Article

Gamma-Glutamyl Hydrolase (GGH) Overexpression in Luminal B Breast Cancer: A New Marker of Aggression and a Therapeutic Target

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Abstract: Introduction. Breast cancer is the most common malignant tumor in women, particularly in Algeria, where it ranks first in terms of incidence. It is classified into molecular subtypes (Luminal A, Luminal B, HER2, and Triple-Negative), which guide therapeutic decisions. Gamma-glutamyl hydrolase (GGH), an enzyme involved in folate metabolism, has been proposed as a biomarker in certain hormone-dependent cancers, but its role in breast cancer, especially the Luminal B subtype, remains poorly understood. This first Algerian study aims to evaluate the immunohistochemical expression of GGH in invasive breast cancer tissues and to analyze its correlation with clinicopathological features, particularly molecular subtypes. Materials and Methods. GGH expression was analyzed by immunohistochemistry on paraffin-embedded tissue samples, and the H-score was calculated based on the intensity and proportion of stained tumor cells. Results were correlated with clinicopathological parameters using the Chi-square test. Results. GGH overexpression was observed in 61.66% of cases, while 38.33% showed no expression. A significant association was found between GGH overexpression and estrogen receptor-positive tumors (89.18%, $p = 0.011$), high proliferation index (94.59%, $p < 0.001$) and the Luminal B molecular subtype (83.78%, $p < 0.001$). Conclusion. GGH expression is significantly increased in Luminal B breast cancers, suggesting its involvement in tumor progression. These findings highlight the potential of GGH as a prognostic biomarker and a promising therapeutic target, particularly for patients with the Luminal B subtype. Further studies are needed to validate these results on a larger scale and to explore the underlying molecular mechanisms.

Keywords: breast cancer; luminal B; GGH; overexpression; H score

1. Introduction

Breast cancer is the most common malignant tumor among women worldwide and represents a major public health concern. Its incidence has increased significantly over the past two decades [1]. In Algeria, breast cancer ranks as the leading cancer in women, highlighting the urgent need for improved screening, diagnosis, and management strategies [2].

Based on a pioneering study by Perou et al., later validated by several transcriptomic analyses, breast tumors are classified into intrinsic molecular subtypes: Luminal A, Luminal B, HER2-enriched, and triple-negative. This classification plays a critical role in guiding therapeutic decisions and predicting response to treatment [3]. Several well-established markers, including axillary lymph node status, tumor size, histological grade, hormone receptor status, and HER2 status, are used for prognostic evaluation, diagnosis, and treatment decisions. However, it should be noted that several new prognostic markers highlight the heterogeneity of breast cancer, particularly luminal B breast cancer, which is in greater need of therapeutic advances. One of the objectives of research on luminal B cancer is the identification of new biological markers and new therapeutic targets to improve patient survival. Gamma-glutamyl hydrolase (GGH) is a lysosomal enzyme involved in the metabolism of folates and anti-folates. It acts as an endo- and/or exo-peptidase to cleave the gamma-polyglutamate chains that are attached to folates and

anti-folates after they enter a mammalian cell. While the addition of multiple glutamates is necessary to allow the cell to retain folates and anti-folates [4], GGH plays an important role in cellular folate homeostasis. By cleaving polyglutamates from extracellular folate-polyglutamates, it enables their import into the cell [5]. Studies have demonstrated a higher GGH expression in certain subtypes of breast cancer, particularly in more aggressive and hormone receptor-negative tumors, where it has been associated with reduced responsiveness to folate-based chemotherapy agents such as methotrexate [6]. Lung Cancer: In non-small cell lung cancer (NSCLC), elevated GGH levels have been linked to increased resistance to pemetrexed, a chemotherapy drug that works through folate metabolism [7]. Some studies have explored GGH inhibition as a strategy to overcome resistance. *In vitro* and *in vivo* models have shown that GGH inhibitors, when combined with chemotherapy, can restore sensitivity to methotrexate and other antifolate drugs [8]. A previous study demonstrated that GGH activity is epigenetically regulated through promoter methylation in acute lymphoblastic leukemia (ALL) cells. Specifically, hypermethylation of CpG1 and CpG2 islands within the GGH promoter region was associated with a marked downregulation of GGH expression, leading to a significant accumulation of methotrexate (MTX) polyglutamates. This alteration in drug metabolism may have important implications for treatment efficacy and resistance mechanisms in ALL [9]. Dysregulated glutamatergic signaling has been implicated in tumor proliferation and survival through the activation of MAPK and PI3K/Akt pathways, particularly in gliomas [10].

We hypothesize that GGH overexpression is significantly associated with the Luminal B molecular subtype in breast cancer and correlates with poor prognosis markers, including high proliferation index (Ki67), estrogen receptor (ER) positivity, and lymph node infiltration. Furthermore, we believe that GGH could serve as a valuable prognostic marker and a potential therapeutic target for patients with Luminal B subtype breast cancer.

This study aims to evaluate the immunohistochemical expression of GGH in breast cancer tissues and its correlation with clinicopathological features, focusing on the Luminal B subtype. To our knowledge, this is the first cohort study in Algeria to explore the relationship between GGH expression and breast cancer molecular subtypes, addressing a significant gap in the literature and providing valuable insights into its potential role in breast cancer management.

