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Clinicopathological analysis of GLUT-1, GLS1, and GLS2 expression in breast cancer tissues from Jordanian patients

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Breast cancer remains the most common malignancy among women, with significant heterogeneity in molecular subtypes and clinical outcomes. This study examines the clinicopathological significance of GLUT-1, GLS1, and GLS2 expression in breast cancer tissues from Jordanian patients, focusing on their role in metabolic reprogramming and potential as therapeutic targets. Using tissue microarray analysis and immunohistochemistry, we evaluated 306 invasive breast cancer cases and 52 normal tissue samples. Overexpression of all three markers was observed in tumor tissues compared to normal samples ($p \leq .01$). GLUT-1 and GLS2 showed significant associations with higher tumor grades and triple-negative breast cancer (TNBC) subtypes, highlighting their potential role in aggressive tumor biology. Conversely, GLS1 expression was consistently elevated in cancer tissues but did not vary significantly across grades or subtypes. Strong correlations between high GLUT-1/GLS2 expression and Ki-67 proliferative index underscore their contributions to tumor proliferation and metabolic adaptation. Population-specific patterns, such as the higher GLS2 expression in HER2-negative cases, reflect potential genetic or environmental influences unique to Jordanian patients. These findings emphasize the critical role of metabolic reprogramming in breast cancer progression and underscore the translational potential of targeting GLUT-1 and GLS2, particularly in aggressive subtypes like TNBC. Further research is warranted to explore functional mechanisms and validate these markers in diverse populations. This study provides novel insights into the metabolic dynamics of breast cancer, offering a foundation for regionally tailored therapeutic strategies.

Keywords Breast cancer, Metabolic reprogramming, GLUT-1, Glutaminases, Triple-negative breast cancer

Breast cancer is the most prevalent form of cancer in women worldwide, representing a substantial public health challenge[1]. In Jordan, the incidence of breast cancer continues to reflect the same patterns observed worldwide. It is the most frequently diagnosed cancer and has become the second most prevalent cause of cancer-related mortality, after lung cancer[2].

Furthermore, Breast cancer is a clinically and molecularly heterogeneous disease, characterized by diverse subtypes with distinct biological behaviours and treatment responses[3,4]. This heterogeneity is primarily classified based on the expression of three key receptors: estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal Growth Factor receptor 2 (HER2). These markers serve as the foundation for categorizing breast cancer into several clinically relevant subtypes. Hormone receptor-positive (HR+) breast cancers are defined by the presence of ER and/or PR and are further stratified into luminal A and luminal B subtypes. Luminal A tumors, which express high levels of hormone receptors and low levels of the proliferation marker Ki-67, are generally associated with a more favourable prognosis and are primarily treated with endocrine therapies. In contrast, luminal B tumors exhibit higher proliferation rates, may co-express HER2, and often require a combination of endocrine therapy and chemotherapy due to their more aggressive nature. HER2-positive breast cancers are characterized by the amplification or overexpression of the HER2 gene, leading to enhanced tumor growth and aggressiveness. This has historically been associated with a poor prognosis. However, the advent of

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targeted therapies such as trastuzumab has significantly improved outcomes for patients with HER2-positive tumors.

These cancers can be further divided based on the receptors' status, influencing the therapeutic approach. Triple-negative breast cancer (TNBC), which lacks expression of ER, PR, and HER2, accounts for approximately 15–20% of all breast cancers. TNBC is more common in younger women and certain ethnic groups, particularly those of African or Hispanic descent. This subtype is known for its aggressive clinical course, high rates of metastasis, and limited treatment options, as it does not respond to hormone therapies or HER2-targeted treatments. Chemotherapy remains the mainstay of treatment, although research into targeted therapies is ongoing. Within TNBC, the basal-like subtype is particularly notable, characterized by the expression of basal cytokeratins (such as CK5/6 and CK14) and other markers like EGFR. Basal-like cancers often overlap with BRCA1-mutated breast cancers and are associated with a poor prognosis. Despite their initial sensitivity to chemotherapy, these tumors frequently recur, posing significant treatment challenges.

Metabolic reprogramming is acknowledged as a fundamental hallmark of cancer[5]. Unlike normal cells, which rely on glycolysis primarily under anaerobic conditions, cancer cells exhibit persistent glucose uptake and utilization regardless of oxygen availability, a phenomenon known as the Warburg effect. This metabolic shift is further increased in hypoxic environments and during cancer progression[6].

Central to this metabolic alteration is the glucose transporter 1 (GLUT-1), which facilitates glucose transport into cancer cells, supporting their energy demands and biosynthetic processes. Crucially, GLUT-1 is implicated in various cancers, including breast cancer, where its overexpression is associated with aggressive tumor behaviour and poor prognosis[7].

Understanding the role of GLUT-1 in breast cancer is vital, as prior studies have linked its expression to high histologic grade, increased proliferative activity, negative ER and PR expression, and basal cytokeratin expression. These findings underscore GLUT-1's potential as a diagnostic marker and therapeutic target in breast cancer management[8].

Additionally, cancer cells modify their metabolism through various enzymes, including glutaminase. This enzyme catalyzes the conversion of glutamine to glutamate, supporting energy production, biomass synthesis, and redox balance, all of which are crucial for cancer cell survival and growth. Glutaminase has two main isoforms—kidney-type (GLS) and liver-type (GLS2)—each with distinct functions. GLS, or GLS1, facilitates glutaminolysis, fueling the tricarboxylic acid (TCA) cycle and providing substrates for nucleotide and lipid biosynthesis, thereby supporting the rapid proliferation of breast cancer cells and their adaptation to hypoxic and nutrient-limited conditions[9].

GLS-mediated glutaminolysis fuels the TCA cycle and provides essential substrates for nucleotide and lipid biosynthesis, thereby facilitating the rapid proliferation of breast cancer cells. Furthermore, this enzymatic process enables cancer cells to adapt to adverse microenvironmental conditions such as hypoxia and nutrient scarcity. Enhanced GLS1 activity promotes glutamine metabolism, fostering cancer cell survival, proliferation, and aggressiveness. Upregulation of GLS1 expression has been consistently associated with breast cancer progression, therapy resistance, and poor clinical outcomes[10–13].

GLS2 is postulated to attenuate cell proliferation via the modulation of reactive oxygen species (ROS) homeostasis within tissues, where oxidative stress-induced tissue damage is implicated in carcinogenesis[14]. The impact of altered levels of GLS1 and GLS2 on breast cancer cell behaviour and tumor growth in different subtypes of breast cancer cells was investigated. A significant reduction in cell growth was observed in luminal-subtype breast cancer cells upon the decrease of GLS2 levels, while a similar effect was observed in basal-subtype cells upon the reduction of GLS levels. These findings suggest that targeting GLS2 could hold promise as an approach to limit tumor growth in breast cancer, with potential implications for further research and clinical applications[15].

In TNBC, GLS2 exhibits a dual role: it can act as a tumor suppressor, inhibiting cancer cell proliferation and growth. However, despite the presence of elevated GLS levels, the concurrent expression of endogenous GLS2 suggests a compensatory mechanism. Notably, overexpression of GLS2 restores cell proliferation and metabolic function after GLS inhibition, indicating its involvement in metabolic adaptation and cell survival. Therefore, while GLS2 may suppress tumors, its expression paradoxically supports cell survival and growth in TN breast cancer[14]. Patients with tumors expressing GLS but lacking GLS2 display significantly poorer survival outcomes[16].

