Original Article

Evaluation of Circulating miRNA-145 Gene Expression as a Potential Biomarker in Breast Cancer: A Comprehensive Correlational Study with Hormonal Receptors, Clinical Parameters and Therapeutic Response

microRNA-145 Gene Expression Levels in the Blood of Breast Cancer

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ABSTRACT

Objective: To investigate microRNA-145 gene expression levels in the blood of breast cancer patients versus healthy subjects and examine its relationship with molecular, clinical, and therapeutic features.

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Imam Hussein (AS) Center for Oncology and Hematology, Karbala, Iraq, from 1st December 2024 to 31st July 2025.

Methods: The samples comprised two groups of women (patients and a control group). The gene expression levels were quantified using quantitative reverse transcription-polymerase chain reaction, with U6 being the normalization gene. Statistical analysis was conducted to establish the correlation between gene levels and hormone receptors (estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2), histological features (tumor size, metastasis) and treatments (chemotherapy, biological, radiation).

Results: Significant differences were evident in the microRNA-145 expression levels between patients and controls (0.37 vs. 1.00; p=0.0001). A significant difference existed between the expression level and the hormone receptor status (estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2), tumor size and lymph node invasion. The radiation group exhibited the lowest expression levels of the gene (0.361) as opposed to the chemotherapy and biological groups.

Conclusion: The reduced microRNA-145 expression in breast cancer patients and supports its role as a diagnostic and prognostic biomarker.

Key Words: Breast cancer, MicroRNA-145, Hormone receptor, Chemotherapy

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INTRODUCTION

The issue of breast cancer development is one of the biggest challenges that affect the health of women today, because the frequency of developing such tumors and mortality rate are high and lead among all other cancer.[1]

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With advances in diagnostics and treatment approaches, there is still a necessity to develop non-invasive biomarkers which will be able to detect breast cancer in early stages predict the disease course, and evaluate the effectiveness of treatment. Traditional biomarkers do not fully explain tumor progression, increasing interest in molecular biomarkers such as microRNAs.[2]

MicroRNAs are small nucleic acids that are involved in post-transcriptional regulation of gene expression in physiological and pathological conditions associated with cell division, differentiation, and apoptosis. These molecules can be divided into two groups, oncogenic miRNAs and tumor suppressor miRNAs. MiRNA-145 is known as a tumor suppressor gene, whose decreased expression contributes to the development of aggressive breast cancer due to increased proliferation and metastatic capacity of tumor cells. While most previous works were devoted to investigating the role of miRNA-145 at tissue level, the analysis of its concentration in circulating blood samples (circulating miRNA) is an underdeveloped aspect that deserves further exploration in combination with clinical data on breast cancer patients.[3-5]

Breast cancer pathology is characterized by the interconnection of multiple factors: hormonal background (ER, PR, HER2 receptor), pathology features, medical and reproductive history of patients, and treatment types (chemotherapy, biological therapy, and radiotherapy).[6] Thus, understanding the interaction of miRNA-145 with these aspects could help to identify molecular mechanisms of breast cancer and the effect of different treatments on this process.[7] This research aims to address the problem and examine the expression of miRNA-145 gene in blood samples of breast cancer patients in comparison with control healthy samples. Specifically, we plan to analyze the correlation of this gene level with hormone receptors and other features described above. The expected results of the study may contribute to the use of miRNA-145 as a biomarker.

METHODS

This case-control study was conducted at the Imam Hussein (AS) Center for Oncology and Hematology, Karbala, Iraq, from 1st December 2024 to 31st July 2025 vide letter 2024/Issue 3783/Approval/JSDJNEHU Dated November 22, 2024. A total of 100 women were enrolled and stratified into two groups; case group (n=60) with histopathologically confirmed breast cancer, and control group (n=40) age-matched healthy women with no personal or family history of malignancy.

Venous blood samples (2 mL) were collected from each participant into EDTA-containing tubes to preserve RNA integrity. Samples were immediately mixed with TRIzol® Reagent (TransGen, China). At a 1:1 (v/v) ratio, vortexed thoroughly, incubated at room temperature for 5 min, and stored at −80°C until RNA extraction. Total RNA, including microRNAs, was isolated following the manufacturer's protocol with minor modifications: samples were phase-separated with chloroform, RNA was precipitated with isopropanol, washed with 75% ethanol, and resuspended in RNase-free water. RNA concentration and purity were assessed using a UV-Vis Spectrophotometer (Shimadzu, Japan) ; only samples with A260/A280 ratios between 1.8–2.0 were included in downstream analyses.

