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The Relationship of GATA3 and Ki-67 With Histopathological Prognostic Parameters, Locoregional Recurrence and Disease-free Survival in Invasive Ductal Carcinoma of the Breast

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Abstract. *Background: In recent years, GATA-binding protein 3 (GATA3) has been indicated as a marker showing good prognosis in breast cancer. In luminal breast cancer, which has good a prognosis, it shows more significant elevation in small-sized and low-grade tumors. In contrast, Ki-67 is defined as a poor prognostic factor. The aim of this study was to emphasise the prognostic importance of GATA3 and the inverse relationship with Ki-67. Materials and Methods: In our study, 90 patients with invasive ductal breast cancer were immunohistochemically evaluated for Ki-67 and GATA3 expression. The relationship between GATA3 and Ki-67 expression was examined. In addition, the relationship between these two factors with estrogen, progesterone, human epidermal growth factor 2 receptor antibodies and other prognostic parameters such as disease-free survival and local recurrence was investigated. We accepted the level of ≥5% nüclear reaction as positive for GATA 3. A Ki-67 cut-off value of 20% was accepted as positive. Results: In GATA3 positive breast cancers, good prognostic parameters were seen including high estrogen receptor (ER) positivity, progesterone receptor (PR) positivity, small tumor size and low histological grade as well as low Ki-67 expression. In breast cancers showing high Ki-67 expression, ER, PR, and GATA3 positivity were lower and there was higher human epidermal growth factor receptor 2 (HER2) positivity and high histological grade while the tumor size was larger. Conclusion: Our study has revealed that GATA3 has an inverse relationship with Ki-67, whereas it has a positive releationship with good prognostic factors.*

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Breast cancer remains the cancer most affecting women throughout the world**.** Despite it increases with age, the risk of breast cancer development in women is 12% and the risk of disease-related death is up to 5% (1).

Prognostic factors predicting recurrence of breast cancer and mortality and affecting treatment are primarily axillary lymph node involvement and number, tumor size, grade, morphology and stage, the presence of lymphovascular invasion (LVI) and perineural invasion (PNI), tumor subtype, and the status of estrogen receptor (ER) and progesterone receptor (PR) (2). Over-expression of human epidermal growth factor receptor 2 (HER2) is a routine part of diagnosis and shows a negative prognosis (3). Other than the known prognostic factors, new factors affecting prognosis have also been identified. Several factors such as GATA-binding protein 3 (GATA3) and pS2 protein regulate ER expression (4). GATA3, which is a proto-oncogene group nuclear transcription factor, has started to be considered as a marker. There are 6 sub-groups, known as GATA 1-6. GATA 1-2-3 are especially connected to hematopoietic cells, whereas GATA 4-5-6 play a key role in the mesoderm, endoderm, lungs and kidneys (5). GATA3 is also necessary for the differentiation of luminal cells in the breast glands, and a deficiency is associated with estrogen and progesterone receptor negativity, HER2/neu over-expression and poor

prognosis. Breast cancer has been reported to increase GATA3 expression in hyperplastic tissue and *in situ* lesions, compared to normal breast tissue. GATA3 expression decreases as tumor grade increases while low nuclear GATA3 immunoexpression is a poor prognostic factor for invasive breast cancer (5-6).

In a meta-analysis by Guo *et al*., a high GATA3 level was found to be related to ER and PR positivity, nuclear grade and tumor size, but not related to lymph node metastasis. According to that study, high GATA3 expression can be related to a better clinicopathological course (5). In another study, low GATA3 expression was strongly correlated with a high grade, ER and PR negativity while HER2 over-expression and a poor prognosis and was also determined to be associated with lymph node metastasis (7).

Ki-67 is a generally accepted poor prognostic marker in breast cancer. Ki-67 was first identified as a nuclear non-histone protein by Gerdes *et al*. in 1991 (8). Expressed by all proliferating cells, Ki-67 is a nuclear antigen which is increased by mitosis throughout the G1 phase, reaches a maximum level in the G2 phase, and falls rapidly following mitosis (9). In the active phase of the cell cycle, it is expressed during cell repair or with the initiation of the cell cycle. Ki-67 is an antibody which can show proliferation in cells. In patients with axillary lymph node positive breast cancer, tumors showing high Ki-67 expression show more aggressive clinical progression than tumors showing low Ki-67 expression, have a worse prognosis, and are more metastasic (10). Ki-67 is one of the most frequently used markers for the evaluation of the proliferative index in breast cancer cells. High Ki-67 levels are associated with a higher risk of recurrence and shorter survival in both patients with and without lymph node involvement (11).

