Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.2025122171-180

The Impact of *miRNA-425* and *miRNA-373* on the Pathogenesis of Breast Cancer

Hanan Jawad Nayyef[a,b], Ismail Hussien Aziz[b]

[a]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq*

[b]*Tropical Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq*

ARTICLE INFO

Received:
16 November 2024
Revised:
18 December 2024
Accepted:
1 February 2025

Keywords:
BC, CEA, EETN, miR-373, miR-425

ABSTRACT

Background: Biomarkers that monitor treatment efficacy could be very useful for early response evaluation, therapy direction, and prognosis prediction. MicroRNAs (miRNAs), a type of noncoding RNA that is only 22 nucleotides long regulate genes after transcription has taken place. miRNAs are essential regulators of cancer biology and are involved in the regulation of key processes such as cell proliferation, apoptosis, migration, and metastasis.

Methods: This study included 100 women with breast cancer (BC) who attended Al-Amal Hospital in Baghdad, Iraq between June 2023 and October 2023. The parameters were evaluated using questionnaires and medical records, including human epidermal growth factor receptor 2 (HER2) receptors, disease progesterone (PR), grade, age, tumor stage, family history, type, location, and estrogen (ER).

Results: The serum Sera' levels (median (IQR)) were significantly higher in patients with BC than in healthy controls (HCs), 104.05pg/ml *versus* 26.85 pg/ml ($P< 0.001$). Sera' levels (median (IQR)) of RETN were significantly higher in the BC group in comparison with HCs, 1.16 ng/ml *versus* 0.41 ng/ml, respectively ($P< 0.001$). The expression levels of miR-373 were significantly higher in the BC group than in the HCs (median (IQR)), 3.54 (8.23) fold change *versus* 1 fold change, respectively ($P< 0.001$). The expression levels of miR-425 were significantly higher in the BC group than in the HCs (median (IQR)), 4.80 (10.22) versus 1 fold change, respectively (P< 0.001).

Conclusion: The biomarkers CEA, RETN, miR-373, and miR-425 were identified as promising candidates for enhancing the diagnostic workup, prognosis, and therapy of BC. CEA and RETN are established markers that aid in the early detection and monitoring of tumor progression, whereas miR-373 and miR-425 are involved in critical processes such as tumor metastasis, EMT, and chemo resistance.

INTRODUCTION

Breast cancer (BC) is the second most common cause of death in women worldwide.[1–3] It arises from uncontrollably altered cell proliferation or function in breast tissue. These alterations cause these cells to become malignant and capable of spreading.[4,5] Biomarkers that aid in monitoring treatment effectiveness may be particularly beneficial.[6] Regulating genes after transcription is the job of microRNAs, a type of noncoding RNA that is only 22 nucleotides long. Differentiation, cell proliferation,

***Address for correspondence:**
Hanan Jawad Nayyef,
Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq
Email: hanan.nayyef@uobaghdad.edu.iq

metabolic processes, and cell death are all affected by the ability to impede mRNA translation. Recent studies have shown a connection between abnormal miRNA expression and the onset of many disorders including cancer.[7,8] A member of a similar family of cell surface glycoproteins is a clinically significant tumor marker. Tumor tissue extracts and normal fetal gastrointestinal tract epithelial cells contain this tumor marker, which is useful for identifying colorectal, gastrointestinal, lung, and breast cancers. Lysine is located at the N-terminus of CEA, which is a glycoprotein. CEA consists of one polypeptide chain with 641 amino acids and 45-50% carbohydrates.[9,10]

Serum CEA levels tend to increase in patients with advanced BC. The clinical use of blood CEA in tracking treatment efficacy in metastatic BC patients, particularly those with bone metastases, has been shown in our research.[3,11,12] Human macrophages and mouse adipocytes release the hormone protein resistin, which has a molecular weight of 12 kDa and is rich in cysteine. It has 108 amino acid peptides and is a progenitor of the resistin-like molecule (RELM) hormone family. In humans, it circulates as a dimer of two 92-amino acid polypeptides.[13] Resistin enhances epithelial-mesenchymal transition and stemness in BC cells via a signalling pathway involving TLR4, NF-κB, STAT3, and TLR4-specific antibodies and antagonists. These pathways are crucial for metastasis and tumorigenesis.[14] Resistin enhances MCF-7 cell migration and invasion to varying degrees, and its role (MCF-7 cell line) is a key model for studying BC due to its well-characterized estrogen receptor-positive (ER+) status, its ability to form tumors in vivo, and its use in drug resistance and stem cell research. Research has showed that resistin encourages BC cells to invade and migrate[15] in Iraqi women diagnosed with BC. in a study aimed to evaluate the expression of *miRNA-425* and *miRNA-373* genes as well as the blood levels of CEA and resistin.

METHODS

This study included 100 womens with BC who attended Al-Amal Hospital in Baghdad, Iraq, for the period from June 2023 to October 2023. The experimental work was carried out at the Institute for Genetic Engineering and Biotechnology Institute for Postgraduate Studies at the University of Baghdad and laboratories of Al-Amal Hospital. The parameters were evaluated using questionnaires and medical records, including disease grade, human epidermal growthfactorreceptor2 (HER2) receptors, age, tumor stage, family history, type, location, estrogen (ER), and progesterone (PR). Women who were 20 years or older and had a history of BC without treatment were included. Individuals with other malignancies, chronic inflammatory illnesses, history of exposure to hormone treatment, radiation, or chemotherapy, and individuals with other cancers were excluded from the study. Overall, 100 healthy women were age-matched with BC patients to rule out their effect on genetic results.