2. Material and Methods

This study included a total of 60 cases of invasive breast carcinoma collected from female patients who underwent mastectomy and were diagnosed with breast cancer at the Pathology Department of the University Hospital of Sidi Bel Abbes, between 1 January 2018, and 31 December 2023. The selection was made to ensure high quality and completeness of clinical and pathological data.

Cases were excluded from the study based on several criteria to ensure data quality and consistency. These included the absence of sufficient tumor tissue for analysis, incomplete or missing clinical records, and male breast cancer, which was excluded to maintain a homogeneous cohort focused exclusively on female breast pathology. Additionally, patients who had received chemotherapy prior to tissue sampling were excluded in order to avoid any potential bias in the immunohistochemical assessment of GGH expression, as treatment could alter the natural expression profile of the protein

The final study cohort consisted of female patients aged between 25 and 89 years, with a median age of 59 years. Key histoclinical features—such as histological subtype, tumor size, lymph node involvement, SBR grade, and metastatic status—were retrieved from patient medical records.

All data were collected with strict adherence to patient confidentiality, and the study was conducted under ethical approval granted by the relevant institutional review board.

GGH expression was assessed by immunohistochemistry (IHC) using tissue sections from formalin-fixed, paraffin-embedded breast tumor samples. To ensure the reliability and specificity of the immunostaining procedure, several internal validation steps were implemented:

The anti-GGH antibody was previously validated on human tissues known to express GGH, such as liver, kidney, and breast cancer tissues.

All immunostained slides were independently reviewed by two experienced pathologists, blinded to clinical data, to minimize observer bias. Discrepancies in scoring were resolved by consensus.

2.1. Immunohistochemistry (IHC)

Tissue imaging using immunohistochemical (IHC) staining is the most common approach to characterize the expression of a specific protein in tissues [11]. IHC staining for GGH was performed on 2-µm tissue sections prepared from formalin-fixed, paraffin-embedded tissue blocks. The slides were deparaffinized in xylene baths, rehydrated in acetone baths, and then incubated in a boiling antigen retrieval solution (Citrate Buffer, (10×, pH

6.0）for 40 min. Cooling was done at room temperature for 20 min, followed by rinsing with a washing solution (Phosphate-Buffered Saline, PBS, Ref. SLCB9214; Sigma-Aldrich, Saint Louis, MO, USA). The primary antibody used was rabbit GGH antibody (anti-GGH C-terminal, 1:100, Sigma-Aldrich, Saint Louis, MO, USA), and the secondary antibody was rabbit HRP (1:1000, Sigma-Aldrich, Saint Louis, MO, USA). Peroxidase activity was revealed by applying the chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB) for 15 min, followed by rinsing with water and counterstaining with hematoxylin. The slides were mounted with coverslips, and the readings were performed separately by two pathologists.

Immunoreactivity was defined as negative when no staining was observed. Cytoplasmic staining in less than 10% of cells was also considered negative. Immunoreactivity was considered positive when observed in more than 10% of invasive tumor cells. Cytoplasmic staining was classified as positive or negative for subsequent statistical analyses. Therefore, GGH overexpression was defined as positive cytoplasmic staining in more than 10% of invasive tumor cells. The H-score was calculated to provide a semi-quantitative assessment of staining intensity and distribution and was used for descriptive purposes only.

Immunostained slides were evaluated for cytoplasmic staining by intensity (1 = weak, 2 = moderate, 3 = strong) and percentage of invasive tumor cells stained for each intensity. The H-score was calculated as follows: (1 × percentage of weak staining) + (2 × percentage of moderate staining) + (3 × percentage of strong staining), yielding a score ranging from 0 to 300 [12].

GGH expression was evaluated by immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections. For statistical analysis, GGH positivity was defined as cytoplasmic staining in more than 10% of invasive tumor cells. The H-score (range 0–300) was calculated to provide a semi-quantitative assessment of staining intensity and distribution [13], listed as follows:

- Luminal A: ER+, PR+/−, HER2−, Ki67 < 20%
- Luminal B: ER+, PR+/−, HER2−, Ki67 ≥ 20% or ER+, PR+/−, HER2+, regardless of Ki67 status
- Triple-negative breast cancer (TNBC): ER−, PR−, HER2−
- HER2 subtype: ER−, PR−, HER2+

2.2. Statistical Analyses

All variables were summarized using descriptive statistics. Qualitative variables are presented in terms of proportions. Initially, a descriptive analysis was conducted regarding: epidemiological characteristics, lesion location, histological type, SBR grade, TNM classification, and lymph node infiltration. The second step involved a univariate analysis to determine the correlation between the expression of GGH protein and various clinicopathological parameters using the standard chi-square test and Fisher's exact test. Statistical significance was determined with $p < 0.05$

Survival data were retrospectively collected from patient medical records. Overall survival (OS) was defined as the time from diagnosis to death from any cause or last follow-up. Kaplan–Meier survival curves were generated and compared using the Log-rank test. Patients who were alive at the last follow-up were censored.

3. Results

Evaluation of the Immunohistochemical Status of the GGH Protein in Breast Cancer For this study, primary invasive breast cancers were included in the cohort, with 60 cases providing detailed clinical and pathological information, as summarized in Table 1.

Missing values were mainly due to unavailable information after review of the pathological reports. The mean age was 55 years (range 25–89), and the median age was 59 years.

