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Assessment of interleukin-6 and cathepsin-B gene expression in breast cancer women

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Abstract

Background Breast cancer (BC) is the most prevalent cancer and the leading cause of cancer-related deaths in women globally. Cysteine protease cathepsin-B has been implicated in various human malignancies and is involved in malignancy progression and metastasis. This study aimed to evaluate the circulating levels of cathepsin-B, interleukin-6 (IL-6), and CA15-3, a cancer antigen, as biomarkers for tumors in women with both localized and metastatic BC. The study employed a case-control design, enrolling 108 participants categorized into three groups: healthy individuals, those with localized BC, and those with metastatic BC. The relative mRNA expression of cathepsin-B in blood samples was assessed using qRT-PCR. Additionally, serum levels of IL-6 and CA15-3 were quantified using ELISA.

Results The relative mRNA expression of cathepsin-B, IL-6 levels, and CA15-3 levels were significantly higher in metastatic BC cases than in localized BC cases and the control group (p-value < 0.001). A statistically significant positive correlation was also found between cathepsin-B and both IL-6 and CA15-3 ($r = 0.905$, $r = 0.667$, and $p < 0.001$), respectively.

Conclusions The findings indicate a strong correlation between the interaction of the proteolytic enzyme cathepsin-B and IL-6 with the unfavorable prognosis of BC. This relationship may serve as a potential indicator and a promising target for therapy in BC treatment.

Keywords CA15-3, Cathepsins, Interleukins, Mammary neoplasm, Metastasis

Background

Breast cancer (BC) is a major global health concern, with approximately 2.3 million new cases reported annually, accounting for 24.2% of all cancers diagnosed in women, making it the most frequently diagnosed cancer in females [1]. BC accounts for one in four cases in women worldwide and contributes to 15% of mortality, highlighting the need to find new diagnostic biomarkers and approaches to enhance prognosis and lower mortality [1].

Cathepsins are a group of enzymes that break down proteins inside most cells, facilitating cell self-destruction and tissue breakdown. They function optimally in acidic environments, such as lysosomes, and are involved in energy production, protein recycling, and immune response. Cathepsins can modulate the activity of cytokines and chemokines, key mediators of inflammation, and contribute to the regulation of inflammatory processes by controlling the production and release of these signaling molecules [2]. They can be categorized into three groups based on their composition and method of protein cleavage: cysteine cathepsins (including cathepsins B, C, F, H, K, L, O, S, V, W, and X), aspartic cathepsins (such as cathepsins D and E), and serine cathepsins (comprising cathepsins A and G)

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[3]. Cathepsins promote BC by aiding tumor growth, invasion, and metastasis through the degradation of the extracellular matrix and activation of other enzymes [4]. Although not all categories of cathepsins are associated with BC, cathepsin B, L, S, and D have been linked to BC progression due to their proteolytic activity and involvement in various cellular processes that support tumor development [5].

Cathepsin-B, located on chromosome 8 (8p22), has been proposed as an effective biomarker for various malignancies [6]. The overexpression of cathepsin-B has been observed in numerous types of malignancies, including prostate and pancreatic tumors, melanoma, and kidney carcinoma, suggesting its potential as a therapeutic target. Decreased cathepsin-B expression has been shown to reduce the aggressiveness of glioma, osteosarcoma, and mammary cancer cells [7].

Mechanistically, cathepsin-B is localized on the surface of cancer cells, facilitating the initiation of proteolytic cascades that ultimately activate downstream proteases, such as urokinase-type plasminogen activator (uPA), pro-matrix metalloproteinases (MMP)-2 and -9, which degrade extracellular matrix (ECM) constituents and adhesion particles, including E-cadherin [8].

Interleukin-6 (IL-6) is a pro-inflammatory cytokine composed of 184 amino acids. It has been demonstrated that IL-6 increases aromatase expression in adipose tissue, leading to increased estrogen production and enhanced breast cancer development. Moreover, IL-6 activates multiple signaling pathways to increase cathepsin-B expression [9, 10].