In conclusion, the intricate interplay between glutaminase isoforms and GLUT-1 underscores the diverse metabolic dynamics and adaptability of breast cancer cells, presenting promising avenues for targeted therapy to disrupt metabolic dependencies and enhance treatment efficacy[17,18]. This study aims at understanding these interconnected expression patterns within the Jordanian population, to aid in the development of precise therapeutic strategies to improve patient outcomes across different subtypes of breast cancer.

Materials and methods

Specimen and data collection

A comprehensive retrospective evaluation was carried out on a total of 306 cases with the diagnosis of invasive breast cancer and 52 cases of normal tissues. The data was collected from departmental archives and institutional databases at King Abdullah University Hospital (KAUH) in Irbid, Jordan, spanning a period between January 2016 and November 2022, after obtaining the institutional review board (IRB) approval at Jordan University of Science and Technology/King Abdullah University Hospital (IRB # 26/2022). All procedures were performed in accordance with the relevant institutional and national guidelines and regulations, including the principles outlined in the Declaration of Helsinki.

As this was a retrospective study involving anonymized archived tissue samples and associated clinical data, the requirement for obtaining individual informed consent was waived by the Institutional Review Board (IRB) at Jordan University of Science and Technology/King Abdullah University Hospital (IRB #26/2022). No

identifying information, such as patient names or other personal identifiers, was included at any stage of the research, ensuring full compliance with ethical and confidentiality standards. The study included samples from patients who had undergone modified radical mastectomies, simple mastectomies, or wide local excisions, with or without axillary clearance.

The study obtained data on tumor characteristics such as histologic type, grade, tumor size, lymph node status, and the respective status of ER, PR, and HER2, determined by immunohistochemistry, from the original histopathology reports. These parameters were previously assessed by certified pathologists using the College of American Pathologists (CAP) standardized data sets. Subject's demographics and metastatic status were retrieved from clinical and initial radiological records.

Tissue microarray production

A fully automated tissue microarrayer (TMA Master II 3DHISTECH) was utilized to generate forty-four TMA cassettes. From each specimen, three 0.6-mm-diameter cores were taken. Thereafter, these three cores were inserted into a TMA paraffin cassette. This approach ensured that the samples were accurately represented by minimizing tissue loss and accommodating tumor heterogeneity.

Immunohistochemistry staining protocol

Four-micrometer-thick sections were produced using a Leica rotary microtome. These sections were then placed on slides that had been coated with a charged compound. Subsequently, the slides were heated for an hour at 62 degrees Celsius in an oven and then they were allowed to cool down at room temperature. The staining process was then carried out using the Ventana Bench Mark ULTRA IHC/ISH automated staining system, following the manufacturer's standard protocol. In this study, primary antibodies for GLUT-1 (Abcam; ab115730; dilution 1:40), GLS (Abcam; ab260047; dilution 1:200), GLS2 (Abcam; ab113509; dilution 1:200), and Ki-67 (Abcam; ab16667; dilution 1:200) were utilized.

Positive controls were included for GLUT-1, GLS, and GLS2 markers from placental tissue, renal tubules, and hepatic parenchyma, respectively. Benign mammary tissue was used as negative control.

Immunohistochemistry interpretation and scoring

The expression of GLUT-1 was designated as positive if any membranous and cytoplasmic staining was observed. Positivity for GLS and GLS2 was defined as any detectable cytoplasmic staining in tumor cells.

A modified histochemical score (H-score) system was employed to evaluate the expression of GLUT-1, GLS1, and GLS2 markers in tumor cells. The scoring system was based on two factors, the staining intensity and percentage. Using a four-tiered system (0 for negative, 1 for weak, 2 for moderate, and 3 for strong), the staining intensity was given (concurring to pre-agreed staining level intensity). The percentage of positively stained tumor cells for each intensity was subjectively estimated. The H-score was calculated by multiplying the percentage of positively stained cells (0–100) by the intensity (0–3), producing a total range of 0–300. The final score for each case was achieved by averaging the H-scores of the three cores within the specific case/specimen. High expression levels were assigned H-score ≥ median for each protein; GLS1 ≥ 75, GLS2 ≥ 150, and GLUT-1 ≥ 131.

Ki67 proliferative index was determined by assessing the percentage of nuclei with positive staining in tumor cells among the total number of tumor cells. Proliferative index was considered high if the positive staining is > 15%.

To ensure the utmost accuracy of the study, all cases were individually interpreted by three pathologists (SMA, SA, QS) using a light microscope (Olympus CX23 microscope). The three reviewers were blinded to clinical data, tumor characteristics, and the results of other markers. The overall concordance rate between the three reviewers was approximately 90.2%. In cases where there was a major discrepancy in interpretation, a consensus was achieved through a collaborative multiheaded microscopic review (using Olympus BX50 microscope).

Statistical analysis

Analyses were performed using IBM Statistical Package for Social Sciences (SPSS©) Statistics for Windows, Version 22, (IBM Corp., Armonk, N.Y., USA). The difference in expression between normal and cancer tissue or differences within different clinicopathologic characteristics were tested via Mann-Whitney U-test or Kruskal–Wallis H-test as appropriate. Pearson correlation test χ^2 and Fisher exact tests were used to test associations for categorical variables. Correlations between variables were evaluated by Spearman's rank correlation test, where a value of 0.70 indicates a very strong relationship, 0.40–0.69 indicates a strong relationship, 0.30–0.39 indicates a moderate relationship, 0.20–0.29 indicates a weak relationship, and 0.01–0.19 indicates no or negligible relationship[19]. P-values ≤ 0.05 were deemed statistically significant.

Results

Differential expression of GLUT-1, GLS1, and GLS2 in breast cancer

Figure 1 illustrates representative immunohistochemical staining patterns and the scoring system used to evaluate GLUT-1, GLS1, and GLS2 expression in breast cancer and adjacent normal tissues. The images depict varying levels of staining intensity and distribution.We observed a significant overexpression of GLUT-1, GLS1, and GLS2 in tumor tissues compared to normal tissues (Fig. 2a and b, and 2c). Both GLS1 and GLS2 exhibited markedly higher expression in cancer tissues with a highly significant difference ($P < .01$), highlighting alterations in glutamine utilization in tumor cells In addition, GLUT-1, showed significantly elevated levels in cancer tissues ($P \leq .01$), indicating an increase in glucose uptake by tumor cells. These findings suggest a notable shift in metabolic processes within breast cancer cells, underscoring the potential role of these proteins in cancer development and progression.