Collection of blood samples

Four mL of peripheral blood from the patient and HCss was drawn into a gel tube and coagulated for approximately 15-20 minutes. The coagulated blood was placed in a centrifuge at a speed of 3000 rpm for 15 min, and the sera obtained for each individual were divided into an aliquot of 0.4 mL of serum which was added to TRIzol™ (0.5 mL) reagent for RNA extraction and the residual serum which was used for the ELISA test (to detect CEA and resistin serum levels).

Gene expression of miRNA-373 and miRNA- 425

Total RNA extraction

The TRIzol RNA isolation kit (ELK Biotechnology, China) was used to separate total RNA from whole blood samples of both patients and healthy controls, in accordance with the protocols provided by the manufacturer. A Quantus Fluorometer (Promega) was used to evaluate the purity and concentration of the extracted RNA to determine the quality of samples for subsequent RT-qPCR analysis. RNA samples were stored at (-80°C) until processing for downstream applications.

Primer design for microRNA-373and microRNA-425 gene expression

Primer sequences for the microRNA-373 gene, microRNA-425, and microRNA-16 (a housekeeping gene) were obtained from the NCBI GenBank database (Table 1).

Synthesis of cDNA from microRNA

An EntiLinkTM Reverse Transcriptase kit (EQ002) was used. The process for reverse transcription of cDNA in a thermal cycler included an initial incubation at 25°C for 5 min, an hour of incubation at 42°C for the cDNA synthesis reaction, and finally 5 min of inactivation of the enzyme. When preparing cDNA for further PCR amplification, it is essential to preserve the results at -20°C.

qRT-PCR for microRNA

miRNA expression levels were estimated using qRT-PCR. The EnTurboTM SYBR Green PCR SuperMix, manufactured by ELK Biotechnology in China, is a 2X reaction mix that has been fine-tuned for use with qRT-PCR equipment that uses the SYBR®/ROX channel to detect and quantify target miRNAs. An innovative passive reference dye (New England Biolabs, UK) was used in its formulation,

which was compatible with hot-start Taq DNA polymerase. Two times the recommended amount of SYBR Green PCR Master Mix was used in the reaction mixture. The total volume was 20µL, and the components were as follows: ROX Dye 0.4µL, Forward primer 0.4µL, Reverse primer 0.4µL, Nuclease-Free Water 5.8µL, cDNA 3µL. A thermocycling procedure was used to configure the RT-PCR protocol.

Table 1. The primers sequences included in this work.

Primers	Sequence (5 – 3)	Band Size (bp)	Optimized temperature
miR-425 RT-primer	GTTGGCTCTGGTGCAGGGTCCGA GGTATTCGCACCAGAGCCAACTCAACG	50	60
miR-425 F	GGTTTTTTTTAGCAGCACGTAAAT	24	60
miR-373 RT-primer	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACGGAAAG	50	62
miR-373 F	GGTTTTTTTACTCAAAATGGGGGCG	25	62
Universal Reverse	GTGCAGGGTCCGAGGT	16	
miR-16-1 RT-primer	GTTGGCTCTGGTGCAGGGTCCGAG GTATTCGCACCAGAGCCAACCGCCAAT	51	59
miR-16-F	GGTTTTTTTTAGCAGCACGTAAAT	24	59

The temperature-dependent gene expression profile

The initial denaturation and denaturation temperatures were 95°C and 45°C, respectively, whereas the annealing and extension temperatures were 59°C and 72°C, respectively.

miRNA425 and miRNA373 Gene Expression Calculation

The technique originally presented by Livak and Schmittgen (2001) was used to measure fold differences in the mature RNAs' quantitative expression, known as the relative cycle threshold ($2^{-\Delta\Delta Ct}$). What establishes this ratio is the relative gene expression ratio between the test group and the HCs. Reduced gene expression or downregulation is indicated by values between 0 and 1, whereas a change of 1 indicates no change. Upregulated or enhanced gene expression is indicated by numbers greater than 1. Target gene expression was normalized by establishing appropriate thresholds to obtain accurate Ct values from RT-PCR. Double delta Ct (threshold cycle) analysis was used to assess the expression of *miRNA425 and miRNA373* genes, in which *miRNA16* was the housekeeping reference gene (HKG). The results of the computations were as follows. Each sample Ct was determined using a real-time cycler program. We estimated the mean values after running each sample twice. For both patients and controls, Ct values were recorded for the housekeeping gene (miRNA16) as well as the target genes (miRNA425 and miRNA373).

Tumor Markers

CEA is a tumor marker that was measured using a kit according to ELK Biotechnology Co., Ltd. (Cat: ELK026ES). RETN is a tumor marker that was measured using a kit according to ELK Biotechnology CO., Ltd.Cat: ELK1225

Statistical Analysis

Data analysis was performed using the SPSS, version 27. When estimating quantitative parametric outcomes, median and interquartile range were used, whereas standard deviations and means were used to derive qualitative non-parametric data. To further compare the data and find the connection between the different research parameters, the Pearson Chi-square test and Spearman relation test were used. A P-value below 0.05 was considered significant at the 95% confidence level. Using ROC analysis, we were able to quantify the AUC that predicted the relevance of a parameter, together with its specificity and sensitivity.

RESULTS

Comparison of mean age between patients and HCs and the clinicopathological characteristics of BC of women enrolled in this study

No significant difference in the mean age was seen between the BC group and HCs, with values of 54.86 ± 10.77 years and 54.61 ± 9.53 years, respectively (P = 0.862) (Table 2).