In human gingival fibroblasts, for example, IL-6 and soluble-IL-6-receptor (sIL-6R) induce the construction of cathepsin-B and cathepsin-L through a pathway involving the c-Jun N-terminal kinase-activator protein-1 (JNK-AP-1) caveolae. Additionally, cyclic adenosine monophosphate (cAMP) and IL-6 have been shown to intensify the release of cathepsin-B from human osteoblasts [11, 12].

CA15-3 is a marker that can help predict the outcome of BC patients and has been extensively studied recently. CA15-3 is a type of mucin, a glycoprotein produced by the MUC-1 gene, and is found on the surface of numerous categories of normal epithelial tissues, including the breast [13]. CA15-3 may be useful for determining the extent of BC spread. After metastatic BC therapy, CA15-3 measurement can be used to monitor disease recurrence. An increase in CA15-3 levels may indicate therapy failure in the absence of detectable disease [14].

Cathepsin-B, IL-6, and CA15-3 are all implicated in the promotion of BC by enhancing tumor growth, invasion, and metastasis [8]. CA15-3 can affect the release of pro-inflammatory cytokines, such as IL-2, IL-6, and TNF-α [15]. IL-6 can also stimulate the expression of cathepsin-B in BC cells, creating a self-reinforcing cycle that drives tumor progression [16]. Cathepsin-B plays a crucial role in tumor progression by degrading the extracellular matrix, which allows cancer cells to invade and metastasize. It also activates pro-angiogenic factors, promoting the formation of new blood vessels that supply the tumor with oxygen and nutrients [17].

This study explored the expression levels of cathepsin-B, IL-6, and CA15-3 in the blood of BC cases. Additionally, it examined the correlation between the expression of these markers and various clinicopathological characteristics of the patients.

Methods

Subjects and clinical data

This case-control research included 108 women categorized into three categories: 36 normal control women, 36 female patients having localized BC, and 36 female patients having metastatic BC. The study was conducted at the Medical Biochemistry & Molecular Biology and General Surgery departments of Zagazig University Hospitals, Egypt, between November 2022 and November 2023. The measured sample size was determined using Epi-Tools Epidemiological calculators to assess the statistical robustness of the findings which indicated a minimum power of 87.1% [18]. The patients were non-obese Egyptian females with histopathologically and clinically confirmed BC, aged between 35 and 56 years, who had not undergone chemotherapy or radiotherapy before surgery. Clinicopathological data were obtained from the pathology and hospital reports. We excluded patients with infectious, inflammatory, autoimmune disorders, or multiple primary tumors, patients with positive BC family history, and those who refused to give consent. The study was authorized by the Zagazig University Institutional Research Board (IRB) (ZU-IRB#10,101/13-11-2022) and conducted according to the ethical guidelines set forth by the World Medical Association's Declaration of Helsinki for human research studies.

RNA extraction and reverse transcription

The blood samples underwent treatment with Trizol reagent obtained from Thermo Fisher Scientific, Inc. to extract total RNA. The RNA quality was evaluated by determining the A260/A280 ratio using a NanoDrop® ND–1000 Spectrophotometer (NanoDrop Technologies; Wilmington, Delaware, United States) with 1.5 µl of RNA. Subsequently, a High-Capacity cDNA Reverse Transcription Kit from Applied Biosystems™, USA, was

utilized for cDNA synthesis as per the manufacturer's guidelines.