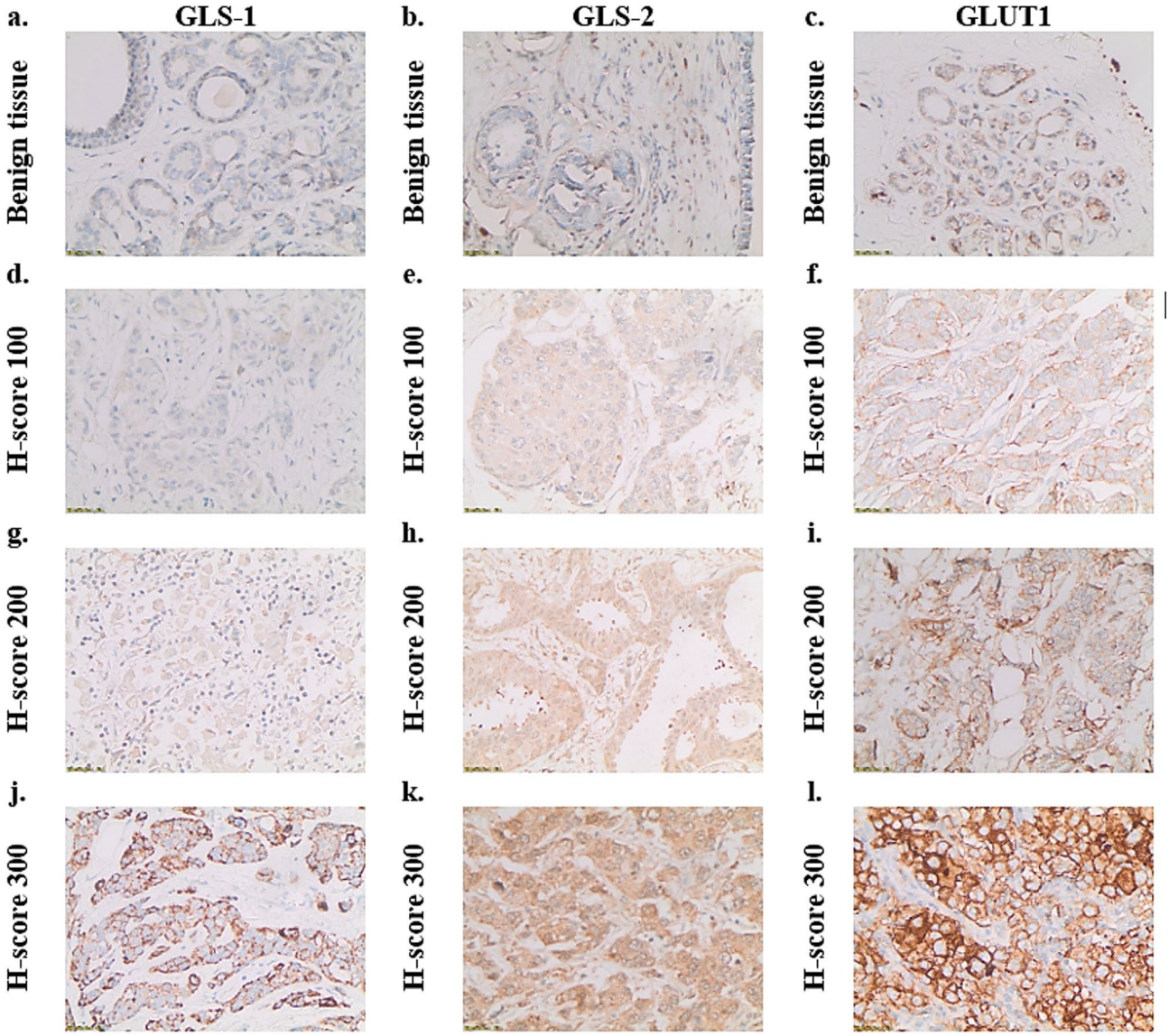

Fig. 1. Representative Immunohistochemical staining patterns of GLS1, GLS2, and GLUT-1 in normal and cancer tissues, **(a-c)** normal tissues with low/negative expression, **(d-f)** cancer tissues with weak expression, **(g-i)** cancer tissues with medium expression, **(j-l)** cancer tissues with strong expression. Scale bar = 50 µm Photomicrographs were taken at 40x magnification from the scanned TMA slides.

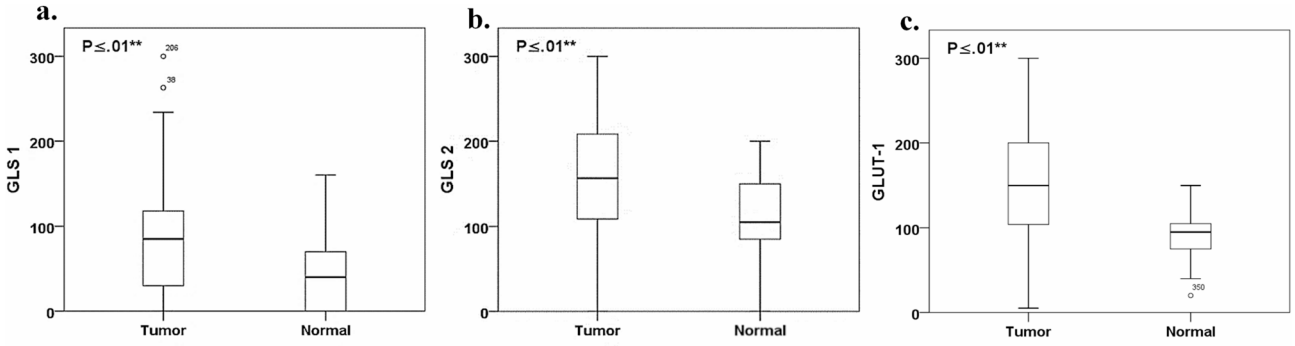

Fig. 2. Expression of GLS1 **(a)**, GLS2 **(b)**, and GLUT-1 **(c)** levels in normal and cancer tissues microarrays. Sample size: 306 breast cancer tissues and 52 adjacent normal tissues. Statistical analysis was performed using the Mann–Whitney U test. *P values ≤ 0.05 and **P values ≤ 0.01 were considered statistically significant.

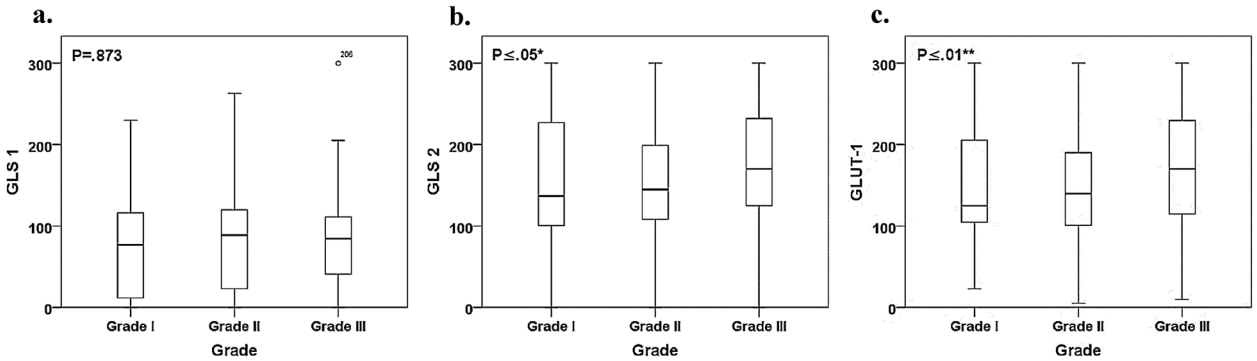

Fig. 3. Expression of GLS1, GLS2, and GLUT-1 among different clinicopathological grades. **(a)**. The expression of GLS1 is not associated with the increased grade level, on the contrary GLS2 and GLUT-1 expression is increased with increased grade level **(b)** and **(c)**. The correlation between the expression and grade level was tested by Kruskal-Wallis test. $^{**}P \leq .01$, $^{*}P \leq .05$ were considered statistically significant.

