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Original article

Breast Cancer Molecular Subtypes in Libyan Women: Incidence and Prognostic Value of Securin and Separase

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Abstract
Breast cancer exhibits molecular heterogeneity, which influences prognosis and treatment response. In Libyan women, the distribution of molecular subtypes and the prognostic significance of proliferation-associated biomarkers such as Securin and Separase remain underexplored. This study aimed to evaluate the incidence of molecular subtypes of breast cancer and assess the prognostic value of Securin and Separase expression. A retrospective analysis was conducted on 162 Libyan female breast cancer patients. Clinicopathological features, demographics, and treatment data were collected. Molecular subtypes were classified using immunohistochemistry and in situ hybridization surrogates. Expression levels of Securin and Separase were assessed in tumor specimens, and associations with clinical outcomes and survival were analyzed using Kaplan–Meier survival curves and statistical correlation tests. The cohort predominantly consisted of premenopausal women under 50 years, with tumors ranging from 2 to 5 cm. Invasive lobular carcinoma was less common, and lymph node involvement was widespread. Molecular subtype distribution was as follows: Luminal A (49%), Luminal B (27%), triple-negative (15%), and HER2-enriched (9%). Hormone receptor-positive tumors were most frequent, suggesting potential responsiveness to endocrine therapy. Survival analysis demonstrated that Luminal A tumors had the highest 5-year survival, followed by Luminal B, whereas triple-negative and HER2-enriched subtypes had poorer outcomes. High cytoplasmic expression of Securin and Separase correlated with reduced survival, whereas nuclear expression showed variable trends. Securin and Separase expression was significantly associated with tumor stage, metastasis, and larger tumor size, but not with age, family history, tumor grade, or molecular subtype. These findings indicate that molecular subtyping in Libyan women aligns with global trends, with Luminal subtypes being predominant. The study highlights the potential of Securin and Separase as adverse prognostic biomarkers linked to tumor progression rather than demographic or subtype characteristics. The poorer outcomes of triple-negative and HER2-enriched tumors underscore the need for tailored therapeutic strategies. Molecular classification and proliferation biomarker assessment provide valuable prognostic insights in Libyan breast cancer patients. Evaluating Securin and Separase expression may improve risk stratification and guide personalized treatment approaches. Further studies are warranted to validate these biomarkers in larger cohorts.
Keywords: Breast cancer, Molecular subtypes, Libyan women, Securin, Separase, Prognosis, Survival.

Introduction

Being the most prevalent malignancy in women, breast cancer (BC) is one of the most researched tumors at all levels. Epidemiologically, it is the sixth greatest cause of cancer mortality globally, with an anticipated 2.3 million new cases and 685,000 deaths in 2020 [1,2]. Moreover, according to an analysis of data on breast cancer outcomes from the 2019 Global Burden of Disease Survey, which covered the years 1990–2019 in five major Asian countries; Brazil, Russia, India, China, and South Africa as well as 30 other Asian countries, there were 900,000 female cases of breast cancer and 350,000 million deaths in these regions in 2019 [3]. The countries with the highest percentage of incident cases and fatalities are China and India, with Pakistan coming in second. In most of these nations, the highest rates of breast cancer deaths in 2019 are caused by a diet heavy in red meat, a high body mass index, and high fasting plasma glucose [4]. Breast cancer still causes one-fifth of fatalities in affluent nations, despite screening programs and effective diagnostic techniques [5]. Currently, there is little and incomplete research on breast cancer in Libya. The distribution of cancer, especially breast cancer, in Libya still needs to be further illustrated because the country's cancer registry system is still in its infancy.

Furthermore, compared to Western nations, breast cancer in North Africa is characterized by more aggressive subtypes, an advanced stage at presentation, and an earlier age [6]. However, data supporting these observations is limited. Breast cancer comprises several subtypes, making it a highly heterogeneous disease. Over time, this diversity has become increasingly well recognized. In the early 2000s, Sørlie et al.,

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using global gene expression profiling, identified five intrinsic subtypes: luminal A, luminal B, normal breast-like, HER2-enriched, and basal-like. These subtypes differ in both treatment response and clinical outcomes [7]. From an immunohistochemistry standpoint, the typical and commonly recognized form of BC is based on the expression of the hormone receptors for human epidermal growth factor (HER2), progesterone (PR), and estrogen (ER). Therefore, it is generally accepted that there are four subtypes of breast cancer: triple-negative, HER2-positive, luminal A, and luminal B (Figure 1). Research indicates that molecular subtypes differ in terms of race, regional distribution, and outcome [8,9].

