J Appl Biotechnol Rep. 2025 December;12(4):1875-1882

Journal of
Applied Biotechnology Reports

doi 10.30491/jabr.2025.483014.1792

Original Article

The Potential of Serum CTHRC1 Expression Changes for Early Breast Cancer Detection: A Clinical Investigation

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Received October 12, 2024; **Accepted** March 1, 2025; **Online Published** December 30, 2025

Abstract

Introduction: This study aimed to evaluate the expression of Collagen Triple Helix Repeat Containing-1 Protein (CTHRC1) in the serum of breast cancer patients, addressing the need for non-invasive biomarkers and assessing its diagnostic potential for early detection of breast cancer.

Materials and Methods: This case-control study involved 24 newly diagnosed breast cancer patients and 24 healthy controls. RNA was extracted from serum and converted into complementary DNA (cDNA), and the expression levels of CTHRC1 were measured. The significance of differences in gene expression was analyzed using GenEx software.

Results: Most patients exhibited metastasis to the axillary lymph nodes and were classified as stage I. There was no statistically significant difference in CTHRC1 expression between the breast cancer patients and the control group. The Area Under the Receiver Operating Characteristic Curve (AUC) for serum CTHRC1 levels was 0.601. The sensitivity and specificity of CTHRC1 were 54.16% and 55.14%, respectively.

Conclusions: While the findings of this study did not align with those from studies on breast cancer tissue, it is anticipated that the sensitivity, specificity, and AUC of the ROC will improve with an increased sample size. The majority of our patients had axillary metastasis and were in stage I. Previous studies suggest that CTHRC1 expression is elevated in advanced stages of metastasis, both in serum and tissue. To establish the diagnostic value of CTHRC1, larger-scale clinical studies with more participants are warranted.

Keywords: Breast Cancer, Neoplasm Metastasis, Diagnosis, Tumor Biomarker, Gene Expression Regulation Neoplastic, Collagen Triple Helix Repeat Containing-1 Protein

Citation: Mombeyni M, Mahmoudian-Sani MR, Sabour H, Karimpourian H, Jorjani E. The Potential of Serum CTHRC1 Expression Changes for Early Breast Cancer Detection: A Clinical Investigation. J Appl Biotechnol Rep. 2025;12(4):1875-1882. doi:10.30491/jabr.2025.483014.1792

Introduction

Breast cancer results from the uncontrolled proliferation of abnormal cells in breast tissue, with the malignant forms leading to significant morbidity and mortality. Among its subtypes, ductal carcinoma (cancer originating in the milk ducts) is the most prevalent. Breast cancer is the leading malignancy among women worldwide, particularly affecting those over the age of 50 in Western populations. However, in Iran, the disease typically manifests at a younger age, presenting unique challenges in its detection and management.[1-3] Approximately 5–10% of breast cancer cases are hereditary, frequently attributed to mutations in the breast cancer type 1 susceptibility protein (*BRCA1)* and *BRCA2* genes, which significantly elevate cancer risk in affected individuals.[4,5] Cancer biomarkers have emerged as a revolutionary approach in oncology, offering critical insights into tumor biology. These biomarkers, including DNA, RNA, proteins, and metabolites, facilitate early diagnosis and provide valuable insights into tumor behavior, such as metastatic potential, proliferation dynamics, and the risk of recurrence.[6,7] Given the relatively young onset age of breast cancer in Iran, early detection strategies are essential. Biomarkers are pivotal in addressing this challenge by identifying measurable changes in cancer cells that could be leveraged for noninvasive diagnosis and personalized treatment strategies. Changes in cancer cells that can be measured can serve as biomarkers.[8] Notably, the earlier the diagnosis, the more favorable the therapeutic outcomes, underscoring the importance of developing reliable and sensitive diagnostic tools.[9] One promising biomarker is Collagen Triple Helix Repeat Containing-1 (CTHRC1)