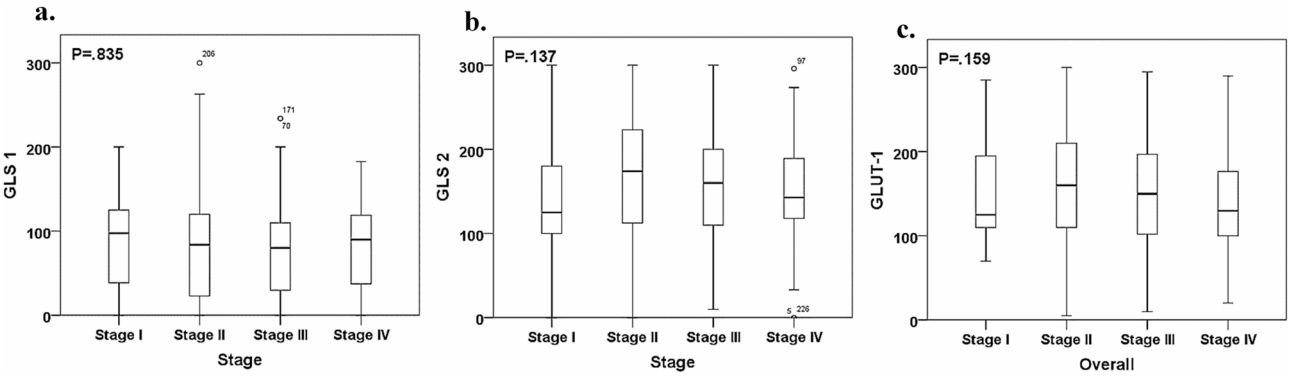

Fig. 4. Expression of GLS1, GLS2, and GLUT-1 among different clinicopathological grades. **(a)**. The expression of GLS1 is not associated with the increased stage level. Similarly, GLS2 and GLUT-1 expression was maintained among breast cancer stages (I to IV) **(b)** and **(c)** respectively. The correlation between the expression and stage level was tested by Kruskal-Wallis Test. $^{**}P \leq .01$, $^{*}P \leq .05$ were considered statistically significant.

Correlation of GLUT-1, GLS1, and GLS2 Expression with Breast Cancer Grade

To explore the potential involvement of GLUT-1, GLS1, and GLS2 in the progression and severity of breast cancer, we examined their expression across different cancer grades (Fig. 3a and b, and 3c).

The analysis of GLS1 expression revealed no statistically significant differences among the various grades of breast cancer ($p = .873$) (Fig. 3a). This lack of correlation suggests that while GLS1 is overexpressed in breast cancer tissues overall, its expression does not appear to increase with cancer grade. This finding may indicate that GLS1 is involved in breast cancer metabolism generally but does not have a grade-dependent association with tumor aggressiveness or progression.

In contrast, GLS2 and GLUT-1 showed statistically significant differences in expression across cancer grades, with $p \leq .05$ for GLS2 and $p \leq .01$ for GLUT-1 (Fig. 3b and c). Notably, higher grades of breast cancer exhibited elevated levels of GLUT-1, suggesting a potential link between increased glucose metabolism and tumor aggressiveness. This is consistent with previous studies reporting that GLUT-1 overexpression is often associated with more aggressive phenotypes and poor prognosis in breast cancer[20]. The observed increase in GLS2 expression in higher grades could reflect enhanced glutamine metabolism in advanced cancer stages, supporting tumor growth and survival under more challenging conditions.

Correlation of GLUT-1, GLS1, and GLS2 expression with breast cancer stage

Figure 4 illustrates the expression levels of GLS1, GLS2, and GLUT-1 across various stages of breast cancer (stages I to IV) and examines whether these markers correlate with disease progression.

The results indicate that GLS1 expression does not significantly change across different stages ($p = .835$), suggesting that GLS1 expression is relatively stable regardless of the breast cancer stage (Fig. 4a). This finding aligns with previous observations that, while GLS1 is generally upregulated in cancer, its expression may not necessarily correlate with the clinical stage or tumor aggressiveness in breast cancer[21]. GLS1 might serve as a general marker of altered metabolism but does not appear to indicate the extent of disease progression.

Similarly, GLS2 and GLUT-1 also did not show significant variation in expression across stages (p=.137 for GLS2 and p=.159 for GLUT-1), as seen in Fig. 4b and c. This lack of correlation suggests that the roles of GLS2 and GLUT-1 in breast cancer may not be directly associated with tumor stage but rather reflect metabolic reprogramming that is consistently required throughout cancer development. The stable expression of GLS2 across stages may imply its role in maintaining redox homeostasis and supporting cellular resilience under metabolic stress, functionsthat are beneficial to tumor cells regardless of the stage of progression.

GLUT-1 expression stability across stages aligns with the hypothesis that glucose uptake remains a critical metabolic adaptation for tumor cells throughout all stages of cancer. While some studies have reported increased GLUT-1 expression in more aggressive subtypes of breast cancer, others suggest that GLUT-1 can be overexpressed in a broad spectrum of tumor stages as an adaptation to the high energy demands of rapidly proliferating cells. Our findings suggest that while GLUT-1 overexpression is a feature of breast cancer, it may not vary significantly with stage, indicating that glucose metabolism is equally crucial across all stages of disease.

Expression patterns of GLS1, GLS2, and GLUT-1 across breast cancer subtypes

We investigated the expression of GLS1, GLS2, and GLUT-1 across breast cancer molecular subtypes (Luminal, Basal, and HER2-enriched). Our analysis revealed insights into their potential roles in tumor biology.

The results indicated that GLS1 expression does not significantly differ across the molecular subtypes (P=.892). suggesting a role of GLS1 in breast cancer pathology irrespective of the subtype (Fig. 5a). In contrast, GLUT-1 expression varied markedly, showing significant overexpression in the Basal subtype compared to Luminal and HER2-enriched subtypes (p<.01), highlighting its potential as a biomarker for aggressive breast cancer behaviour. GLS2 expression exhibited a trend toward higher levels in the Basal subtype, with marginal significance (p=.053), suggesting a potential subtype-specific role that may warrant further investigation (Fig. 5b and c).

Differential expression of GLS1, GLS2, and GLUT-1 in HR + and TNBC

We analyzed the expression patterns of GLS1, GLS2, and GLUT-1 to explore their potential differential roles in HR + versus TNBC, hypothesizing that distinct metabolic pathways may underlie the biological behavior of these subtypes and highlight potential therapeutic targets.

GLS1 expression did not differ significantly between HR + and TNBC (p=.613), suggesting that GLS1's involvement in glutamine metabolism may be a shared feature across breast cancer subtypes, unaffected by hormone receptor status (Fig. 6a). Similarly, GLS2 showed significantly higher expression in TNBC than in HR + tumors ($p \leq .05$), although to a lesser extent than GLUT-1. This differential expression of GLS2 further supports its role in promoting the survival and proliferation of TNBC cells, likely through enhanced glutamine metabolism (Fig. 6b).