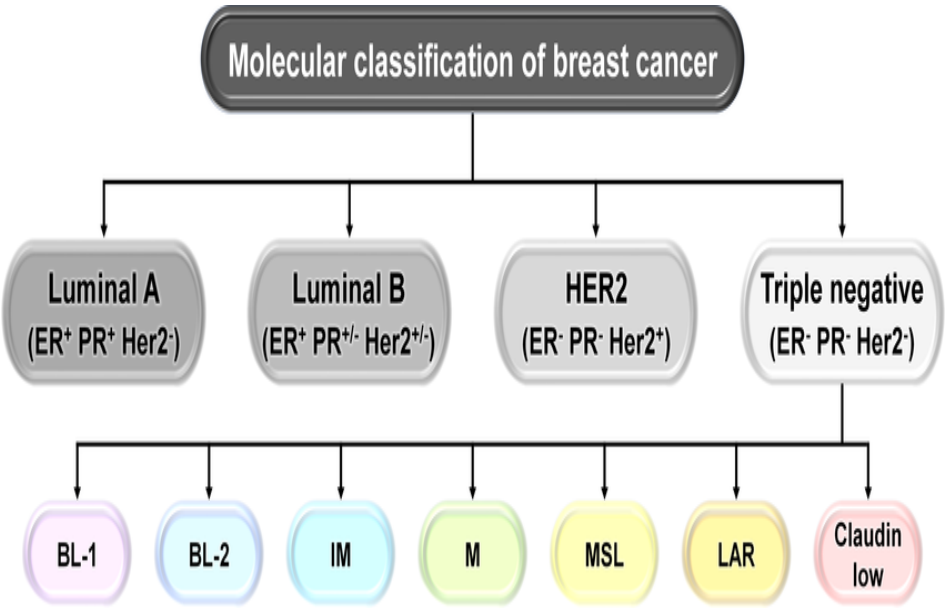

Figure 1. Molecular classification of breast cancer (BC). Human breast carcinoma has been divided into four types based on the presence or absence of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2): luminal A (ER^+, PR^+, $HER2^-$), luminal B (ER^+, PR+/−, HER2+/−), $HER2^+$, and triple negative breast cancer (TNBC) (ER^-, PR^-, $HER2^-$). Basal cell-like type 1 (BL-1), basal cell-like type 2 (BL-2), immune-modulatory (IM), mesenchymal-like (M), mesenchymal stem cell-like (MSL), luminal androgen receptor (LAR), and claudin low are the transcriptome-based subtypes of TNBCs [10].

Accurate molecular classification is a prerequisite for prognostication and treatment selection in women with breast cancer. It identifies patients most likely to benefit from endocrine therapy or HER2-directed therapy [11]. Recent transcriptomic work has revealed subtype-specific RNA-binding proteins, long non-coding RNAs, and differentially expressed genes, providing early evidence for new prognostic biomarkers and potential therapeutic targets tailored to each subtype [12].

In general, breast cancer classification integrates molecular and morphologic criteria. Morphologically, tumors are grouped by site of origin and degree of invasiveness; non-invasive lesions remain confined to ductal or lobular epithelium without stromal invasion [13]. This category includes lobular carcinoma in situ (LCIS), limited to lobular epithelium, and ductal carcinoma in situ (DCIS), confined to the ductal system [14,15]. In routine practice, intrinsic subtype assignment and prognostic assessment rely heavily on measures of proliferation, particularly Ki-67 immunohistochemistry and mitotic counts [16]; underlying these features is cell-cycle control: checkpoints—most notably the spindle assembly checkpoint, acting through the anaphase-promoting complex/cyclosome (APC/C)—govern the metaphase-to-anaphase transition to ensure accurate chromatid segregation [17].

Beyond Ki-67, two clinically relevant proliferation markers are Securin (PTTG1) and Separase (ESPL1). Under normal mitotic control, Securin restrains Separase until APC/C-mediated ubiquitination and proteolytic degradation of Securin activate Separase, which then cleaves cohesin to permit sister-chromatid separation. Disruption of this sequence promotes chromosomal instability, aneuploidy, intratumoral heterogeneity, and carcinogenesis [18]. Separase is frequently overexpressed in breast cancer—particularly in luminal B tumors—and has been reported as an independent predictor of aggressive disease and poor prognosis [19–22]; however, breast cancer research in Libya remains limited, and existing studies are generally considered insufficient to reflect the true status of the disease. In particular, the local distribution of molecular subtypes has not been comprehensively investigated, leaving a significant knowledge gap regarding the biological characteristics of breast cancer in the country. Nevertheless, several regional studies consistently report that breast cancer is relatively common in Libya, often presenting at an advanced stage and at a younger age [23, 24]. This highlights the importance of incorporating molecular subtyping into clinical practice, as it can provide more precise prognostic information, help assess the risk of relapse, and predict the likelihood of achieving a pathological complete response. This study is the first descriptive and

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prognostic investigation of breast cancer molecular subtypes in Libya. Its primary aim was to determine the distribution of these subtypes in the western region of the country, with particular emphasis on Separase and Securin as emerging biomarkers of cancer cell proliferation and prognosis. The study also explored their associations with key clinicopathological features. The findings are expected to enhance healthcare management and deepen the understanding of breast cancer in the Libyan context.

Materials and Methods

Study design

This is a retrospective study established on data retrieved from the Pathology Department at the National Cancer Institute (NCI), Sabratha, Western Libya, across an eight-year study period.

Patients and data collection

The clinical and histological characterization of 170 breast cancer patients from Libya, out of which 162 had complete clinical data, was the primary focus of the investigation. The patient data includes extensive registry information, up to 8-year follow-up, and survival statistics that offer a unique perspective on extraordinary cancer cases. The worldwide criteria for reporting cohort studies in surgery were followed for gathering the data [25]. The cohort consists of female patients with unilateral invasive breast cancer who were diagnosed and treated at NCI Libya. From 2002 to 2010, all clinical data reports of patients with primary invasive breast cancer were used. Every patient received either a mastectomy with axillary evacuation or a surgical resection. In accordance with worldwide standards for the treatment of breast cancer at the time of diagnosis, surgical treatment was followed by radiation therapy and/or adjuvant treatment with anti-estrogenic or cytostatic medicines, depending on the patient's age, hormone receptor, and lymph node status [25]. Complete clinical data were gathered from patient files and pathology reports, and it was registered using the standards set forth by the St. Gallen International Expert Consensus [26] and the WHO [27]. In accordance with worldwide recommendations, IHC was used to match intrinsic subtypes [28].

Inclusion and exclusion criteria

This study included patients with unilateral invasive breast cancer, those with known histological grades and lymph node conditions, patients with full clinical records, and patients with high-quality formalin-fixed, paraffin-embedded specimens (FFPE) available, whereas male patients were excluded.