The aim of this study was to evaluate the correlation between GATA3 and Ki-67, and the relationship between these factors and other receptors (ER, PR, HER2). The relationship was also investigated between other clinicopathological factors and local recurrence, disease-free survival (DFS) metastasis and disease-related mortality.

Materials and Methods

H&E analysis and immunohistochemistry. One hundred and forty-seven patients were operated at General Surgery Clinic of the Health Sciences University Turkey, Gaziosmanpaşa Training and Research Hospital between 2008 and 2015. 27 of them had non-invasive ductal cancer subtypes. 23 patients had neo-adjuvant chemotheraphy and seven patients could not be reached. The remaining 90 patients with a diagnosis of invasive ductal cancer were included in the study. The demographic, clinical and pathological data of the patients were retrospectively reviewed from the patient files.

Breast resection material slices stained with hematoxylene-eosin (H&E) were examined by a single pathologist in respect of histopathological diagnosis, histological grade, lymphovascular invasion (LVI), perineural invasion (PNI), ductal carcinoma *in situ* (DCIS), lymphocytic response, lymph node metastasis and surgical margins. The Bloom-Richardson system Nottingham modification was used in the determination of histological grade. Tumor grade was evaluated according to the 2017 AJCC cancer grading guideline (8th edition) and the 2019 CAP guideline (12, 13). For each patient, immunohistochemical GATA3 antibody was applied by selecting the best block containing the invasive tumor and normal breast tissue. Estrogen receptor antibody, progesterone receptor antibody, HER2 and Ki-67 were evaluated according to the CAP guidelines.

In the ER and PR scoring, <1% was accepted as nuclear reaction negative and >1% as nuclear reaction positive. For the estrogen and progesterone antibodies, benign ductal epithelial cells in the breast tissue were evaluated as positive controls.

In the HER2 scoring, 0 was accepted as no reaction in tumor cells or incomplete reaction in ≤10% of tumor cells, 1 as paleness in >10% of the tumor cells and incomplete membranous reaction that was difficult to differentiate, 2 as incomplete weak or moderate level membranous reaction in >10% of the tumor cells or a complete strong mebranous reaction in ≤10% of the tumor cells, and 3 as a uniform strong membranous reaction in >10% of the tumor cells. Scores 0 and 1 were accepted as negative, Score 2 as suspected positive, and Score 3 as a positive reaction. Patients with Score 2 were then evaluated according to fluorescent *in situ* hybridisation (FISH), and were included in the negative or positive group according to the results. For HER 2, ductal carcinoma *in situ* was used as the positive control.

For Ki-67 scoring, a cut-off value of 20% was used in the evaluation, as revised in the St Gallen international expert consensus (14). For GATA3 antibody evaluation, <5% was classified as a negative nuclear reaction, ≥5% as a positive nuclear reaction. For GATA3 antibody, benign ductal epithelial cells in the breast tissue were evaluated as positive controls (15).

Clinicopathological definitions of breast cancer subtypes were made as follows (14): *Luminal A like:* ER positive, PR positive (>20%), Ki-67 low. *Luminal B like:* ER positive, PR low (<20%), or ER positive, Her2 neu positive (3+on IHC/amplified on FISH), any PR. Ki-67 value or low PR may be used to distinguish between Luminal A like and Luminal B like. *Her2neu positive (non-luminal):* ER and PR negative, Her2neu positive (3+on IHC or amplified on FISH (for 2+IHC results). *Triple negative (TNBC):* ER, PR and Her2neu negative.