Association of high GLS1, GLS2, and GLUT-1 expression with clinicopathological parameters and hormonal receptor status

The analysis of GLS1, GLS2, and GLUT-1 high expression across various clinicopathological parameters revealed distinct patterns (Table 1). Age was not significantly associated with the high expression of GLS1 (P=.88), GLS2 (P=.55), or GLUT-1 (P=.81), suggesting that these metabolic markers are independent of age-related factors. Normal versus Tumor Tissues showed marked differences, with significantly higher expression of GLS1, GLS2, and GLUT-1 in tumor tissues compared to normal samples ($P \leq .01$ for all), indicating their potential involvement in tumor biology and metabolic adaptation.

When considering histological grade, only GLUT-1 exhibited a significant association, with higher expression in more advanced tumor grades ($P \leq .01$), supporting its role in the metabolic needs of aggressive tumors. Clinical stage did not show significant correlations with any of the three markers (P=.77 for GLS1, P=.13 for GLS2, and

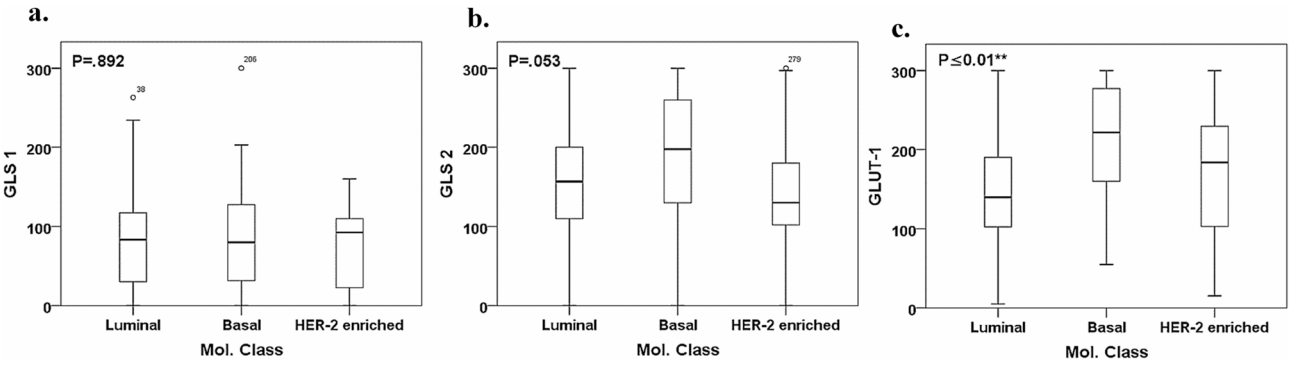

Fig. 5. Expression of GLS1, GLS2, and GLUT-1 among different molecular subtypes (Luminal, Basal, and HER2-enriched). **(a)**: The expression of GLS1 does not significantly differ across the molecular subtypes. While GLS2 was overexpressed with a marginal significancy with Basal subtype **(b)**, GLUT-1 was significantly overexpressed in Basal subtype compared to Luminal and HER2-enriched subtypes **(c)** respectively. **$P \leq .01$, *$P \leq .05$ were considered statistically significant.

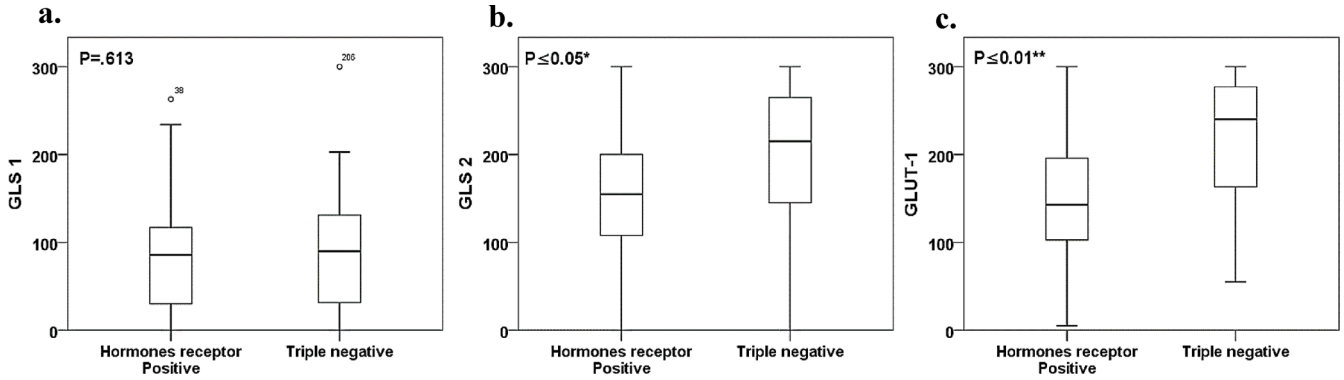

Fig. 6. Differential Expression of GLS1, GLS2, and GLUT-1 in HR + and TNBC. **(a)**: The expression of GLS1 was not significantly altered. GLS2 and GLUT-1 on the other hand were significantly associated with Triple Negative breast cancer. **(b)** and **(c)** respectively. $^{**}P \leq .01$, $^{*}P \leq .05$ were considered statistically significant.