Protein, a regulatory gene initially identified in damaged arterial tissues in rodent models. CTHRC1 is implicated in numerous physiological processes, including vascular remodeling, osteoblast differentiation, wound repair, collagen synthesis, and cellular migration. This protein is highly expressed in the extracellular matrix and is particularly abundant in the tumor microenvironment, contributing to cancer progression and metastasis. Aberrant overexpression of CTHRC1 has been reported in several malignancies, highlighting its clinical significance.[10] The expression of CTHRC1 is regulated by various molecular entities, including miRNAs, lncRNAs, WAIF1, and DPAGT1, which influence its role in tumor biology. Furthermore, CTHRC1 exerts its effects through diverse and complex signaling pathways, such as TGF-β, Wnt, integrin/FAK, Src/FAK, MEK/ERK, PI3K/AKT/ERK, HIF-1, and PKC-α. These pathways underscore its multifaceted role in oncogenesis, contributing to angiogenesis, epithelial-to-mesenchymal transition (EMT), and immune evasion. The widespread involvement of CTHRC1 in these pathways highlights its potential as a diagnostic biomarker and a therapeutic target in breast cancer and other malignancies.[11] A meta-analysis study examined the diagnostic and prognostic significance of CTHRC1 in the progression of various human cancers. The findings revealed that CTHRC1 protein expression is significantly associated with key clinical indicators such as the tumor-node metastasis (TNM) staging system, tumor size (T), nodal involvement (N), metastasis (M), and lymph node metastasis (LN). Elevated CTHRC1 levels were correlated with advanced TNM stage, increased lymph node metastasis, larger tumor size, and poorer clinical outcomes, including overall survival (OS) and disease-free survival (DFS). These associations suggest that CTHRC1 could be a prognostic biomarker in cancer patients.[12] CTHRC1 expression was found to be higher in tumors from patients with stage III/IV cancer compared to those with stage I/II cancer, indicating that CTHRC1 may play a significant role in cancer progression. However, the precise mechanisms by which CTHRC1 influences tumorigenesis are still under investigation.[13] Furthermore, CTHRC1 in the cell membrane has been shown to stabilize the interaction between the Wnt-Frizzled receptor complex, selectively activating the planar cell polarity (PCP) pathway, a critical component of Wnt signaling. This pathway regulates essential processes, including tissue polarity, cell movement, tumor dissemination, and metastasis.[10,14] Increased expressions of CTHRC1 in cancer patients are strongly correlated with node metastasis and advanced TNM stage, suggesting that CTHRC1 may be a crucial factor in tumor progression, cell migration, and the metastatic potential of cancer cells. Tumors from patients with positive LN status and tumor size greater than 5 cm exhibited higher CTHRC1 levels compared to those with negative LN status and tumors smaller than 5 cm, underscoring the role of CTHRC1 in tumor metastasis and proliferation. CTHRC1 has been implicated in the degradation of proteins within the extracellular matrix (ECM), essential for tumor cell proliferation, migration, and metastasis.[15,16] Aberrant CTHRC1 expression may contribute to tumorigenesis by disrupting normal ECM dynamics. Moreover, cancer patients with positive CTHRC1 expression exhibited shorter OS and DFS compared to those with negative CTHRC1 expression, further supporting the notion that elevated CTHRC1 levels in tumors are associated with a poor prognosis.[17,18] This study aims to address the critical need for non-invasive biomarkers by evaluating the diagnostic potential of serum CTHRC1 expression for early detection of breast cancer.

Materials and Methods

Peripheral blood samples (5 ml) were collected from 24 breast cancer patients, aged 20 to 80 years, and 24 healthy controls, aged 20 to 80 years, in this case-control study. All participants provided written informed consent prior to their inclusion in the study. The samples were collected from April to March 2022 at Shafa Hospital, Ahvaz, Iran, under the supervision and guidance of an experienced clinical team. Blood samples were processed in the laboratory using a centrifuge set at 800 g to isolate the serum. The blood serum supernatant was then separated and stored at -80 °C within one hour of collection.

