Result

Patient and Clinicopathological Data: Thirty female cases of invasive breast carcinomas were included in the study. The age at diagnosis of the patients ranged from 24 to 84 years, with a median age of 50 years. Mean age was 51.3±11.38 years. 40% of patients were older than 51 years. Similar to the general patient population, most of the cases were infiltrating ductal carcinoma (96.6%). This cohort of primary invasive ductal carcinomas consisted of 9 luminal A carcinomas (30%), 10 luminal B carcinomas (33.4%), 6 HER2/neu enriched carcinomas (20%), and 5 triple negative carcinomas (16.6%). The important clinicopathological data is presented in Table 2.

GATA3 Immunostaining: Immunoexpression of GATA3 was observed in the nuclei of malignant cells, as well as in some benign luminal epithelial cells from adjacent normal ducts (serving as a positive internal control).

14 of the 30 cases (46.7%) scored positive for GATA3 expression in tumour cells including 63.2% of luminal subtypes, 16.7% of HER2/neu enriched carcinomas and 20% of triple negative carcinomas. Most positive cases (35.7%) showed 3+ staining. The intensity of staining was found to range from moderate to strong in the luminal A and luminal B subgroups, and weak to moderate/strong in the Her-2 and triple-negative subtypes. The distribution of GATA3 scores is displayed in Table 1.

Association with Clinicopathological Characteristics: GATA3 expression showed a significant correlation with luminal subtype of breast cancer (Pearson χ^2 test, P =0.017) as 63.2% of luminal breast cancers were GATA3 positive versus only 18.2% of the non-luminal subtype (Figure 1).

Of the ER positive cases (60% of the patient cohort), 66.7% were GATA3 positive. On the other hand, among the ER negative cases, only 16.7 % were GATA3 positive. A strong association between GATA3 protein expression and positive ER status was observed (Pearson χ^2 test, P =0.007). Altogether, 85.7% of GATA3 positive cases were also ER positive.

Similarly, 68.7% of PR positive cases showed GATA3 positivity, while only 46.7% of PR negative cases exhibited GATA3 positivity. PR status and GATA3 were found to have a significant association (Pearson χ^2 test, P =0.009).

60% of HER2/neu (score 0 and 1) negative cases (66.7%of the patient cohort) were GATA3 positive. In contrast, only 20% of HER2/neu (score 3) positive cases were GATA3 positive .This denotes statistically significant inverse association between GATA3 and HER2/neu (Pearson χ^2 test, P =0.038).

54.5% of cases with Ki-67 < 14% were GATA3 positive as compared to 42.1% of cases with Ki-67 ≥ 14%. However, this difference was statistically insignificant. (Pearson χ^2 test, P =0.51).

There was a strong association between GATA3 positive cases and grade 1 and 2 tumours (Pearson χ^2 test, P = 0.012). 100% of grade 1 tumours, 60% of grade 2 tumours and only 16.7% of grade 3 tumours exhibited GATA3 expression

The mean age in GATA3 positive cases was 48 years versus 54 years in GATA3 negative cases. But no significant association was found between GATA3 positivity and age at diagnosis (Mann-Whitney test, P = 0.224).

GATA3 expression was not significantly associated with laterality of breast (Pearson χ^2 test, P = 0.654), histological type (Pearson χ^2 test, P = 0.276), tumour size (Linear by Linear Association, P =0.639), lymph node status (Linear by Linear Association, P =0.453).

Correlation between clinicopathological variables with GATA3 expression are summarised in Table 2.

Table 1: Immunohistochemical labelling of GATA3 in cases of invasive breast carcinoma (n=30).

Tumor type	n	GATA3					Total Positive
		0	1+	2+	3+	4+	
Luminal A	9 (30%)	4	0	1	2	2	5/9 (55.56%)
Luminal B	10 (33.4%)	3	1	1	3	2	7/10 (70%)
Her 2 Neu enriched	6 (20%)	5	0	1	0	0	1/6 (16.66%)
Triple Negative	5 (16.6%)	4	0	1	0	0	1/5 (20%)
Total	**30**	**16**					**14/30 (46.67%)**

Table 2: Correlation between clinicopathological parameters and GATA3 expression.

Parameter	n(%)	GATA3+(%)	GATA3- (%)	p value
Age				
≤51	18(60)	10(55.6)	8(44.4)	0.231
>51	12(40)	4(33.3)	8(66.7)	