One-Step RT-qPCR for miRNA-145 Quantification: Relative expression of miRNA-145 was quantified using the GoTaq® 1-Step RT-qPCR System (Promega, USA) on an Analytik Jena Real-Time PCR System (Germany). The small nuclear RNA (U6)was selected as the endogenous reference gene based on its stable expression across blood samples.[4,5] Primer sequences (5'→3') were: miRNA-145 Forward:ACACTCCAGCTGGGCAGGTCAAAAGGG TCC, Reverse: GGTGTCGTGGAGTCG) and U6 (Forward: CCTTGTCCTCACGGTCCAGT, Reverse: AACCATGACCTC AAGAACAGTATTT) (Macrogen, South Korea) Each 20 µL reaction contained: 10 µL GoTaq® qPCR Master Mix (2X), 0.4 µL GoScript™ RT Mix (50X), 300 nM each primer, 1.6 µL $MgCl_2$ (25 mM), 100 ng RNA template, and nuclease-free water to volume. Thermal cycling conditions were: reverse transcription at 37°C for 15 min; initial denaturation at 95°C for 10 min; followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. All reactions were performed in triplicate, and amplification specificity was confirmed by melting curve analysis.

Relative quantification of miRNA-145 expression was calculated using the comparative Ct (2^−ΔΔCt) method.[6] Normalization was performed as: ΔCt = Ct(miRNA-145) – Ct(U6); calibration as: ΔΔCt = ΔCt(case) – ΔCt(control); and fold change as: Relative Quantity (RQ) = 2^−ΔΔCt. Expression levels were expressed as fold change relative to the control group.

Data analysis was done with SPSS-23. Comparisons among groups were done through independent t-test or Mann Whitney U test where appropriate. Association between miRNA-145 level and other clinicopathological variables was studied by applying Pearson and/or Spearman correlation coefficient. Significance level was set at two-tailed $p<0.05$.

RESULTS

The decrease in the relative expression level of miRNA-145 in breast cancer patients compared to the control group, with an average expression of 0.37 in the patient group versus 1.00 in the control group, which served as the reference value (Fig. 1). A significant decrease in the relative expression level of miR-145 was observed in breast cancer tissue compared to the control. This indicates reduced gene expression of this microRNA in tumor tissue (Fig. 2).

Table 1 showed significant differences in miRNA-145 gene expression levels between the control group and the groups associated with hormone receptor status (ER and PR) and HER2 receptor status. The mean concentration in the control group was 1.359±0.232, with an LDS of 0.193 and a significance level of p =0.0001. In patients with HER2-positive receptors, the mean concentration was 0.400 ± 0.244, while in patients with HER2-negative receptors it was 0.327±0.124. This confirms a significant difference in miRNA-145 gene expression levels between the groups. Additionally, the results for the ER receptor in the table show that the mean concentration in the control group was 1.378±0.264, with an LDS of 0.308 and a significance level of p=0.0021, while the concentration in patients with ER-positive receptors was 0.328±0.328. The concentration of miRNA-145 was 0.160 and rose relatively to 0.545±0.247 in ER-negative patients. For the PR receptor, the concentration in the control group was 1.358±0.214 with an LDS of 0.189 and a p-value of 0.0001. The concentration level in the positive PR receptor was 0.257±0.123, and in the negative PR receptor it reached 0.523±0.256.

Figure 3 showed negative correlations between the control group and both HER2-positive (r=-0.324) and HER2-negative groups (r=-0.456), while a weak positive correlation was observed between HER2-positive and HER2-negative groups (r=0.218). Figure 4 demonstrated a strong negative correlation between the control group and ER-positive patients (r=-0.734), and a weak negative correlation with ER-negative patients (r=-0.263). A weak positive correlation was observed between ER-positive and ER-negative groups (r=0.262). Figure 5 showed a weak positive correlation between the control group and PR-positive patients (r=0.104), whereas a weak to moderate negative correlation was observed with PR-negative patients (r=-0.403). In addition, a weak inverse correlation was found between PR-positive and PR-negative groups (r=-0.170).

