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Predicting response to neoadjuvant therapy with glucose transporter-1 in breast cancer

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SUMMARY

OBJECTIVE: Glucose transporter-1 is a marker involved in energy transport in cancer cells. It has been shown to be a poor prognostic factor in many cancer types, including breast cancer. However, there is no satisfactory parameter predicting treatment in breast cancer patients receiving neoadjuvant therapy. This study investigated the effect of glucose transporter-1 in predicting the treatment response of patients receiving neoadjuvant therapy.

METHODS: In this study, glucose transporter-1 immunohistochemistry was applied to tru-cut biopsy of patients who were diagnosed with breast cancer and received neoadjuvant therapy between 2010 and 2021. A built-in scoring system was used to evaluate both the pattern and intensity of glucose transporter-1 immunohistochemistry staining. The relationship between glucose transporter-1 immunohistochemistry staining and other clinicopathological parameters was examined. In addition, the relationship of glucose transporter-1 with response to treatment was investigated.

RESULTS: A relationship was found between high glucose transporter-1 expression and other clinicopathological parameters (such as estrogen and progesterone receptor negativity, high Ki-67, triple-negative, and Her2 status). Cases with high glucose transporter-1 expression had either a complete or a partial pathologic response. The result was statistically significant.

CONCLUSION: Glucose transporter-1 has the potential to be a biomarker that can be evaluated more objectively as an alternative to Ki-67 labeling index in evaluating the response to treatment in patients receiving neoadjuvant therapy.

KEYWORDS: Glucose transporter type 1. Breast. Cancer. Immunohistochemistry. Neoadjuvant therapy.

INTRODUCTION

Breast cancer (BC) is the most common tumor worldwide with a high mortality rate among women. Some parameters, such as tumor stage, molecular subtyping, and hormone receptor status, are used in the selection of treatment and in predicting the prognosis[1]. Molecular subtyping is the most important parameter that predicts the response to neoadjuvant therapy (NT)[2]. Molecular subtyping alone is insufficient to predict treatment. However, more parameters are needed. Therefore, it is important to investigate different biomarkers that will shed light on new agents in predicting the prognosis, response of patients, and even in choosing treatment method.

Glucose transporters are membrane transporter proteins that catalyze the facilitative bidirectional transfer of their substrates across membranes[3]. Glucose transporter-1 (Glut-1) is the first identified member of the glucose transporter family as well as the most common of all membrane transport proteins[4]. It is highly expressed in the endothelium of tissues where selective glucose transfer from blood to tissues is important, such as the central nervous system, retina, iris, ciliary muscle, and endoneurium. Moreover, Glut-1 is also expressed in erythrocytes physiologically[5], and pathologically, it mediates basal glucose transport in cancer cells, which require considerably higher energy levels than normal cells, and provides glucose for energy metabolism[6]. Various studies have also investigated whether insulin resistance, which regulates glucose metabolism in the body, is a risk factor in BCs. Some of these studies have defined a high risk of BC in obese and diabetic patients. However, the mechanisms are not clear[7]. As a result, Glut-1 has been found to be overexpressed in various types of cancer, including prostate, stomach, lung, and BC; squamous cell carcinoma of the head and neck[8-11]; and its overexpression is a poor

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prognostic parameter[12,13]. Therefore, it has been thought that tumor progression can be prevented via Glut-1 mechanism.

In the present study, we aimed to investigate the potential use of Glut-1 antibody in tru-cut biopsy (TCB) as a new biomarker to predict the response and prognosis before NT. In addition, we studied the relationship between Glut-1 expression and clinicopathological parameters, such as hormone receptor status and Ki-67 labeling index (LI).

METHODS

Study design and case selection

In our retrospectively planned study, patients with a diagnosis of breast carcinoma and received NT between 2010 and 2021 were retrieved from the hospital electronic system.

Patient data

The age, details of NT protocol, the status of recurrence or distant metastasis, and survival status were retrieved from the hospital and national electronic database. Tumor size, status of hormone receptor and Her2 expression, Ki-67 LI, and the presence of lymphovascular and perineural invasion were obtained from pathological reports.