Table 2. Comparison of mean age between patients and HCs

Characteristic	BC group *n* = 100	HCs *n* = 100	P-Value
Age (years)			
Mean ±SD	54.86 ±10.77	54.61 ±9.53	0.862 I
Range	27 -75	30 -75	NS

NS, not significant; *n*, number of cases; I, independent samples *t*-test

Most patients (84 %) had grade I or grade II disease, whereas 16 % had grade III or grade IV disease. The majority of patients (74 %) had T1 or T2 stage, 10 % of whom had T3 or T4 stage and in 16 % of cases the information about T stage was lacking. In most cases, the N stage was that of N1, accounting for 54 %, followed by N2 stage, which was seen in 24 %,

followed by N3 stage, which was reported in 12 %, and lastly no lymph node involvement (N0) was observed in 10 %. No metastasis was the predominant finding, as it was reported in 70 %, whereas distant metastasis was seen in 4 %; however, information about metastasis was lacking in 26 %. The common type was invasive ductal carcinoma, which was seen in 84 %, whereas invasive lobular carcinomas were seen in 16 %. The results showed that half of the cases involved the left side, 46% the right side, and 4% showed bilateral involvement. Positive immunohistochemical expression of estrogen receptors (ER) was observed in 58 %, the expression of progesterone receptors (PR) was observed in 44 % and Her2 neu was reported in 36 %.

The classification of patients with breast carcinoma based on immunohistochemical profiles is shown in Table 3. Luminal B (ER/PR+, Her2+) was seen in 28 %, luminal A (ER/PR+, Her2-) was seen in 44 %, her2+ (ER/PR−, Her2+) was seen in 8 %, and triple negative (ER/PR−, Her2-) was observed in 20 % of cases.

Table 3. Classification of patients with breast carcinoma based on immunohistochemical profile

Subtype	ER	PR	Her2neu	Number (%)	
Luminal B (ER/PR+, Her2+)	+	+	+	10 (10 %)	28 (28 %)
	+	-	+	16 (16 %)	
	-	+	+	2 (2 %)	
Luminal A (ER/PR+, Her2-)	+	+	-	20 (20 %)	44 (44 %)
	+	-	-	12 (12 %)	
	-	+	-	12 (12 %)	
Triple negative (ER/PR−, Her2-)	-	-	-	20 (20 %)	20 (20 %)
Her2+ (ER/PR−, Her2+)	-	-	+	8 (8 %)	8 (8 %)

PR, progesterone receptor; +, Positive; -: Negative; ER, estrogen receptor.

Comparison of serum markers between BC groups and HCs

A comparison of serum markers between the BC and HCs groups is shown in Table 4. Sera' levels (median (IQR)) of CEA were significantly higher in the BC group in comparison with HCs, 104.05 pg/ml *versus* 26.85 pg/ml, respectively ($P< 0.001$) (Figure 1). Sera levels (median (IQR)) of resistin (RETN) were significantly higher in the BC group than in the HCs, 1.16 ng/ml *versus* 0.41 ng/ml, respectively ($P< 0.001$); Figure 2).

The findings of the receiver operating characteristic (ROC) curve analysis are shown in Table 6 and Figures 3 and 4, in which the diagnostic capacity of blood CEA and serum RETN in the case of BC is assessed. With an area under the curve (AUC) of > 0.7 (0.762) and a cutoff value of >35.71 (pg/ml), CEA demonstrated a satisfactory degree of accuracy of 76.2%, sensitivity of 69.7%, and specificity of 79.2%. With a sensitivity of 89.9% and a specificity of 51.5%, RETN had an excellent accuracy level of 74.2% and a cutoff value of >0.41 (ng/ml). The area under the curve (AUC) was > 0.7 (0.742).

In this study, CEA serum levels in patients with BC averaged 104.05 pg/ml which was significantly higher in the BC group than in the HCs group (Table 4). In this study, with respect to CEA, there was a significant positive correlation with age and a significant negative correlation with tumor location (higher levels with right-sided lesions and lower levels with left-sided lesions), as shown in Table 5. With respect to CEA, there was a significant positive correlation with age and a significant negative correlation with tumor location (higher levels with right-sided lesions and lower levels with left-sided lesions).

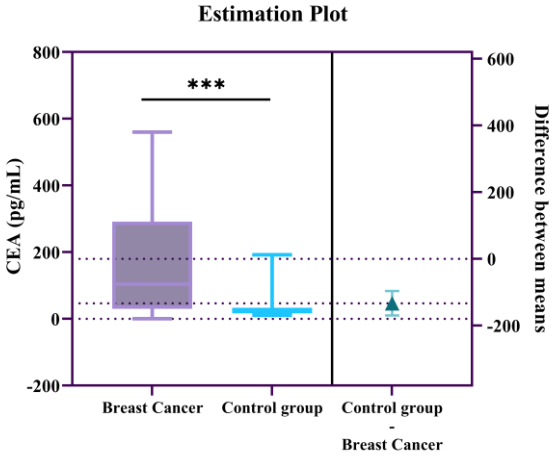

Figure 1. Plot box representing comparisons of serum CEA between BC group and HCs

With respect to RETN, there was a significant positive correlation between tumor grade and ER expression, and a significant negative correlation with family history.

Regarding miR-373, there was a significant positive correlation with ER expression and a significant negative correlation with lymph node involvement and the presence of metastasis (Table 5).