Quantitative real-time PCR

To perform real-time RT-PCR using the TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725 or P750) obtained from Enzynomics, Korea, in an Mx3005P Real-Time PCR System from Agilent Stratagene, USA, following the manufacturer's instructions. The cycling conditions for qRT-PCR consisted of a primary denaturation step at 95 °C (12 min), then forty cycles of denaturation at 95 °C (20 s), annealing at 60 °C (30 s), and extension at 72 °C (30 s). Oligonucleotide-specific primers were custom-synthesized via Sangon Biotech (Beijing, China). After qRT-PCR amplification, melting curve testing was done. The pursue gene expression levels were standardized to the mRNA expression of human GAPDH using the $2^{-\Delta\Delta CT}$ method [19]. Primer sequences were as follows: cathepsin-B (forward); 5′-TGT AAT GGT GGC TAT CCT GCT-3′, (reverse); 5′-AGG CTC ACA GAT CTT GCT ACA-3′. GAPDH (forward) 5′-GGA GTC AAC GGA TTT GGT CGT-3′, (reverse); 5′-ACG GTG CCA TGG AAT TTG C-3′.

ELISA assay

The quantities of human IL-6 and CA15-3 in serum were established using new and optimized ELISA kits (Human IL-6 ELISA kit, Bioneovan. Co. Ltd Beijing, China) and (Human CA15-3 ELISA kit, INNOVA BIOTECH CO., LTD) respectively. The analysis was standardized following the constructor's instructions, and the findings were adjusted to a standard curve.

Statistical analysis

The data were subjected to statistical analysis using IBM's SPSS software, Version 20.0. For the characterization of quantitative data, the mean, range, median, and standard deviation were employed. For categorical variables, the Chi-square test was utilized, followed by a one-way ANOVA test and Tukey's post-hoc test for multiple differences among the three groups. All tests conducted were two-tailed. A p-value less than 0.05 was considered statistically significant.

Results

The studied subjects consisted of 35–56-year-old non-obese females categorized into three groups (control, localized BC, and BC cases with metastasis) with a mean age of 44.3 ± 8.3, 48.5 ± 6.2, and 45.5 ± 7.6, respectively. The clinicopathological attributes of the studied groups are displayed in Table 1.

The relative mRNA expression of cathepsin-B was statistically significantly higher in metastatic BC cases than in localized BC cases and in the control group (9.2 ± 2.1 > 5.1 ± 1.8 > 1.1 ± 0.2, p-value < 0.001) (Table 2).

A statistically significant variance was observed among different stages of the tumor regarding cathepsin-B gene expression in both localized BC and metastatic BC groups ($p = 0.02$). Additionally, cathepsin-B gene expression was significantly higher in patients with lymphatic and distant metastasis than in those with lymph node (LN) metastasis only ($p = 0.04$) (Table 3).

IL-6 and CA15-3 serum levels were statistically significantly higher among the metastatic BC group than the localized BC group and the healthy individuals (9.2 ± 1.6 > 5.4 ± 0.9 > 1.9 ± 0.4) and (30.2 ± 6.8 > 29.4 ± 5.1 > 15.4 ± 2.8), respectively (p-value < 0.001) (Table 4).

Among the studied groups, we revealed a statistically significant positive correlation among cathepsin-B and IL-6 & CA15-3 ($r = 0.905$ & 0.667, respectively, $p < 0.001$) (Table 5).

Cathepsin-B at a cutoff point of ≥ 1.4 can be used as a significant predictor for the occurrence of BC

Table 1 Clinicopathological data of the breast cancer groups

Variable	Group II (localized BC) ($n = 36$)	Group III (metastatic BC) ($n = 36$)
Diagnosis		
Invasive ductal carcinoma	33 (91.7%)	32 (88.9%)
Invasive lobular carcinoma	3 (8.3%)	4 (11.1%)
Site of tumor		
Bilateral	0 (0%)	1 (2.8%)
Left side	15 (41.7%)	16 (44.4%)
Right side	21 (58.3%)	19 (52.8%)
Grade of tumor		
I	4 (11.1%)	4 (11.1%)
II	24 (66.7%)	20 (55.6%)
III	8 (22.2%)	12 (33.3%)
Stage of tumor		
T1&T2	18 (50%)	22 (61.1%)
T3&T4	18 (50%)	14 (38.9%)
Site of metastasis		
Lymphatic metastasis	0 (0%)	13 (36.1%)
Lymphatic with distant metastasis	0 (0%)	23 (63.9%)
Estrogen receptor		
Negative	11 (30.6%)	8 (22.2%)
Positive	25 (69.4%)	28 (77.8%)
Progesterone receptor		
Negative	15 (41.7%)	11 (30.6%)
Positive	21 (58.3%)	25 (69.4%)
Human epidermal growth factor receptor-2		
Negative	24 (66.7%)	27 (75%)
Positive	12 (33.3%)	9 (25%)