Histopathological and immunohistochemical staining

Hematoxylin and eosin-stained slides of both TCB and resection were retrieved from the pathology archive. Cases that did not have tumor slides or clinical data were excluded. H&E and immunohistochemical slides were re-evaluated by three different pathologists (SDÖ, ÇÖ, and GA). All cases were classified according to their molecular and histological subtypes according to the World Health Organization classification[14-16]. The cutoff value for Ki-67 LI was accepted as 14%.

The best representative tumor block was selected from both TCB and resections, and 4-µm sections were obtained. The Ventana Medical Systems (SN: 714592, Ref: 750-700 Arizona, USA) automated immunohistochemistry device was used. Immunohistochemical staining was performed using the Ultra-view Universal DAB Detection Kit (REF: 760-500, Ventana) and Glut-1 antibody (PA1-46152, 1/200 diluted, Glut-1 Rabbit Polyclonal Antibody).

An established scoring system that evaluates both the pattern and intensity of staining was used. Membranous and cytoplasmic staining were considered positive. Briefly, the staining pattern was scored according to the percentage of cells that showed cytoplasmic and/or membranous staining as follows: 0=less than 1%, 1+=1–10%, 2+=11–50%, 3+=51–80%, and 4+=over 80%. The intensity was scored as 1: weak, 2: moderate, and 3: strong. Blinded assessment was done by two different observers (SDO and OO). The overall score was then calculated as (1+intensity/3)×pattern[17]. Tumor cells were scored as negative if no immunopositive cells were present after immunostaining. The total score was based on the percentage of positive tumor cells and the degree of immunostaining intensity[18].

Statistically, the median value for staining score was 3.9. Score <4 was accepted as low, while score ³4 was accepted as high (Figure 1).

ETHICAL APPROVAL

Ethics committee approval for our study was obtained from the ethics committee of the Recep Tayyip Erdogan University Faculty of Medicine, non-interventional clinical research (E-40465587-050.01.04-352). The study was conducted in accordance with the Declaration of Helsinki, the ethical standards of the institutional research committee, and the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guideline[19].

STATISTICAL ANALYSIS

Statistical post-hoc power and effect size were calculated by using the G*Power version 3.1.9.7 software[20]. Statistical analyses were performed using IBM SPSS Statistics, Version 22.0 (SPSS Inc., Chicago, USA). Each group's descriptive statistics were reported as frequency and percentages within the group (n, %). Whether there was a correlation between the groups in terms of categorical variables was evaluated using the chi-square (Pearson's chi-square) and Fisher's exact test. The Kaplan-Meier method was used for survival analysis and was evaluated with the log-rank test. For statistical significance, the p-value was accepted as <0.05.

RESULTS

Clinicopathological parameters

A total of 65 cases were included, and the median age was 58 years (range, 33–84 years). Estrogen receptor (ER) and progesterone receptor (PR) positivity were observed in 45 (69%) and negative in 41 (63%) cases. In all, 50 (77%) cases had high Ki-67 LI (≥15%). Complete and partial pathologic responses were observed in 25 (38%) and 31 (48%) cases, respectively, while 9 (14%) had no response to NT.

Association of glucose transporter-1 expression with clinicopathological parameters in tru-cut biopsy before neoadjuvant therapy

High Glut-1 expression was present in 31 of 65 cases. Glut-1 expression was high in cases that had no expression of ER and PR (p=0.016 and p=0.004, respectively). There was a statistically significant relationship between Glut-1 expression and high Ki-67 LI (p=0.001) (Figure 1). Glut-1 expression was statistically higher in cases that were classified as luminal A and luminal B compared to Her2 and triple-negative (TN) ones (p=0.032). Glut-1 expression was statistically low in cases with lymphovascular invasion (p=0.002) and lymph node metastasis (p=0.017). Cases with high Glut-1 expression had either a complete or a partial pathologic response. The result was statistically significant (p=0.028) (Table 1).

