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Original Research Article

Prevalence of GATA-3 in invasive breast cancer and its significance in predicting response to neoadjuvant chemotherapy: a tertiary center experience

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ABSTRACT

Background: GATA-3 expression was shown to be an independent predictor of overall and disease-free survival in some studies, whereas others showed no difference. We prospectively studied GATA-3 expression in index breast cancer patients and co-related with other immunohistochemical (IHC) markers along with response evaluation in IIIB onwards receiving neoadjuvant chemotherapy. Objective was to determine the expression of GATA-3 in Indian breast cancer tissue specimens and correlating GATA-3 expression with existing clinicopathological, radiological and immunohistochemical markers of breast cancer as well as with clinical response in breast cancer patients receiving neoadjuvant chemotherapy.
Methods: It was a prospective study that was conducted from November 2016 to October 2017. GATA-3 expression in carci-noma breast tissue obtained by tru-cut biopsy was studied by IHC.
Results: The distribution of GATA-3 receptor positivity with age showed higher positivity (p=0.79) with the older age group. The distribution of biological receptors in breast cancer patients had shown the highest presence of GATA-3 (87.5%) followed by HER2/neu (62.5%), ER (60%) and PR (50%). GATA-3 receptor positivity showed maximum positivity with luminal A subtype (50 and 9.11=59.1%) followed by HER2/neu enriched subtype (48.9%). Triple-negative breast cancer patients showed 48% positivity for the GATA-3 receptor. GATA-3 receptor expression was more in the locally advanced stage of breast cancer as compared to the early stage (p=0.02). GATA-3 positive patients showed partial response to chemotherapy (75.8%).
Conclusions: There is a raised possibility that GATA-3 or its downstream genes could be used in the management of luminal breast cancer.

Keywords: GATA-3, Breast cancer, Immunohistochemical markers, Clinical response

INTRODUCTION

Breast cancer is the commonest cancer in urban Indian females and the second commonest in rural Indian women.[1] The rise in the incidence of 0.5–2% per annum has been seen across all regions of India and in all age groups but more so in the younger age groups (<45 years).[2]

While the majority of breast cancer patients in western countries are postmenopausal and in their sixth or seventh decade, the picture is quite different in India with premenopausal patients constituting about 50% of all patients.[3]

The key regulatory mechanisms controlling normal embryonic development (EMT, stem cell differentiation and others) underscore the importance of identifying the overlapping molecular programs that are shared in these cellular processes to understand how cancers develop and metastasize.

GATA-3 is 1 of 6 members of the GATA family of transcription factors, whose members contain zinc-finger deoxyribonucleic acid (DNA) binding domains that bind to consensus 5′-(A/T) GATA (A/G)-3′ motifs.[4,5]

In the breast, GATA-3 plays a specific role in the differentiation of the breast luminal epithelial cells.[6] Immunohistochemistry (IHC) for GATA-3 expression is primarily used in surgical pathology to support a breast or urothelial origin in a carcinoma.[7-10]

In addition to regulating their expression, GATA-3 and ER are in a positive cross-regulatory loop for each other and are frequently co-expressed in luminal A breast cancers although no such correlation was observed in a recent study of male breast cancers.[11,12] Many studies looking at the prognostic use of GATA-3 in breast cancers in adjuvant and neoadjuvant settings showed somewhat conflicting results.[13-17]

Our goal was to determine the significance of GATA-3 expression in outcomes of patients with breast cancer who received neoadjuvant systemic therapy, as well as clinicopathological features of GATA-3–positive tumours in Indian patients.

METHODS

The present study was conducted combined in general surgery and pathology disciplines in King George's Medical University, Lucknow Uttar Pradesh among 100 index cases of all age groups. The study was conducted after informed consent was obtained from the subjects and approved by the ethical committee of the institution.

Study design

The study was a prospective observational study design.

Duration of study

The duration of the study was from November 2016 to October 2017 (one year).