Tissue materials

Standard histological procedures were followed in the preparation of the tissue materials, which included first fixing them in buffered formalin (pH 7.0) and then embedding them in paraffin blocks. Tissue Microarray Technique (TMAs) sections, cut at 3 µm, were subjected to IHC staining using Securin and Separase, as previously described [22, 29]. In summary, TMAs were carried out initially by selecting two representative cancer cell regions using hematoxylin and eosin (H&E) staining. Two tissue cores from each tumor were then extracted by punching the paraffin blocks in these locations. The tissue cores ranged in diameter from 0.6 to 1.5 mm. Sections of normal breast tissue were also included. Additionally, IHC identified Ki-67, E-cadherin, HER2, the estrogen receptor (ER), and the progesterone receptor (PR), among others. The IHC was applied to whole sections cut at 4 µm using Benchmark XT (Roche Diagnostics/Ventana Medical Systems, Tucson, AZ, USA) to prevent tumour heterogeneity. The signals were then identified using the ultra-View Universal DAB Detection Kit (Roche Diagnostics/Ventana Medical Systems, Tucson, AZ, USA).

IHC results interpretation

IHC was carried out in compliance with internationally recognized standards. The Quick scoring method (a semi-quantitative rating system), was used for ER and PR estimation. The ratio of labeled tumor cells was determined solely by nuclear staining and intensity. Scores on the HER2 test ranged from 0 to 3 +, where 0 or 1 indicates a negative result, 2 + indicates equivocal results, and 3 + indicates a positive result. When more than 10% of invasive tumor cells exhibit severe, full circumferential cytoplasmic membrane staining, the case is assigned a 3 + score. Cases were chosen for in situ hybridization (ISH) gene amplification; their verification was based on HER2-IHC (intensity score 2+). Benchmark XT (Roche Diagnostics/Ventana Medical Systems, Tucson, AZ, USA), the HER2 DNA and the Inform Chromosome 17 probe set, the Ultra-View SISH Detection Kit (Roche/Ventana) for HER2, and the Ultra-View Alkaline Phosphatase Red ISH Detection Kit (Roche/Ventana) for Chr17 were used to perform HER2/Chr17 double ISH. 73 tumor microarrays of invasive breast cancer were used to assess the expression of the proteins Securin and Separase in breast cancer cells. Insufficient tissue material was used in the remaining cases to complete the staining. Using previously documented methods, immune expressions for Securin and Separase were detected as nuclear and/or cytoplasmic staining and recorded as average fractions of cancer cells that were positively stained.

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Ethical considerations

Each patient's identity and confidentiality were safeguarded by giving them a unique serial number. The study protocol was accepted by the NCI ethical committee and registered under registration number 1/2010.

Statistical analysis

Fisher's exact test or chi-squared test (if Fisher's test was not practical) was used to compare the distributions of class variables in various molecular subtypes or Separase/Securin classes. Survival in each group was compared using Kaplan-Meier curves. The groups' differences in survival were evaluated using the log-rank test. Furthermore, the 5-year survival rates were computed along with their confidence ranges. P-values below 0.05 are regarded as significant, and all tests were two-tailed. The R program (version 4.2.1) and the "survival" R-software package (version 3.5-5) are used for analysis.

Results

The clinicopathological features and demographics of the 162 Libyan women who were part of this retrospective analysis are listed in Table 1. With tumors ranging from 2 to 5 cm, the majority of patients were diagnosed before the age of 50. Only a tiny percentage of respondents had a positive family history of breast cancer, and premenopausal status predominated. Compared to other histological categories, invasive lobular carcinoma (ILC) accounted for a small percentage of instances. At presentation, there was widespread involvement of lymph nodes, suggesting advanced regional illness. Only a small percentage of cases occurred in the early clinical phases (I–II), and grade II tumors were the most common. Nearly every patient had surgery, and everyone had chemotherapy. A comparatively significant disease burden was indicated by the 39% breast cancer-related mortality rate over the median follow-up period.

Table 1. Demographics and clinical characteristics of Libyan female breast cancer patients (n = 162).

Variable	Group	N	%
Age	< 50	104	64
	≥ 50	58	36
Tumor size	< 2 cm	16	10
	2-5 cm	66	41
	> 5 cm	80	49
Menopausal status	Premenopausal	101	62
	Postmenopausal	61	38
Family history	Positive	13	8
	Negative	127	78
	Unknown	22	14
Histological type	IDC	151	93
	ILC	19	12
	Others	11	7
Lymph node status	Positive	127	78
	Negative	32	20
	Unknown	3	2
Histological grade	Grade I	19	12
	Grade II	77	48
	Grade III	66	41
Stage	Stage I-II	13	8 %
	Stage II-IV	108	67 %
Surgery	Yes	146	90
	No	16	10
Chemotherapy	Yes	162	96
	No	7	4
Median follow-up	Death from breast cancer	24	39 %
	Alive with disease	46	29 %

**The 162 tumors with subtype included only. IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma*

Immunohistochemistry analysis showed distinct cytoplasmic and nuclear staining for Securin and Separase. Relative to adjacent normal epithelium, breast cancers displayed consistently stronger staining in both compartments. Staining intensity tracked histologic grade: Securin was minimal/absent in normal and low-grade tumors but prominent in high-grade lesions; Separase showed a similar pattern with

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conspicuous nuclear staining in high-grade tumors and little to none in low-grade cases. These findings indicate upregulation in more aggressive diseases and support the prognostic utility of both markers. Representative micrographs in Figure 2.