Relationship between glucose transporter-1expression and prognosis

The median follow-up for the entire cohort was 36 months (range, 1–88 months). Notably, seven (11%) cases were died of

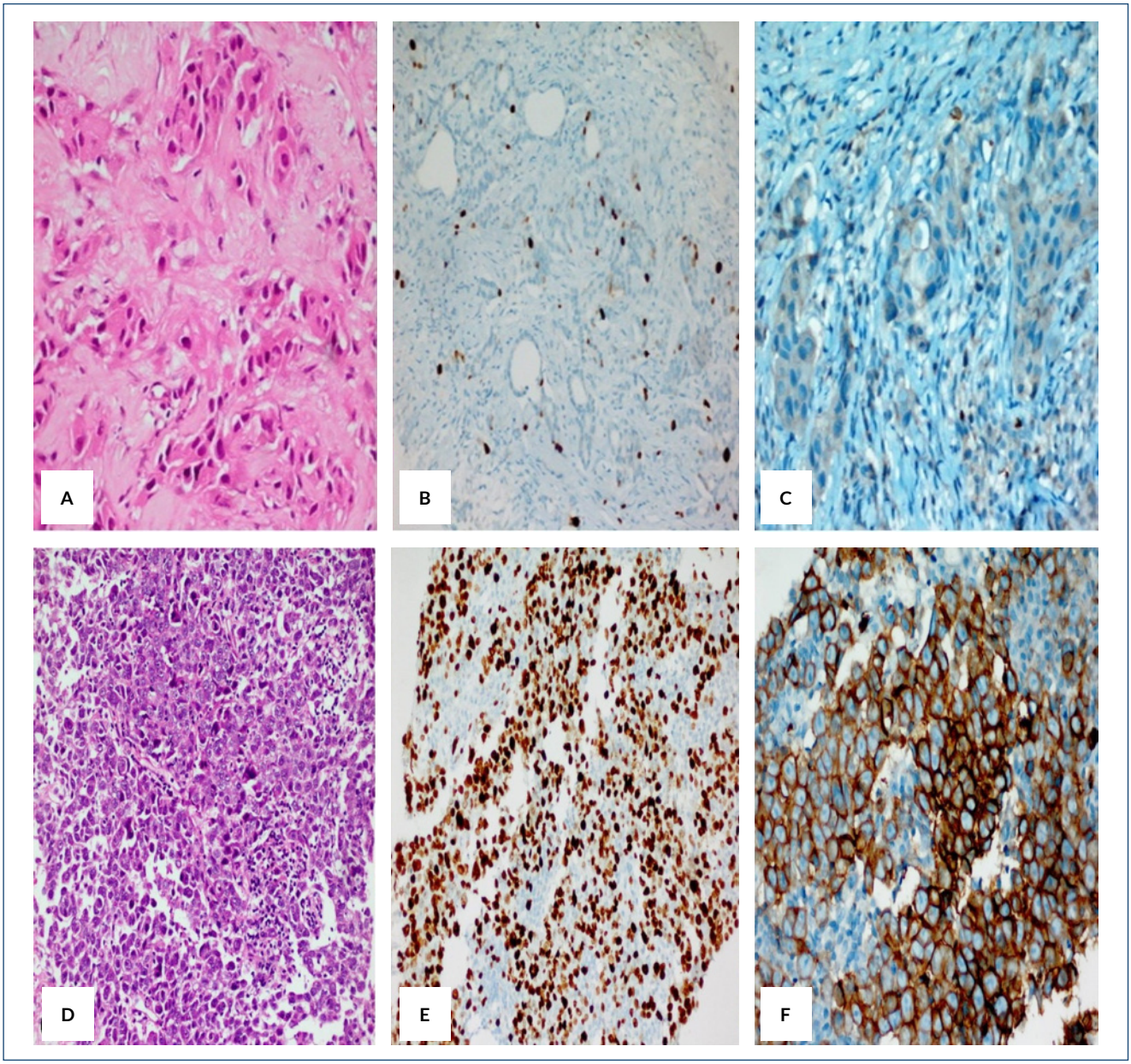

Figure 1. **(a)** Microscopic image of invasive breast carcinoma (H&E 200×). **(b)** Tumor labeling index (200×) with Ki-67. **(c)** Low glucose transporter-1 expression (400×). **(d)** Microscopic view of invasive breast cancer (H&E 200×). **(e)** Tumor labeling index (200×) with Ki-67. **(f)** High glucose transporter-1 expression (200×).

disease, and two (29%) had high Glut-1 expression. Distant organ metastases were observed in 14 (22%) cases, and Glut-1 expression was low in 12 (86%) of them. Statistically, Glut-1 expression was found to be associated with disease-free survival (DFS), but no correlation was found with overall survival (OS) (log-rank p=0.014 and p=0.469, respectively) (Figure 2).