RNA Isolation and Real-time PCR

The expression levels of CTHRC1 were quantified using real-time PCR. Total RNA was extracted from the samples using the FavorPrep™ RNA Kit (Favorgen, Taiwan) according to the manufacturer's protocol. RNA quantity and purity were assessed using a NanoDrop® spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) synthesis was performed using the Revert Aid RT Reverse Transcription Kit (Thermo Fisher Scientific, Inc.), following the manufacturer's guidelines. All cDNA samples were stored at -20°C until subsequent analysis. For gene expression analysis, SYBR® Green PCR Master Mix (Takara Bio, Japan) was used, with a final reaction volume of 20 µl. The Light Cycler Real-Time PCR system (Roche, Germany) was used for all PCR reactions, performed according to the manufacturer's specifications. The thermal cycling conditions were denaturation at 95 °C for 3 minutes, followed by 40 cycles of 95 °C for 15 seconds and 72 °C for 25 seconds. The relative expression of CTHRC1 was normalized to the reference gene *GAPDH* in each sample. All experiments were performed in triplicate to ensure reproducibility. The data were analyzed using the $2^{-\Delta\Delta CT}$ method, and fold changes in gene expression were calculated relative to the control group. The primer sequences used in this study are provided in Table 1.

Table 1. Sequences of Primers Used in real-time PCR

Gene	Primer sequence	Product Size (bp)	Identification Code
CTHRC1	F:5′-GGCAGAGGGAGGTGGTGGAC-3′	138	XM_011516824.3
CTHRC1	R: 5′-CTCCTTTGAATCCATCCCGACC-3′		
GAPDH	F: 5`CAGAGCACAAGAGGAAGAGAGAG-3`	102	NM_001289745.3
GAPDH	R: 5`TCTACATGGCAACTGTGAGGAG-3`		

Statistical Analysis

The significance of differences in *CTHRC1* gene expression was assessed using GenEx software. The receiver operating characteristic (ROC) curve was generated, and the AUC was calculated to evaluate the diagnostic performance of the CTHRC1 biomarker, including its sensitivity and specificity. To assess the distribution of the data, the Shapiro-Wilk test was performed to check for normality. Given the non-normal distribution of the data, the Mann-Whitney U test was employed for group comparisons. This non-parametric test compares the mean ranks between two independent groups. A significance level was established at $p < 0.05$ for all statistical analyses.

Results

The analysis revealed a statistically significant difference in ethnicity and age among participants in the two groups. In contrast, no significant differences were observed for other variables, including gender, religion, family history of cancer, marital status, and allergies. The notable difference in ethnicity and age suggests that factors such as cultural background, living environment, and other socio-environ mental influences may contribute to this variation. On the other hand, the findings indicate that gender, religion, family history of cancer, marital status, and allergies had minimal impact on the differences in participants' responses or conditions (Table 2).

Table 2. Demographic Characteristics of Breast Cancer Patients and Control Subjects Participating in the Study