Inclusion criteria

All newly detected cases of breast cancer (T1-T4, N1-3, and M0) of all age groups were included in the study.

Exclusion criteria

Patients with M1 disease and patients receiving adjuvant chemotherapy were excluded from the study.

Sample size calculation used=4pq/d2 (p=prevalence; q=1-p; d=allowable error) [alpha error=0.05; beta error=0.2]. Required sample size of 100 subjects.

However, only 40 cases could be evaluated in the study because some of the tissue samples got exhausted in the evaluation of ER/PR/HER2 NEU and Ki67 and secondly, some of the tissues that were found to be >2 times floating sections during the procedure were not used for analysis.

Tru-cut/incisional biopsies were performed to obtain tissue samples from breast lumps and subjected to immunohistochemistry (IHC).

Hematoxylin and eosin (H and E) stained slides and paraffin block were retrieved from the records. 3-4 micrometer thick sections were taken from each block for H and E staining and; immunohistochemical staining was done on (3-aminopropyl triethoxy silane) coated slides with the ER, PR, HER2, Ki67 and GATA-3 markers. Both positive and negative, tissue control and reagent control were performed with the immunohistochemistry panel.

Positive controls were: ER/PR- adjacent breast containing normal glands; HER2- ductal carcinoma in situ (DCIS) component; Ki67- ductal carcinoma breast; and GATA-3- human breast tumour tissue.

For negative control, the primary antibody was omitted while performing immunohistochemical staining.

Histopathological categorization of breast carcinoma was done under the College of American Pathologists (CAP) protocol. Histopathological grading was done of all cases according to Nottingham's modification of the Bloom Richardson system. Immunohistochemical evaluation using streptavidin biotin immunoperoxidase method was done**.**

Immunohistochemical staining

More than/equal to 1% moderate to strong nuclear positivity was considered positive for ER/PR status. HER2/neu status was calculated according to ASCO CAP protocol 2014.

Ki-67 index

Immunostaining was quantitatively evaluated by using light microscopy, in which the entire section was scanned at low-power magnification to determine areas with the highest numbers of positive nuclei (hot spot) within the invasive component. These were usually found at the periphery of tumours. Ki-67 labelling index (Ki-67LI) was expressed as the percentage of MIB1-positive cells among a total number of 1,000 malignant cells at high-power magnification.

GATA-3

Staining and evaluation using specific rabbit monoclonal antibody to GATA-3 were done as per standard protocol.

The percentage of tumor cells labelled by GATA-3 were scored as follows - 0: no tumor cells stained, 1: 1-10%, 2: 11-50%, 3: 51-80% and 4: 81-100%.

The staining intensity of tumour cells labelled by GATA-3 was scored as follows: 0: no tumour cells stained, 1: weak, 2: moderate and 3: strong.

Immunoreactivity scores for GATA-3 expression were calculated by multiplying the number representing the percentage of immunoreactive cells by the number representing staining intensity and were considered as follows - 0–1: negative, 2–4: weakly positive, 5–8: moderately positive, and 9–12: strongly positive.

The presence of brown coloured end product at the site of target antigen was indicative of positive reactivity.

The intensity of staining was recorded separately as weak, moderate or strong.

Any intensity of staining of greater than 5% distribution was considered positive - <5%: negative for GATA-3, 5-75%: low GATA-3 expression, and >75%: high GATA-3 expression.

Statistical analysis

Data were analysed using statistical package for social sciences (SPSS) version 24.0. Descriptive summary using frequencies, percentages, chi-square, graphs, diagrams were used in the present study protocols.

RESULTS

The distribution of GATA-3 receptor positivity with age showed higher positivity (p=0.79) with the older age group. The distribution of biological receptors in breast cancer patients had shown the highest presence of GATA-3 (87.5%) followed by HER2 neu (62.5%), ER (60%) and PR (50%) as shown in Table 2.