Statistical analysis. Data obtained in the study were analysed statistically using SPSS 15 software. Differences in frequency of prognostic variables in the GATA3 and Ki-67 groups were compared using the Chi-square or Fisher tests. The relationship between GATA3 positivity and Ki-67 negativity was examined with Pearson correlation analysis. The effects of GATA3 and Ki-67 on survival were examined using the Log Rank test. Rates of recurrence-free survival (RFS), distant metastasis-free survival (DMFS), DFS, and mean overall survival (OS) were calculated using Kaplan-Meier survival analysis. A value of $p<0.05$ was accepted as statistically significant.

Multivariate Cox regression analysis was used for the variables whose p was smaller than 0.25 in univariate regression analysis in evaluation of variables including death and recurrence outcome.

Ethical approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable

ethical standards. Approval for this study was granted from Gaziosmanpasa Training and Research Hospital Ethics Committee for Clinical Studies in July 2018 (reg:59). Informed consent was not required due to the retrospective use of de-identifed administrative data.

Results

The mean age of patients was 61.9 years (range=36-90 years). Breast conserving surgery (BCS) was applied to 40 (44.4%) patients and modified radical mastectomy (MRM) was applied to 50 (55.6%). We applied radiotherapy (RT) to the 35 (87%) patients with BCS and 33 (66%) patients with MRM. The number of patients with BCS who didn't apply RT is 5, these patients refused to complete RT after surgery. The 24 (60%) of patients with BCS and 29 (58%) of patients with MRM have been given adjuvant chemotherapy. The patients have been given doxorubicin plus cyclophosphamide followed by paclitaxel treatment as chemotherapy. Additionally the patients with HER 2 positivity have been applied trastuzumab treatment for 1 year. The treatment continued with hormonotherapy (tamoxifen in most cases) in 43 (86%) patients with MRM and 32 (80%) patients with BCS. Both of the 2 groups were ER positive. RT has been applied as a total dose of 45-50 Gy during 4.5-5 weeks. The patients given both RT and chemotherapy spread evenly among the groups with both GATA3 positive and negative and Ki-67 with low and high expressions. The mean follow-up period was 82.3 months (range=20-135 months). Subtypes were defined as luminal A in 55 (58.9%) patients, luminal B in 24 (26.7%), HER2 enriched type in 6 (6.7%) and TNBC in 7 (7.8%). Tumor size ranged from 0.3 cm to 6.5 cm, with tumor size <2 cm in 52 (57.8%) patients and >2 cm in 38 (42.2%). Histological grade 1 was determined in 8 (8.9%) patients, grade 2 in 59 (65.6%) and grade 3 in 23 (25.6%). Perineural invasion was determined in 22 (24.4%) patients. Tumor stage 1 was determined in 40 (44.4%) patients, stage 2 in 38 (42.2%), stage 3 in 11 (12.2%) and stage 4 in 1 (1.11%). During the follow-up period, local recurrence (LR) developed in 3 (3.3%) patients in the first 5 years. Distant metastasis (DM) developed in 12 (13.3%) patients. Disease-related mortality developed in the first 5 years in 10 (11.1%) patients. Disease-free survival (DFS) was recorded in a total of 73 (81.1%) patients. Other clinicopathological variables are shown in Table I.

In the evaluation of the findings, ER positivity, PR positivity, and small tumor size were seen more frequently in GATA3 positive patients ($p<0.05$). High GATA3 expression was more frequent in grade 1 and 2 patients than in grade 3 patients ($p<0.001$). Necrosis was seen less in tumors with high GATA3 expression ($p=0.002$). These differences were statistically significant. GATA3 positive tumors were mostly luminal A molecular subtype ($p=0.011$). A weak negative correlation was determined between GATA3 positivity and high Ki-67 expression ($p=0.036$). No statistically significant difference was determined in GATA3 positivity in respect of lymph node metastasis.

Table I. *Clinicopathological variables*.

	Positive N (%)	Negative N(%)
Nodal status	37 (41.1)	53 (58.9)
ER status	75 (83.3)	15 (16.7)
PR status	61 (67.8)	29 (32.2)
HER 2 status	25 (27.8)	65 (72.2)
Ki-67	22 (24.4)	68 (75.6)
GATA3	72 (80)	18 (20)
LVI	40 (44.4)	50 (55.6)
PNI	22 (24.4)	68 (75.6)
Necrosis	19 (21.1)	71 (78.8)
DCIS status	59 (65.6)	31 (34.4)

ER: Estrogen receptor; PR: progesterone receptor; HER 2: human epidermal growth factor receptor 2; GATA3: GATA-binding protein 3; LVI: lymphovascular invasion; PNI: perineural invasion; DCIS: ductal carcinoma *in situ*.