Parameter	n(%)	GATA3+(%)	GATA3- (%)	p value
Laterality of Breast				
Right Breast Cancer	12(40)	5(41.7)	7(38.3)	0.654
Left Breast Cancer	18(60)	9(50)	9(50)	
Histological Grade				
1	3(10)	3(100)	0(0)	**0.012**
2	15(50)	9(60)	6(40)	
3	12(40)	2(16.7)	10(83.3)	
ER				
Positive	18(60)	12(66.7)	6(33.3)	**0.007**
Negative	12(40)	2(16.7)	10(83.3)	
PR				
Positive	16(53.3)	11(68.7)	5(31.2)	**0.009**
Negative	14(46.7)	3(21.4)	11(78.6)	
HER2/neu				
Positive	10(33.3)	2(20)	8(80)	**0.038**
Negative	20(66.7)	12(60)	8(40)	
Ki 67				
<14%	11(36.7)	6(54.5)	5(45.5)	0.51
≥14%	19(63.3)	8(42.1)	11(57.9)	
Histological Type				
IDC	29(96.7)	13(44.8)	16(55.2)	0.276
Mucinous Ca	1(2.3)	1(100)	0	
T Category				
T1	0	0	0	0.639[a]
T2	4(13.3)	1(25)	3(75)	
T3	5(16.7)	1(20)	4(80)	
T4	1(3.3)	0	1(100)	
Not assessed	20(66.7)			
N Category				
N0	4(13.3)	1(25)	3(75)	0.453[a]
N1	3(10)	1(33.3)	2(66.7)	
N2	2(6.7)	0	2(100)	
N3	1(3.3)	0	1(100)	
Not assessed	20(66.7)			

ER: Estrogen Receptor, PR: Progesterone Receptor, Her2 neu: Human Epidermal Growth Factor Receptor. Pearson's $\chi 2$ test was used. P values <0.05 are shown in bold. [a] Linear by linear association

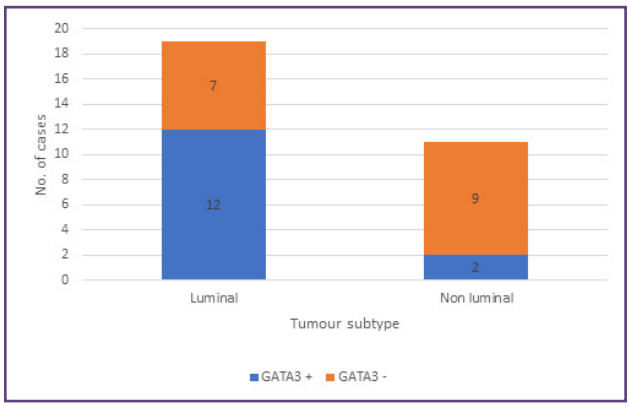

Fig. 1: GATA3 status according to tumour subtype among cases of invasive breast carcinoma (n=30).

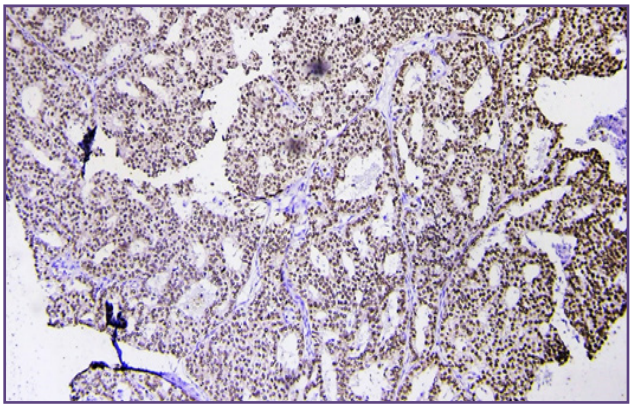

Fig. 2: GATA3 staining in invasive carcinoma breast (Nuclear;3+)(IHC;10X).

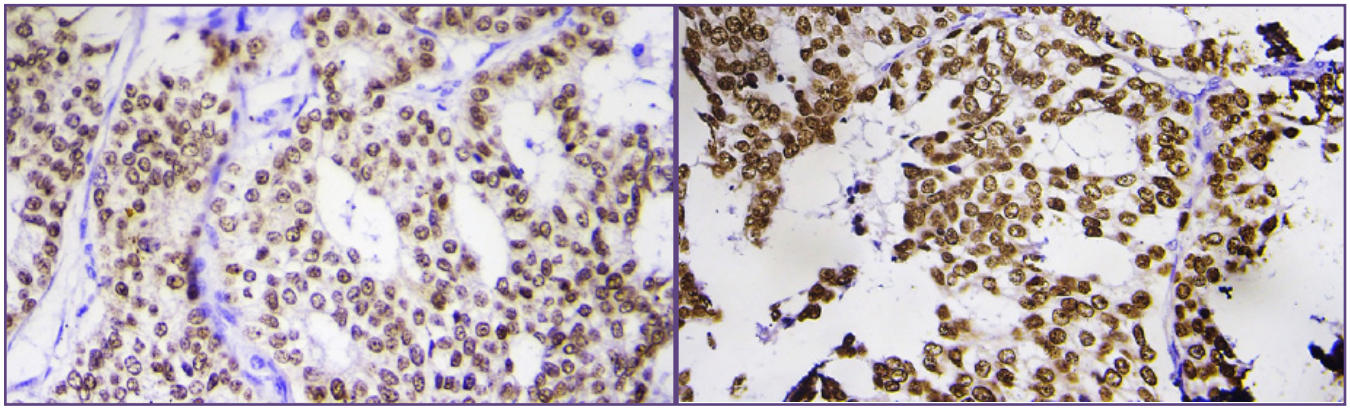

Fig. 3 A&B: GATA3 staining in invasive carcinoma breast (Nuclear;3+)(IHC;40X).

