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Title Evaluation Of Mir-375 Expression As Epigenetic And Progesterone Hormone Levels In Women with Breast Carcinoma

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Abstract

Breast cancer is one of the most common malignancies affecting women worldwide and is associated with hormonal and molecular alterations. This study aimed to evaluate the role of progesterone hormone levels and miR-375 expression in breast cancer (BC) patients and to assess their potential as diagnostic biomarkers. Ninety blood samples from participant were obtained, including (60) samples of patients with BC and (30) samples of healthy individuals. Progesterone levels were measured using ELISA, while miR-375 expression was analyzed using quantitative real-time PCR.

The results showed a significant increase in progesterone levels in patients compared to controls, while miR-375 expression was significantly decreased. In addition, ROC analysis demonstrated that miR-375 has moderate diagnostic accuracy, with an area under the curve (AUC) of 0.762, indicating its ability to differentiate between patients and healthy individuals.

In conclusion, our findings suggest that both progesterone and miR-375 play a role in breast cancer development. While progesterone may contribute through hormonal pathways, miR-375 appears to be involved in gene regulation. The combination of hormonal and molecular markers may improve diagnostic accuracy and provide better understanding of the disease.

Keywords: Breast cancer, Progesterone, Gene expression, miR-375,

Introduction:

Breast carcinoma (BC) is one of the common cancers that occur in breast tissues[1], which results from un controlled growth of abnormal cells and it often affects the tissues responsible for milk production (lobules and ducts)[2].its most common cancer among women and represents major cause of cancer-related mortality worldwide[3] .

According to the Iraqi Ministry of Health cancer Registry Report[4] ,breast cancer Represented 34.8% of all cancer cases diagnosed among females ranks and the second most common cause of cancer -related death compared to other types of cancer.

Hormonal factors play crucial role in the development and progression of breast cancer[5] . The Progesterone hormone consider important hormone for regulates normal breast tissue growth [6] ,alterations in progesterone levels may contribute to abnormal cell proliferation and tumor progression[7]

In addition to hormonal factors, (miRNAs) have emerged as key regulators of gene expression at the post-transcriptional level[8]. These small non-coding RNAs are involved in various biological processes including cell proliferation, apoptosis, and cancer development[9].

So, miR-375 has attracted considerable attention due to its role in breast cancer. Several studies [10] and [11] reported that miR-375 expression is dysregulated in breast cancer patients, suggesting its potential role as a diagnostic biomarker[12]. The changes that occur in miRNA expression by hormones driven can affect the whole body by altering the breast cancer cells behave lead to spread to other tissues [13].

Furthermore, recent evidence suggests a possible interaction between hormonal changes and microRNA expression. In particular, progesterone levels may influence the expression of miR-375, which could contribute to breast cancer progression[14].

Materials and method

2.1 Study design

The present study was a case -control study that included 60 patients of breast cancer who were diagnostic with specialized physician in the Oncology Unit at the AL Hussein Teaching Hospital, Al Muthanna, Iraq. In addition, 30 healthy control individuals had no history or clinical evidence of breast cancer. All samples were females; their ages ranged between 25 and 65).

2.2 Sample collection

Five ml of blood was obtain from each participant placed in a gel tube which contains a clot activator and a serum gel separator at room temperature (20- 25 ºC) for 30minutes, The serum was separated by a centrifuge at a speed of 4000 rpm for 10 minutes, then store at -40 ˚C to Measurement the levels of progesterone by using ELIZA assays and determine the gene expression of microRNA by by using the (qPCR) technique.

2.3 Measurement of progesterone

Enzyme-linked immunosorbent assay (ELISA) kits were used for measuring serum levels progesterone hormone according to the manufacturer's instructions. A microplate reader was used to detect the absorbance, and the standard curves included with the assay kits were used to compute the concentrations.