Variable		Group		*P*
		Patient	Control	
Gender	Female	24 (100%)	23 (95.8)	0.268
	Male	0 (0%)	1 (4.2%)	
Total		24 (100%)	24 (100%)	48
	20-30	0 (0%)	6 (25%)	0.023
	30-40	6 (25%)	8 (33.33%)	
	40-50	11 (45.8%)	6 (25%)	
	50-60	4 (16.7%)	2 (8.3%)	
	60-70	1 (4.2%)	1 (4.2%)	
	70-80	2 (8.3%)	1 (4.2%)	
Total		24 (100%)	24 (100%)	48
Ethnicity	Bakhtiari	7 (29.2%)	13 (54.17%)	0.004
	Arab	15 (62.5%)	2 (8.3%)	
	Fars	1 (4.2%)	6 (25%)	
	Kurd	0 (0.0%)	1 (4.2%)	
	Lor	1 (4.2%)	1 (4.2%)	
	Turk	0 (0.0%)	1 (4.2%)	
Total		24 (100.0%)	24 (100.0%)	20 (100%)
Religion	Shia	22 (95.7%)	24 (100%)	0.345
	Sunni	2 (4.3%)	0 (0%)	
Total		24 (100%)	24(100%)	48 (100%)
Family history of cancer	Yes	9 (37.5%)	12 (50%)	0.405
	No	15 (62.5%)	12 (50%)	
Total		24 (100%)	24 (100%)	48 (100%)
Allergy	Yes	2 (8.3%)	8 (33.33%)	0.064
	No	22 (91.7%)	16 (66.67%)	
Total		24 (100%)	24 (100%)	48 (100%)
Marital Status	Single	3 (12.5%)	6 (25%)	0.498
	Married	21 (87.5%)	18 (75%)	
Total		24 (100%)	24 (100%)	48 (100%)

Table 3 presents key clinical data regarding metastasis status, cancer stage, and chemotherapy treatment in breast cancer patients. The metastasis status section records the extent to which cancer has spread to other organs or tissues. Metastasis is categorized into three groups: "none," indicating no spread; "local," indicating cancer has spread to tissues surrounding the breast; and "distant," indicating cancer has spread to distant organs or tissues beyond the breast. The stage of breast cancer is also recorded, typically classified from 0 to IV. Stage 0 signifies the presence of precancerous changes, while Stage IV indicates that the cancer has metastasized to distant organs or tissues. Additionally, the chemotherapy column contains information regarding the chemotherapy treatments administered to the patients. Chemotherapy involves the use of anti-cancer drugs, and the data in this section can be used to explore the relationship between cancer stages, metastasis, and treatment outcomes. This table offers valuable insights for researchers and clinicians,

Table 3. Characteristics of Breast Cancer Patients, Including Stage, Metastasis Status, and Receiving Chemotherapy

Variable		Frequency	Percent	Valid Percent
Metastasis	Yes	19	79.2	79.2
	No	5	20.8	20.8
	Total	24	100.0	100.0
Stage	Stage 0	1	4.2	4.2
	Stage I	17	70.8	70.8
	Stage II	4	16.7	16.7
	Stage III	2	8.3	8.3
	Total	24	100.0	100.0
Chemotherapy	No	17	70.8	70.8
	Yes	7	29.2	29.2
	Total	24	100.0	100.0

enabling them to identify patterns and treatment needs among breast cancer patients, thereby supporting informed decision-making in breast cancer management.

Evaluation of RNA Quality and Purity

RNA quality and purity were assessed using a NanoDrop spectrophotometer. All samples had A260/A280 ratios between 1.8 and 2.0, indicating acceptable purity for downstream applications.

Amplification Curve and Melting Curve for the Expression of CTHRC1

The amplification curve (Figure 1A) represents the increase in fluorescence signal during PCR cycles, indicating successful amplification of the *CTHRC1* gene. In addition, a distinct peak in the melting curve (Figure 1B) suggests the specificity of the amplification product and the absence of non-specific products or primer dimers.

In Table 4, the expression of the *CTHRC1* gene was compared between the control and the cancer groups using the Mann-Whitney U test. The *p*-value (2-tailed) obtained was 0.3. The Mann-Whitney U test is a statistical test used to compare the mean ranks of two independent groups. A *p*-value of 0.3 indicates a 30% probability of observing a significant difference in the mean ranks between the two groups. Based on this result, we cannot conclude that there is a substantial difference in CTHRC1 gene expression between the two groups. A *p*-value of 0.3 does not provide sufficient evidence to affirm a significant difference in CTHRC1 gene expression between normal and cancerous breast tissues. Therefore, further investigation, potentially with a larger sample size, may be necessary to thoroughly explore this relationship.