Table 2 Cathepsin-B gene expression among the studied groups

Variable	Group I (control) ($n=36$)	Group II (localized BC) ($n=36$)	Group III (metastatic BC) ($n=36$)	*P**	LSD
Cathepsin-B				< 0.001	< 0.05 [1]
Mean ± SD	1.1 ± 0.2	5.1 ± 1.8	9.2 ± 2.1	(HS)	< 0.05 [2]
Range	0.8–1.5	1.4–7.9	5.5–16.7		< 0.05 [3]

*Test; ANOVA test, *LSD* least significant difference post-hoc test, *HS* highly significant difference ($p<0.001$). 1; Group I vs. Group II, 2; Group I vs. Group III, 3; Group II vs. Group III

Table 3 Correlation among the clinicopathological data and cathepsin-B relative expression in breast cancer patients

Variable	*N*	Cathepsin-B	*P*
Menopause			
Premenopausal	55	5.1 ± 3.6	0.35
Postmenopausal	53	5.8 ± 4	(NS)
Diagnosis			
Invasive ductal carcinoma	65	7.7 ± 2.6	0.553
Invasive lobular carcinoma	7	7.1 ± 1.8	(NS)
Site of tumor			
Bilateral	1	9.2 ± 0	
Left	31	7.7 ± 2.8	0.838
Right	40	7.6 ± 2.4	(NS)
Grade of tumor			
Grade I	8	6.8 ± 2.1	
Grade II	44	7.5 ± 2.4	0.239
Grade III	20	8.5 ± 2.9	(NS)
Stage of tumor			
T1 + T2	40	6.9 ± 1.8	**0.02**
T3 + T4	32	8.3 ± 1.7	**(S)**
Metastasis			
Lymphatic metastasis	13	9 ± 1.2	**0.04**
Lymphatic with distant metastasis	23	9.9 ± 1.2	**(S)**
Estrogen receptor			
Negative	19	8.1 ± 3.3	0.445
Positive	53	7.5 ± 2.2	(NS)
Progesterone receptor			
Negative	26	7.3 ± 2.5	0.53
Positive	46	7.9 ± 2.6	(NS)
Human epidermal growth factor receptor-2			
Negative	51	7.7 ± 2.1	0.858
Positive	21	7.6 ± 3.4	(NS)

Bold indicates a significant and highly significant difference between the study groups

with a sensitivity of 95.8% and specificity of 94.4%. Additionally, at a cutoff point of ≥ 6.1, it can be used as a significant predictor for the presence of metastasis in BC women with a sensitivity of 97.2% and specificity of 70% (Table 6) and (Figs. 1, 2).

Discussion

Breast cancer (BC) is the most common cancer among females and poses a significant public health concern. Therefore, identifying circulating biomarkers is crucial for the early detection and diagnosis of BC [20].

The current study revealed that the relative mRNA expression of cathepsin-B was statistically significantly elevated in metastatic BC cases (9.2 ± 2.1-fold change) compared to localized BC cases (5.1 ± 1.8-fold change) and normal individuals (1.1 ± 0.2-fold change) ($p<0.001$) (Table 2). A highly significant expression was found among patients with higher tumor stages (T3 and T4) ($p=0.02$). Additionally, cathepsin-B gene expression was observed to be notably elevated among cases with lymphatic and distant metastasis compared to patients with lymph node metastasis only ($p=0.04$) (Table 3).