	GLS1			GLS2			GLUT-1		
	low	high		low	high		low	high	
	N (%)	*N* (%)	*p*	*N* (%)	*N* (%)	*p*	*N* (%)	*N* (%)	*p*
Age			0.88			0.55			0.81
≤ 52	61 (44.5)	76 (55.5)		70 (50.0)	70 (50.0)		60 (43.5)	78 (56.5)	
≥ 52	69 (45.4)	83 (54.6)		74 (46.5)	85 (53.5)		67 (42.1)	92 (57.9)	
Normal vs. tumor			≤ 0.01**			≤ 0.01**			≤ 0.01**
Normal	33 (80.5)	8 (19.5)		36 (80.0)	9 (20.0)		44 (97.8)	1 (2.2)	
Tumor	130 (44.8)	160 (55.2)		145 (48.3)	155 (51.7)		127 (42.6)	171 (57.4)	
Histological grade			0.99			0.10			≤ 0.01**
1	14 (45.2)	17 (54.8)		17 (54.8)	14 (45.2)		17 (54.8)	14 (54.2)	
2	65 (44.8)	80 (55.2)		78 (53.1)	69 (46.9)		73 (49.0)	76 (51.0)	
3	51 (45.5)	61 (54.5)		49 (40.8)	71 (59.2)		36 (31.0)	80 (69.0)	
Clinical stage			0.77			0.13			0.37
1	11 (39.3)	17 (60.7)		18 (62.1)	11 (37.9)		15 (51.7)	14 (48.3)	
2	65 (47.4)	72 (52.6)		58 (41.7)	81 (58.3)		54 (39.4)	83 (60.6)	
3	36 (44.4)	45 (55.6)		41 (48.2)	44 (51.8)		34 (40.0)	51 (60.0)	
4	16 (40.0)	24 (60.0)		24 (55.8)	19 (44.2)		22 (51.2)	21 (48.8)	
ER			0.88			0.84			≤ 0.05*
Negative	26 (44.1)	33 (55.9)		31 (49.2)	32 (50.8)		18 (29.5)	43 (70.5)	
Positive	101 (45.1)	123 (54.9)		110 (47.8)	120 (52.2)		108 (46.8)	123 (53.2)	
PR			0.74			0.87			≤ 0.05*
Negative	33 (43.4)	43 (56.6)		39 (49.4)	40 (50.6)		25 (32.5)	52 (67.5)	
Positive	94 (45.6)	112 (54.4)		103 (48.4)	110 (51.6)		99 (46.3)	115 (53.7)	
HER2 Receptor			0.90			≤ 0.01**			0.73
Negative	84 (43.8)	108 (56.3)		87 (43.9)	111 (56.1)		87 (43.7)	112 (56.3)	
Positive	29 (44.6)	36 (55.4)		42 (63.6)	24 (36.4)		26 (41.3)	37 (58.7)	
Molecular Class			0.95			0.12			≤ 0.05*
Luminal	101 (45.1)	123 (54.9)		110 (47.8)	120 (52.2)		108 (46.8)	123 (53.2)	
Basal	15 (46.9)	17 (53.1)		13 (38.2)	21 (61.8)		8 (23.5)	26 (76.5)	
HER-2 enriched	12 (42.9)	16 (57.1)		19 (63.3)	11 (36.7)		10 (35.7)	18 (64.3)	
Outcome			0.95			0.45			0.87
Dead	10 (38.5)	16 (61.5)		14 (51.9)	13 (48.1)		12 (44.4)	15 (55.6)	
Alive	59 (39.1)	92 (60.9)		68 (44.2)	86 (55.8)		71 (46.1)	83 (53.9)	
Hormonal Status			0.70			0.06			≤ 0.05*
Hormones Receptor Positive	113 (44.5)	141 (55.5)		129 (49.2)	133 (50.8)		118 (45.4)	142 (54.6)	
Triple Negative	11 (40.7)	16 (59.3)		9 (31.0)	20 (69.0)		6 (20.7)	23 (79.3)	

Table 1. Association of GLS1, GLS2, and GLUT-1 high expression and different clinicopathological parameters. $^{**}P \leq .01$, $^{*}P \leq .05$ were considered statistically significant.

P=.37 for GLUT-1), suggesting that these proteins' expressions are more closely related to tumor grade than to clinical stage.

Hormone Receptor Status analysis indicated that GLS2 expression was significantly higher in HER2-negative cases ($P \leq .01$), while no such association was observed for GLS1 (P=.90) or GLUT-1 (P=.73). Additionally, GLUT-1 expression was significantly associated with ER-negative ($P \leq .05$) and PR-negative ($P \leq .05$) statuses, suggesting a possible role in hormone receptor-negative breast cancer subtypes.

In terms of molecular classification, GLUT-1 expression was significantly higher in the Basal subtype compared to Luminal and HER2-enriched subtypes ($P \leq .05$), indicating a potential subtype-specific metabolic function. TNBCs showed notable expression patterns, with higher GLS2 (P=.06) and significantly higher GLUT-1 ($P \leq .05$) expression, underscoring the increased metabolic activity in this aggressive subtype.

Outcome analysis (alive versus dead) did not reveal significant associations between high expression levels of GLS1 (P=.95), GLS2 (P=.45), or GLUT-1 (P=.87) and patient survival, suggesting that while these markers are involved in tumor metabolism, they may not serve as direct predictors of patient outcomes.

The correlation analysis of high expression levels among GLS1, GLS2, GLUT-1, and their association with Ki-67 revealed significant relationships across multiple comparisons, indicating coordinated roles in tumor metabolism and cell proliferation.

There was a significant association between GLS1 and GLUT-1 expression levels ($P \leq .05$), with 57.2% of cases with high GLS1 expression also exhibiting high GLUT-1 expression, compared to 45.5% of cases with low GLS1 expression. This suggests a possible co-regulatory or synergistic role between GLS1 and GLUT-1, potentially enhancing glucose uptake and utilization in tumor cells. Similarly, GLS2 expression was strongly correlated with GLUT-1 ($P \leq .01$), as 60.7% of high GLS2 cases also showed high GLUT-1 expression, while only 40.7% of low GLS2 cases exhibited high GLUT-1 levels. This strong association implies that GLS2 and GLUT-1 may function together to support increased metabolic activity in tumors, specifically through the coordination of glutamine and glucose metabolism.

Additionally, GLS1 and GLS2 showed a significant correlation ($P \leq .01$), with 65.5% of high GLS2 cases also expressing high levels of GLS1, compared to 34.5% in low GLS2 cases. This finding suggests that GLS1 and GLS2 may be jointly involved in glutamine metabolism, facilitating metabolic flexibility to support tumor growth and survival.

The association between these markers and Ki-67, a proliferation marker, further underscores their role in tumor cell proliferation. High GLS1 expression was significantly associated with elevated Ki-67 levels ($P \leq .05$), with 71.4% of high GLS1 cases also exhibiting high Ki-67 expression, compared to only 49.1% of low GLS1 cases. Similarly, high GLS2 expression correlated with increased Ki-67 ($P \leq .05$), with 67.6% of high GLS2 cases showing elevated Ki-67 compared to 47.1% of low GLS2 cases. GLUT-1 also showed a strong association with Ki-67 ($P \leq .01$), where 75.7% of high GLUT-1 cases displayed elevated Ki-67 expression, compared to 48.8% in low GLUT-1 cases. These associations suggest that high levels of GLS1, GLS2, and GLUT-1 contribute to the increased proliferative capacity of tumor cells, likely by meeting the metabolic demands of rapid cell division.

Correlation between GLS1, GLS2, GLUT-1, and Ki-67 in tumor tissues

The correlation analysis among GLS1, GLS2, GLUT-1, and Ki-67 expression within various clinicopathological subgroups provided deeper insights into the functional roles and interactions of these biomarkers in different tumor characteristics and patient outcomes (Table 2).

In patients with poorer outcomes (deceased group), there was a very strong positive correlation between GLS1 and GLS2 (Rho = 0.709, $P \leq .01$), indicating a high level of interdependence between these two glutaminases in severe cases. This suggests that GLS1 and GLS2 may work synergistically to meet the heightened glutamine metabolism demands of aggressive tumors. Additionally, in this subgroup, GLS2 showed a significant correlation with Ki-67 (Rho = 0.619, $P \leq .05$), linking GLS2 expression directly to cellular proliferation in tumors associated with poorer survival. This finding highlights GLS2's potential role in driving both metabolic and proliferative processes in advanced or aggressive cancer phenotypes.

In the Luminal molecular subtype, which is typically less aggressive than other subtypes, a significant positive correlation was found between GLS2 and GLUT-1 (Rho = 0.500, $P \leq .01$) and between GLS2 and Ki-67 (Rho = 0.577, $P \leq .05$). This suggests that, even in less aggressive cancers, GLS2 may contribute to both glutamine and glucose metabolism, facilitating energy production and supporting tumor cell proliferation.