The Results of the CTHRC1 Gene Expression

When the levels of CTHRC1 expression were compared

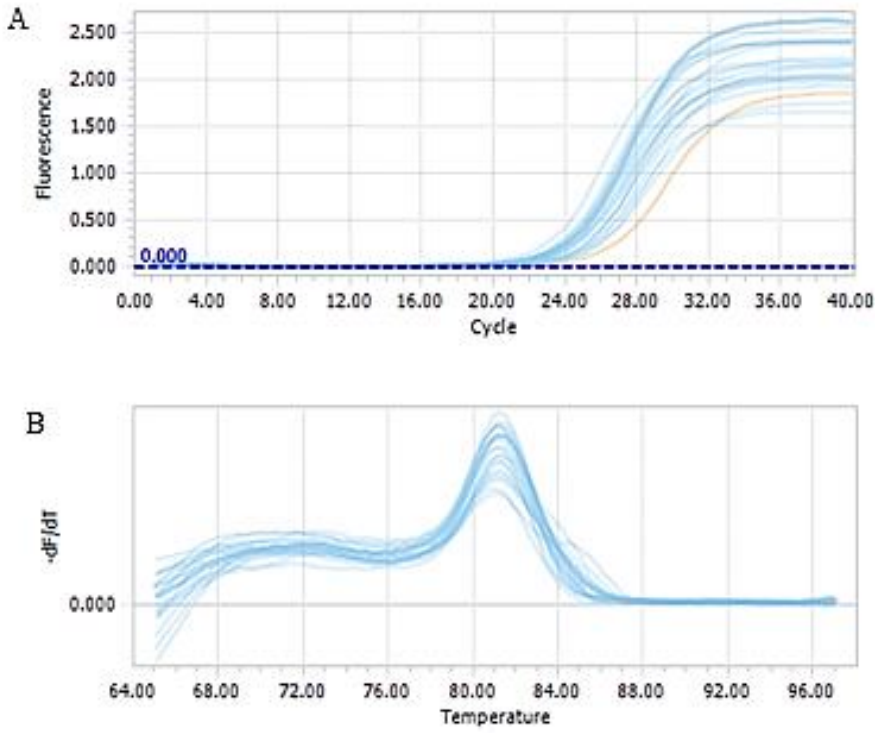

Figure 1. Amplification (A) and Melting (B) Curves from RT-qPCR Analysis of Serum CTHRC1 Expression in Breast Cancer Patients and Healthy Controls. The amplification curve shows an increase in fluorescence during PCR cycles, confirming successful amplification, while the melting curve exhibits a single sharp peak, indicating the formation of a specific product without primer dimers.

Table 4. Comparison of CTHRC1 Gene Expression in the Serum of Breast Cancer Patients and Healthy Controls Using the Mann-Whitney U Test

	CTHRC1 (normal)	CTHRC1 (tumor)
Mean Rank A/B	24.37	20.93
U		277.5
U´		202.5
Z		0.872098363
Total Rank	487.5	502.5
P (2-tail)		0.3

between the groups, the cancer group exhibited lower levels of expression than the control group, though this difference was not statistically significant. Figure 2 illustrates the results of this comparison. GAPDH was used as a reference gene (housekeeping gene) in this study to normalize the data. The plot in the figure indicates whether the fold change in *CTHRC1* expression has increased or decreased, highlighting the relative changes between the two groups.