In high Ki-67 expression, ER, PR and GATA3 positivity were seen at statistically significantly lower rates than in those with low Ki-67 expression ($p<0.05$) (Figures 1, 2). Tumor size was seen to be larger in high Ki-67 expression ($p<0.001$). Patients with high Ki-67 expression were mostly luminal B subtype ($p<0.001$). In Grade 3 patients, statistically significantly higher Ki-67 expression was determined ($p=0.22$). Tumor necrosis was seen statistically significantly more frequent in those with high Ki-67 expression ($p=0.003$). A lymphocytic response was found to be statistically signifcantly higher in patients with high Ki-67 expression. Lymph node metastasis was seen more frequently in patients with high Ki-67 expression but not at a statistically significant level.

According to the univariate cox regresyon analysis, the relationships of GATA3 and Ki-67 with histopathological prognostic parameters, locoregional recurrence and disease-free survival are shown in Table II.

In the Cox multivariate regression analysis for recurrence, hazard ratio (HR) was found 11.7 [95% CI=0.9-139.2, $p=0.05$)] for Ki-67, HR was found 16.7 [95% CI=1.37-203.9, $p=0.03$] for perineural invasion. In this model, Ki-67 and Perineural invasion were independent predictors for recurrence.

In the Cox multivariate regression analysis, parameters of age, metastasis, GATA3, Ki67 and lymphocytic response were found significant for the variable of death outcome. Although ER and PR were significant in univariate analysis, there was no significant effect in multivariate analysis (Table III).

In the 5-year survival analysis, DFS and OS were seen to be proportionally better in the GATA3 positive group, but

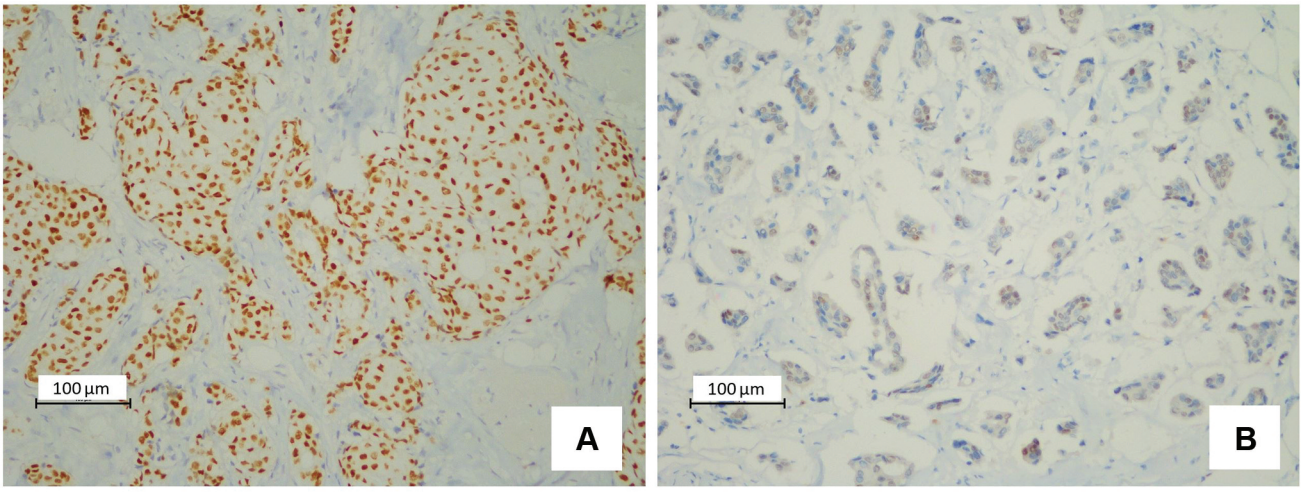

Figure 1. *A Positive nuclear reaction was observed with GATA3 in invasive ductal carcinoma areas (≥5) with ımmunohystochemical staining and original magnification ×200 (IHC, ×200), B. <5 nuclear reaction with GATA3 antibody was observed in invasive ductal carcinoma areas (IHC, ×200).*