DISCUSSION

Glut-1, a member of the glucose transporter family, expression is controlled by different transcription factors. For example, hypoxia-inducible factor (HIF-1 alpha) has been reported to regulate Glut-1 expression in hypoxic conditions. Moreover, c-Myc plays a role in Glut-1 expression in many different tumors[21]. Abnormal expression of Glut-1 is also affected by the PI3K/Akt pathway. Changes in the stability of Glut-1 transcription are associated with changes in glucose concentration, the structure of growth factors, cytokines, and some hormones[22]. The Glut-1 expression reflects increased glycolytic metabolism, so there is Glut-1 upregulation in many cancers to maintain high glucose levels in neoplastic cells[23].

Glut-1 has been shown as an optimal biomarker in various types of cancer[24], and it has been reported that agents providing Glut-1 inhibition in BC can be used in targeted therapy in different studies[21-27]. Moreover, this is the first study regarding Glut-1 expression in BC patients receiving NT.

BC is the most common type of cancer with a high mortality rate among women[11]. Some parameters, such as tumor

Table 1. The relationship between glucose transporter-1 expression and clinicopathological parameters in tru-cut biopsies before neoadjuvant therapy.

		Glut-1		
		<4	≥4	p-value
		n (%)	n (%)	
Histological subtypes	Invasive ductal carcinoma	32 (94.1)	29 (93.5)	1.000
	Invasive lobular carcinoma	2 (5.9)	2 (6.5)	
Response to treatment	No response	8 (23.5)	1 (3.2)	0.028
	Partial/complete response	26 (76.5)	30 (96.8)	
Molecular subtypes	Luminal A + Luminal B	28 (82.4)	18 (58.1)	0.032
	Her2 + Triple negative	6 (17.6)	13 (41.9)	
Estrogen receptor	Negative	6 (17.6)	14 (45.2)	0.016
	Positive	28 (82,4)	17 (54.8)	
Progesterone receptor	Negative	7 (20.6)	17 (54.8)	0.004
	Positive	27 (79.4)	14 (45.2)	
Her2	Negative	19 (55.9)	19 (61.3)	0.141
	Positive	11 (32.4)	12 (38.7)	
	Unknown	4 (11.8)	0 (0)	
Ki-67 proliferation index	Low	12 (36.4)	1 (3.3)	0.001
	High	21 (63.6)	29 (96.7)	
Lymphovascular invasion	Absent	16 (47.1)	26 (83.9)	0.002
	Present	18 (52.9)	5 (16.1)	
Perineural invasion	Absent	28 (82.4)	29 (93.5)	0.262
	Present	6 (17.6)	2 (6.5)	
Axillary lymph node metastasis	Absent	13 (38.2)	21 (67.7)	0.017
	Present	21 (61.8)	10 (32.3)	
Distant organ metastasis	Absent	22 (64.7)	29 (93.5)	0.005
	Present	12 (35.3)	2 (6.5)	
Dead of disease	Alive	29 (85.3)	29 (93.5)	0.43
	Exitus	5 (14.7)	2 (6.5)	