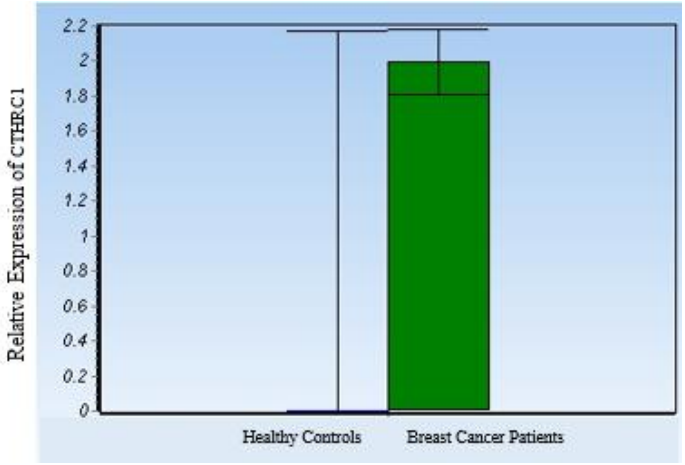

Figure 2. Box Plot Showing Relative Serum CTHRC1 Expression Levels in Breast Cancer Patients and Healthy Controls. No significant difference was observed between the two groups ($p = 0.30$).

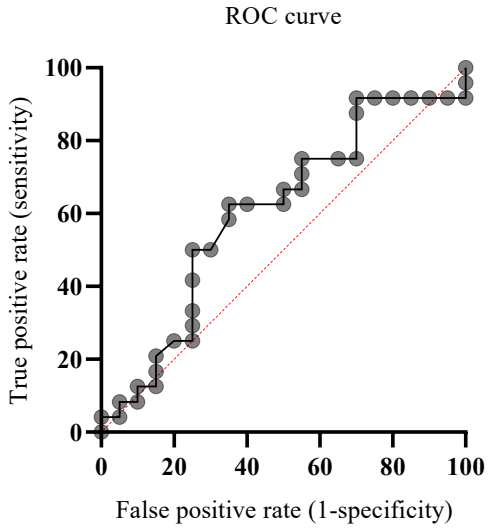

Figure 3. ROC Curve Assessing the Diagnostic Performance of Serum CTHRC1 in Distinguishing Breast Cancer Patients from Healthy Controls.

A box plot was constructed to visualize the distribution of data in the two groups. The box plot reveals that *CTHRC1* gene expression changes are similar in the breast cancer and control groups. Given the considerable overlap between the boxes of the two groups in the box plot, there is minimal interference when comparing *CTHRC1* gene expression changes between the two groups. As a result, we conclude that there is no statistically significant difference in the changes in *CTHRC1* gene expression between individuals with breast cancer and healthy controls.

The area under the curve (AUC) in Figure 3 is 0.601, indicating the divergence between the two groups. The AUC

for CTHRC1 serum levels was found to be 0.601. The AUC, ranging from 0 to 1, measures how effectively a test distinguishes between positive and negative samples. A value closer to one suggests better discrimination between the two groups.

In terms of sensitivity and specificity, CTHRC1 demonstrated a sensitivity of 54.16% and a specificity of 55.14% in this study. Sensitivity reflects the test's ability to correctly identify positive samples, while specificity measures its ability to correctly identify negative samples. Higher values for both metrics indicate a more substantial capacity to differentiate between the two groups (Table 5).

Table 5. Sensitivity, Specificity, Cut-off Point, and Area under the ROC Curve of CTHRC1 for Detecting Breast Cancer from Control Subjects

Gene	(Cut off)	(Specificity)	(Sensitivity)	(AUC)
CTHRC1	3.03	55.14 %	54.16 %	0.601

Discussion

This study represents the first investigation into the serum expression levels of CTHRC1 in newly diagnosed breast cancer patients compared to healthy controls. The findings revealed no statistically significant difference in CTHRC1 serum levels between breast cancer patients and the control group. The ROC analysis showed an AUC of 0.601 for CTHRC1, with sensitivity and specificity values of 54.16% and 55.14%, respectively. Previous research has primarily focused on the expression of CTHRC1 in breast cancer tissues compared to normal controls, with limited attention given to its role as a biomarker in other types of cancer.[17] For instance, only one prior study examined the serum expression levels of CTHRC1 in cervical cancer patients. That study, like ours, utilized enzyme-linked immunosorbent assay (ELISA) to quantify CTHRC1 expression at the protein level. These studies discuss the role of biomarkers in other cancers less; only one study in cervical cancer examined the level of CTHRC1 expression in the serum, and the AUC and sensitivity obtained were comparable to our results. CTHRC1 expression was measured at the protein level using an ELISA assay.[19]