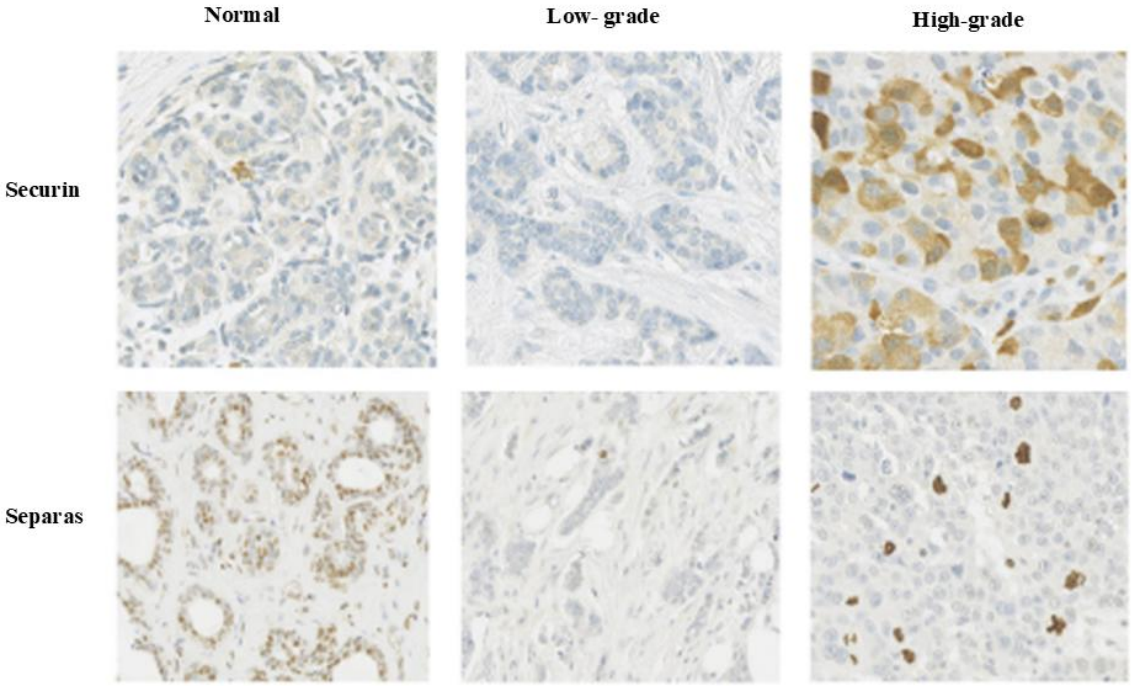

Figure 2. Representative microscopic images showing cytoplasmic and nuclear staining of Securin and Separase in breast cancer and normal breast tissue. High-grade tumors exhibited strong staining, whereas normal and low-grade tumors showed little to no expression.

Based on immunohistochemical and in situ-hyperdiazation surrogate classification, the distribution of breast cancer molecular subtypes among 162 female Libyan patients is displayed in Our study showed that the distribution of molecular subtypes among Libyan breast cancer patients was dominated by Luminal A (49%), followed by Luminal B (27%), triple-negative (15%), and HER2-enriched (9%). The predominance of hormone receptor–positive subtypes (Luminal A and B) suggests that many patients may benefit from endocrine therapy, consistent with global patterns. By contrast, the presence of triple-negative and HER2-enriched tumors highlights the burden of more aggressive disease and supports the evaluation of proliferation biomarkers such as Securin and Separase for improved prognostic assessment. Similarly, most tumors expressed estrogen and progesterone receptors, reinforcing the potential benefit of endocrine therapy, while a smaller proportion were HER2-positive, in line with international prevalence rates. Together, these findings provide insight into the molecular characteristics of breast cancer in western Libya and emphasize the importance of biomarker evaluation in guiding prognosis and treatment strategies. These results are shown in Figure 3.

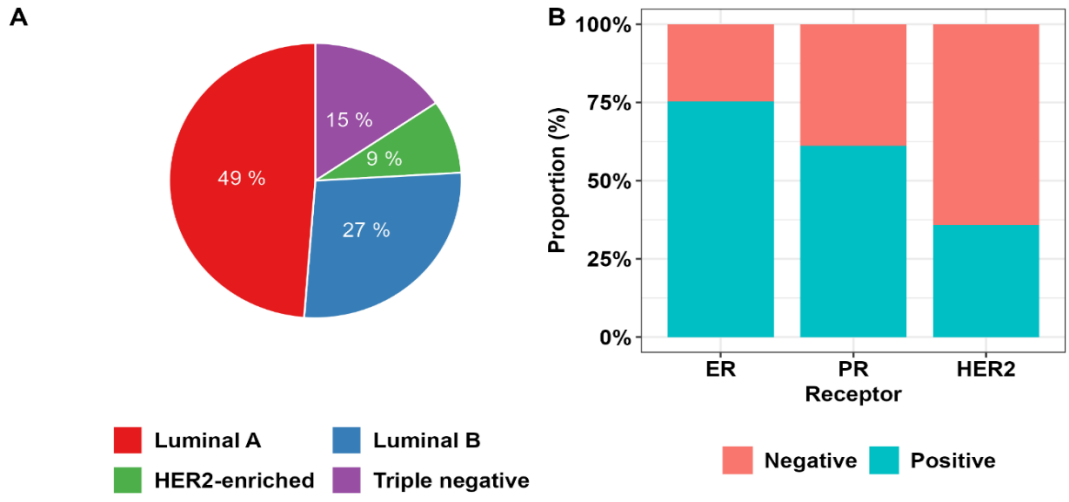

Figure 3. A. Distribution of breast cancer molecular subtypes in Libyan female patients. B. Hormone receptor (ER, PR) and HER2 status in Libyan female breast cancer patients. Luminal A was the predominant subtype, reflecting a high proportion of hormone receptor–positive tumors with potential responsiveness to endocrine therapy.