Molecular classification of breast cancer was determined by IHC staining in 60 invasive cancers with complete data. Of these, 11 (18.33%) were Luminal A, 36 (60%) were Luminal B, 6 (10%) were HER2-positive, and 7 (11.66%) were Triple-negative, as shown in Figure 1.

Different levels of GGH (Gamma-glutamyl hydrolase) cytoplasmic staining in invasive carcinomas are shown in Figure 2.

In our cohort of 60 invasive breast carcinomas, GGH overexpression was observed in 61.66% of cases ($n = 37$), as shown in Figure 3 and detailed in Table 2. A detailed analysis of the clinicopathological data revealed several important associations. GGH expression was significantly correlated with estrogen receptor (ER) positivity, with 89.18% of GGH-positive tumors expressing ER, compared to 60.86% of GGH-negative tumors ($p = 0.011$), indicating a strong link between hormonal sensitivity and GGH overexpression.

A particularly notable finding was the strong association between GGH expression and tumor proliferation. Among GGH-positive tumors, 94.59% exhibited a high Ki-67 index (≥20%), in contrast to only 43.47% of

GGH-negative tumors ($p < 0.001$). This suggests that GGH overexpression is linked to increased cellular proliferation and a more aggressive tumor phenotype.

Table 1. Clinicopathological characteristics of patients with invasive breast carcinoma.

Histoclinical Characteristics		Number of Cases (%)
Age	<50	20 (33.33)
	≥50	40 (66.66)
Breast localization	Left	29 (48.33)
	Right	31 (51.66)
Histological types	IC-NST	53 (88.33)
	ILC	6 (10)
	Other	1 (1.66)
Tumor size	<2 cm	19 (31.66)
	[2–5] cm	34 (56.66)
	≥5 cm	7 (11.66)
Pathological axillary lymph node	Negative	24 (40)
	Positive	36 (60)
SBR grade	I	3 (5)
	II	54 (90)
	III	3 (5)
Estrogen receptor status	Negative	13 (21.66)
	Positive	47 (78.33)
Progesterone receptor status	Negative	19 (31.66)
	Positive	41 (68.33)
HER2 receptor status	Negative	42 (70)
	Positive	18 (30)
Ki 67	<20%	15 (25)
	≥20%	45 (75)
Molecular classification	Luminal A	11 (18.33)
	Luminal B	36 (60)
	HER 2 like	6 (10)
	Triple negative	7 (11.66)

Table 2. Association of GGH expression and clinicopathological features of our patients.

Clinical Features		GGH^- 23 Number (%)	GGH^+ 37 Number (%)	*p* Value
Age	<50 years old	5 (21.73)	15 (40.54)	0.133
	≥50 years old	18 (78.26)	22 (59.45)	
Breast localization	Left	13 (56.52)	16 (43.24)	0.317
	Right	10 (43.47)	21 (56.76)	
Histological type	IC-NST	21 (91.30)	32 (86.48)	0.697
	ILC	2 (8.69)	4 (10.81)	
	OTHER TYPE	0	1 (2.70)	
Tumor size	<2 cm	5 (21.73)	14 (37.83)	0.111
	[2–5] cm	13 (56.52)	21 (56.75)	
	≥5 cm	5 (21.73)	2 (5.41)	
Pathological axillary lymph node	Negative	12 (52.17)	12 (32.43)	0.129
	Positive	11 (47.82)	25 (67.57)	
SBR grade	I	2 (8.69)	1 (2.70)	0.580
	II	20 (86.95)	34 (91.89)	
	III	1 (4.34)	2 (5.40)	
Estrogen receptor status	Negative	9 (39.13)	4 (10.81)	0.011
	Positive	14 (60.86)	33 (89.18)	
Progesterone receptor status	Negative	10 (43.47)	9 (24.32)	0.121
	Positive	13 (56.52)	28 (75.67)	
HER2 receptor status	Negative	14 (60.86)	28 (75.67)	0.224
	Positive	9 (39.13)	9 (24.32)	
Ki 67	<20%	13 (56.52)	2 (5.40)	< 0.001
	≥20%	10 (43.47)	35 (94.59)	
Molecular classification	Luminal A	9 (39.13)	2 (5.40)	< 0.001
	Luminal B	5 (21.73)	31 (83.78)	
	HER 2 like	4 (17.39)	2 (5.40)	
	Triple negative	5 (21.73)	2 (5.40)	