Kharaishvili et al. (2011) explored the expression of CTHRC1 in breast cancer and its association with clinico pathological characteristics. They used specific antibodies to analyze CTHRC1 expression in formalin-fixed paraffin-embedded tissue samples, including invasive carcinoma, normal breast tissue, precursor lesions, and metastatic lymph nodes. The study employed semi-quantitative methods to evaluate expression levels.[20]

Kim and colleagues (2013) examined the clinical relevance of CTHRC1 expression in breast cancer. Their findings indicated that significant upregulation of CTHRC1 is correlated with clinicopathological factors and a poor prognosis.[17] In 2017, Lai and colleagues investigated CTHRC1 expression in breast cancer tissues and cell lines. They identified the pivotal role of the miR-30c/CTHRC1 axis in breast cancer progression and demonstrated that CTHRC1 holds potential as a prognostic biomarker and therapeutic target.[21] Data derived from The Cancer Genome Atlas (TCGA) were utilized in one study, while the Oncomine online database validated differential expression of CTHRC1 between gastric cancer (GC) and adjacent normal tissues. Survival analysis using the Kaplan-Meier method illustrated the impact of CTHRC1 expression on GC survival. The results revealed that CTHRC1 expression is significantly higher in GC tissues than in adjacent non-tumor tissues. Moreover, the Kaplan-Meier curve showed that patients with elevated CTHRC1 expression exhibit poorer prognoses.[22] Microarray analysis of hepatocellular carcinoma (HCC) revealed overexpression of the *CTHRC1* gene, which is associated with tumor size and progression stage. Suppression of CTHRC1 inhibited tumor migration and invasion, whereas increased expression accelerated tumor invasion. Consequently, CTHRC1 emerges as a promising biomarker for predicting the prognosis of HCC.[23] Colon adenocarcinoma (COAD), one of the most prevalent cancers globally, ranks third in incidence and second in mortality. The early stages of COAD are frequently asymptomatic, and the absence of effective screening methods or diagnostic markers with high sensitivity and specificity often leads to missed therapeutic opportunities. In one study, researchers investigated the potential of CTHRC1 as a diagnostic and prognostic biomarker for early detection of COAD. CTHRC1 mRNA and protein expression analyses using various tumor databases revealed significantly elevated CTHRC1 levels in COAD, independent of clinical stage, age, gender, or race. Additionally, elevated CTHRC1 expression was associated with poorer prognoses.[24] CTHRC1 promotes tumorigenesis by modulating the tumor microenvironment; however, research has been limited to a few cancer subtypes. In a comprehensive study, changes in CTHRC1 expression across 24 different human cancer tissues were analyzed and validated using available databases. The study found that CTHRC1 is overexpressed in all 24 major human cancer subtypes. This overexpression was significantly linked to decreased OS in human neural stem cells (HNSC), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC). This evidence suggests that CTHRC1 plays a critical role in the development and progression of various cancers. For patients with HNSC, KIRC, LIHC, LUAD, STAD, and UCEC, CTHRC1 may serve as a

