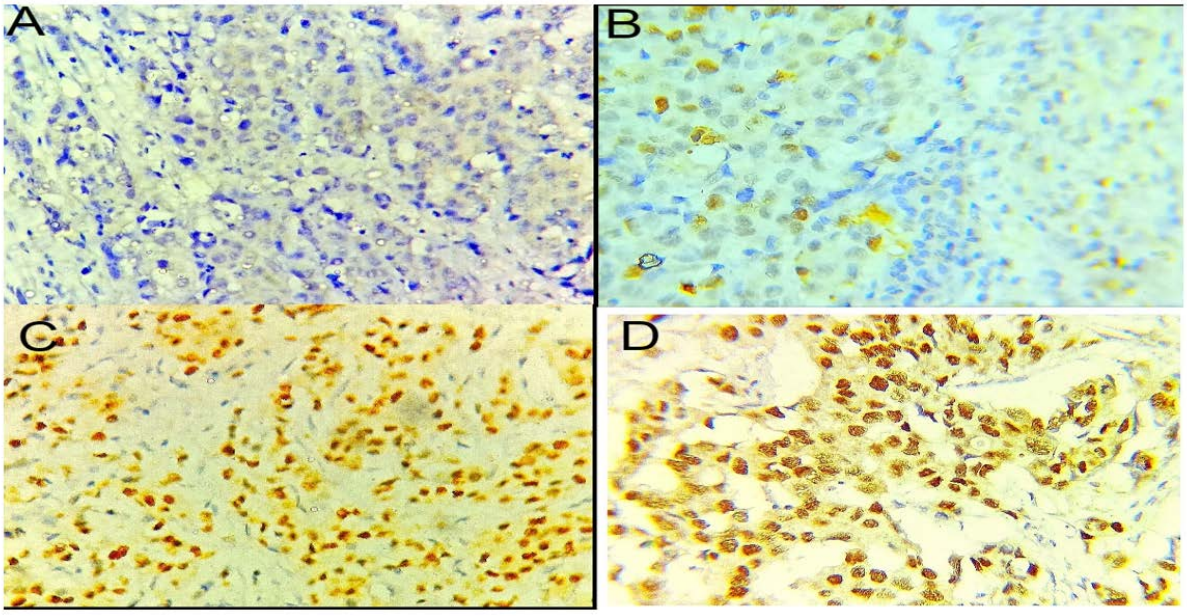

Figure 1. Expression of GATA-3 by IHC (A) GATA-3 Group 1 (1X1) = Negative [40X] ; (B) GATA-3 Group 2 (2X1) = weakly + [40X] ; (C) GATA-3 Group 3 (4X2) = moderately + [40X] ; (D) GATA-3 Group 3 (4X3) = strongly + [40X].

RESULTS

Out of 110 cases, 107 were female (97.3%) aged between 32 to 80 years (Mean age=49.5 years). Overall, 90% cases presented with breast lump and 85.5% cases underwent Modified Radical Mastectomy. Majority of cases comprised invasive ductal Carcinoma, no special type (IDC NST (83.6%), histology grade 2 (71.8%), $T_2N_0M_x$ stage and luminal A subtype (42.7%). Lymphovascular invasion and an associated in-situ component were present in 62.7% and 78.2% cases, respectively. Surgical margin involvement was present only in 6.4% cases.

GATA-3 Expression in breast carcinoma

Out of 97 cases positive for GATA-3 immunostaining, 35% cases were weak positive (score 2), 35% moderately positive (score 3) and 30% strong positive (score 4) for GATA-3.

GATA-3 Expression and clinical parameters

GATA-3 expression was not significantly associated with age (P=0.539), sex (P=0.358), tumor laterality (P=0.797), history of menopause (P=0.740), history of breast feeding (P=0.216) and history of chemotherapy (P=0.143). Interestingly, a significant association was found between GATA-3 expression and family history of breast carcinoma with lower expression of GATA-3 in cases with positive family history (P=0.019), although this analysis might have been compromised by the small number of family history positive cases (Table 2).