2.4 RNA extraction and qRT-PCR

The microRNAs, was extracted from serum samples using Trizol reagent according to the manufacturer's protocol and the concentration purity of the isolated RNA were determined spectrophotometrically. Complementary DNA (cDNA) was subsequently synthesized through reverse transcription. Quantitative real-time polymerase chain reaction (qRT-PCR) was then performed using SYBR Green detection chemistry to evaluate the expression levels of miR-375. The relative expression levels were normalized to an internal control and calculated using the comparative Ct (2^−ΔΔCt) method [15]This specific primers for target microRNA miR-375)[16] and the internal reference gene (RNU6) https://www.ncbi.nlm.nih.gov/ were utilized (Table 1)[17].

Table 1: Primer sequences of the miRNA- 375 and internal reference gene.

Primer	**Sequence 5'-------3'**	
RNU6	forward (5′ -3′)	CGCTTCGGCAGCACATATAC
	reverse (5′ -3′)	AGGGGCCATGCTAATCTTCT
mir-375	forward (5′ -3′)	AGTTTGTTCGTTCGGCTC
	reverse (5′ -3′)	GGTCCAGTTTTTTTTTTTTTTCAC

2.5 Statical analysis

All data statistical analyses were performed using SPSS software (version 25.0). Data were expressed as mean ± standard deviation (SD). Differences between breast cancer patients and healthy controls were analyzed using the independent samples t-test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the studied biomarkers. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 progesterone levels in Breast Cancer patients and Healthy control

The comparison of female hormones progesterone in BC patients and healthy controls has been carried out and the results were demonstrated in **(table 2)**. the mean of progesterone levels was 565.54 ± 57.6 and 341.24 ± 32.8, in BC patients and healthy control group respectively; the mean levels was higher in patients' group in compared to other groups (**figure 1)**

Table 2: Comparison of female hormones progesterone levels (pg/ml) in BC patients and healthy controls

Groups		Progesterone pg/ml
BC patients	Mean ± SD	565.54 ± 57.6
	Range	368.00-753.13
Control	Mean ± SD	341.24 ± 32.8
	Range	264.45-435.43
p-value		0.001**

(SD: standard deviation; †: Independent t Test; **: significant at P < 0.05

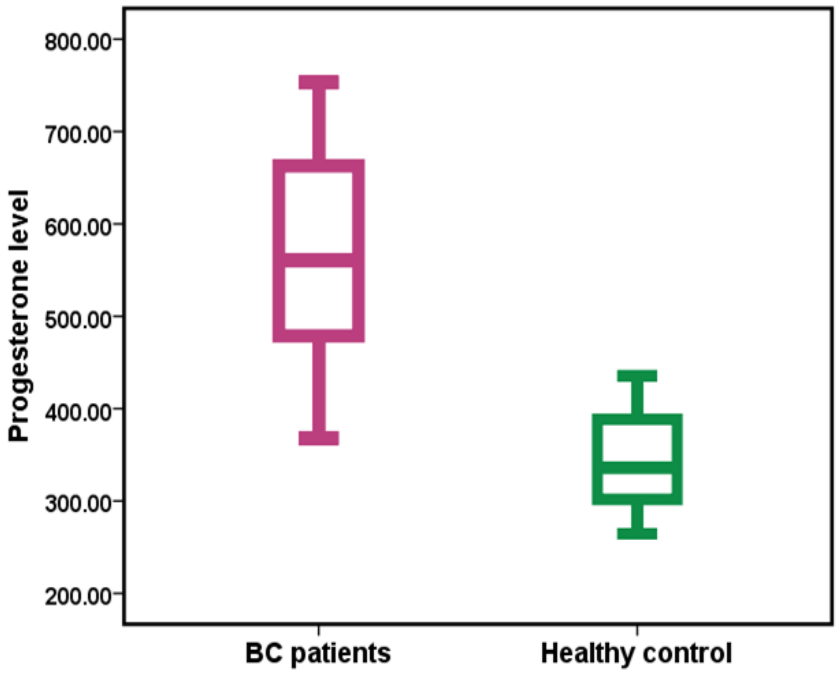

Figure 1: The means level of Progesterone in BC patients and control groups

3.2 Efficiency of the amplification analysis of MicroRNA-375

The Efficiency of the amplification assay for miR-375is demonstrated by the results of melting curve analysis which show highly specific amplification for miR-375 and the internal reference genes Additionally, the curves of amplification of the analysis samples point to the successful amplification of the for miR-375 alongside the internal reference gene. This indicated that selected samples demonstrating the typical sigmoidal amplification pattern across PCR cycles.