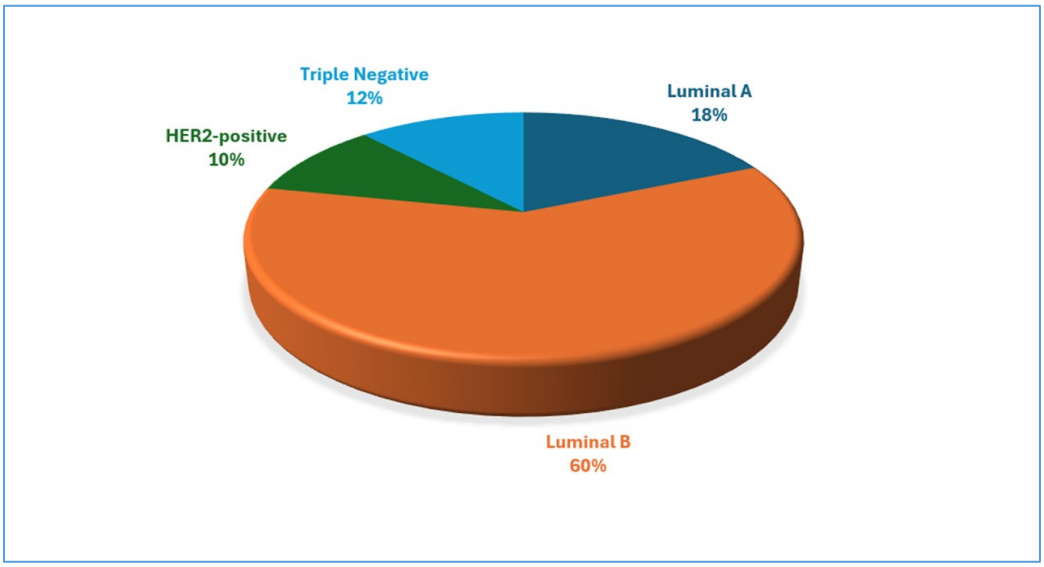

Figure 1. Distribution of molecular subtypes in our cohort of 60 invasive breast carcinomas.

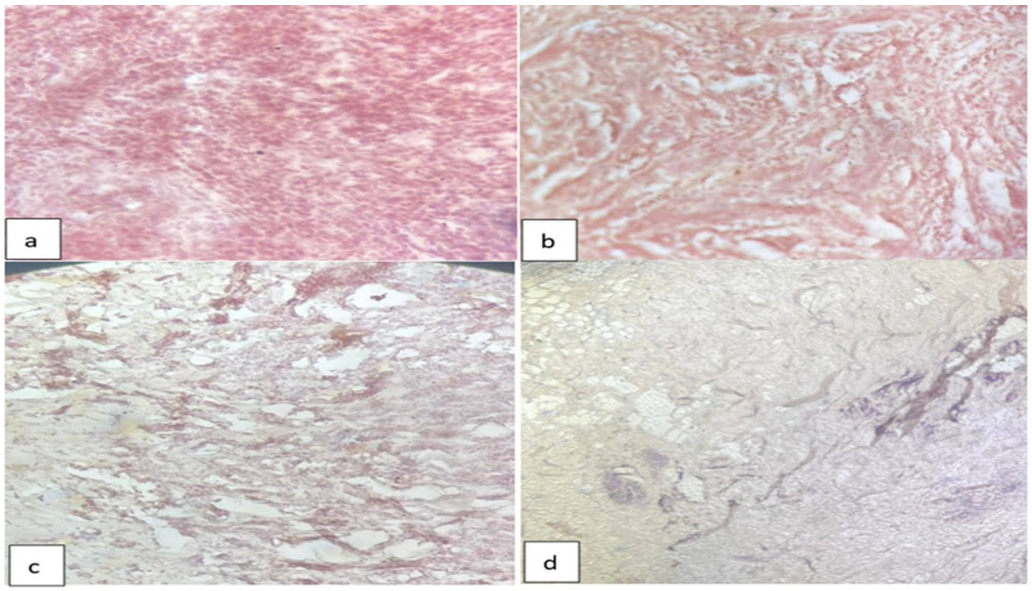

Figure 2. Cytoplasmic immunohistochemical staining of GGH in invasive breast carcinomas: (**a**) strong staining; (**b**) moderate staining; (**c**) moderate to weak staining; (**d**) weak staining.

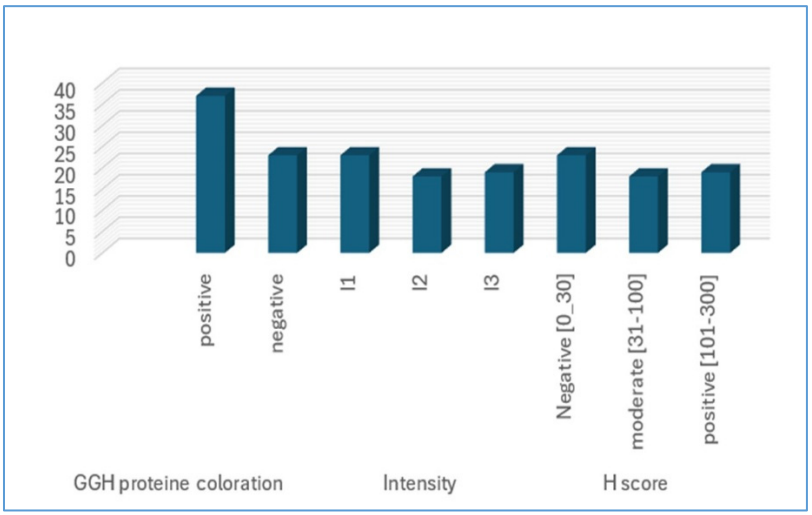

Figure 3. Distribution of GGH immunostaining according to H-score thresholds used to define positivity and overexpression.

No significant association was observed between GGH expression and tumor size ($p > 0.05$). Regarding lymph node involvement, 67.57% of GGH-positive tumors showed axillary lymph node infiltration, compared to 47.82% of GGH-negative tumors. While this difference was not statistically significant ($p = 0.129$), it suggests a potential pattern worth further investigation in larger cohorts. No significant associations were found between GGH expression and SBR grade or tumor laterality.