Table 2. Association of GATA-3 with the clinical characteristics of the patients

Clinical Characteristics	Total [N (%)]
Age (years)	
<50	58(52.7)
≥50	52(47.2)
Sex	
Female	107(97.3)
Male	03(2.7%)
Tumor laterality	
Right sided	54(49.0%)
Left sided	56(50.9%)
Family history	
Present	06(5.5%)
Absent	104(94.5%)
Menopausal status	
Pre-menopausal	28(25.5%)
Post-menopausal	79(72%)
Breast feeding history	
Present	97(88.1%)
Absent	10(09%)
History of chemotherapy	
Present	06(5.5%)
Absent	104(94.5%)

Table 3. Association of GATA-3 with relevant histological characteristics of the tumor

Histological Characteristics	GATA-3 Grade 1	GATA-3 Grade 2	GATA-3 Grade 3	GATA-3 Grade 4	Association with GATA-3
Histologic Grade					
1	00	00	05	02	P<0.001
2	03	26	25	25	
3	10	08	04	02	
Tumor size					
T1	00	01	06	02	P=0.002
T2	04	18	20	00	
T3	07	14	06	02	
T4	02	01	02	00	
Axillary lymph node status					
N0	02	10	14	17	P=0.014
N1	00	03	05	04	
N2	03	10	10	05	
N3	08	11	05	03	
LVI					
Present	11	27	18	13	P=0.007
Absent	02	07	16	13	
CIS					
Present	09	21	30	26	P=0.017
Absent	04	13	04	03	
Margin Status					
Involved	03	04	00	00	P=0.007
Uninvolved	10	30	34	29	

GATA-3 Expression and histological parameters

GATA-3 expression was significantly lower with larger tumor size (P=0.002), number of axillary lymph nodes involved (P=0.014), higher histological grade (P=0.014), presence of lymphovascular invasion (P=0.007), in-situ carcinoma component (P=0.017) and positive margins (P=0.007) (Table 3). However, GATA-3 expression was not significantly associated with the tumor morphological type (P=0.063).

GATA-3 Expression and immunohistochemical parameters

GATA 3 expression was significantly associated with ER/PR/HER2neu and ki67 expression with P value<0.001 for each variable. Higher GATA-3 expression was noted in positive ER/PR and low ki67 cases while lower expression was seen in Her-2-neu positive and triple negative cases (Table 4).

Table 4. Cross-tabulation of GATA-3 expression with different biomarkers (P=<0.001)

Prognostic marker	GATA-3 score 1 [N (%)]	GATA-3 score 2 [N (%)]	GATA-3 score 3 [N (%)]	GATA-3 score 4 [N (%)]	Total
ER positive	00	13(19.4%)	28(41.7%)	26(38.8%)*	67
ER negative	13(30.2%)	21(48.8%)	06(13.9%)	03(6.9%)	43
PR positive	00	10(16.1%)	26(41.9%)	26(41.9%)*	62
PR negative	13(27%)	24(50%)	08(16.6%)	03(6.2%)	48
HER2-neu positive	03(9%)	22(66.6%)	07(21.2%)	01(3%)	33
HER2-neu negative	10(12.9%)	12(15.5%)	27(35%)	28(36.3%)*	77
Ki67 low	00	03(6.5%)	17(36.9%)	26(56.5%)*	46
Ki67 high	13(20.3%)	31(48.4%)	17(26.5%)	03(4.6%)	64

*Higher expression of GATA-3 noted in cases with positive ER/PR, negative Her-2-neu expression and low ki67 status

DISCUSSION

In this study, 97 out of 110 breast carcinoma cases (88.1%) expressed GATA-3. No statistically significant association of GATA-3 was found with age and sex of patient, tumor laterality, history of menopause, breast-feeding or chemotherapy. These findings corroborate the findings of the study of Ismail *et al.* [17] where nuclear expression of GATA-3 was detected in 36 (72%) cases and no association was found between GATA-3 expression and the age of the patients or tumor laterality. However, in our study, there was statistically significant lower expression of GATA-3 in the six cases (5.5%) with a positive family history (P=0.019).