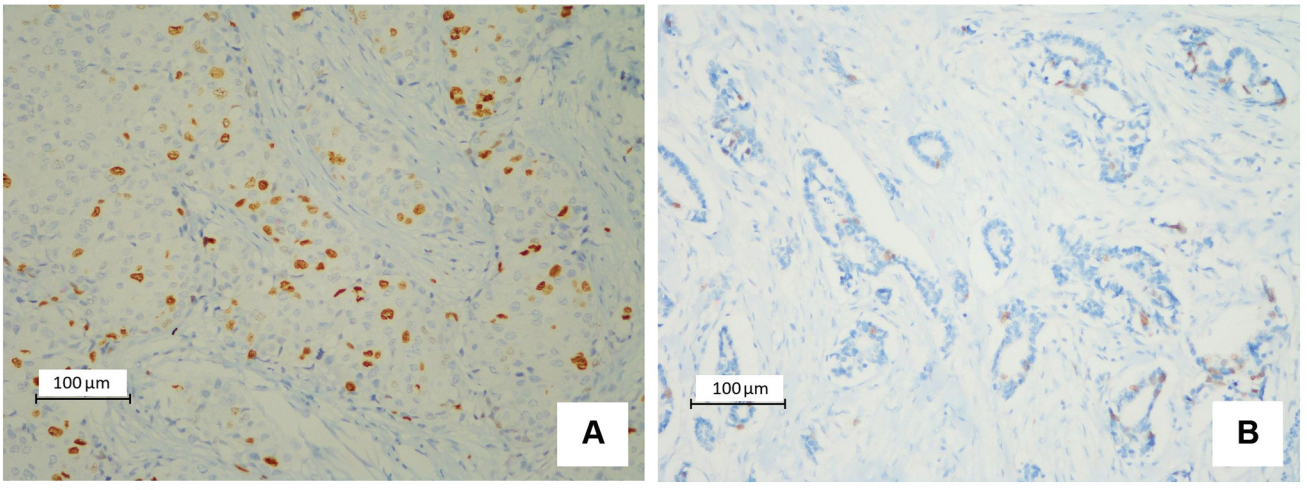

Figure 2. *In the invasive ductal carcinoma areas, a low proliferation index was observed with Ki-67 with ımmunohystochemical staining and original magnification ×200 (IHC, ×200), B. In the invasive ductal carcinoma areas, a high proliferation index was observed with Ki-67 (IHC, ×200).*

no statistically significant difference was determined in respect of DMFS, RFS, DFS and OS. In the Ki-67 positive group, DFS and OS were statistically significantly lower *(p<0.05)*. In those with high Ki-67 expression, DMFS and RFS rates were worse, but not at a statistically significant level (Table IV).

Discussion

In the current study an evaluation was made on the relationship between GATA3 and Ki-67 and the association between this and other prognostic parameters. Ki-67 is a marker of poor prognosis and that has been emphasised in literature with many examples. However, the aim of the current study was to highlight the negative relationship of GATA3 and Ki-67 with other prognostic parameters, breast cancer subtypes and with each other.

GATA3 is associated with a less aggressive phenotype in breast cancer patients and with a better prognosis. Kouros-Mehr *et al*. stated that GATA3 was a strong, independent predictor of the clinical result in human luminal breast cancer (16). In a 2008 study of rats, the results showed that high GATA3 expression in breast cancer cells caused differentiation and thereby supressed tumor spread. That