GATA-3 receptor positivity when correlated with various molecular subtypes of breast cancer showed maximum positivity with luminal A subtype (50 and 9.11=59.1%) followed by HER2 neu enriched subtype (48.9%). Triple-negative breast cancer patients showed 48% positivity for the GATA-3 receptor thus raising the hope for future hormonal targeted therapies in these subgroups of patients. GATA-3 receptor expression was more in the locally advanced stage of breast cancer as compared to the early stage; on comparing this data statistically, this difference was significant (p=0.02).

Since most of the patients at the time of presentation were locally advanced breast cancer so neoadjuvant chemotherapy (92.5%) was offered while surgically operable patients were 7.5%. In patients who were offered neoadjuvant chemotherapy, response grading was done with the majority of patients showing partial response (75.7%). Complete response was seen in 10.8%. 8.1% of patients showed stable response while 5.4% showed no response.

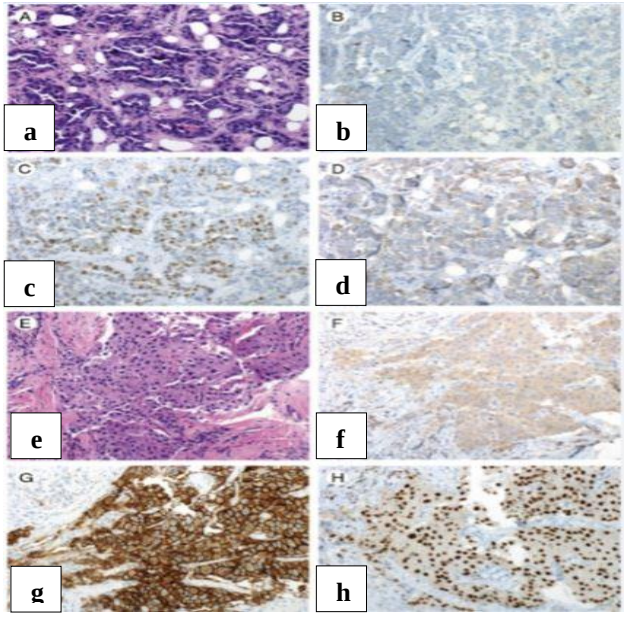

Figure 1: GATA-3 labelling in ER-negative primary breast carcinoma (a) a female patient presents with a new breast mass, (b) on core biopsy, the mass consists of irregular atypical glands without an in situ component, (c) the carcinoma is triple-negative for ER, progesterone receptor (PR), and HER2 (not shown), the carcinoma is positive for GATA-3, and (d) negative for TTF1, PAX8, and CDX2 (not shown). The immunoprofile and clinical presentation support classification as primary breast carcinoma; (e) and (f) a female patient presents with a new breast mass (e) on core biopsy, the mass consists of sheets of atypical cells with apocrine cytoplasm, the carcinoma is negative for ER (F) and PR (not shown) but is positive for HER2 with an IHC score of 3+, (g) and (h) the carcinoma is positive for GATA-3, hematoxylin and eosin, original magnification×100.

Table 1: Association of GATA-3 according to their age groups (N=40).

Age (years)	GATA-3			Total
	High N (%)	Low N (%)	Negative N (%)	
≤40	6 (25.0)	3 (27.3)	2 (40.0)	11 (27.5)
>40	18 (75)	8 (72.7)	3 (60)	29 (72.5)
Total	24 (100)	11 (100)	5 (100)	40 (100)

Table 2: The distribution of biological receptor in breast cancer patients (N=40).

Hormone receptor	Number	Positivity (%)
Estrogen receptor	24	60
Progesterone receptor	20	50
HER2 neu	25	62.5
GATA-3 (high+low)	35	87.5

c^2 value=0.467; p value=0.792

Table 3: Molecular subtypes of breast cancer and GATA-3 receptor status.