Table 4. Comparison of serum markers between BC groups and HCs

Characteristic	BC group *n* = 100	HCs *n* = 100	P
CEA (pg/mL)			
Median (IQR)	104.05 (261.24)	26.85 (16.47)	<0.001 M
Range	0.48 -559.76	10.91 -191.67	***
RETN (ng/mL)			
Median (IQR)	1.16 (1.46)	0.41 (0.99)	< 0.001 M
Range	0.06 -6.38	0.02 -1.7	***

***: significant at $P \leq 0.001$; M: Mann Whitney U test; CEA: carcinoembryonic antigen; RETN: resistin; *n*: number of cases; IQR: inter-quartile range

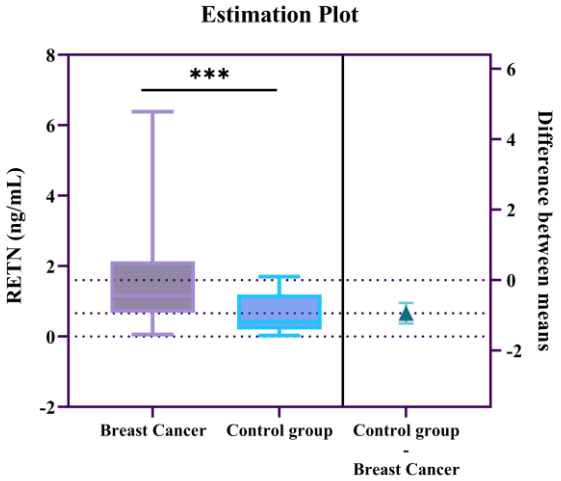

Figure 2. Plot box representing comparisons of serum RETN between BC group and HCs

Comparison of gene expression of miR-373 and miR-425 between BC groups and HCs

In contrast to HCs, the BC group showed considerably greater levels of miR-373 expression, with a median (IQR) fold change of 3.54 (8.23) vs 1 fold change (P<0.001), as seen in Figure 5. Figure 6 shows that there was a considerably greater expression level of miR-425 in the BC group than in the HCs, with a median (IQR) of 4.80 (10.22) fold change and a 1 fold change, respectively (P< 0.001).

Table 5. Correlations of CEA, RETN, miR-373 and miR-425 to clinicopathological characteristics of patients with breast carcinoma

Parameter	CEA (pg/ml)		RETN (ng/ml)		miR-373 fold change		miR-425 fold change	
	p	*r*	*p*	*r*	*p*	*r*	*p*	*r*
Age	0.024 *	0.226	0.351	0.094	0.850	0.019	0.113	-0.160
Grade	0.074	0.180	0.003 **	0.293	0.133	-0.151	0.709	-0.038
Tumor size	0.075	-0.179	0.350	-0.094	0.742	0.033	0.256	0.115
Lymph node	0.994	0.000	0.301	-0.105	0.018 *	-0.236	0.961	-0.005
Metastasis	0.559	0.059	0.544	0.061	0.024 *	-0.226	0.439	-0.078
Family history	0.190	-0.132	0.001 **	-0.313	0.597	0.053	0.860	-0.018
Type of BC	0.177	0.136	0.867	-0.017	0.390	0.087	0.312	-0.102
Location of BC	0.001 ***	-0.326	0.070	-0.182	0.570	-0.057	0.590	0.055
1= Left, 2= Right, 3= Bilateral								
ER	0.934	0.008	0.047 *	0.199	0.002 **	0.305	0.297	-0.105
PR	0.234	-0.120	0.090	-0.170	0.659	-0.045	0.815	-0.024
HER2	0.271	-0.111	1.000	0.000	0.512	0.066	0.921	-0.010

***: significant at $P \leq 0.001$; CEA: carcinoembryonic antigen; ER: estrogen receptors; PR: progesterone; RETN: resistin; BC: BC; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$;

Table 6. The diagnostic potential of serum CEA and serum RETN in case of BC

Characteristic	CEA	RETN
Cutoff	>35.71	> 0.41
AUC (95 % CI)	0.762 (0.697 to 0.820)	0.742 (0.676 to 0.801)
P	<0.001*	<0.001*
Sensitivity %	69.7	89.9
Specificity %	79.2	51.5
Accuracy %	76.2	74.2

*: significant at $P \leq 0.001$; CEA: carcinoembryonic antigen; RETN: resistin.

DISCUSSION

There were different results in this study regarding the presence of luminal B, luminal A, her2+, ER/PR−, and triple negative. Luminal B was seen in 28% of patients, luminal A in 44%, her2+ in 8%, and triple negative in 20%. In 2024, Mohsin and Mohamad (2024) found that 46.67 percent of hormone receptors were positive, 42.22 percent were negative, 62.2 percent were negative for HER2, and 46.67 percent of all molecular subtypes belonged to luminal A and B.[16]

The CEA level was 104.05 (261.24) in the control compared to 26.85 (16.47) in the HCs group, which was close to a study which found that metastatic and recurring BCs were associated with significantly higher blood CEA levels in patients.[17] Serum CEA positive rates have been reported in investigations ranging from 36% to 70%. These heightened levels are recognized to be positively correlated with tumor burden, tumor grade, and metastatic site, consequently leading to reduced overall survival (OS) and progression-free survival.[18]