Table 2 showed the significant differences in the expression level of miRNA-145 between the control group and breast cancer patients based on certain clinical and pathological characteristics. The gene expression concentration in the control group was 0.214±1.358, while this level was lower in breast cancer patients. Analysis of the relationship between tumor size and gene expression level revealed that patients with tumors larger than 5 cm had a level of 0.82±0.66, while patients with tumors smaller than 5 cm had a lower level of 0.372±0.168 compared to the control group. The p-value was 0.00001, and the LSD value was 0.161. Regarding lymph node metastasis, the results showed a significant increase in gene expression levels in patients with positive lymph node metastasis (0.241±0.466), while a significant decrease was observed in patients without lymph node metastasis compared to the control group. The lymphatic system, where the value was 0.114±0.357 and the p-value was 0.0001 and the LSD value was 0.228. When comparing the type of surgical excision, a significant increase in the level of gene expression was found in cases of total tumor removal, where it reached 0.291±0.786, and a significant decrease was recorded in partial tumor removal, where it reached 0.121±0.288, compared to the control group, whose mean was 0.214±1.358, and the p-value was 0.00001 and the LSD value was 0.161.

Table No. 1 Gene expression level for miRNA145 and its relationship to the condition of HER2, ER and PR receptors in breast cancer patients compared to the control group

Variables	Parameters	Mean±SD	p-value	LDS value
miRNA145	Control	0.232±1.359	0.0001	0.193
	Her 2 Positive	0.244±0.400		
	Her 2 Negative	0.124±0.327		
	Control	0.246±1.378	0.0021	0.308
	ER Positive	0.160±0.328		
	ER Negative	0.247±0.545		
	Control	0.214±1.358	0.0001	0.189
	PR Positive	0.257±0.123		
	PR Negative	0.256±0.523		

Table No. 2: Comparison of miR-145 gene expression levels according to tumor size, lymph node involvement, and type of surgical resection in breast cancer patients

Variables	Parameters	Mean±SD	p-value	LDS value
miRNA145	Control	0.214±1.358	0.0001	0.161
	Tumor size >5 cm	0.66±0.82		
	Tumor size <5 cm	0.168±0.327		
	Control	0.214±1.358	0.0001	0.228
	Lymph node positive (positive lymph node involvement)	0.241±0.466		
	Lymph node negative (negative lymph node involvement)	0.114±0.355		
	Control	0.214±1.358	0.0001	0.161
	Total tumor resection	0.291±0.786		
	Partial tumor resection	0.121±0.288		

Table No. 3: Impact of various forms of treatment on the levels of miRNA in women suffering from breast cancer as compared to the control group

Variables	Parameters	Mean±SD	p-value	LDS value
miRNA145	Control	0.214±1.358	0.0001	0.294
	Chemotherapy	0.339±0.643		
	Biological treatment	0.186±0.467		
	Radiotherapy	0.340±0.631		