This coordinated interaction between GLS2 and GLUT-1 in the Luminal subtype implies a metabolic flexibility that may be exploited for therapeutic interventions targeting these pathways.

In the HER2-enriched subtype, which is known for its aggressive behavior and poor prognosis, there was a strong correlation between GLS1 and GLS2 (Rho = 0.456, $P \leq .01$). This relationship underscores the potential reliance on glutamine metabolism in HER2-enriched tumors, where both GLS1 and GLS2 may play crucial roles in supporting the increased metabolic needs of these rapidly growing cells. Targeting glutamine metabolism in this subtype may therefore be a promising therapeutic approach, especially for patients with high GLS1 and GLS2 expression.

For hormone receptor-negative tumors, distinct patterns emerged. In ER-negative cases, a significant positive correlation was observed between GLS2 and GLUT-1 (Rho = 0.324, $P \leq .05$) and between GLS2 and Ki-67 (Rho = 0.451, $P \leq .01$). These correlations suggest that GLS2 is particularly active in hormone receptor-negative environments, where it may support the proliferative and metabolic demands through coordinated interactions with GLUT-1. In PR-negative cases, GLS1 and GLS2 exhibited a strong correlation (Rho = 0.471, $P \leq .01$), indicating that these glutaminases may have a critical, interlinked role in glutamine metabolism within this subgroup. Additionally, PR-negative tumors showed a significant correlation between GLS2 and Ki-67 (Rho = 0.490, $P \leq .01$), further associating GLS2 with increased cell proliferation in hormone receptor-negative breast cancers.

	GLS1 and GLS2		GLS1 and GLUT-1		GLS1 and Ki-67		GLS2 and GLUT-1		GLS2 and Ki-67		GLUT-1 and Ki-67	
	Rho	*P*	Rho	*P*	Rho	*P*	Rho	*P*	Rho	*P*	Rho	*P*
Histological grade												
1	0.387	0.079	0.048	0.898	0.148	0.986	0.525	≤ 0.01**	0.448	0.245	0.237	0.605
2	0.384	≤ 0.01**	0.094	0.396	0.186	0.070	0.196	≤ 0.05*	0.196	≤ 0.05*	0.109	0.119
3	0.391	≤ 0.01**	0.087	0.421	0.142	0.277	0.275	≤ 0.01**	0.286	≤ 0.01**	0.365	≤ 0.01**
Clinical stage												
1	0.287	0.224	0.350	≤ 0.05*	0.058	0.058	0.412	0.075	0.392	≤ 0.05*	0.486	≤ 0.05*
2	0.400	≤ 0.01**	−0.024	0.476	0.186	0.336	0.312	≤ 0.01**	0.373	≤ 0.01**	0.329	≤ 0.01**
3	0.385	≤ 0.01**	0.160	0.153	0.013	0.869	0.279	≤ 0.05*	0.036	0.454	0.040	0.089
4	0.371	≤ 0.05*	0.093	0.776	0.306	0.201	0.192	0.357	0.360	0.063	0.331	≤ 0.05*
ER												
Negative	0.444	≤ 0.01**	−0.017	0.795	0.267	0.262	0.324	≤ 0.05*	0.451	≤ 0.01**	0.338	≤ 0.05*
Positive	0.345	≤ 0.01**	0.114	0.171	0.140	0.098	0.287	≤ 0.01**	0.229	≤ 0.05*	0.142	≤ 0.05*
PR												
Negative	0.471	≤ 0.01**	0.025	0.958	0.322	0.133	0.285	≤ 0.05*	0.490	≤ 0.01**	0.328	≤ 0.01**
Positive	0.330	≤ 0.01**	0.097	0.296	0.081	0.723	0.297	≤ 0.01**	0.209	≤ 0.05*	0.160	≤ 0.01**
HER2 Receptor												
Negative	0.349	≤ 0.01**	0.123	0.154	0.183	0.173	0.320	≤ 0.01**	0.424	≤ 0.01**	0.352	≤ 0.01**
Positive	0.392	≤ 0.01**	−0.071	0.588	0.036	0.552	0.356	≤ 0.01**	0.120	≤ 0.05*	0.107	0.359
Molecular Class												
Luminal	0.714	0.171	−0.100	≤ 0.01**	0.273	0.098	0.500	≤ 0.01**	0.577	≤ 0.05*	0.564	≤ 0.05*
Basal	0.345	≤ 0.05*	0.114	0.780	0.140	0.399	0.287	0.164	0.229	≤ 0.01**	0.142	≤ 0.05*
HER-2 enriched	0.456	≤ 0.01**	−0.041	0.598	0.366	0.851	0.255	0.133	0.528	0.420	0.450	0.766
Outcome												
Dead	0.709	≤ 0.01**	0.127	0.667	0.349	0.414	0.266	0.139	0.619	≤ 0.05*	0.316	≤ 0.01**
Alive	0.358	≤ 0.01**	0.089	0.366	0.141	0.093	0.285	≤ 0.01**	0.330	≤ 0.01**	0.373	≤ 0.01**
Hormonal status												
Hormones Receptor Positive	0.369	≤ 0.01**	0.092	0.281	0.113	0.167	0.285	≤ 0.01**	0.191	≤ 0.05*	0.187	≤ 0.01**
Triple Negative	0.519	≤ 0.05*	−0.079	0.613	0.335	0.664	0.232	0.202	0.527	≤ 0.05*	0.411	≤ 0.05*

Table 2. Correlation between GLS1, GLS2, GLUT-1, and Ki-67 in tumor tissues. $^{**}P \leq .01$, $^{*}P \leq .05$ were considered statistically significant.

In HER2-negative tumors, there were notable interactions between GLS2 and both GLUT-1 and Ki-67. GLS2 and GLUT-1 were positively correlated (Rho = 0.320, $P \leq .01$), indicating that these markers may work together to fulfill the metabolic demands of HER2-negative tumor cells. Additionally, GLS2 and Ki-67 were significantly correlated (Rho = 0.424, $P \leq .01$), suggesting that GLS2 is linked to cell proliferation in HER2-negative breast cancer, potentially supporting tumor growth by facilitating metabolic adaptability.

Among clinical stages, specific patterns were evident. In stage 2 tumors, strong correlations were identified between GLS2 and GLUT-1 (Rho = 0.312, $P \leq .01$), GLS2 and Ki-67 (Rho = 0.373, $P \leq .01$), and GLUT-1 and Ki-67 (Rho = 0.329, $P \leq .01$). These relationships in stage 2 tumors indicate that GLS2 and GLUT-1 may collectively contribute to the metabolic flexibility and proliferative potential of tumors as they progress, underscoring the role of these metabolic markers early in disease advancement. This suggests that targeting GLS2 and GLUT-1 in early-stage tumors may be effective in slowing tumor progression by disrupting essential metabolic pathways.

In HR + tumors, significant correlations were also observed, emphasizing the potential role of GLS2 in supporting both metabolic and proliferative demands. In this subgroup, GLS2 showed a positive correlation with GLUT-1 (Rho = 0.285, $P \leq .01$) and Ki-67 (Rho = 0.191, $P \leq .05$), suggesting that, even in HR + tumors, GLS2 may drive key metabolic processes. This finding supports the hypothesis that, regardless of hormone receptor status, GLS2 may enhance glucose metabolism through GLUT-1 and contribute to tumor cell proliferation.