The serum IL-6 level was statistically significantly higher among metastatic BC cases than localized BC cases and healthy individuals ($p<0.001$). While CA15-3 levels were statistically significantly elevated among BC groups compared to the healthy group ($p<0.001$), there was no significant difference between cases with localized or metastatic BC ($p>0.05$) (Table 4).

Our results showed a strong positive association between cathepsin-B and both IL-6 and CA15-3 ($r=0.905$ and 0.667, respectively), ($p<0.001$) (Table 5).

When we plotted the ROC curve to investigate the ability of cathepsin-B in predicting BC and metastasis, the test showed 95.8% and 97.2% sensitivity and 94.4% and 70% specificity at a cut-off value of ≥ 1.4- and ≥ 6.1-fold change, with an area under the curve of 0.996 and 0.958, respectively (Table 6) and (Figs. 1, 2). These findings suggest that monitoring cathepsin-B expression in the blood could be a non-invasive and easy diagnostic procedure.

Although CA15-3 is widely used in clinical practice, its role in managing BC remains disputed. The most recent recommendations from both the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) advise against the use of CA15-3 levels assessment for screening, diagnosis, or post-therapy monitoring [21].

Table 4 IL-6 and CA15-3 levels among the studied groups

Variable	Group I (control) (n = 36)	Group II (localized BC) (*n* = 36)	Group III (metastatic BC) (*n* = 36)	*P**	LSD
IL-6 (pg/mL):					**< 0.05** [1]
Mean ± SD	1.9 ± 0.4	5.4 ± 0.9	9.2 ± 1.6	**< 0.001**	**< 0.05** [2]
Range	1.1–2.8	4.1–7.5	6.5–12.1	**(HS)**	**< 0.05** [3]
CA15-3 (U/mL):					**< 0.05** [1]
Mean ± SD	15.4 ± 2.8	29.4 ± 5.1	30.2 ± 6.8	**< 0.001**	**< 0.05** [2]
Range	10–19.8	19–40.2	18.5–43.3	**(HS)**	> 0.05 [3] (NS)

pg/mL; picogram per milliliter, U/mL; unit per milliliter, *test; ANOVA test, LSD; Least significant difference post-hoc test, NS; non-significant difference ($p > 0.05$), HS; highly significant difference ($p < 0.001$). 1; Group I vs. Group II, 2; Group I vs. Group III, 3; Group II vs. Group III.

Bold indicates a significant and highly significant difference between the study groups

Table 5 Correlation between cathepsin-B expression and both IL-6 and CA15-3 among the studied groups

Variable	Cathepsin-B	
	R	*p*
IL-6	0.905	< 0.001
CA15-3	0.667	< 0.001

r; Pearson correlation coefficient

CA15-3 levels can be elevated in healthy individuals, benign diseases, malignant illnesses, and various non-malignant conditions, including cirrhosis, hepatitis, lupus, sarcoidosis, and tuberculosis, as well as during pregnancy and breastfeeding [22].

In contrast to our results, Zhao et al. found that individuals with metastatic BC experienced more frequent CA15-3 increases than those with early BC [23].

According to Berruti et al., CA15-3 levels were found to be elevated in various metastatic sites, with patients who had visceral metastasis showing higher rates of elevated CA15-3 levels compared to those with soft tissue and bone metastases [24]. He et al. suggested that patients with higher CA15-3 levels had a higher chance of developing abdominal and bone metastases. In agreement with our results, some studies did not discover significant alterations in CA15-3 levels among various metastatic positions [25].

Cathepsin-B, a lysosomal cysteine protease, is regarded as a "multifunctional enzyme in cancer" and primarily contributes to the proteolytic cascades involved in cancer progression, invasion, and metastasis. Cathepsins are typically found in lysosomes, but when cancer occurs, they often move to the cell surface or are even secreted [5]. In BC, cathepsin-B overexpression can break down the extracellular matrix, allowing cancer cells to migrate and invade surrounding tissues. Additionally, it can activate pro-angiogenic factors, promoting the formation of new blood vessels that feed the tumor [16, 17].