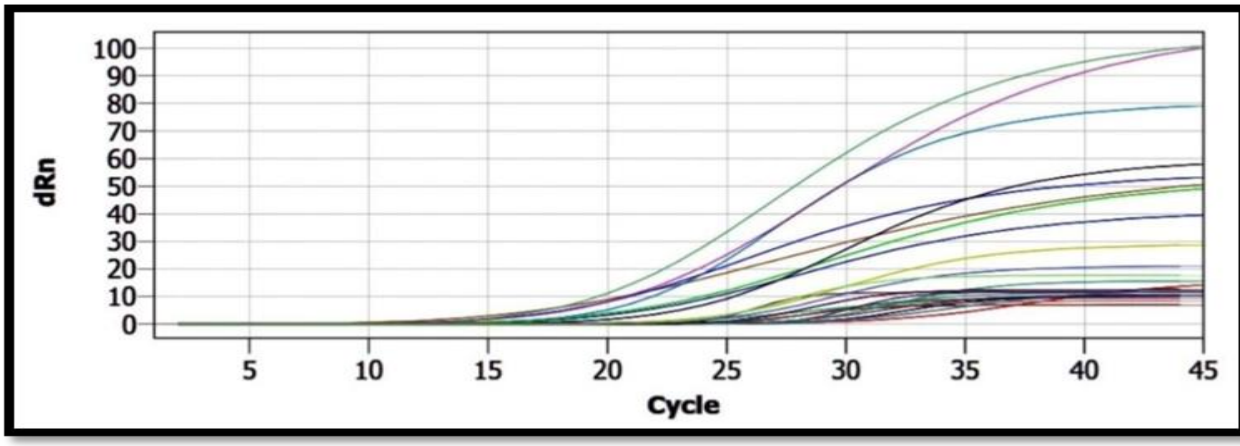

Figure No. 1: Amplification Curves for qPCR analysis of miRNA-145 expression in breast cancer patient samples and control group, and the CT value for both miRNA145 and U6 genes

Table 3 showed that the average level of miRNA gene expression in the control group was (1.358±0.214), which is higher than the values recorded in the treated groups.

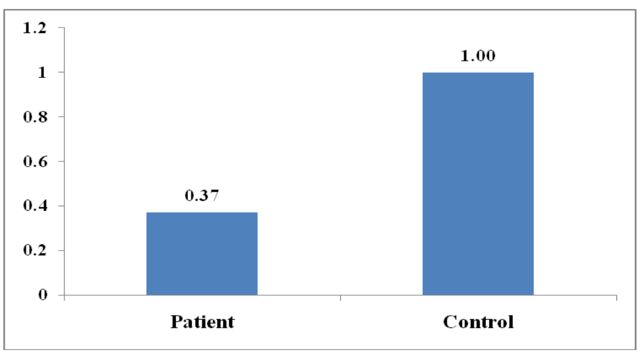

Figure No. 2: Relative expression level of miRNA-145 in the patient group compared to the control group

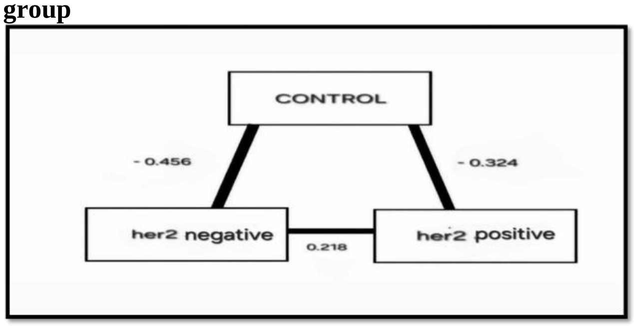

Figure No. 3: Relationships between the three miRNA-145 levels in the control group and breast cancer patients according to HER2 receptor status

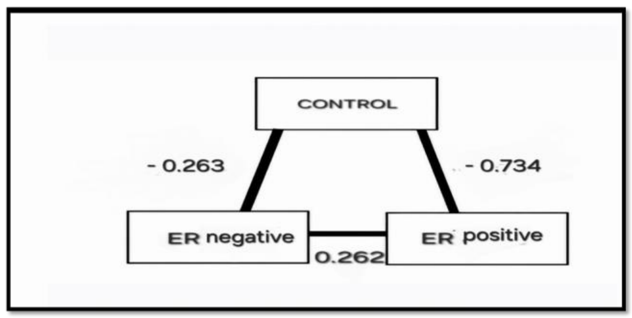

Figure No. 4: Relationships between the three miRNA-145 levels in the control group and breast cancer patients according to ER receptor status

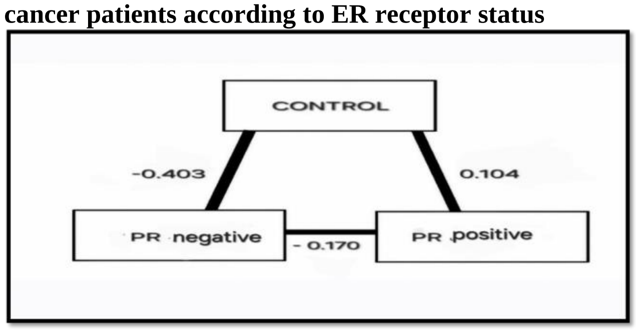

Figure No. 5: Relationships between the three miRNA-145 levels in the control group and breast cancer patients according to PR receptor status

The chemotherapy group recorded (0.634±0.339), while the biological therapy group recorded (0.467±0.186), and the radiotherapy group recorded (0.361±0.234). Statistical analysis revealed highly significant differences between the groups at a significance level of

($p \leq 0.0001$). The lowest significant difference (LSD=0.294) indicates that the differences between the group means are real and not random. It is clear that the control group differs significantly from all treatment groups. Significant differences were also found between chemotherapy and both biological and radiotherapy, as well as between biological and radiotherapy.