When examining molecular subtypes, GGH expression displayed a particularly strong association with the Luminal B subtype. Of all GGH-positive tumors, 83.78% belonged to this category, compared to only 21.73% among GGH-negative tumors ($p < 0.001$). Conversely, Luminal A (39.13%) and Triple Negative (21.73%) subtypes were more frequently represented in GGH-negative tumors, suggesting that GGH overexpression is characteristic of more aggressive, highly proliferative tumors, particularly within the Luminal B group.

As shown in Figure 4, a Kaplan–Meier survival analysis was performed to evaluate overall survival (OS) according to GGH expression. The Log-rank test showed a statistically significant difference between the two groups ($p = 0.009$), indicating that patients with GGH overexpression had reduced overall survival. These results suggest that GGH overexpression may be associated with poorer clinical outcomes.

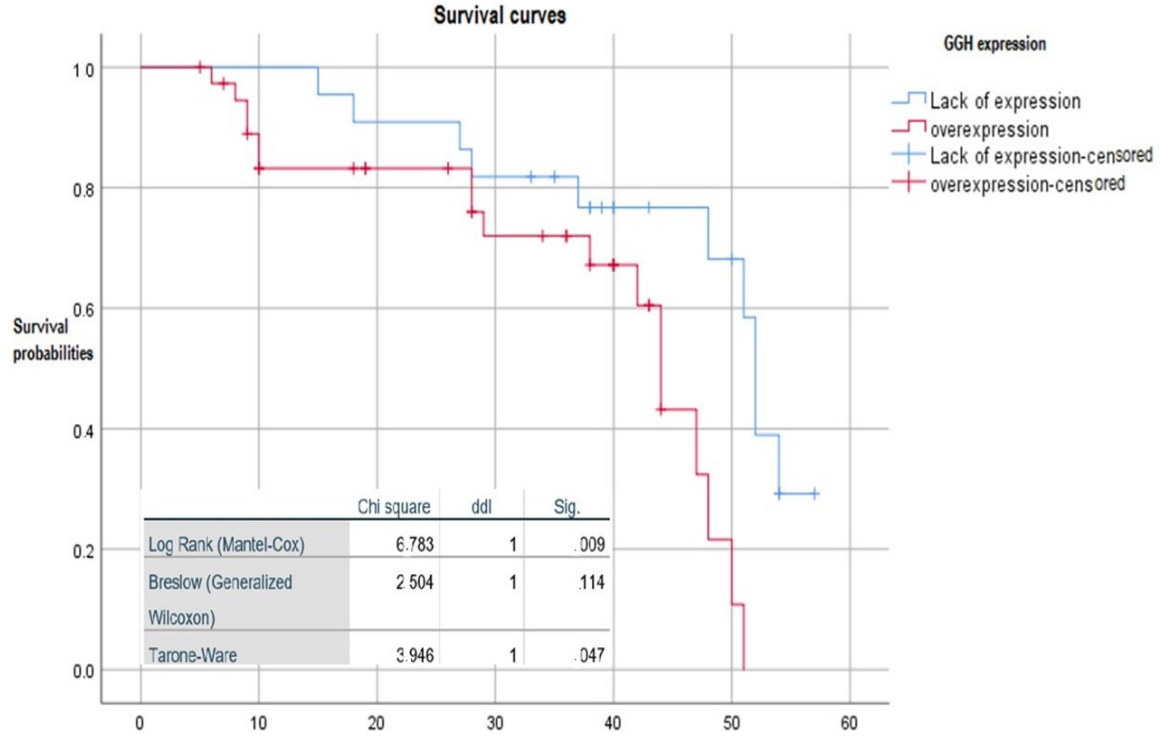

Figure 4. Kaplan–Meier curves of overall survival (OS) in breast cancer patients according to GGH expression status.

4. Discussion

This study highlights several key epidemiological and biological features of breast cancer within the Algerian female population. The mean age at diagnosis was 55 years, with most cases occurring in women aged between 50 and 59 years. This trend is consistent with international epidemiological data showing that breast cancer incidence increases with age, particularly after menopause, suggesting a potential role of hormonal changes in tumor development [14]. A high proportion of tumors were large at diagnosis (>2 cm in 68.33% of cases), suggesting delayed diagnosis, likely related to limited access to organized screening programs and late presentation. Histologically, SBR grade II tumors were most frequent (90%), indicating moderately differentiated tumors with intermediate aggressiveness. However, the presence of 5% grade III tumors indicates a smaller subset of poorly differentiated and more aggressive lesions.

Lymph node dissection revealed a high rate of nodal involvement, further supporting the hypothesis of late-stage presentation. As described in the literature, lymph node status remains one of the most critical prognostic indicators, with a direct impact on recurrence risk and survival [15]. Our findings of frequent nodal infiltration underscore the urgency of implementing more robust screening and early intervention strategies.

Molecular profiling revealed that GGH (gamma-glutamyl hydrolase) was overexpressed in a significant number of cases. Notably, overexpression was more commonly observed in ER/PR-positive tumors and HER2-

negative subtypes, with a strong correlation with high Ki-67 proliferation index, indicating enhanced tumor cell proliferation. These observations are consistent with the growing recognition of breast cancer as a heterogeneous disease driven by multiple molecular alterations that shape its phenotype and clinical behavior [16,17].