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Our results showed no significant associations were observed between molecular subtypes and patient age groups, family history of breast cancer, tumor histological type (ductal/lobular vs. others), or tumor grade. Similarly, no clear correlations emerged with tumor size classification or metastatic status (M). A borderline association was noted for tumor stage (T, p = 0.3*), suggesting some variation across subtypes. The strongest association was observed with hormonal treatment (p < 0.001), as patients with Luminal A and Luminal B subtypes received endocrine therapy, whereas those with HER2-enriched and triple-negative tumors did not. At the last follow-up, survival outcomes did not differ significantly across subtypes. However, a trend was noted (p = 0.056), with Luminal A patients more frequently disease-free and HER2-enriched or triple-negative cases more often associated with death or metastasis. In addition, Securin and Separase expression showed no significant associations with patient age (<50 vs. ≥50 years), family history, histological grade (Grades 1–3), or molecular subtype (Luminal A, Luminal B, HER2-enriched, and triple-negative). In contrast, significant associations were identified with tumor stage (T; p = 0.009 and 0.027), metastatic status (M; p = 0.024), and tumor size, particularly in tumors larger than 5 cm (p = 0.002). These findings suggest that Securin and Separase expression are more closely linked to tumor progression and burden than to demographic or subtype-related factors. These findings are summarized in Table 2.

Table 2. Clinicopathologic characteristics by molecular subtype (Luminal A/B, HER2-enriched, Triple-negative) for cases with complete data (n=162). *** p-values from x^2 tests.**

Variable	Luminal A	Luminal B	HER2-enriched	Triple negative	Total	P*
Age groups						
< 50	50	25	8	21	104	0.12
≥ 50	29	19	6	4	58	
Menopausal status						
Premenopausal	30	19	6	6	61	0.4
Postmenopausal	49	25	8	19	101	
Family history of breast cancer						
No	65	31	12	19	127	0.4
Yes	5	4	0	4	13	
Unknown	9	9	0	7	25	
Histological type of the tumor						
IDC(Ductal)	64	34	11	19	128	
ILC (lobular)	12	8	1	2	23	0.3
Others	3	1	2	1	7	
Histological grade of the tumor						
Grade 1	12	4	1	2	19	0.3
Grade 2	42	20	7	12	66	
Grade 3	25	20	9	12	66	
Lymph node status						
At least 3	29	16	4	4	53	0.2
1–2	29	15	4	4	52	
T category						
T1	10	4	0	0	14	0.3
T2	29	13	5	7	54	
T3	25	16	8	13	62	
T4	15	11	1	5	32	
N category						
Unknown	2	0	0	1	3	
N0	72	37	13	24	146	0.5
N1	7	7	1	1	16	
M category						
M0	72	37	13	24	146	0.5
M1	7	7	1	1	16	
Size classification						
< 2 cm	11	5	0	0	16	0.3
2–5 cm	31	15	6	14	66	
> 5 cm	37	24	8	11	80	
Hormonal treatment						
No	0	0	14	25	39	< 0.001
Yes	79	44	0	0	123	
Status at last follow-up						
Death	24	17	9	15	65	0.056
Free	44	23	3	8	78	
Metastasis	11	4	2	2	19	

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We also performed Kaplan–Meier survival analysis to compare breast cancer–specific survival across molecular subtypes in 162 Libyan female patients (Figure 4). The curves demonstrated significant variation in survival outcomes. Luminal A tumors showed the most favorable prognosis, with the highest survival rates over 60 months, followed by Luminal B. In contrast, triple-negative and HER2-enriched subtypes were associated with poorer survival, reflecting their aggressive biological behavior and limited therapeutic options. These findings underscore the prognostic value of molecular classification and the need for tailored treatment strategies, particularly for high-risk subtypes. Moreover, they highlight the potential role of additional biomarkers, such as Securin and Separase, in refining prognostic evaluation. Kaplan–Meier analyses comparing negative (0) versus positive (1) protein expression across breast cancer molecular subtypes showed no statistically significant differences in survival across cellular localizations (Figure 5). Securin, cytoplasmic (Fig. 5A): 5-year survival 67.7% (95% CI 53.5–85.6) for 0 vs 54.8% (39.8–75.6) for 1; log-rank p=0.20. Securin, nuclear (Fig. 5B): 64.2% (42.9–95.9) vs 60.1% (47.9–75.4); p=0.60. Separase, cytoplasmic (Fig. 5C): 60.5% (47.6–76.8) vs 63.8% (45.5–89.3); p=1.00. Separase, nuclear (Fig. 5D): 72.0% (51.9–99.8) vs 59.0% (46.9–74.2); p=0.30. Median survival was not reached within 60 months for any comparison. Although some contrasts showed numerical differences (e.g., lower 5-year survival with positive Securin/cytoplasmic and positive Separase/nuclear), confidence intervals overlapped and none reached statistical significance.

On the other hand, Kaplan–Meier curves demonstrated clear survival stratification by clinicopathological factors. Lymph-node status (Figure 6A) showed the strongest separation (log-rank $p < 0.001$), with node-positive cases faring worse than node-negative. Tumor size (Figure 6B) was similarly associated ($p < 0.001$), with larger tumors showing inferior outcomes. Histological grading (Figure 6C) also discriminated survival ($p < 0.001$), with a higher grade associated with poorer prognosis. Ki-67 immunopositivity (Figure 6D) further stratified risk ($p = 0.010$), with Ki-67–positive/high cases exhibiting lower survival than negative/low cases. Curves generally diverged early and remained separated across follow-up, consistent with the expected direction of effect for each adverse feature.