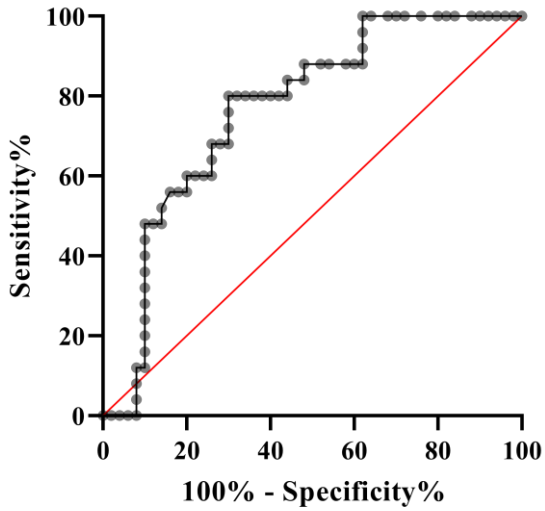

Figure 3. Curve analysis of ROC to detect the value of cutoff of sera CEA level that can predict a diagnosis of breast carcinoma with best accuracy

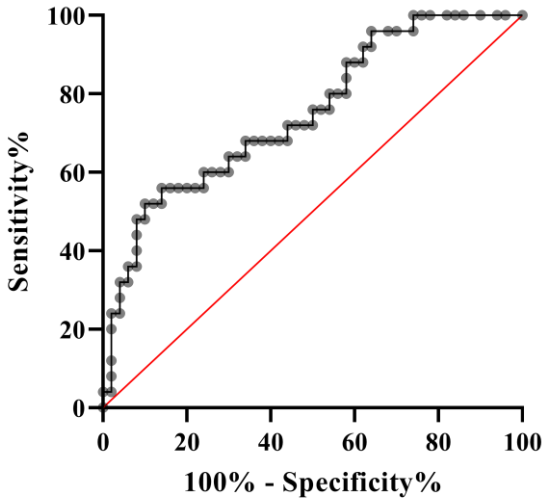

Figure 4. Curve analysis of ROC to detect the value of cutoff of sera RETN level that can predict a diagnosis of breast carcinoma with best accuracy

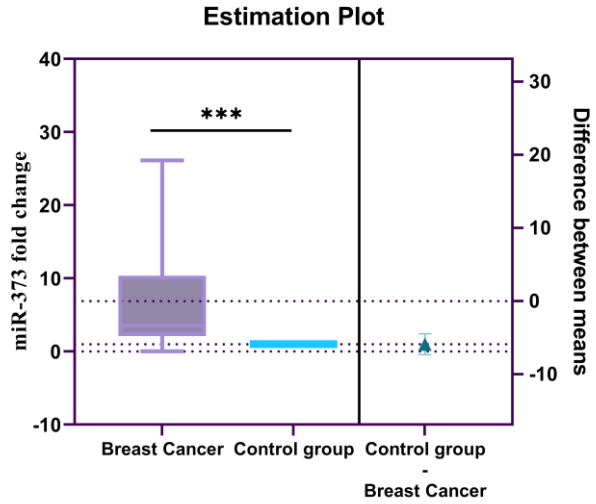

Figure 5. Plot box representing comparisons of miR-373 expression between BC group and HCs

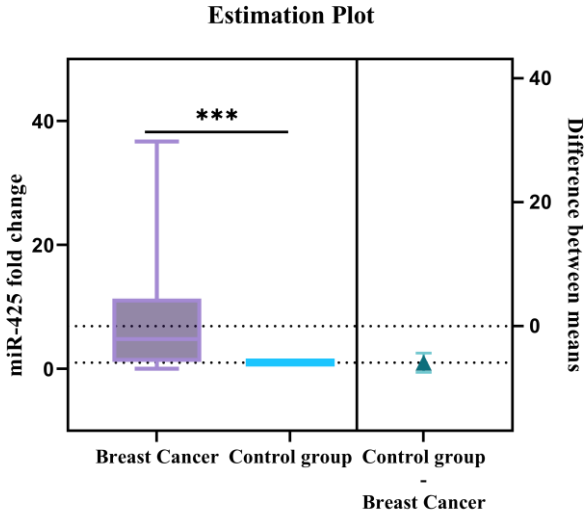

Figure 6. Plot box representing comparisons of miR-425 expression between BC group and HCs

The recommendation for serial monitoring of tumor markers is not supported in asymptomatic individuals after BC treatment.[19,20]

The commonly used serum tumor markers for BC are CA15-3 and CEA.[21,22]. Therefore, the estimation of serum CEA levels can be considered an adjunctive method for evaluating treatment response, monitoring progress, and obtaining prognostic insights. Nevertheless, the clinical applicability of these markers remains uncertain because of conflicting outcomes.[23,24]

In the present study, Sera' levels of RETN were significantly higher in BC group in comparison with HCs. Our findings are in agreement with the results of several previous articles.[25–27] The study conducted by Hou *et al.* (2007) collected blood samples from 80 recently diagnosed BC patients who had received histological confirmation, along with 50 age-matched healthy controls.[25] The findings indicated that the serum concentrations of resistin were elevated in BC patients compared to healthy controls, with levels of (26.35±5.36) μg/L *versus* (23.32±4.75)μg/L, showing

a statistically significant difference (P=0.000). Dalamaga *et al.* observed that the average serum resistin levels were notably higher in the cases than in HCs (P<0.001).[26] Furthermore, in a study conducted by Assiri *et al.*, a cohort of 82 newly diagnosed BC patients who had histological confirmation and 68 healthy controls matched in terms of age and BMI were included.[27] The results revealed significantly elevated levels of resistin in BC patients compared with their respective control counterparts. However, the results of Georgiou *et al.*, were inconsistent with those of the present study as they reported that women with breast carcinoma exhibited resistin levels (6.11±4.49ng/ml), whereas control subjects showed a mean level of 6.14±1.83ng/ml and according to the statistical analysis, there was no notable variation in serum resistin levels between the groups (P=0.064).[28]