The role of GGH in cancer has been increasingly explored. Physiologically, GGH contributes to folate and antifolate metabolism, maintaining intracellular folate homeostasis. Its dysregulation has been documented in a variety of cancers, including urothelial bladder carcinoma [18], pulmonary neuroendocrine tumors [19], and hepatocellular carcinoma, where elevated GGH levels were associated with poor clinical outcomes.

Our results echo previous studies showing that GGH expression is significantly elevated in malignant breast tissue compared to adjacent non-cancerous tissue [20], and that this overexpression may represent a key event in breast carcinogenesis [21].

No statistically significant association was observed between GGH expression and histological type (p = 0.697), although a higher frequency of expression was noted in invasive carcinoma of no special type (IC-NST). Additionally, GGH overexpression was more frequently observed in high-grade tumors. These findings are consistent with those reported by Shubbar et al. [22], who also observed increased GGH levels in aggressive tumor phenotypes.

From a molecular standpoint, GGH overexpression was most significantly associated with the luminal B subtype, followed by triple-negative and luminal A subtypes. This distribution is important, as luminal B tumors are known for their aggressive behavior and poorer prognosis compared to luminal A, despite hormone receptor positivity. Interestingly, our results indicate that tumors overexpressing GGH are more proliferative and potentially more aggressive. This observation reinforces the idea that GGH could serve not only as a biomarker for aggressive disease but also as a potential therapeutic target, particularly in luminal B tumors, where treatment options remain limited [23].

These findings carry several clinical implications. First, GGH may serve as a useful prognostic biomarker, identifying patients at risk for more aggressive disease and poor outcomes. Second, its expression could aid in refining molecular classification and informing treatment decisions, especially in distinguishing between luminal A and B subtypes. Finally, targeting GGH may open new therapeutic avenues, particularly for patients with high-proliferation, hormone receptor-positive tumors.

Despite these promising results, several limitations must be acknowledged. The study sample was relatively small (n = 60) and drawn from a single institution, which may limit generalizability. Additionally, while immunohistochemistry provided reliable expression data, functional studies are necessary to validate the biological role of GGH in tumor progression. Finally, further research involving larger, multi-center cohorts and diverse populations is needed to confirm the clinical utility of GGH as a biomarker or therapeutic target in breast cancer.

The observed association between GGH overexpression and aggressive breast tumor characteristics—such as high proliferation index (Ki67), Luminal B subtype predominance, and ER positivity—may be explained by several biochemical and signaling mechanisms.

GGH is a key enzyme in folate homeostasis, catalyzing the hydrolysis of polyglutamated folates into monoglutamate forms that can exit the cell or be used in nucleotide biosynthesis. Cancer cells have a high demand for nucleotides to support rapid proliferation. By modulating intracellular folate pools, GGH facilitates increased thymidine and purine synthesis, essential for DNA replication and repair [24,25].

GGH has been implicated in resistance to antifolate chemotherapy agents, such as methotrexate and pemetrexed. By deconjugating polyglutamated antifolates, GGH reduces their retention and efficacy within tumor cells [26,27].

GGH activity increases the extracellular concentration of glutamate, a byproduct of polyglutamate hydrolysis. High levels of extracellular glutamate can activate glutamate receptors on cancer cells, triggering MAPK/ERK and PI3K/Akt signaling pathways [28].

There is evidence that GGH expression may be regulated by epigenetic modifications in breast cancer, particularly in ER-positive tumors [28,29].

Through its role in folate metabolism, GGH may also influence redox balance and one-carbon metabolism, processes crucial for rapidly dividing cancer cells [29].

Several published studies support the findings of our analysis, showing that GGH overexpression is associated with reduced overall survival in various cancer types.

In endometrial cancer, GGH was associated with poor prognosis [30]. In gastric cancer, high GGH expression was an independent prognostic factor [31]. In prostate cancer, GGH was associated with more aggressive tumor features in ERG-negative patients [32].

Taken together, these mechanisms suggest that GGH overexpression not only marks aggressive biological behavior but may actively contribute to it. The strong correlation with Luminal B subtype supports its potential role in therapy resistance and tumor progression.

Our findings position GGH as a potential biomarker for aggressiveness in breast cancer, especially in Luminal B tumors, which are known for their poorer prognosis and lower sensitivity to endocrine therapy compared to Luminal A. If validated in larger cohorts, GGH could serve as a stratification marker to guide more personalized therapeutic decisions. It may also offer a target for novel therapies aimed at disrupting folate metabolism or glutamate signaling in aggressive subtypes.

5. Conclusions

In conclusion, this study focused on the potential involvement of the GGH protein in breast cancer. We demonstrated, for the first time, that GGH expression is significantly elevated in the luminal B subtype of breast cancer. Furthermore, we showed that GGH overexpression is associated with aggressive clinicopathological features.

These findings suggest an association between GGH overexpression and primary invasive tumors, as well as highly proliferative and aggressive tumor characteristics.

These findings suggest that GGH may serve as both a prognostic biomarker and a promising therapeutic target for luminal B breast cancer. To enhance the clinical relevance of these results, future studies should emphasize the functional validation of GGH through *in vitro* and *in vivo* models. Such investigations are essential to determine whether GGH plays a causal role in tumor progression and to evaluate its potential for therapeutic targeting.