Lah et al. conducted one of the first studies on cathepsin-B in a large group of BC patients. They demonstrated that cathepsin-B levels were significantly higher in breast carcinomas compared to healthy breast tissues, which is consistent with our findings [26].

Decock et al. discovered that early-stage BC patients with poorly differentiated tumors had lower blood cathepsin-B levels than patients with well or moderately differentiated carcinomas [27].

Maguire et al. observed that cathepsin-B levels were significantly higher in both primary carcinomas and metastatic (nodal metastasis) tissues compared to benign tumors (fibroadenomas), aligning with our study results. However, they found no significant difference between cathepsin-B levels in primary cancers and metastatic specimens, and these levels did not correlate with tumor stage or nodal status unlike our findings [28].

Rudzinska-Radecka et al. reported significantly higher cathepsin-B expression in laryngeal cancer compared to nearby normal tissue, with no notable differences between patients with or without lymph node involvement or cancer stage [7].

Cathepsin-B serum concentrations were elevated in hepatoma and cirrhotic liver cases compared to normal

Table 6 Performance of cathepsin-B in breast cancer and metastasis prediction among the studied groups

Cathepsin-B	Cutoff-point	AUC	Sensitivity (%)	Specificity (%)	PVP (%)	PVN (%)	Accuracy (%)
Breast cancer prediction	≥ 1.4	0.996	95.8	94.4	89.5	97.1	96.2
Metastasis prediction	≥ 6.1	0.958	97.2	70	76.1	96.2	83.3

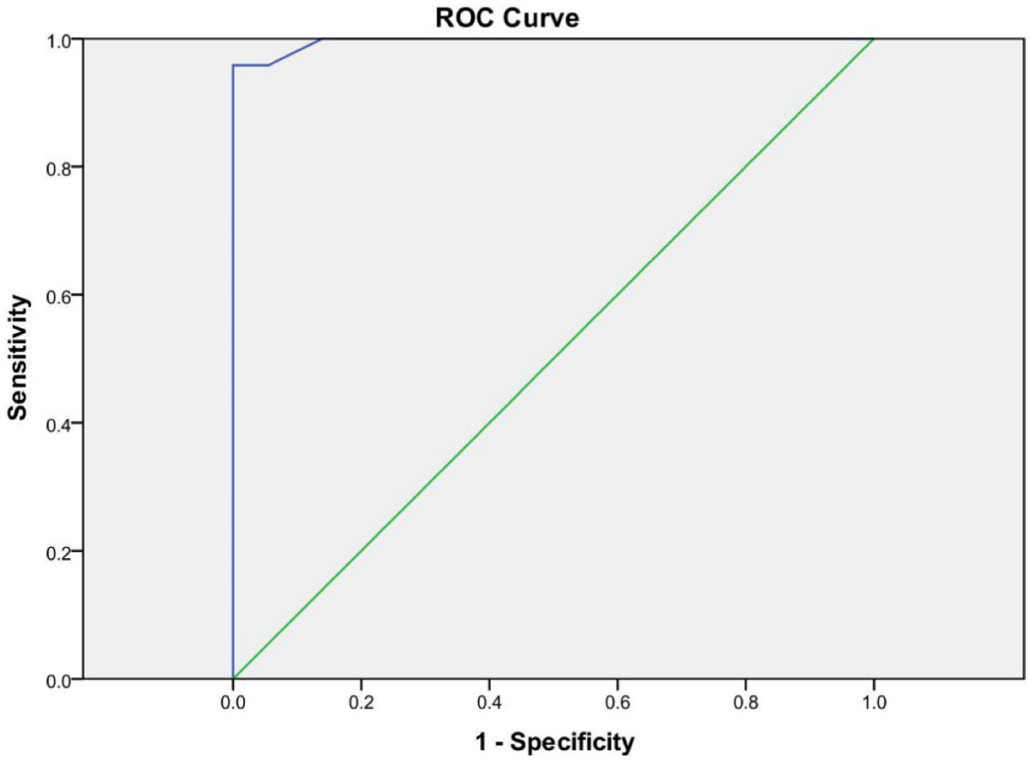

Fig. 1 Receiver operating characteristic (ROC) curve for using cathepsin-B as a predictor for cancer breast