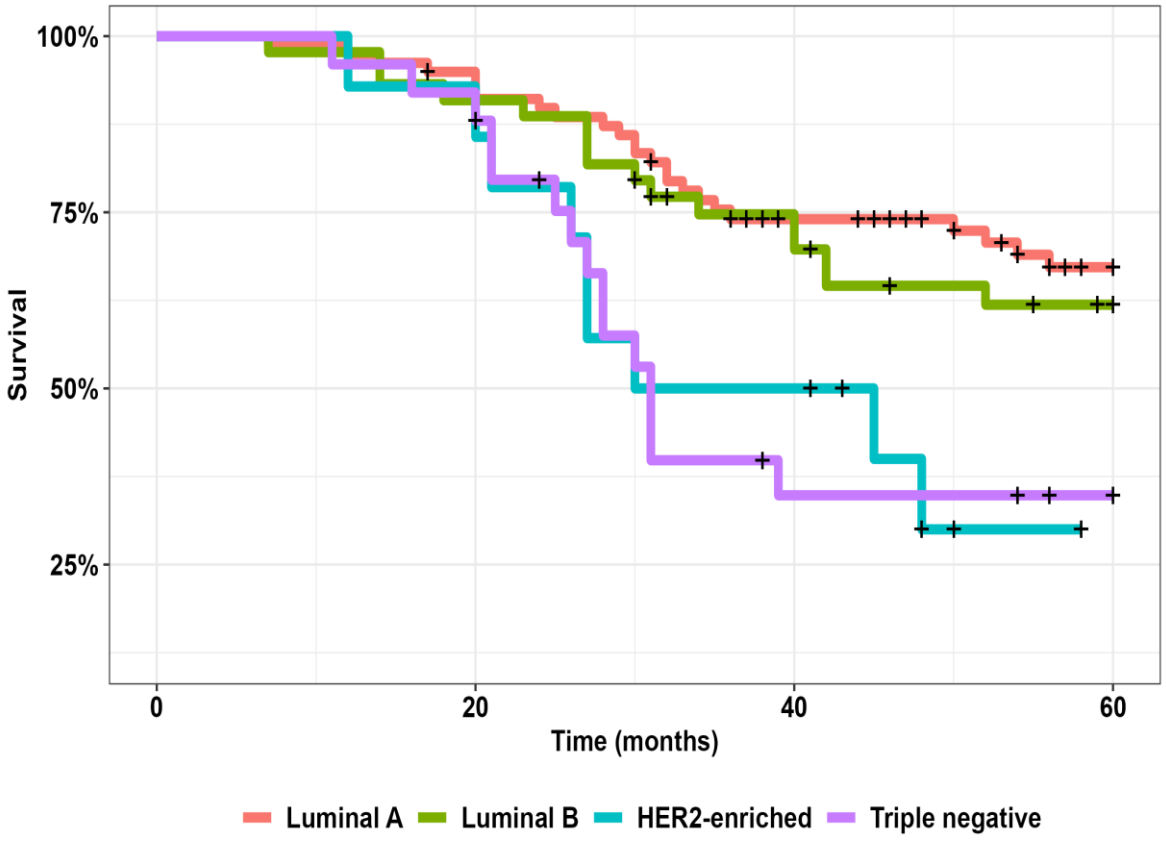

Figure 4. Kaplan–Meier survival curves by molecular subtype of breast cancer in Libyan female patients (n = 162).

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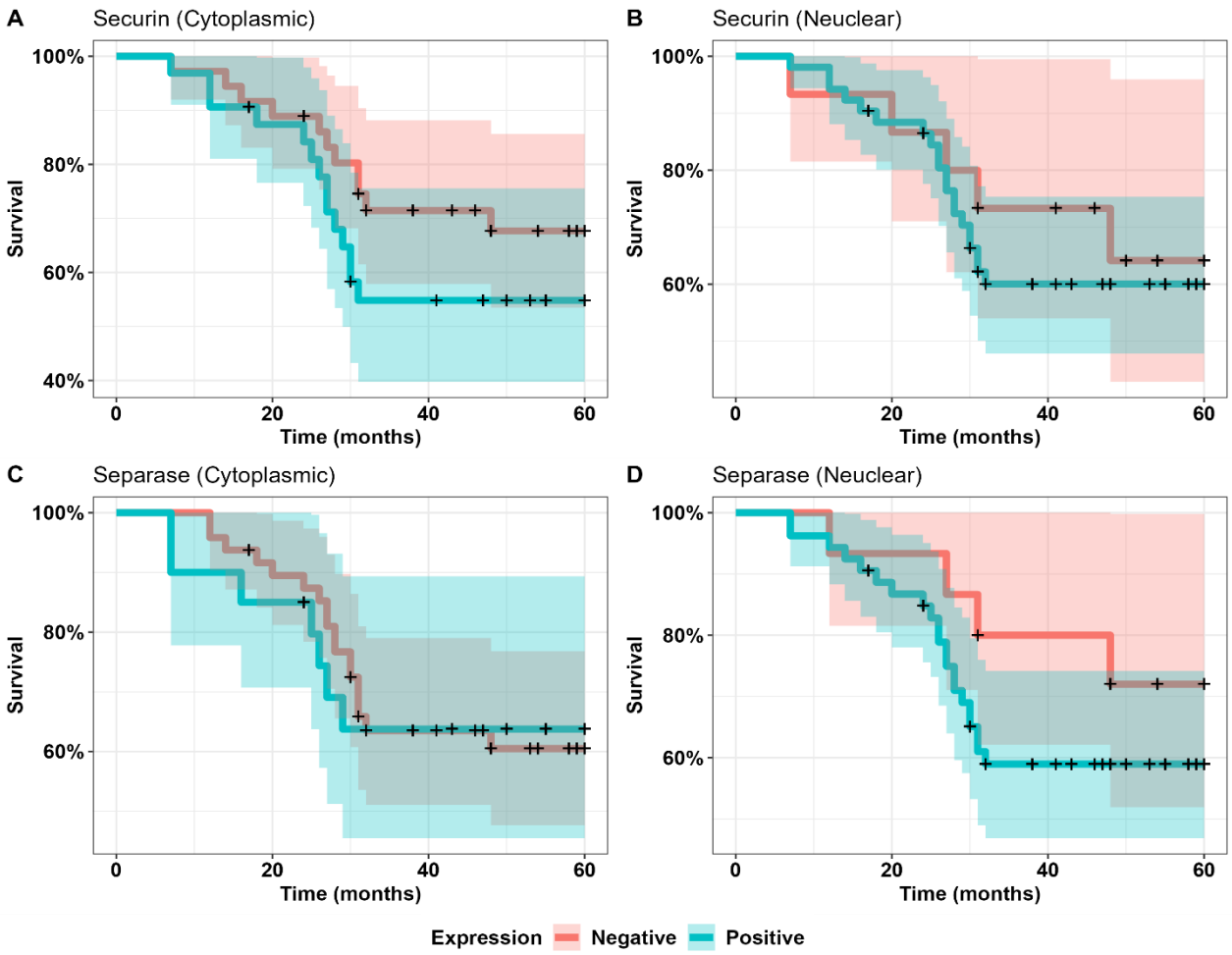