Discussion

GATA3, a highly conserved and essential transcription factor, plays an integral role in cell fate specification. It is an important regulator of the development and commitment of luminal epithelial cells in the mammary gland.[3] Since GATA3 promotes and specifies luminal cell identity in the mammary gland, it is involved in the pathogenesis of the luminal subtype in breast cancer too. GATA3 and ER proteins as components of the luminal transcriptional program may dictate the phenotype of hormonal-dependent breast cancer. [20]

The aim of this study was to determine whether expression of GATA3 correlated with pathological prognostic parameters in breast cancer. Previous works have highlighted the prognostic and predictive value of expression of GATA3 in breast carcinoma and shown using immunohistochemistry that it is closely correlated with ERα expression. [6-8,10,20] In the present study too, the expression of GATA3 was directly associated with ER, PR and the luminal subtype. But some researches suggest the relationship between GATA3 and the ER stimulated pathways is not direct and may be bridged by the forkhead family transcription factor, FOXA1. [3,20]

Despite the strong association between GATA3 and ER expression in breast carcinomas, we found that there was a group of ER positive tumours expressing low GATA3. This may indicate that GATA3 levels may have prognostic value in patients with ER positive tumours.

GATA3 expression was inversely correlated with HER2/neu overexpression [7,12] and histological grade [7,8,10-12,14,19] in our cohort as well as in others. Our data demonstrated that the highest GATA3 levels are seen in the well-differentiated/ER positive/luminal breast cancers and its expression is inversely correlated with histologic grade/tumour differentiation. This is in contrast to studies by McCleskey et al. [11] , Cimino-Mathews et al. [15] and Byrne et al. [16] , who observed significant GATA3 expression in non-luminal breast carcinomas (including triple negative) as well.

In our patient cohort, GATA3 immunopositivity was observed in 46.7% cases. The proportion of GATA3 expression varies widely among different studies. This discrepancy could be explained by geographic variability, sample size, choice of the antibody used, differences in

immunohistochemical protocols and different scoring methods. Therefore, multicentric studies are required to confirm GATA3 expression.

The current study found that common clinicopathologic parameters including patient age at diagnosis, laterality of breast, histological type, tumour size, nodal status were all statistically insignificant. Similar to our findings, Voduc et al. concluded no significant association with lymph node status. But they did find a linear association between age at diagnosis/tumour size and GATA3 expression although the actual difference between means was small. [12] Some studies did show a statistically significant association between tumour size [7,8,12], lymph node status [7] and even metastasis. [11,16,17] pTNM data of only 10 of the 30 cases included in this study was available, which may have contributed to the incongruity of our results with those of other studies.

Based on our results, we can consider GATA3 as a transcription factor that regulates the ER pathway which may be important to the luminal phenotype of breast cancer. This is an independent validation of many similar studies and opens up avenues to gauge the prognostic value of GATA3 by assessing relapse, disease free survival and response to therapeutic strategies.

Conclusion

Based on immunohistochemical evaluation of GATA3 in patients of invasive breast carcinoma, we found GATA3 to be a breast cancer marker almost exclusively expressed among ER positive and PR positive tumours. Similar to the ER, it is associated with favourable prognostic features like lower tumour grade and the absence of HER2 overexpression.

Presently, prediction of tumour progression and selection of individualised therapies based on molecular targets are the major obstacles in oncology. Nowadays, ER expression alone is being used to assess patient prognosis and guide hormone-based treatment in breast cancer. However, not all ER positive carcinomas react similarly to such therapies or show comparable prognosis. Therefore, there is a need to refine the molecular classification of tumours in order to better predict their clinical course and the patient's response to current therapies. GATA3 might be useful in this respect because, as stated earlier, GATA3 expression was found to be low in some ER positive cases.

The expression of GATA3 as an ER associated gene provides a chance to identify patients that will have a good prognosis. Although our results are promising, they need to be validated in relationship to outcome in a larger prospective cohort. But assessment of the value of GATA3 in the therapeutic response setting and in metastatic cases was beyond the scope of this study. Further investigation into GATA3 levels in multiple separate and independent patient populations will greatly strengthens its potential utility as a prognostic indicator and will be crucial to our understanding of breast cancer dissemination and recurrence early in the progression of breast cancer. It may be significant as predictor of pathological complete remission (pCR). This would help in better patient management and enable us to reliably assess the prognosis of patients diagnosed with breast cancer allowing accurate treatment modalities, efficient adjustment and monitoring of neoadjuvant/chemotherapy/hormonal therapy and decrease in treatment failure, risks of therapies, side effects and remission.

Nonetheless, the present study already offers some headway in the overview of breast cancer; GATA3 was significantly associated with ER positive tumours and low tumour grade. But this marker should not be used in isolation. Careful evaluation of the cytomorphology, clinical history, and radiologic findings, as well as the use of a panel of immunomarkers are important.

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Competing Interests

None

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