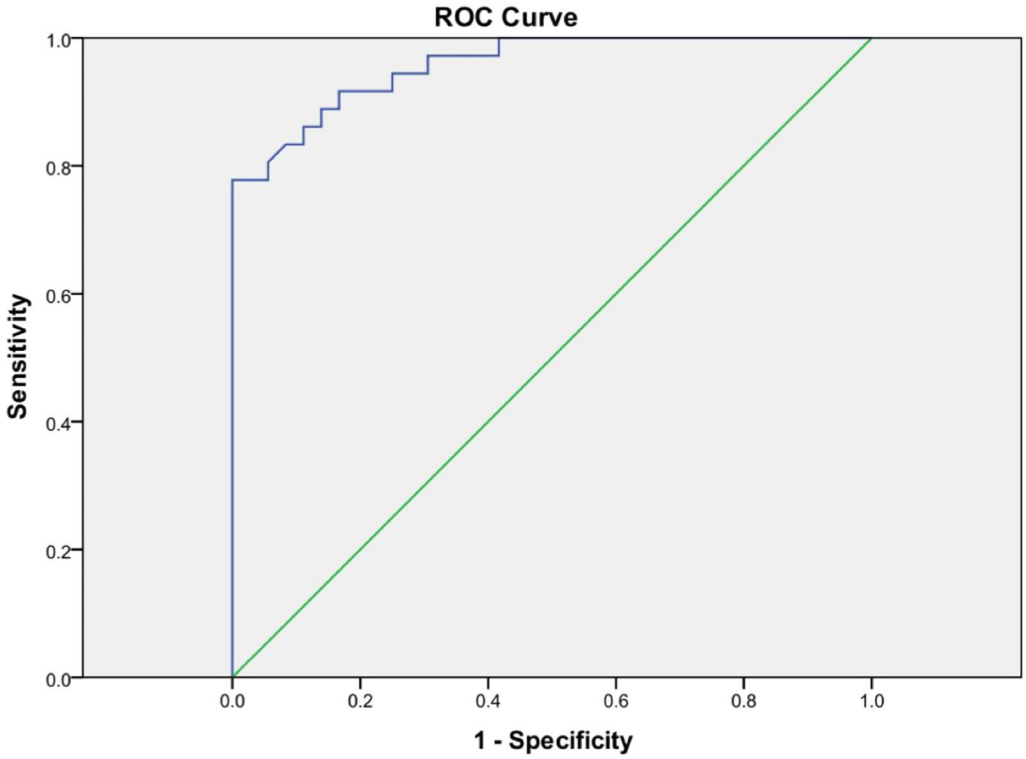

Fig. 2 Receiver operating characteristic (ROC) curve for using cathepsin-B as a predictor for breast cancer metastasis

individuals, but no distinction was observed between hepatoma and cirrhotic liver cases [29].

IL-6 is a key cytokine present in the cancer microenvironment produced by adipocytes and monocytes that are linked to BC tissues. The effect of IL-6 on BC cell growth varies based on the activation level in the Jak/Stat3 signaling pathway and hormone receptor status. In some instances, IL-6 has been shown to stimulate BC cell growth, while in others, it can inhibit growth. Studies have indicated that IL-6 can play various tumor-promoting roles, including enhancing proliferation and suppressing apoptosis in BC cells [30]. IL-6 can stimulate the production of vascular endothelial growth factor (VEGF), which induces angiogenesis and tumor vascularization [31]. Furthermore, IL-6 can induce the expression of genes involved in epithelial-to-mesenchymal transition (EMT), which enables cancer cells to acquire a more aggressive, invasive phenotype [32].

In accordance with our findings, Kozlowski et al. reported that patients with BC exhibited significantly elevated levels of interleukin-6 (IL-6) in their bloodstream compared to healthy women, which was consistent with the patient's clinical information [33].