Consistent with the results of the present study, ROC curve analysis showed that resistin had poor diagnostic performance, with a total accuracy level of 72%.[26] According to a number of studies, resistin is essential for cancer cell metabolic regulation, angiogenesis, inflammation, proliferation, and metastasis.[29]. Resistin may serve as a biomarker for BC, indicating an advanced disease stage and inflammatory state, even if its diagnostic performance was poor according to ROC curve study [0.72, 95% CI: 0.64-0.79].[26] Research has identified resistin as a possible biomarker for cancer diagnosis and prognosis.[25]

Researchers have found that resistin levels were higher in BC patients (80 total) than in healthy controls (50 total). Additionally, resistin levels have been shown to be higher in individuals with lymph node metastasis than in those without it.[30] Age, menopausal status, blood glucose, body mass index (BMI), and adiponectin levels had no effect on the association between resistin levels and an increased risk of BC. The study conducted by Dalamaga *et al.* and Assiri *et al.* found a strong association between tumors and inflammatory markers, tumor size, malignancy grade, stage, and lymph node invasion.[26,27]

Hou *et al.* documented that serum concentrations of resistin exhibited notable discrepancies between individuals afflicted with lymph node metastasis and those devoid of such metastasis.[25] Also, Dalamaga *et al.* noted in their study that resistin was significantly correlated with various parameters in BC patients, including cancer stage, tumor dimensions, grade, and lymph node infiltration, while exhibiting no apparent relationship with hormone receptor status.[26] In addition, according to Assiri *et al.* (2015), histological grading, tumor size, lymph node metastasis, and TNM staging were positively correlated with serum resistin levels.[27] Our study found a strong negative link between family medical history and a strong positive relationship between RETN, tumor grade, and ER expression. Research suggests that microRNAs play important roles in a variety of cellular processes, and patterns of miRNA expression might be useful indicators for the diagnosis of different cancers and patient outcomes. Additionally, multiple studies have shown that miRNA-373 has oncogenic functions in human cancers by targeting certain genes.[17,24,31,32] Nevertheless, the functions of *miRNA-373* in BC have been debated by Wei and Wang, who motivated us to examine the role of *miRNA-373* in BC.[33]

The current study demonstrated a notable increase in the expression levels of miR-373 among patients compared to HCs. This observation aligns with the results reported by Bakr *et al.*, who noted a heightened presence of *miRNA-373* in BC patients and those with benign breast lesions, in contrast to reduced levels in the HCs. These results suggest that *miRNA-373* could serve as a valuable molecular biomarker for the early detection and diagnosis of BC, facilitating the differentiation between cancerous and non-cancerous instances.[34]

Interestingly, these findings contradict the observations of Bakr *et al.*, who identified a significant link between the expression level of *miRNA-373* and adverse prognostic factors in BC.[34] In addition, ̲Raheem *et al.* found elevated levels of MiRNA-373 in BC pataints (-12.20 ± 1.11). *miRNA-373*, which is unique to human embryonic stem cells (ESCs), exerts novel oncogenic effects by regulating the growth and formation of tumors in primary human cells containing oncogenic factors such as rat sarcoma (RAS) and wild-type p53.[33] Its distinctive expression patterns and strong relationship with cancer cell invasion and metastasis make it a promising candidate as a potential biomarker for BC, as suggested by findings from studies on cancer cells and tissue samples.[35,36] This study examined miR-425 expression levels, which were much higher in patients than in healthy controls. The role of miR-425 in the development of certain cancers was suggested by Zhang *et al.*[10] The results of this study are in agreement with those of Xiao *et al.*, who used RT-qPCR to show that miR-425 expression was higher in BC tissues than in neighboring tissues and cell lines. In addition, compared to human mammary epithelial cells, BC cell lines exhibit higher expressions of miR-425. To our knowledge, our study is the first to compare miR-425 expression levels in healthy individuals with BC patients in Iraqi women. The significant increase in miR-425 levels observed in our study highlights the promising role of this biomarker as a non-invasive method for detecting early stage.[37]

While miR-425-5p's involvement in the oncogenesis of other neoplasms has been established, the exact nature of its function in BC remains unknown.[10] This study found that miR-425-5p is greatly upregulated in BC cells and is associated with poor prognosis in BC patients. Zhang *et al.* (2020) found that miR-425-5p was significantly upregulated in BC cells and predicted a poor prognosis in BC patients.[38] BC cell growth was greatly enhanced by an increase in miR-425-5p levels. Following this, researchers found that cyclin-dependent kinases (CDK4), Cyclin D3 (cyclin D3), and miR-425-5p protein levels were all upregulated. Conversely, cell cycle arrest in the G0/G1 phase was the end outcome of miR-425-5p suppression, which led to reduced expression of these genes.[10] From a mechanistic standpoint, silencing miR-425-5p had the opposite effect as overexpression, leading to increased phosphorylation of phosphoinositide 3-kinase-regulatory subunit -p85 (PI3K- p85) and protein kinase B (AKT). Additionally, miR-425-5p binds to the 3'UTR of Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) mRNA, which in turn reduces the production of PTEN in BC cells, both at the mRNA and protein levels. In summary, the results demonstrate that miR-425-5p is involved in BC carcinogenesis via PI3K/AKT pathway activation, implying that it may be a therapeutic target for BC. This study provides important insights into the expression of miR-373, miR-425, and CEA, and serum levels. These findings may be more applicable to the specific patient population and healthcare setting studied, but they lay the groundwork for further research. Larger multicenter studies with more diverse patient populations are essential to confirm and extend these results. Future research should be conducted by increasing the sample size, including more diverse demographic groups, and incorporating multi-center designs. This would enhance the external validity of the findings and provide a clearer picture of how the results can be applied to different settings and populations.