Table II. *The relationship of GATA 3 and Ki-67 with prognostic variables.*

N (%)	GATA 3+	GATA 3–	*p*-Value (X^2)	Ki-67+	Ki-67–	*p*-Value (X^2)
Age (years) (SD)	61.5 (11.7)	63.8 (15.7)	NA	63.5 (14.8)	61.4 (11.8)	NA
ER positive (%)	66 (91.7)	9 (50)	<0.0001	15 (68.2)	60 (88.2)	0.045
PR positive (%)	55 (76.4)	6 (33.3)	<0.001	9 (40.9)	52 (76.5)	0.002
HER2 Negative (%)	53 (73.6)	12 (66.7)	0.556	10 (45.5)	55 (80.9)	0.001
Ki-67 Negative (%)	58 (80.6)	10 (55.6)	0.036	–	–	
GATA 3 Positivity (%)	–	–		14 (63.6)	58 (85.3)	0.036
Tumor size (SD)	2.1 (1.18)	3.25 (1.96)	<0.001	3.25 (1.52)	2.04 (1.29)	<0.001
Molecular subtype (%)						
Luminal A	46 (63.9)	7 (38.9)	0.011	0 (0)	53 (77.9)	<0.001
Luminal B	20 (27.8)	4 (22.2)		16 (72.7)	8 (11.8)	
HER2 enriched type	3 (4.2)	3 (16.7)		3 (13.6)	3 (4.4)	
Triple negative	3 (4.2)	4 (22.2)		3 (13.6)	4 (5.9)	
Histological grade (%)						
Good	8 (11.1)	0 (0)	<0.001	0 (0)	8 (11.8)	0.022
Moderate	53 (73.6)	6 (33.3)		12 (54.5)	47 (69.1)	
Poor	11 (15.3)	12 (66.6)		10 (45.5)	13 (19.1)	
Lymphovascular invasion negative (%)	31 (43.1)	9 (50)	0.596	7 (31.8)	33 (48.5)	0.17
Perineural invasion present (%)	21 (29.2)	1 (5.6)	0.06	3 (13.6)	19 (27.9)	0.175
Necrosis + (%)	10 (13.9)	9 (50)	0.002	10 (45.5)	9 (13.2)	0.003
Lymph node metastasis + (%)	30 (41.7)	7 (38.9)	0.83	12 (54.5)	25 (36.8)	0.141
Lymphocytic response + (%)	30 (41.7)	10 (55.6)	0.289	16 (72.7)	24 (35.3)	0.002
DCIS positivity (%)	49 (68.1)	10 (55.6)	0.32	13 (59.1)	46 (67.6)	0.463
Local recurrence (%)	3 (4.2)	0 (0)	1	2 (9.1)	1 (1.5)	0.147
Metastasis (%)	11 (15.3)	1 (5.6)	0.447	4 (18.2)	8 (11.8)	0.478
Mortality (%)	6 (8.3)	4 (22.2)	0.108	6 (27.3)	4 (5.9)	0.012
Event-free survival (%)	59 (82)	14 (77.8)	0.739	15 (68.2)	58 (85.3)	0.114

N: Number of patients; SD: standard deviation; ER: estrogen receptor; PR: progesterone receptor; HER 2: human epidermal growth factor receptor 2; GATA3: GATA-binding protein.

study showed that GATA3 in experimentally-induced breast cancer directly regulated luminal cell differentiation (16). In a meta-analysis of 10 studies including 5080 patients by Gou *et al*., high GATA3 expression was seen to be related to time to tumor progression (TTP) in breast cancer (5).

Four studies in the same meta-analysis included a comparison of ER, PR and HER2 expression. While high GATA3 expression was seen to be related to ER and PR positive expression, there was no relationship with HER2. In another 4 studies included in the meta-analysis, nuclear grade and GATA3 expression were compared and GATA3 positivity was found to be associated with a low nuclear grade. There were 3 studies in the meta-analysis that included tumor size (<2 cm *vs*. >2 cm) and lymph node metastasis. This meta-analysis stated that GATA3 was a critical biomarker for the prediction of recovery in breast cancer patients (5). In a study by Yu *et al*., GATA3 expression was found to be lower in basal subtypes and significantly higher in the luminal A subtype and in cases of ER positivity, a higher level of GATA3 was seen. When tumor grading was examined, relatively lower expression was seen in grade 3 tumors compared to grade 2 and grade 1. No significant difference was determined between clinical grades in respect of GATA3 expression (17). In another study, a relationship was found between GATA3 expression and a low histological grade, ER positivity, PR positivity, HER2 negativity and a low Ki-67 index (18). Ni *et al*. showed variable GATA3 expression in molecular subtypes.