Author Contributions: R.R.: Conceptualization, methodological design, investigation, sample collection, data analysis, and manuscript writing. A.B.: Data interpretation, statistical analysis, and critical review of the manuscript. S.A.S.: Methodological support, data curation, statistical analysis, and manuscript editing. S.K.: Investigation, data analysis. Z.M.: Histopathological evaluation, IHC score validation, and clinical data acquisition. S.H.B.: Histopathological evaluation, IHC score validation, and clinical data acquisition. T.S.: Supervision, project administration, funding search, and final manuscript approval. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of the University Hospital of Sidi Bel Abbès, Algeria (Ref: No. 10/for 4 November 2023). All patient data were anonymized to ensure confidentiality. Informed consent was not required as the study involved a retrospective analysis of archived medical records and tissue samples, in line with institutional ethical guidelines for secondary use of patient data in biomedical research.

Informed Consent Statement: Patient consent was waived due to the retrospective nature of the study and the use of anonymized archived tissue samples, in accordance with the ethical guidelines of the University Hospital of Sidi Bel Abbès, Algeria.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. https://doi.org/10.3322/caac.21660.
2. International Agency for Research on Cancer (IARC). Available online: https://gco.iarc.who.int/media/globocan/factsheets/populations/12-algeria-fact-sheet.pdf (accessed on 22 June 2025).
3. Perou, C.M.; Sørlie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S.; Rees, C.A.; Pollack, J.R.; Ross, D.T.; Johnsen, H.; Akslen, L.A.; et al. Molecular Portraits of Human Breast Tumours. *Nature* **2000**, *406*, 747–752. https://doi.org/10.1038/35021093.

4. Galivan, J.; Ryan, T.J.; Chave, K.; Rhee, M.; Yao, R.; Yin, D. Gamma-glutamyl hydrolase: Pharmacological role and enzymatic characterization. *Pharmacol. Ther.* **2000**, *85*, 207–215.
5. Zhao, R.; Goldman, I.D. Resistance to antifolates. *Oncogene* **2003**, *22*, 7431–7457.
6. Cole, P.D.; Kamen, B.A.; Gorlick, R.; Banerjee, D.; Smith, A.K.; Magill, E.; Bertino, J.R. Effects of overexpression of γ-glutamyl hydrolase on methotrexate metabolism and resistance. *Cancer Res.* **2001**, *61*, 4599–4604.
7. Liang, J.; Lu, T.; Chen, Z.; Zhan, C.; Wang, Q. Mechanisms of resistance to pemetrexed in non-small cell lung cancer. *Transl. Lung Cancer Res.* **2019**, *8*, 1107–1118.
8. Kim, S.E.; Cole, P.D.; Cho, R.C.; Ly, A.; Ishiguro, L.; Sohn, K.J.; Croxford, R.; Kamen, B.A. γ-Glutamyl hydrolase modulation and folate influence chemosensitivity of cancer cells to 5-fluorouracil and methotrexate. *Br. J. Cancer* **2013**, *109*, 2175–2188.
9. Cheng, Q.; Cheng, C.; Crews, K.R.; Ribeiro, R.C.; Pui, C.H.; Relling, M.V.; Evans, W.E. Epigenetic regulation of human gamma-glutamyl hydrolase activity in acute lymphoblastic leukemia cells. *Am. J. Hum. Genet.* **2006**, *79*, 264–274.
10. Prickett, T.D.; Samuels, Y. Molecular pathways: Dysregulated glutamatergic signaling pathways in cancer. *Clin. Cancer Res.* **2012**, *18*, 4240–4246.
11. Graham, R.C., Jr.; Karnovsky, M.J. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **1966**, *14*, 291–302.
12. McCarty, K.S., Jr.; Miller, L.S.; Cox, E.B.; Konrath, J.; McCarty, K.S., Sr. Estrogen receptor analyses: Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch. Pathol. Lab. Med.* **1985**, *109*, 716–721.
13. Dowsett, M.; Nielsen, T.O.; A'Hern, R.; Bartlett, J.; Coombes, R.C.; Cuzick, J.; Ellis, M.; Henry, N.L.; Hugh, J.C.; Lively, T.; et al. Assessment of Ki67 in breast cancer: Recommendations from the International Ki67 in Breast Cancer Working Group. *J. Natl. Cancer Inst.* **2011**, *103*, 1656–1664.
14. Guerin, S.; Doyon, F.; Hill, C. The frequency of cancer in France in 2006, mortality trends since 1950, incidence trends since 1980 and analysis of the discrepancies between these trends. *Bull.* Cancer **2009**, *96*, 51–57. https://doi.org/10.1684/bdc.2008.0795.
15. Carter, C.L.; Allen, C.; Henson, D.E. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. *Cancer* **1989**, *63*, 181–187.
16. Guinebretière, J.M.; Menet, E.; Tardivon, A.; Cherel, P.; Vanel, D. Normal and pathological breast: Histological basis. *Eur. J. Radiol.* **2005**, *54*, 6–14.
17. Chen, D.-T.; Nasir, A.; Venkataramu, C.; Fulp, W.J.; Gruidl, M.; Yeatman, T.J. Evaluation of Malignancy-Risk Gene Signature in Breast Cancer Patients. *Breast Cancer Res. Treat.* **2010**, *120*, 25–34. https://doi.org/10.1007/s10549-009-0357-6.
18. Pollard, C.; Nitz, M.; Baras, A.; Williams, P.; Moskaluk, C.; Theodorescu, D. Genoproteomic Mining of Urothelial Cancer Suggests γ-Glutamyl Hydrolase and Diazepam-Binding Inhibitor as Putative Urinary Markers of Outcome after Chemotherapy. *Am. J. Pathol.* **2009**, *175*, 1824–1830.
19. He, P.; Varticovski, L.; Bowman, E.D.; Fukuoka, J.; Welsh, J.A.; Kovatich, A.; De Togni, P.; Kemp, C.; Cuttitta, F.; Harris, C.C. Identification of carboxypeptidase E and γ-glutamyl hydrolase as biomarkers for pulmonary neuroendocrine tumors. *Hum. Pathol.* **2004**, *35*, 1196–1209. https://doi.org/10.1016/j.humpath.2004.06.014.
20. van't Veer, L.J.; Dai, H.; van de Vijver, M.J.; He, Y.D.; Hart, A.A.; Mao, M.; Peterse, H.L.; van der Kooy, K.; Marton, M.J.; Witteveen, A.T.; et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* **2002**, *415*, 530–536.
21. Andersen, J.N.; Sathyanarayanan, S.; Di Bacco, A.; et al. Pathway-Based Identification of Biomarkers for Targeted Therapeutics: Personalized Oncology with PI3K Pathway Inhibitors. *Sci. Transl. Med.* **2010**, *2*, 43ra55. https://doi.org/10.1126/scitranslmed.3001065.
22. Shubbar, E.; Hélou, K.; Kovács, A.; Nemes, S.; Hajizadeh, S.; Enerbäck, C.; Borg, Å. High levels of γ-glutamyl hydrolase (GGH) are associated with poor prognosis and unfavorable clinical outcomes in invasive breast cancer. *BMC Cancer* **2013**, *13*, 47. https://doi.org/10.1186/1471-2407-13-47.
23. Longley, D.B.; Harkin, D.P.; Johnston, P.G. 5-fluorouracil: Mechanisms of action and clinical strategies. *Nat. Rev. Cancer* **2003**, *3*, 330–338.
24. Trihia, H.; Murray, S.; Price, K.; Gelber, R.D.; Golouh, R.; Goldhirsch, A.; Coates, A.S.; Collins, J.; Castiglione-Gertsch, M.; Gusterson, B.A. Ki-67 expression in breast carcinoma. *Cancer* **2003**, *97*, 1321–1331. https://doi.org/10.1002/cncr.11188.
25. Karlsson, E.; Delle, U.; Danielsson, A.; Olsson, B.; Karlsson, P.; Helou, K. Gene expression variation to predict 10-year survival in lymph-node-negative breast cancer. *BMC Cancer* **2008**, *8*, 254. https://doi.org/10.1186/1471-2407-8-254.
26. Matherly, L.H.; Hou, Z.; Deng, Y. Human reduced folate carrier: Translation of basic biology to cancer therapy. *Cancer Metastasis Rev.* **2007**, *26*, 111–128.
27. Schneider, E.; Ryan, T.J. Gamma-glutamyl hydrolase and drug resistance. *Clin. Chim. Acta* **2006**, *374*, 25–32. https://doi.org/10.1016/j.cca.2006.05.044.