CONCLUSION

The biomarkers CEA, RETN, miR-373, and miR-425 were identified as promising candidates for enhancing the diagnostic workup, prognosis, and therapy of BC. CEA and RETN are established markers that aid in the early detection and monitoring of tumor progression, whereas miR-373 and miR-425 are involved in critical processes such as tumor metastasis, EMT, and chemoresistance. Their potential to regulate CD44 expression highlights their roles in inhibiting cancer cell invasion and reducing metastasis.

ETHICAL CONSIDERATIONS

Each patient and control participant provided descriptive information to complete a questionnaire designed for this purpose. The study was conducted based on the ethics code of Al-Amal Hospital in the Medical City in Baghdad, Iraq, under the document number (11581) on (19/03/2023).

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

FUNDING

No funding was received to conduct this work.

ACKNOWLEDGEMENTS

We are truly grateful to the patients who supplied samples for our study despite their debilitating medical conditions. The staff members at Al-Amal Hospital made considerable efforts to make the process of collecting samples as simple as possible. We thank the laboratories of the Institute for Genetic Engineering and Biotechnology for Postgraduate Studies/ University of Baghdad for their contribution to accomplishing the practical part of this study.

REFERENCES

1. Abdallah M, Aziz IH, Alsammarraie AZ. (2024) "Assessment of miRNA-10b Expression Levels as a Potential Precursor to Metastasis in Localized and Locally Advanced/Metastatic Breast Cancer among Iraqi Patients." *Int J Breast Cancer*. 2024;2024(1):2408355.
2. Hussain AM, Ali AH, Mohammed HL. (2022) "Correlation between serum and tissue markers in breast cancer Iraqi patients." *Baghdad Sci J*. 2022;19(3):501.
3. Sahan KA, Aziz IH, Dawood SN, Al Qazzaz H. (2022) "The role of resistin gene polymorphism in Iraqi breast cancer patients." *Biomedicine*. 2022;42(6):1296-1300.
4. Siegel RL, Miller KD, Fuchs HE, Jemal A. (2022)