In our study, 92 cases (83.6%) were diagnosed as invasive ductal carcinoma, no special type (IDCNST) and no significant association was found between GATA-3 expression and the morphological subtype of breast cancer (P=0.063). This is also similar to the study of Ismail *et al.*[17] where GATA-3 expression between cases of invasive ductal and lobular carcinoma breast was statistically insignificant (t=0.625).

Similar to the study by Albergaria *et al.*[18] and Voduc *et al.*[19] in our study, reduced GATA-3 expression was found in higher histological grade 3. GATA-3 expression was significantly associated with tumor grades (P<0.001), CIS (P=0.017) and LVI (P=0.007). All the cases with higher GATA-3 expression had uninvolved margin status while the cases with lower expression of GATA-3 had a statistically significant positive margin involvement (P=0.007). This may be related to the fact that GATA-3 is associated with reversal of epithelial-mesenchymal transition and inhibits tumor metastasis.[20]

It was seen that with an increase in the tumor size, there was reduced expression of GATA-3 (P=0.002). This finding corroborates the findings of the study of Mehra *et al.*[5] who found low GATA-3 expression was associated with larger tumor size (P=0.03). A significant association was found between GATA-3 expression and pN in our study (P=0.014) unlike the study of Voduc *et al.*[19] where there was no significant association between GATA-3 and lymph node status (Pearson chi-square test, P=0.47).

In our study, a significant association was found between expression of GATA-3 and the molecular types of breast cancer (P<0.001) with higher GATA-3 expression in the luminal subtypes and lower GATA-3 expression in the triple negative cases. A study by Ismail *et al.*[17] through the analysis of KW test demonstrated a significant association between GATA-3 expression in both luminal A and B-HER2-negative tumors when compared with GATA-3 expression in the triple-negative subtype. The same finding was reported by Jiang *et al.*[21] These observations agree with the hypothesis that GATA-3 mutations might be important in the aetiology of luminal-like breast cancers.

Individually, GATA-3 expression showed a significant association with ER, PR and Her-2-neu expression with P-value<0.001 in all cases. More cases of triple negative breast cancer were in the group that expressed low levels of GATA-3 and a significant association was found between the two in

this study (P=<0.001). A high Ki-67value was found in all cases with low GATA-3 expression and a low ki67 value was observed for the majority of GATA-3- Grade 3 tumors. This finding is consistent with the study of Yildirim *et al.*[22] which revealed that GATA-3 has an inverse relationship with Ki-67. There was a significant association of GATA 3 expression with the ki67 status in the Grade 2 tumors (P<0.001).

Limitations

We have inferred statistical significance on the basis of the chi-square test. However, the trends are to be interpreted as only preliminary in view of the limited numbers in some of the cross-tabulation categories. Moreover, as no data on follow-up was available, the prognostic significance of GATA-3 in breast carcinoma could not be assessed.

CONCLUSION

GATA-3 is expressed in most cases of breast carcinoma. GATA-3 expression is significantly associated with different known prognostic factors in breast cancer such as the size and histology grade of tumor, axillary lymph node status, LVI, CIS and the molecular subtypes of breast cancer including ki67 index. GATA-3 expression was higher in the luminal subtypes and lower in the triple negative cases. Ki-67 was high in all low GATA-3 expressing tumors. Further studies on GATA-3 could be undertaken to assess its potential to predict prognosis in breast carcinoma or develop novel therapeutic approaches in the future.

CONFLICTS OF INTEREST

None.

ETHICAL CONSIDERATIONS:

Institutional Ethics Committee approval no/NMC/10018; dated: 08-01-19.

FUNDING

None.

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How to Cite This Article

Banik L, Pal M, Mayur N. Study of GATA-3 Expression in Breast Carcinoma in a Tertiary Care Hospital in Eastern India. Arch Breast Cancer. 2022; 9(4): 450-5.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/593