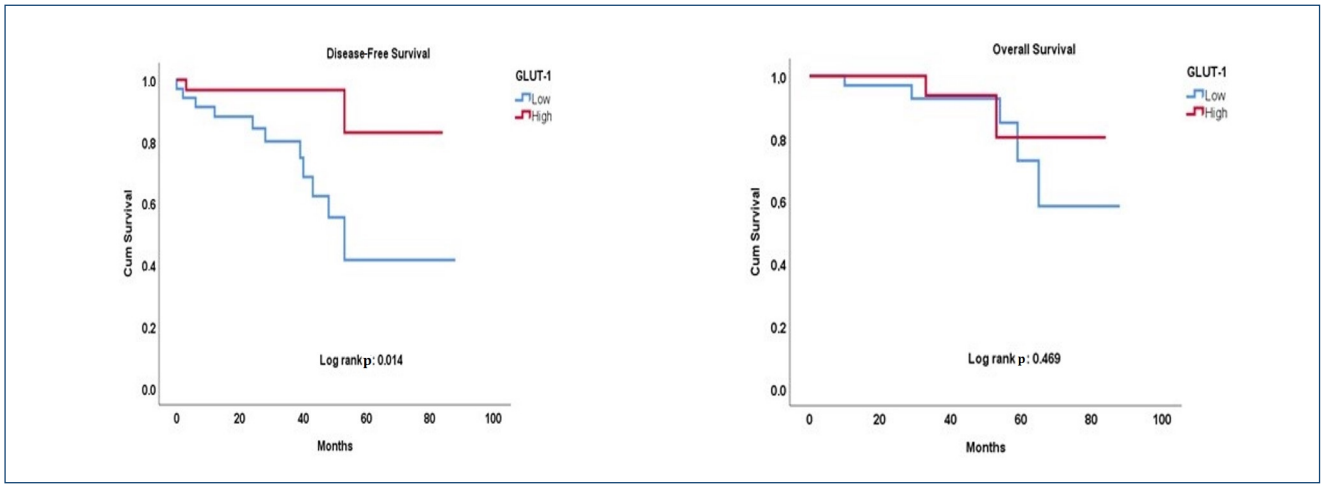

Figure 2. The relationship of glucose transporter-1 expression of cases with disease-free survival and overall survival by Kaplan-Meier analysis.