"Cancer statistics, 2022." *CA Cancer J Clin*. 2022;72(1):7-33. doi:10.3322/caac.21708.
5. Abbas R, Aziz I. (2022) "A study comparing the oncogenic microRNA-21-5p and the CA15-3 characteristics as an effective tumor marker in breast cancer patients from Iraq." *Bionatura*. 2022;7:1-7. doi:10.21931/RB/2022.07.04.10.
6. Bodaghi A, Fattahi N, Ramazani A. (2023) "Biomarkers: Promising and valuable tools towards diagnosis, prognosis and treatment of Covid-19 and other diseases." *Heliyon*. 2023;9(2).
7. Abed RM, Yaaqoob LA. (2023) "Novel single nucleotide polymorphism (rs1600485907) of IL-41 gene associated with systemic lupus erythematous." *Asia-Pacific J Mol Biol Biotechnol*. 2023;31(4):1-8.
8. AL-saqabi ANI, Aziz IH. (2023) "The expression miRNA-195 in a sample of Iraqi breast cancer patients." *Bionatura*. 2023;8(3):1-6.
9. Salih AM, Aziz IH, Mohsin FY. (2023) "Role of miRNA 199a-5p Expression in Iraqi Women with Breast Cancer." *Al-Rafidain J Med Sci (ISSN 2789-3219)*. 2023;5(1S):S94-99.
10. Zhang Y, Zhao J, Wang Y, et al. (2022) "Changes of tumor markers in patients with breast cancer during postoperative adjuvant chemotherapy." *Dis Markers*. 2022;2022(1):7739777.
11. Anoop TM, Soman S, Chacko S, Mathew M. (2022) "Significance of serum carcinoembryonic antigen in metastatic breast cancer patients: A prospective study." *World J Clin Oncol*. 2022;13(6):529.
12. Sahan KA, Aziz IH, Dawood SN, Razzaq SSA. (2023) "Evaluation of miR-146 and miR-196 as potential biomarkers in a sample of Iraqi breast cancer patients." *Bionatura*. 2023;8(2):1-2.
13. Shin JH, Park S, Cho H, Kim JH, Choi H. (2022) "Adipokine human Resistin promotes obesity-associated inflammatory intervertebral disc degeneration via pro-inflammatory cytokine cascade activation." *Sci Rep*. 2022;12(1):8936.
14. Wang CH, Wang PJ, Hsieh YC, et al. (2018) "Resistin facilitates breast cancer progression via TLR4-mediated induction of mesenchymal phenotypes and stemness properties." *Oncogene*. 2018;37(5):589-600.
15. Lee JO, Kim N, Lee HJ, et al. (2016) "Resistin, a fat-derived secretory factor, promotes metastasis of MDA-MB-231 human breast cancer cells through ERM activation." *Sci Rep*. 2016;6(1):18923.
16. Mohsin RN, Mohamad BJ. (2024) "Clinical and Histopathological Features of Breast Cancer in Iraqi Patients between 2018-2021." *Iraqi J Sci*. Published online 2024:90-107.
17. Yang Y, Zhang H, Zhang M, Meng Q, Cai L, Zhang Q. (2017) "Elevation of serum CEA and CA15-3 levels during antitumor therapy predicts poor therapeutic response in advanced breast cancer patients." *Oncol Lett*. 2017;14(6):7549-7556.
18. Khushk M, Khan A, Rehman A, et al. (2021) "The role of tumor markers: carcinoembryonic antigen and cancer antigen 15-3 in patients with breast cancer." *Cureus*. 2021;13(7).
19. Puglisi F, Fontanella C, Numico G, et al. (2014) "Follow-up of patients with early breast cancer: is it time to rewrite the story?" *Crit Rev Oncol Hematol*. 2014;91(2):130-141.
20. Rasmy A, Abozeed W, Elsamany S, et al. (2016) "Correlation of preoperative Ki67 and serum CA15. 3 levels with outcome in early breast cancers a multi institutional study." *Asian Pacific J Cancer Prev*. 2016;17(7):3595-3600.
21. Bhindi B, Leibovich BC. (2018) "Re: Robotic versus Open Level I-II Inferior Vena Cava Thrombectomy: A Matched Group Comparative Analysis: L. Gu, X. Ma, Y. Gao, H. Li, X. Li, L. Chen, B. Wang, Y. Xie, Y. Fan and X. Zhang: J Urol 2017;: 198:: 1241–1246." *J Urol*. 2018;199(5):1351-1352.
22. Araz M, Beypinar I, Kazan S, Inci F, Celiker M, Uysal M. (2019) "Are preoperative serum CA15-3 levels different in breast cancer subgroups?" *Curr Probl Cancer*. 2019;43(2):115-122.
23. Nam S eun, Lim W, Jeong J, et al. (2019) "The prognostic significance of preoperative tumor marker (CEA, CA15-3) elevation in breast cancer patients: data from the Korean Breast Cancer Society Registry." *Breast Cancer Res Treat*. 2019;177:669-678.
24. Li J, Liu L, Feng Z, et al. (2020) "Tumor markers CA15-3, CA125, CEA and breast cancer survival by molecular subtype: a cohort study." *Breast Cancer*. 2020;27:621-630.
25. Hou W-K, Xu Y-X, Yu T, et al. (2007) "Adipocytokines and breast cancer risk." *Chin Med J (Engl)*. 2007;120(18):1592-1596.
26. Dalamaga M, Sotiropoulos G, Karmaniolas K, Pelekanos N, Papadavid E, Lekka A. (2013) "Serum resistin: a biomarker of breast cancer in postmenopausal women? Association with clinicopathological characteristics, tumor markers, inflammatory and metabolic parameters." *Clin Biochem*. 2013;46(7-8):584-590.
27. Assiri AMA, Kamel HFM, Hassanien MFR. (2015) "Resistin, visfatin, adiponectin, and leptin: risk of breast cancer in pre-and postmenopausal saudi females and their possible diagnostic and predictive implications as novel biomarkers." *Dis Markers*. 2015;2015(1):253519.
28. Georgiou GP, Provatopoulou X, Kalogera E, et al. (2016) "Serum resistin is inversely related to breast cancer risk in premenopausal women." *The Breast*. 2016;29:163-169.
29. Deb A, Deshmukh B, Ramteke P, Bhati FK, Bhat MK. (2021) "Resistin: A journey from metabolism to cancer." *Transl Oncol*. 2021;14(10):101178.
30. Hlavna M, Kohút L, Lipková J, et al. (2011) "Relationship of resistin levels with endometrial cancer risk." *Neoplasma*. 2011;58(2):124-128.
31. Liu W, Li M, Chen X, et al. (2015) "MicroRNA-373 promotes migration and invasion in human esophageal squamous cell carcinoma by inhibiting TIMP3 expression." *Am J Cancer Res*. 2015;6(1):1.
32. Tu H, Chang K, Cheng H, Liu C. (2015) "Upregulation of mi R-372 and-373 associates with lymph node metastasis and poor prognosis of oral carcinomas." *Laryngoscope*. 2015;125(11):E365-E370.
33. Wei F, Cao C, Xu X, Wang J. (2015) "Diverse functions of miR-373 in cancer." *J Transl Med*.

2015;13:1-8.
34. Bakr NM, Mahmoud MS, Nabil R, Boushnak H, Swellam M. (2021) "Impact of circulating miRNA-373 on breast cancer diagnosis through targeting VEGF and cyclin D1 genes." *J Genet Eng Biotechnol*. 2021;19(1):84.
35. Huang Q, Gumireddy K, Schrier M, et al. (2008) "The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis." *Nat Cell Biol*. 2008;10(2):202-210.
36. Tavazoie SF, Alarcón C, Oskarsson T, et al. (2008) "Endogenous human microRNAs that suppress breast cancer metastasis." *Nature*. 2008;451(7175):147-152.
37. Xiao S, Zhu H, Luo J, Wu Z, Xie M. (2019) "miR-425-5p is associated with poor prognosis in patients with breast cancer and promotes cancer cell progression by targeting PTEN." *Oncol Rep*. 2019;42(6):2550-2560.
38. Zhang L, Zhang J, Zhou H, et al. (2020) "MicroRNA-425-5p promotes breast cancer cell growth by inducing PI3K/AKT signaling." *Kaohsiung J Med Sci*. 2020;36(4):250-256.
39. Raheem AR, Abdul-Rasheed OF, Khattab OS, Alsammarraie AZ, Al-Aubaidy H, Abid HA. (2024) "Circulating miRNA-373 and Vascular Endothelial Growth Factor as Potential Biomarkers for Early Detection of Breast Cancer." *Indian J Clin Biochem*. Published online 2024:1-10.

How to Cite This Article

Nayyef HJ, Aziz IH. The Impact of miRNA- 425 and miRNA- 373 on the Pathogenesis of Breast Cancer. Arch Breast Cancer. 2025; 12(2):171-80.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/1051