Table III. *Multivariate cox regression analysis for the variable of death outcome.*

Variable	Hazard ratio (HR)	95% Confidence interval	*p*-Value
Age	1.124	1.05-1.21	0.002
Metastasis	36.8	3.76-360.04	0.002
ER	1.129	0.112-11.4	0.92
PR	0.65	0.093-4.51	0.66
GATA3	0.061	0.005-0.695	0.024
Ki-67	15.83	1.68-148.9	0.016
Lymphocyctic response	7.03	1.01-48.92	0.05

ER: Estrogen receptor; PR: progesterone receptor; GATA3: GATA-binding protein 3.

Table IV. *Five-year survival statistics.*

N (%)	GATA3+ (%)	GATA3– (%)	*p*-Value	Ki67+ (%)	Ki67– (%)	*p*-Value
Recurrence-free survival (RFS)	95.8	100	0.38	90.9	98.5	0.09
Distant metastasis-free survival (DMFS)	84.7	94.4	0.233	81.8	88.2	0.49
Disease-free survival (DFS)	81.9	77.8	0.68	68.2	85.3	0.05
Overall survival (OS)	91.7	77.8	0.09	72.7	94.1	0.002

GATA3: GATA-binding protein 3.

Expression was determined to be high in luminal subtypes and low in non-luminal types (19).

In the current study a significant negative relationship was determined between high GATA3 expression and high Ki-67 expression. A statistically significant relationship was also found between high GATA3 expression and ER positivity, PR positivity, small tumor size, and low histological grade. Tumors with high GATA3 expression were statistically significantly more often luminal A subtype. In patients with high GATA3 expression, even if HER2 positivity was lower, the difference was not statistically significant. No significant correlation was found between lymph node metastasis and GATA3 expression. Tumor necrosis was observed less in patients with high GATA3 expression. The study findings were consistent with previous findings in literature and support the view that GATA3 is a good prognostic marker.

A study published in 2019 reported a negative correlation between high GATA3 expression, ER and PR expression, and positive nuclear grade and Ki-67 index. A small number of HER2 enriched and triple negative tumors showed high GATA3 expression (17). In the current study, GATA3 positivity was found to be low in the TNBC and HER2 enriched subgroups and when there was GATA3 positivity in these groups, the positivity level was determined to be very low. In the same study it was stated that GATA3 positive tumors were less aggressive than tumors with low expression. GATA3 expression was not found to be related to lymph node metastasis. High GATA3 expression was determined to be associated with a significantly better prognosis compared to low GATA3 expression and high GATA3 expression was also related to significantly longer DFS (20). The OS and DFS have been determined better in the GATA3 positive group although it is not statistically significant. A meaningful relationship hasn't been found between the metastasis and local recurrence, but the RFS and DMSF was lower in GATA3 positive group. The level of GATA3 positivity was lower in the patients with metastasis and recurrence. The level of Ki-67 was higher in these patients; also these patients were at a more advanced stage on presentation and chemotherapy had been refused by half of the patients with metastasis and by 2 of the 3 patients with local recurrence. The inclusion of patients with different stages of breast cancer in the study made comparisons more difficult.

As high Ki-67 expression is associated with a higher tumor grade, it is a valuable biomarker of breast cancer. In a study by Hashmi *et al.* (21), it was reported that TNBC showed the highest Ki-67 index, followed by HER2 positive and luminal B subtypes. When compared with ductal carcinoma, metaplastic and medullary breast cancer showed significantly higher Ki-67 index values. No significant relationship has been recorded between the Ki-67 index and any of the expected histological parameters for tumor grade in different subtypes of breast cancer (21). In the current study, Ki-67 was seen more in luminal B subtype, and the highest values were reached in the TNBC subgroup.

The reason that high Ki-67 expression was seen more in luminal B subtype could be that there were fewer patients in the TNBC and HER2 positive subgroups. In the majority of studies, a relationship has been determined between Ki-67 and histological grade, lymph node status, patient age, tumor diameter, hormone receptor status, ploidy and p53 status (22-24). In the current study, high Ki-67 expression was found to be lower in ER and PR positivity and greater in HER2 positivity and lymph node metastasis. In patients with high Ki-67 expression, tumor size was found to be large, and histological grade, tumor necrosis and lymphocyte response were higher. In another study, a correlation was shown between Ki-67 and tumor grade and diameter in lymph node negative patients, and between Ki-67 and tumor grade, hormone receptor status and HER2 expression in lymph node positive patients (25). The disease-free survival and overall survival rates have also been shown to be worse in patients with higher Ki-67 expression (25).