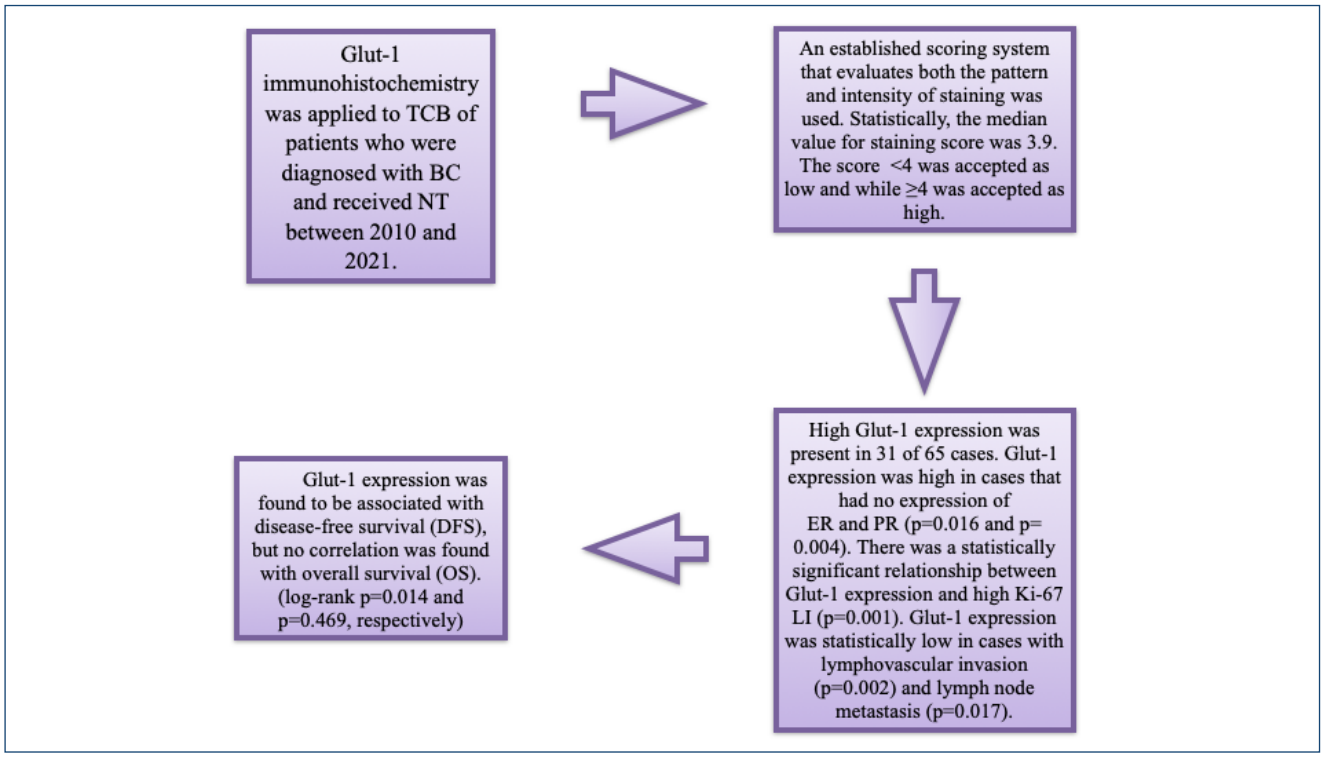

Figure 3. Flowchart of the research.

stage, molecular subtype, and hormone receptor status, have been used in daily practice to choose the treatment method and predict the prognosis.

To the best of our knowledge, there has been no study regarding Glut-1 expression in BC patients receiving NT.

According to Deng Y et al., Glut-1 expression was associated with higher tumor grade, ER, and PR negativity in BC patients who did not receive NT (1). In the current study, overexpression of Glut-1 was significantly related to the negative hormone receptor. In addition, higher expression was found in Her2 and TN BCs compared to luminal subtype. As a result, high expression of Glut-1 may indirectly be a sign of poor prognosis, since it is associated with hormone receptor negativity.

In our study, there was a statistically significant relationship between high Glut-1 expression and high Ki-67 LI (Figure 3). In a study by Alba et al., BC patients with a high LI had a complete response to NT. As in the studies of Alba et al., other studies advocate the predictive use of the Ki-67 LI to predict response

to chemotherapy in identifying patients with pathological complete response. In this way, the use of Ki-67 is very useful in determining the patient group with a long prognosis[25]. On the contrary, Ki-67 LI in breast carcinomas is assessed by eyeballing method by choosing three hotspot areas, counting 10 different high-magnification areas, and taking the average of the values. Therefore, this assessment is highly subjective among pathologists. In our study, Glut-1 expression was high in almost all of the cases with complete response to treatment. With these results, we can suggest that the evaluation of Glut-1 expression, which is an objective parameter that can be easily done in routine practice, can be used to predict response to treatment, as well as Ki-67.

In the meta-analysis by Yu Deng et al., the prognostic role of Glut-1 in BC was widely investigated but the results are reported to be inconsistent[1]. Hussein et al. reported that Glut-1 expression was not associated with OS in BC[26]. However, other researchers have presented significant associations between Glut-1 expression and poor prognosis in BC[1,27]. In our study, there was a statistically significant relationship between Glut-1 expression and DFS, but no relationship was found between its expression and OS. Glut-1 expression has not been studied in neoadjuvant patients before, and we think that higher expression can be used as a good prognostic marker in patients receiving NT. A significant correlation was found between low Glut-1 expression and lymphovascular invasion, perineural invasion, lymph node metastasis, and distant organ metastasis in patients receiving NT. This result also supports that high Glut-1 expression can be indirectly used as an indicator of good prognosis in patients receiving NT.

There were some limitations in our study; for example, our cases did not show a homogeneous distribution in terms of molecular subtype, hormone receptor status, response to treatment and had a short follow-up time. Another limitation of our study is the small number of cases.

In conclusion, cancer with high Glut-1 expression has a better response to NT. This is the first and pioneering study regarding Glut-1 expression in BC patients receiving NT. As a result, we suggest that Glut-1 could be used as an alternative biomarker to Ki-67 in objective evaluation of treatment response among BC patients.

AUTHORS' CONTRIBUTIONS

SDÖ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. **ÇÖ:** Conceptualization, Data curation, Methodology, Resources, Supervision, Writing – review & editing. **OO:** Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **GA:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **RB:** Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **BŞ:** Data curation, Formal Analysis, Software, Validation, Writing – review & editing.

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