Discussion

In this study, we evaluated the expression of key metabolic markers-GLUT-1, GLS1, and GLS2 in breast cancer tissues from Jordanian patients. Our findings demonstrate significant overexpression of all three markers in tumor tissues compared to adjacent normal tissue, consistent with the established hallmark of metabolic reprogramming in cancer[22]. This highlights the pivotal shift in energy metabolism that cancer cells undergo to sustain rapid proliferation and survival, and underscores the well-documented phenomenon of altered glucose and glutamine metabolism in cancer[9,10]. Furthermore, this work provides new insights into metabolic behavior in breast cancers from a Middle Eastern population that is underrepresented in the literature.

GLUT-1 overexpression was associated with higher tumor grade and was most prominent in TNBC and basal-like subtypes, which are known for their aggressive nature. These results are consistent with previous reports linking GLUT-1 to hypoxia tolerance, increased glycolytic activity, and poor clinical outcomes[23,24]. Interestingly, in our study, HER2-enriched tumors showed the second-highest GLUT-1 levels, suggesting a possible role for GLUT-1 in supporting HER2-driven tumor progression, although no significant association with HER2 status alone was observed. Luminal subtypes, by contrast, demonstrated relatively lower GLUT-1 expression. The significant correlation between GLUT-1 and negative ER/PR status further emphasizes its preferential upregulation in hormone receptor-negative cancers, where increased glycolysis may compensate for the lack of hormone-driven growth. Similar patterns of GLUT-1 overexpression in high-grade, hormone receptor-negative tumors have been described in other populations, including those from Saudi Arabia and French Guiana[6,25]. These studies confirm that the role of GLUT-1 as a marker of aggressiveness extends beyond Western cohorts and is observable in ethnically and regionally diverse populations, including our Jordanian cohort. This consistency across populations strengthens the case for GLUT-1 as a robust prognostic and potentially therapeutic marker in breast cancer.

Regarding glutaminase isoforms, both GLS1 and GLS2 were significantly overexpressed in breast cancer tissues compared to normal tissues, with high expression thresholds set at an H-score of 75 for GLS1 and 150 for GLS2. However, in contrast to several published studies that report an association between GLS1 expression and tumor aggressiveness, particularly in MYC-driven cancer[26], our results showed no significant correlation between GLS1 expression and clinicopathological features such as tumor stage, hormone receptor status, or molecular subtype. This discrepancy may reflect a population-specific metabolic adaptation, where GLS1 serves a baseline anabolic function rather than acting as a progression marker. This interpretation is supported by studies showing that certain metabolic regulators vary by ethnicity. For example, Shuch et al. reported distinct frequencies of fumarate hydratase mutations among patients of different ancestries, with clinical implications for renal tumor biology[27]. Similarly, Bardella et al. highlighted variability in SDH mutation patterns and metabolic impact across populations[28].

More directly related to glutamine metabolism, population-specific differences in metabolic profiles have been documented by Terunuma et al., who employed an unbiased metabolomics approach to compare metabolic profiles between African-American and European-American breast cancer patients[29]. Their analysis revealed that 2-hydroxyglutarate, a well-characterized oncometabolite associated with MYC activation and glutamine dependency was significantly elevated in tumors from African-American patients. This metabolic alteration was accompanied by a distinct DNA methylation signature and stem cell–like transcriptional program, both of which are indicative of aggressive tumor behavior and poor prognosis. Importantly, this phenotype was not observed at the same frequency in tumors from European-American patients, suggesting a population-specific metabolic vulnerability. Additionally, differences in glutamine addiction and the reverse Warburg effect have also been reported to vary by population and tumor type, suggesting that metabolic dependencies are not uniform across ethnic backgrounds[30]. Taken together, these findings emphasize that tumor metabolic behavior is shaped not only by oncogenic programs but also by population-specific factors. In this light, our finding that GLS1 is consistently overexpressed in Jordanian breast cancer tissues but not associated with clinical aggressiveness may reflect a region-specific metabolic phenotype. This reinforces the importance of studying metabolic biomarkers across diverse populations to inform more accurate biomarker interpretation and therapeutic targeting.

In contrast, GLS2 emerged as a critical isoform with specific relevance in breast cancer. High GLS2 expression was associated with HER2-negative and TNBCs, reflecting a greater dependency on glutamine metabolism in these subtypes[14]. This pattern is significant in TNBC, where the lack of hormone receptors drives cells to adopt alternative metabolic pathways. Moreover, our finding that GLS2 expression is strongly associated with HER2-negative cases highlights its compensatory role in fueling tumor growth in the absence of HER2 signalling, contrasting with other studies where GLS2 was predominantly elevated in HER2-positive cases. This discrepancy suggests that population-specific factors may influence the metabolic adaptations in breast cancer, underscoring the need for region-specific therapeutic These findings suggest that GLS2's functional role may shift depending on the tumor's signaling and metabolic context, and that ethnic-specific differences may further influence these associations. Such population-specific insights are critical for developing precision oncology approaches tailored to the metabolic profiles of diverse patient populations.

The significant correlations observed between GLS2, GLUT-1, and Ki-67 further underline their roles in tumor proliferation and aggressiveness. The strong association of GLS2 and GLUT-1 with Ki-67 in TNBC and basal-like subtypes suggests that these markers contribute to the high proliferative capacity of these tumors by meeting their metabolic demands. Additionally, the positive correlation between GLS2 and GLUT-1 in Luminal subtypes suggests a coordinated interaction between glutaminolysis and glycolysis, facilitating metabolic flexibility and tumor growth. These findings are supported by prior research indicating that enhanced metabolic activity through these pathways is a hallmark of aggressive breast cancers.

In summary, this study highlights significant overexpression of GLUT-1, GLS1, and GLS2 in Jordanian breast cancer tissues, with subtype-specific associations observed for GLUT-1 and GLS2. The lack of prognostic correlation for GLS1 and the population-specific patterns seen in GLS2 expression suggest the presence of distinct metabolic phenotypes in this cohort.

The clinical impact of this work lies in its support for metabolic profiling as a valuable adjunct to molecular classification, particularly in underrepresented populations. Future studies should incorporate integrated omics approaches and examine the therapeutic response to metabolic inhibitors across diverse populations. Such efforts will be critical for advancing precision oncology strategies tailored not only to tumor subtype but also to the unique metabolic landscapes shaped by population background.

Data availability

The data supporting the findings of this study are available upon reasonable request. Researchers interested in accessing the data should contact the corresponding author, Dr. Haneen A. Basheer, at hbasheer@zu.edu.jo.

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Author contributions

H.A.B. and A.Z.A. conceptualized the study. H.A.B., S.A.B., M.A.A., S.B.A., and Q.M.S. performed the meth-

odology and data analysis. Supervision was carried out by H.A.B., A.Z.A., and S.A.B. H.A.B., A.Z.A., S.A.B., M.A.A., S.B.A., Q.M.S., L.E., and K.A. wrote the original draft. H.A.B., A.Z.A., S.A.B., M.A.A., S.B.A., Q.M.S., L.E., and K.A. reviewed and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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