Luminal subtypes	GATA-3			Total n (%)
	High N (%)	Low N (%)	Negative N (%)	
A	12 (50)	1 (9.1)	2 (40)	15 (37.5)
B	5 (20.8)	3 (27.3)	1 (20)	9 (22.5)
HER2 neu	3 (12.5)	4 (36.4)	0 (0)	7 (17.5)
TNB	4 (16.7)	3 (27.3)	2 (40)	9 (22.5)
Total	24 (100)	11 (100)	5 (100)	40 (100)

c^2 value=8.140; p value=0.228

Table 4: Molecular subtypes of breast cancer and GATA-3 receptor status in patients, age correlated.

Luminal subtypes at age intervals (years)	GATA-3			Total n (%)
	High N (%)	Low N (%)	Negative N (%)	
≤40				
A	2 (33.3)	0 (0)	0 (0)	2 (18.2)
B	1 (16.7)	0 (0)	1 (50)	2 (18.2)
HER2 neu	2 (33.3)	1 (33.3)	0 (0)	3 (27.3)
TNB	1 (16.7)	2 (66.7)	1 (50)	4 (36.4)
Total	6 (100)	3 (100)	2 (100)	11 (100)
>40				
A	10 (55.6)	1 (12.5)	2 (6.7)	13 (44.8)
B	4 (22.2)	3 (37.5)	0 (0)	7 (24.1)
HER2 neu	1 (5.6)	3 (37.5)	0 (0)	4 (13.8)
TNB	3 (16.7)	1 (12.5)	1 (33.3)	5 (17.2)
Total	18 (100.0)	8 (100.0)	3 (100.0)	29 (100.0)

c^2 value=5.550; p value=0.481 for <40 years of age,c^2 value=9.107; p value=0.168 for >40 years of age

Table 5: Relation of overall stage of disease with GATA-3 receptor expression.

Stage	GATA-3			Total n (%)
	High N (%)	Low N (%)	Negative N (%)	
II	1 (4.2)	2 (18.2)	0 (0)	3 (7.5)
III	22 (91.7)	5 (45.5)	5 (100)	32 (80)
IV	1 (4.2)	4 (36.4)	0 (0)	5 (12.5)
Total	24 (100)	11 (100)	5 (100)	40 (100)

c^2 value=11.673; p value=0.020

Table 6: Response to neoadjuvant chemotherapy and its relation with GATA-3 receptor expression (n=37).

Response	GATA-3			Total n (%)
	High N (%)	Low N (%)	Negative N (%)	
Partial	18 (78.3)	5 (55.6)	5 (100)	28 (75.7)
Progressive	0 (0)	2 (22.2)	0 (0)	2 (5.4)
Stable	2 (8.7)	1 (11.1)	0 (0)	3 (8.1)
Complete	3 (13)	1 (11.1)	0 (0)	4 (10.8)
Total	23 (100)	9 (100)	5 (100)	37 (100)

c^2 value=8.278; p value=0.218

DISCUSSION

Present study results clearly showed that GATA-3 expression was a common feature of invasive breast cancer in the Indian female population. Among 40 interpretable cases with invasive ductal breast carcinoma in females, 87.5% were positive for GATA-3 expression. To the best of our knowledge, this study for the first time in published English literature reports the expression of GATA-3 in Indian breast cancer patients. Voduc et al analyzed 3119 cases of female breast cancer, determining that 31.4% were positive for GATA-3 by IHC.[17] 70.8% of estrogen receptor-positive breast cancer patients expressed high GATA-3 receptor expression and 4% of estrogen receptor-

positive breast cancer patients expressed low GATA-3 receptor expression as compared to only 43.8% of estrogen receptor-negative breast cancer patients who expressed high GATA-3 receptor expression and 43.8% of estrogen receptor-negative breast cancer patients expressed low GATA-3 receptor expression. This data when compared statistically was non-significant (p=0.155). In a study conducted by Gonzalez et al statistically, significant correlations were observed when comparing rates of GATA-3 positivity to rates of ER and PR positivity in female breast cancers.[18] In women, 93.3% of GATA-3 positive breast cancers were also ER-positive, whereas only 6.9% of GATA-3 negative breast cancers expressed ER (2/29; p<0.001).