28. Yi, H.; Talmon, G.; Wang, J. Glutamate in Cancers: From Metabolism to Signaling. *J. Biomed. Res.* **2020**, *34*, 260–270. https://doi.org/10.7555/JBR.34.20190037.
29. Ducker, G.S.; Rabinowitz, J.D. One-carbon metabolism in health and disease. *Cell Metab.* **2017**, *25*, 27–42.
30. Yu, C.; Qi, H.; Zhang, Y.; Zhao, W.; Wu, G. Elevated Expression of Gamma-Glutamyl Hydrolase Is Associated with Poor Prognosis and Altered Immune Signature in Uterine Corpus Endometrial Carcinoma. *Front. Genet.* **2021**, *12*, 764194. https://doi.org/10.3389/fgene.2021.764194.
31. Maezawa, Y.; Sakamaki, K.; Oue, N.; Kimura, Y.; Hashimoto, I.; Hara, K.; Kano, K.; Aoyama, T.; Hiroshima, Y.; Yamada, T.; et al. High gamma-glutamyl hydrolase and low folylpolyglutamate synthetase expression as prognostic biomarkers in patients with locally advanced gastric cancer who were administrated postoperative adjuvant chemotherapy with S-1. *J. Cancer Res. Clin. Oncol.* **2020**, *146*, 75–86. https://doi.org/10.1007/s00432-019-03087-8.
32. Melling, N.; Rashed, M.; Schroeder, C.; Hube-Magg, C.; Kluth, M.; Lang, D.; Simon, R.; Möller-Koop, C.; Steurer, S.; Sauter, G.; et al. High-Level γ-Glutamyl-Hydrolase (GGH) Expression is Linked to Poor Prognosis in ERG Negative Prostate Cancer. *Int. J. Mol. Sci.* **2017**, *18*, 286. https://doi.org/10.3390/ijms18020286.