In the group with high Ki-67 expression in the current study, DFS and OS were seen to be low and the difference was determined to be statistically significant. In addition, the DMFS and RFS rates were worse in this group, but not at a statistically significant level. In another study, there was found to be a significant negative correlation between Ki-67 levels and ER and PR, and the Ki-67 values were seen to be directly proportional to the tumor grade and HER2/neu status. No significant relationship was found between Ki-67 and tumor size and the nodal status (26). Viale *et al.*,

reported that Ki-67 expression was directly correlated to tumor grade and the presence of PNI and there was a negative correlation with ER and PR receptor expression. High Ki-67 expression was identified especially in patients with poorly differentiated tumors. Ki-67 was found to be significantly related to DFS (27).

Although Ki-67 is a cheap and can be easily and routinely used method, the available guidelines of the American Society of Clinical Oncology (ASCO) do not include it in the biological marker list because of the problems in standartisation (28). One of the probems in the standartisation is the inconsistency of the evaluation of antigen, staining procedures and the cell count studies. The other problem is the discussion about the cut off level of Ki-67(29). But Ki-67 in most of the studies has been accepted as a prognostic marker; it is used to discriminate the subtypes of luminal A and luminal B. The high expression of Ki-67 has a relation with the high recurrence rate and bed survival in breast cancer patients (14).

In our study, to be able to minimise this standartisation differences both preoperative biopsy and postoperative surgical specimen was examined by the same pathologist and the cut off level was accepted as 20%. Also, for Ki-67 proliferation score, the nuclear staining has been determined by counting at least 500 malignant cells as in the similar studies (30). At result, the prognostic markers were worse in the patients with the high Ki-67 expressions as expected.

When we look at the treatment strategies of the chemotherapy protocols in the Ki-67 and GATA 3 positive patients, additional drug advice hasn't been found in the literature; but we saw that the prognosis was better after the systemic treatment in the luminal breast cancer patients with low Ki-67 expression (31).

The high level of Ki-67 expression has been associated with a good response to neoadjuvant chemotherapy. But also the studies didn't Show us that the Ki-67 is an independent predictors of pCR (28). Furthermore, in the other studies Ki-6 7 alone has not been shown to predict the benefit of adjuvant cyclophosphamide, methotrexate and fluorouracil (CMF) chemotherapy to adjuvant endocrine therapy in the patients with the negative lymph nodes, but this has not been supported by the results (32).

In another study it has been defended that Ki-67 expression in ER positive breast cancer patients may be a predictive markers for the effectiveness of the adjuvant docetaxel (33).

In a metaanalysis of 35 studies including the TNBC patients, all the patients has been given system treatment in addition to surgery. In this study independent of surgical treatment, it has been determined that in the patients with Ki-67 expression ≥40%, DFS and OS were lower (34). The studies on the GATA3 showed that there is a bad response to neoadjuvant chemotherapy in the patients with GATA3 positivity (35). Nevertheless, there isn't much more study about this topic. Limitations of this study were the retrospective design and the relatively low number of patients

In conclusion, the results of the study showed that there was a negative correlation between GATA3 expression and Ki-67. While a significant relationship between Ki-67 and poor prognostic parameters was identified, GATA3 was observed to be related to ER positivity, PR positivity, small tumor size, and low histological grade. The mortality rate was seen to be lower in high GATA3 expression and higher in high Ki-67 expression. No relationship was determined between high GATA3 expression and lymph node metastasis. In patients with high Ki-67 expression, lymph node metastasis was identified more frequently but not to a statistically significant level.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

KOE performed the literature search, database set up and contributed to the writing of the manuscript. SB reviewed the manuscript. OG, DF contributed to the study design and reviewed the manuscript. EY, SA, MY contributed to the study design, the writing of the manuscript and reviewed the manuscript. All authors are in agreement will all aspects of the final manuscript.

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