In the present study, 65% of progesterone receptor-positive breast cancer patients expressed high GATA-3 receptor expression and 20% of progesterone receptor-positive breast cancer patients expressed low GATA-3 receptor expression as compared to 55% of progesterone receptor-negative breast cancer patients who expressed high GATA-3 receptor expression and 35% of progesterone receptor-negative breast cancer patients expressed low GATA-3 receptor expression. This data when compared statistically was non-significant (p=0.553). The 65% of HER2 neu receptor-positive breast cancer patients expressed high GATA-3 receptor expression and 32% of HER2 neu receptor-positive breast cancer patients expressed low GATA-3 receptor expression as compared to 60% of HER2 neu receptor-negative breast cancer patients expressed high GATA-3 receptor expression and 20% of HER2 neu receptor-negative breast cancer patients expressed low GATA-3 receptor expression. This data when compared statistically was non-significant (p=0.456).

A Ki-67 cut-off point of 15% was defined according to the experience of different pathologists as well as national and international recommendations at present.

63.2% of Ki67 receptor-positive breast cancer patients expressed high GATA-3 receptor expression and 23.7% of Ki67 receptor-positive breast cancer patients expressed low GATA-3 receptor expression as compared to none of the Ki67 receptor-negative breast cancer patients expressed high GATA-3 receptor expression and 100% of Ki67 receptor-negative breast cancer patients expressed low GATA-3 receptor expression. This data when compared statistically was non-significant (p=5.550).

GATA-3 receptor positivity when correlated with various molecular subtypes of breast cancer showed maximum positivity with Luminal A subtype (59.1%) followed by HER2 neu enriched subtype (48.9%). Triple-negative breast cancer patients showed 48% positivity for the GATA-3 receptor thus raising the hope for future hormonal targeted therapies in these subgroups of patients. Gulbahce et al, 8% had shown positivity for GATA-3 receptor in TNB cancer.

GATA-3 receptor positivity in these subgroups was different with 83.4% positivity in the TNB breast cancer subtype and 66.6% positivity in HER2 neu enriched subtype. In patients with advanced age percentage of GATA-3 receptor expression, positive tumors were 68.1% in luminal A subtype and 59.7% in luminal B subtype.

GATA-3 receptor expression significantly decreased as stage advanced (100% in stage II versus 84.37% in stage III) but a further increase from stage III to stage IV could not be explained due to the small sample size. Our data did not correlate with reported literature where expression of GATA-3 receptor is decreased with systemic spread of the disease. A large number of patients (67.5%) presented with positive nodal status. 60% of GATA-3 positive cases belonged to N1; 5% GATA-3 positive cases belonged to N2 and only 2.5% GATA-3 positive cases belonged to N3. GATA-3 receptor expression was more in low nodal status as compared to high nodal status however the difference was not statistically significant (p=6.366). Gonzalez et al reported only 15.5% of GATA-3-positive cancers demonstrated nodal disease compared to 28.6% GATA-3-negative cancers (p=0.102).

In the present study, for patients who were offered neoadjuvant chemotherapy, response grading was done using WHO clinical criteria with the majority of patients showing partial response (75.7%). All five patients with no GATA-3 expression showed partial response.

There is supporting data on the negative effect of GATA-3 positivity on tumour response to treatment in a neoadjuvant setting, as determined by lower complete pathologic response.

CONCLUSION

In conclusion, there is a raised possibility that GATA-3 or its downstream genes could be used in the management of luminal breast cancer. GATA-3 receptor expression was found to be more in the locally advanced stage of breast cancer as compared to early-stage with a statistically significant difference. There was a correlation in GATA-3 expression with existing clinicopathological, radiological and immunohistochemical markers of breast cancer as well as with clinical response in breast cancer patients receiving neoadjuvant chemotherapy, however the difference with a statistically insignificant difference.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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