DISCUSSION

In the present study, the decrease in the gene expression of miRNA-145 in breast cancer tissue, suggesting a possible loss of its regulatory role in inhibiting cell growth. The findings are consistent with Zheng et al8 and Campos-Parra & Sánchez-Marín[9] that have suggested miR-145 acts as tumor suppressor microRNA in breast cancer, where it was expressed at lower levels in cancerous tissue compared to normal tissue.

This study showed that the mean concentration in the control group was 1.359±0.232. In patients with HER2-positive receptors, the mean concentration was 0.400±0.244, while in patients with HER2-negative receptors it was 0.327 ± 0.124. The mean concentration of ER receptor in control group was 1.378±0.264, while the concentration in patients with ER-positive receptors was 0.328±0.328. PR receptor, the concentration in the control group was 1.358±0.214. The concentration level in the positive PR receptor was 0.257±0.123, and in the negative PR receptor it reached 0.523±0.256. These results show a clear decrease in the gene expression level of miRNA-145 in breast cancer patients compared to the control group, with some differences related to the status of hormone receptors and the HER2 receptor. This indicates the regulatory role of this gene in tumor development and progression.[10-13]

In the current study, negative correlations between the control group and both HER2-positive and negative groups, while a weak positive correlation was observed between HER2-positive and negative groups. The strong negative correlation between control group, ER-positive was in patients group and weak negative correlation with ER-negative patients. A weak positive correlation was observed between ER-positive and ER-negative groups. The weak positive correlation between the control group and PR-positive patients, whereas a weak to moderate negative correlation with PR-negative patients. When its expression is reduced, the activity of signaling pathways associated with receptor family increases, leading to increased proliferation of cancer cells and their ability to invade and spread.[14-16]

The results of the present study support the findings of other studies showing that miRNA-145 plays a crucial role in inhibiting cancer cell migration and metastasis, and that its reduced levels may contribute to tumor progression and increased aggressiveness.[17-21] The results of this study show that low levels of miRNA-145 in breast cancer patient samples are associated with tumor metastasis to lymph nodes. This association is based on the molecular targets of this miRNA, most notably SOX2 (Sex Determining Region Y-Box[2]) and FSCN1 (Fascin Actin-Bundling Protein 1). miRNA-145 normally acts as an inhibitor of SOX2 expression, a transcription factor that promotes stem cell characteristics and proliferative capacity, and also prevents apoptosis. Therefore, low levels of miRNA-145 lead to increased SOX2 expression, which facilitates tumor growth. Moreover, miRNA-145 also targets the FSCN1 protein, which is involved in the control of cell architecture and motility. High concentrations of FSCN1 due to the reduction of miRNA-145 contribute to increased capacity of tumor cells to penetrate and migrate to lymph nodes.[22] The results obtained in the current study correspond to the data obtained previously.[23] This paper shows that reduction of miRNA-145 leads to increased proliferation through increased expression of SOX2 and FSCN1 genes, thus emphasizing the importance of the considered miRNA as a marker of breast cancer progression and metastasis.

CONCLUSION

There is a statistically significant decrease in circulating miRNA-145 in breast cancer patients in comparison with the healthy control group. Circulating miRNA-145 expression levels correlated significantly with hormonal receptors, clinical parameters of tumors, lymph node invasion, and methods of therapy used. The reduction in expression of miRNA-145 was associated with higher aggression in tumor development and various responses to therapies. In general, circulating miRNA-145 can be considered a promising biomarker for the diagnosis, prognosis, and monitoring of breast cancer patients.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Intisar Mohammed Kashash, Kiaser Abdulsajjad M. Hussain
Drafting or Revising Critically:	Intisar Mohammed Kashash, Alaa Hussein Mahdi
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

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