Figure 5. Kaplan–Meier survival curves illustrating the prognostic impact of Scurin and Separase expression by cellular localization. (A) Securin, cytoplasmic; (B) Securin, nuclear; (C) Searase, cytoplasmic; (D) Separase, nuclear. Curves compare negative (0) vs positive (1) expression; shaded bands indicate 95% confidence intervals, and "+" marks denote censored observations.

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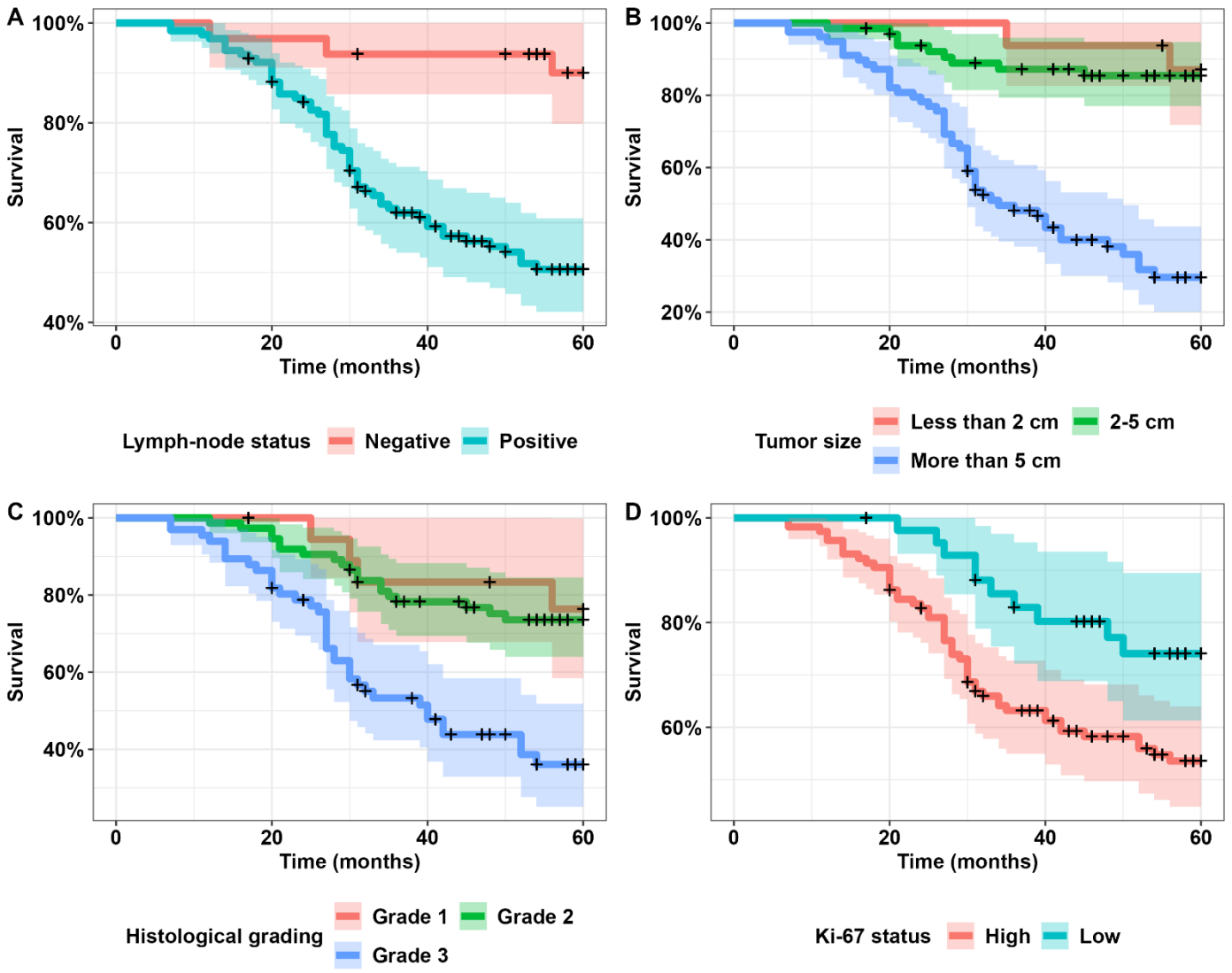

Figure 6. Kaplan–Meier survival curves stratified by clinicopathological factors. (A) Lymph-node status (node-negative vs node-positive). (B) Tumor size (clinical/pathological size categories). (C) Histological grade (G1–G3). (D) Ki-67 immunopositivity (low/negative vs high/positive). Curves show survival probability over time; tick marks denote censored observations. Group differences were assessed with the log-rank test.