Furthermore, Mohamed et al. demonstrated that IL-6 can increase the expression of cathepsin-B in response to soluble factors released by BC cells, and showed, through western blotting and enzymatic activity analyses, that counteracting antibodies against IL-6 could reduce cathepsin-B secretion and activity stimulated by 231-conditioned medium [16].

Ibrahim et al. investigated how the cathepsin-B protein expression in BC cells was influenced by IL-6 at different concentrations. They revealed that IL-6 is higher in carcinoma tissues of human hormonal receptor-positive (HRP) BC and that it correlates with the expression of cathepsin-B. Moreover, they found that IL-6, either alone or together with cathepsin-B, are significant therapeutic targets for patients with HRP-BC and positive lymph node patients [11]. Additionally, Knüpfer and Preiss revealed that cathepsin-B and IL-6 were markers associated with poor prognosis in BC patients [34].

Our results, in line with the outcomes of previous studies, suggest that elevated levels of both cathepsin-B and IL-6 are associated with more aggressive BC, poorer prognosis, and resistance to treatment. Consequently, targeting either cathepsin-B or IL-6 has been explored as a promising therapeutic approach in BC. It is influential to note that the association between cathepsin-B and IL-6 in BC is complex and context-dependent, with other factors, such as other proteases, signaling pathways, and interactions with the immune system, also influencing their interplay [16]. Further research is needed to fully elucidate the intricacies of their relationship and its therapeutic implications. Moreover, the current study has key limitations that should be acknowledged, including a small sample size and the fact that the study comprised only Egyptian participants. We suggest that subsequent genetic assessment of the cathepsin-B/IL-6 axis with other proteases, such as matrix metalloproteinases (MMPs), and signaling pathways, such as NF-κB and STAT3, in diverse molecular subtypes of BC with larger sample sizes is necessary to gain a better understanding of their role in BC development and to evaluate the significance between them.

Conclusions

A significant positive correlation between cathepsin-B and IL-6 in BC Egyptian female cases was found. The levels of cathepsin-B gene expression and IL-6 concentrations were higher in metastatic BC cases than in localized BC patients, and both were higher than in healthy individuals. Furthermore, higher cathepsin-B expression was also associated with higher tumor stages and lymphatic with distant metastasis, suggesting that the IL-6-cathepsin-B interaction may play a role in BC development, progression, and metastasis. Therefore, they can be applied as new non-invasive biomarkers for BC detection and assist in the identification of BC cases in need of prompt treatment to reduce mortality and increase life expectancy. Additionally, they can provide valuable insights into the development of novel therapeutic strategies for BC treatment.

Abbreviations

BC	Breast cancer
IL-6	Interleukin-6
CA15-3	Cancer antigen 15-3
qRT-PCR	Quantitative real-time polymerase chain reaction
uPA	Urokinase-type plasminogen activator
MMP	Matrix metalloproteinase
ECM	Extracellular matrix
sIL-6R	Soluble-IL-6-receptor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
LN	Lymph node
HRP	Hormonal receptor-positive
JNK-AP-1	C-Jun N-terminal kinase-activator protein-1
ASCO	American society of clinical oncology
NCCN	National comprehensive cancer network

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Author contributions

BAI and ENE created the idea and designed the research. ESN conducted the statistical analysis of the data. BAI, and ESN accomplished all the laboratory tests and interpreted the patients' data. AMIK and AKT chose the patients. All authors wrote, read, and approved the final manuscript.

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Availability of data and materials

Data and materials are available upon request.

Declarations

Ethical approval and consent to participate

The research conducted in this study was approved by the Ethical Board of the University of Zagazig, Faculty of Medicine, with reference number Zu-IRB# 10101/13-11-2022, and all participants signed the consent form for participation in the study.

Consent for publication

All the authors declared their consent for publication of the manuscript in this journal.

Competing interests

The authors declare no conflict of interest in this work.

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