3. 3 Analysis of the qRT-PCR expression data of miRNA-375

The comparison of MicroRNA-375 gene expression between BC patient and healthy controls has been carried out and the results were demonstrated in table 3, Mean of miRNA-375 gene expression were 0.658 ± 0.04 and 1.00, in BC patients, and healthy control group respectively; the mean levels was significantly higher in BC patients in compared healthy control (P< 0.05)**(figure 2)**

Table 3: MicroRNA-375 gene expression in BC patients and healthy controls.

Groups		***MicroRNA-375 gene expression***
BC patients	**Mean ± SE**	**0.658 ± 0.04**
	Range	**0.02-2.30**
Healthy Control	**Mean ± SE**	**1.0**
	Range	**1.0-1.0**
p-value		**0.001****

SD: standard deviation; †: Independent T test; ******: significant at $P < 0.05$

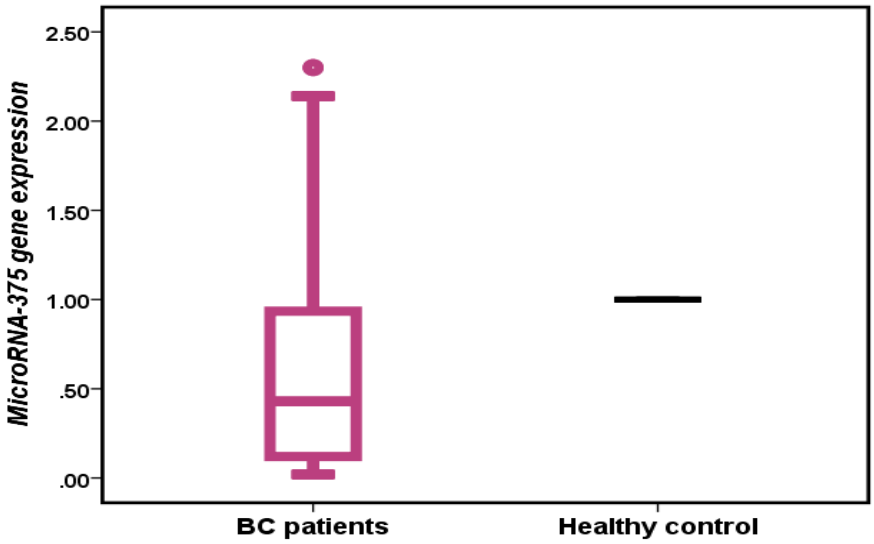

Figure 2: Mean MicroRNA-375 gene expression of patients and healthy controls.

3. 4 Diagnostic accuracy of *MicroRNA-375 gene expression of patients and healthy controls.*

Receiver operating characteristic (ROC) analysis was performed to reveal the diagnostic accuracy of using *miRNA-375 gene expression* to distinguish BC patients from healthy control subjects and the results are shown in table 4, and figure 3 the *miRNA-375 gene* cutoff value was less than 0.934-fold with sensitivity,77.5% specificity 100%, positive predictive value (PPV)100% , negative predictive value (NPV)61.7%, and area under curve of 0.762 (0.670- 0.855). The present results indicate *miRNA-375* is considered as acceptable diagnostic marker.

Table 4 : Sensitivity and specificity of *miRNA-375 gene* level (< 0.934-fold) in BC disease

miRNA-375 gene	**BC patients *n* = 80**	**Healthy control *n* = 30**
< 0.934	**58 (%)**	**0 (%)**
> 0.934	**22 (%)**	**30 (%)**
Sensitivity %	**77.5 %**	
Specificity %	**100.0 %**	
PPV %	**100.0 %**	
NPV %	**61.7%**	

AUC (95% CI)	**0.762 (0.670- 0.855)**

CI: Confidence interval, AUC: Area under curve.