Discussion

This study includes a Libyan cohort of 162 patients diagnosed with breast cancer women with ~8-year follow-up. The molecular subtypes were distributed as Luminal A 49%, Luminal B 27%, TNBC 15%, and HER2-enriched 9%—a pattern consistent with global and regional reports, with Luminal A predominance reflecting the high burden of hormone-receptor–positive disease [7,23,30,31]. Survival differed by subtype as expected: Luminal A best, Luminal B intermediate, and TNBC/HER2-enriched worse, underscoring the therapeutic relevance of endocrine and anti-HER2 strategies where appropriate [34–39]. The proportion of triple-negative breast cancers (15%) in this study is comparable to the global average (10–20%), which is associated with more aggressive clinical behavior, lack of targeted therapies, and poorer survival outcomes [33]. In our cohort, HER2-enriched tumors accounted for 9% of cases, slightly lower than reports from Western countries (12–15%) but consistent with some regional studies [34]. The presence of these aggressive subtypes highlights the need for multimodal treatment strategies and reinforces the importance of early detection and molecular profiling to guide therapy.

Regarding proliferation markers, Securin (PTTG1) and Separase (ESPL1) were more highly expressed in aggressive tumours, in line with prior studies reporting their prognostic significance [32,40]; although our cohort did not show statistically significant survival differences by high versus low expression of Securin and Separas proteins, the direction of effect was consistent—poorer outcomes with higher expression, most evident for cytoplasmic Securin and nuclear Separase. Biologically plausible given their roles in cohesion cleavage and chromosomal instability. The lack of significance likely reflects limited power and residual confounding., the Kaplan–Meier curves show a consistent trend toward poorer survival with higher expression—most clearly for cytoplasmic Securin and nuclear Separase, where the high-expression curves

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lie below their low-expression counterparts across much of follow-up; this direction is biologically plausible: dysregulation of the Securin–Separase axis can disrupt cohesion cleavage, drive chromosomal instability, and promote aggressive behaviour. The lack of statistical significance likely reflects limited power due to the modest sample size and event count. Overall, our findings are consistent with a worse prognosis in tumors exhibiting higher Securin/Separase expression, and they highlight the need for validation in larger, uniformly treated cohorts using multivariable and time-dependent models, with particular attention to localization-specific effects and standardized scoring cut-offs. In addition, Kaplan–Meier analysis of 162 Libyan women showed clear subtype-specific survival. Luminal A had the most favorable outcomes over 60 months, followed by Luminal B. This aligns with international data: hormone-receptor–positive tumors generally fare better due to endocrine responsiveness and slower growth [7,30]. In contrast, triple-negative and HER2-enriched cancers showed poorer survival, reflecting aggressive biology and limited therapeutic options—particularly for triple-negative disease [33,38]—mirroring global recognition of these subtypes as high-risk with inferior long-term outcomes [34,39]. The prognostic value of molecular classification observed in this cohort underlines the clinical relevance of tailoring therapy to subtype-specific characteristics. The predominance of hormone receptor–positive cases support the use of endocrine therapy, while the aggressive outcomes seen in triple-negative and HER2-enriched subtypes highlight the need for multimodal approaches and the integration of novel therapeutic strategies. Furthermore, these results suggest that incorporating proliferation biomarkers such as Securin and Separase may enhance prognostic assessment within each molecular subtype and help identify patients at increased risk of poor outcomes [32]. In summary, our data and prior studies support Securin (PTTG1) and Separase (ESPL1) as prognostic markers in breast cancer, with stronger predictive value when assessed together or in combination with other cell-cycle regulators such as Ki-67 or CDK1 rather than individually [41]. Subcellular localisation is also important—particularly the adverse association of cytoplasmic Securin—indicating that both nuclear and cytoplasmic staining should be reported. While we observed no clear subtype-specific effects, other reports highlight greater relevance for Separase in luminal B/high-proliferative disease and broadly adverse signals for Securin [42,43]. Variability across studies likely reflects differences in cohort composition, scoring thresholds, and treatment eras, underscoring the need for validated cut-offs and larger, subtype-stratified analyses. Overall, combined and localisation-aware evaluation of Securin and Separase, particularly alongside CDK1 and proliferation markers such as Ki-67, may enhance risk stratification beyond standard clinicopathological factors and warrants further clinical validation.

Conclusion

To our knowledge, this is the first study to characterize the distribution of molecular subtypes and evaluate proliferation markers in a Libyan breast cancer cohort with complete clinical follow-up. Luminal A and B emerged as the most prevalent subtypes, consistent with global patterns, whereas triple-negative and HER2-enriched tumors were less common but clinically more aggressive. Both Securin (PTTG1) and Separase (ESPL1) were associated with adverse clinicopathological features, including higher stage and metastasis, and showed a directional trend toward poorer survival, supporting their potential as negative prognostic biomarkers. These findings underscore the value of integrating molecular subtyping and proliferation-marker assessment into routine care to enhance risk stratification and guide personalized treatment for Libyan patients. Validation in larger, uniformly treated cohorts remains warranted.

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Conflicts of Interest

The authors declare no conflicts of interest.

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