universal diagnostic and prognostic biomarker, applicable across diverse clinicopathological features.[25] Although our study's findings diverge from previous research, the sensitivity, specificity, and AUC will likely improve with an increased sample size. mRNA biomarkers in serum have garnered significant interest from researchers and clinicians due to their potential for non-invasive cancer diagnosis and monitoring. Serum samples can be easily obtained through routine blood draws, offering a patient-friendly alternative to invasive tissue biopsies.[26] Numerous studies in breast cancer have focused on identifying serum-based mRNA biomarkers for early detection, prognosis, treatment response, and prediction of disease recurrence. While mRNA biomarkers show great promise, further investigation and validation are essential. Developing reliable and clinically applicable mRNA-based diagnostic tools requires comprehensive research and large-scale clinical trials to assess sensitivity, specificity, and clinical utility.[27] Detecting mRNA biomarkers in serum remains challenging due to their low abundance relative to other blood components, such as circulating proteins and tumor cells. To address this, highly sensitive and specific detection techniques are required. Standardizing protocols for sample collection, RNA extraction, and detection methods is critical for ensuring the reproducibility and comparability of findings across research and clinical settings.[28] Breast cancer is a heterogeneous disease characterized by diverse mRNA expression profiles. Identifying a single mRNA biomarker that is universally applicable across all breast cancer subtypes is inherently challenging. Extensive validation is required before mRNA biomarkers can be introduced into clinical practice. RNA extraction, reverse transcription, quantitative PCR, and other detection methods are integral to the analysis of mRNA biomarkers.[28] However, these techniques are technically demanding and require expert knowledge to ensure accurate and reproducible results. Errors, including false positives or negatives, can arise from factors such as sample variability, technical inconsistencies, or biological variation, potentially leading to misinterpretations with clinical consequences.

mRNA biomarkers can provide valuable insights into the molecular characteristics of breast tumors, such as hormone receptor status or HER2/neu expression. This information can be leveraged to tailor treatment strategies and identify novel therapeutic targets.[29] Despite their potential, serum mRNA biomarkers remain in the developmental phase. Significant challenges must be addressed for widespread adoption in routine clinical practice, including the validation of their clinical utility and overcoming technical hurdles. To establish the diagnostic value of CTHRC1 in breast cancer, large-scale clinical studies with greater sample sizes are necessary. This endeavor requires significant time and resources. Moreover, the clinical application of CTHRC1 necessitates the development of standardized detection methodologies and cutoff values to ensure consistency and accuracy in evaluations. Future studies should focus on measuring CTHRC1 protein expression using ELISA or western blotting and investigating its relationship to metastasis and disease stages in breast cancer, utilizing a sufficiently large patient cohort.

Conclusion

In this study, the expression levels of CTHRC1 did not show significant differences between breast cancer patients and healthy controls. Therefore, CTHRC1 expression alone may not be a reliable biomarker for the early detection of breast cancer. ROC curve analysis was also performed to assess the diagnostic potential of CTHRC1, yielding a modest diagnostic value of 0.60 for serum CTHRC1 levels. The study compared CTHRC1 expression in breast cancer tissues with that in other forms of cancer, using results from previous studies. Consequently, it is essential to replicate and validate research findings across various study populations. Further clinical studies involving a larger number of participants are recommended, as well as the use of ELISA or Western blots to measure CTHRC1 protein expression in future studies. According to the study, there were no significant differences in CTHRC1 expression levels between healthy controls and breast cancer patients. Although CTHRC1 may be a helpful biomarker for breast cancer, the study's results suggest that it may not be a highly sensitive or specific marker on its own.

Authors' Contributions

MM conducted the experiments, analyzed the data, and drafted the manuscript; HK and EJ supervised the study and provided critical revisions; MMS advised on molecular methodologies and manuscript preparation, while HS contributed to statistical analysis and ensured clinical relevance.

Ethical Approval

The ethics committee for research at the Ahvaz Jundishapur University of Medical Sciences follows the code of ethics of IR.AJUMS.REC.1403.064 approved our work. All participants signed informed written consent, and this study was conducted in accordance with the Declaration of Helsinki.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgment

The authors sincerely thank their esteemed colleagues at the Clinical Research Development Unit, Golestan Hospital and Ahvaz Jundishapur University of Medical Sciences, for their invaluable collaboration and support.

Disclosure of AI Tool Use

This manuscript has utilized Generative AI tools in its

preparation. Specifically, OpenAI's ChatGPT (version December 2024) was employed for language refinement and generating summaries. This use aimed to enhance the clarity and coherence of the text while preserving the integrity and originality of the research content.

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