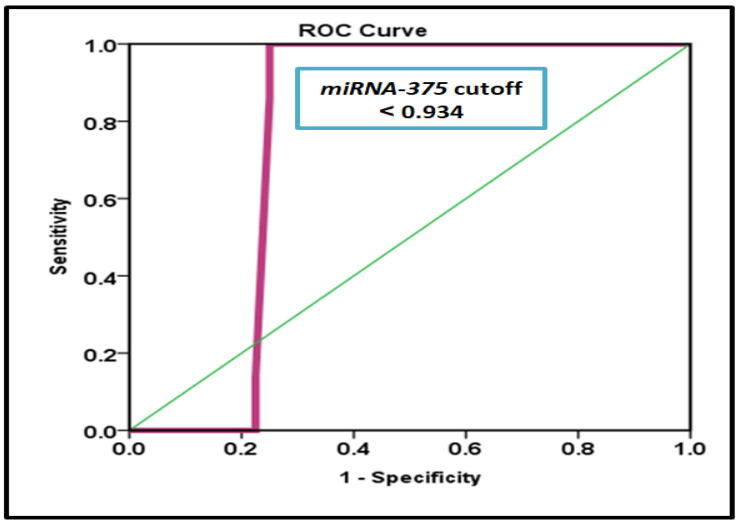

Figure 3 : Receiver operator characteristic curve analysis of miRNA-375 for the calculation of possible diagnostic cutoff value.

Discussion

The progesterone levels were noticeably higher in breast cancer patient compared to healthy controls, the distribution of values figure 1, shows a wider range and higher median levels that mean variability in hormone expression among breast cancer patients so, many studies like[18] have shown that the progesterone hormone and its receptor play a key role in breast cancer development and progression.

These results agreement with Hadizadeh et al, which have been previously demonstrated that the increasing levels of progesterone in the body may actively fuel cancer progression[19] .

The present study demonstrated a significant and increase progesterone levels in breast cancer patients compared with healthy controls, these results may suggest potential role of hormonal imbalance and in the development or progression of breast cancer[20] .

These results correspond to other studies like[21] that show the role of progesterone is known to influence breast tissue through its interaction with progesterone receptors and contributing with cell proliferation and differentiation and could be linked to increased hormonal stimulation that may promote tumor growth[20][22] .

In addition, the increasing of progesterone levels among breast cancer patient may be attributed to many physiology factors like age, hormonal status particularly before and after menopause that the hormonal changes in these stages can significantly influence circulation sex hormone levels[23] .

On the other hand, our results demonstrated a significant decrease in miR-375 gene expression in breast cancer patient this reduction may indicate the potential role of miR-375 as a tumor suppressor so, its downregulation contributes to the progression of breast cancer, this agreement with many studies like[10] ;[24]who reported that miR-375 is involved in regulating cell proliferation, apoptosis and differentiation these decrease expression has been associated with increased tumor growth and aggressiveness[25].

The results in this study with sensitivity (77.5%) observed indicates that miR-375 can correctly identify a considerable proportion of breast cancer cases table 4, while the specificity (100%) demonstrates a strong ability to correctly classify healthy individuals, minimizing false positive results. This high specificity is particularly important in clinical settings, where overdiagnosis should be avoided[26],

in addition, The ROC curve analysis in the present study suggests that miR-375 has a reasonable diagnostic ability to differentiate breast cancer patients from healthy individuals[27] reported AUC value (0.762) reflects a moderate level of accuracy, indicating that although miR-375 alone may not be sufficient as a standalone marker, it still provides meaningful diagnostic value[28].

Moreover, recent reviews have highlighted that microRNAs like [29] including miR-375, play a crucial role in cancer biology and may serve as promising diagnostic biomarkers.

Conclusion

This study showed that progesterone levels were elevated in breast cancer patients, while miR-375 expression was reduced compared to healthy controls. These findings suggest that both hormonal imbalance and microRNA dysregulation may play a role in the development of breast cancer.

Also, miR-375 has a moderate ability to distinguish between BC patients and healthy individuals, supporting its potential use as a diagnostic biomarker. Overall, combining hormonal and molecular markers may improve diagnostic accuracy and provide better understanding of breast cancer mechanisms.

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