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Evaluation of microRNA-9 expression levels in Iraqi women patients with breast cancer

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Abstract

More information about the mechanisms underlying the pathophysiology of breast cancer (BC) is needed given the widespread epidemic of the disease and its detrimental effects on women's health. The relevance of microRNAs, also known as miRNAs or miRs, for breast cancer research, has lately become known due to the extensive number of risk agents connected to this cancer. The purpose of this study was to investigate whether microRNA-9 molecules expressed as early prognostic biomarkers for breast cancer detection.

Twenty-five serum samples were obtained from women who had just been diagnosed with BC, and 25 serum samples were obtained from healthy women. On the other hand, qPCR was used to look at the expression of miR-9 in study groups.

The miR-9 results revealed that serum BC patients had significantly higher ($P<0.05$) expression when compared to controls, with fold changes of 2.8 and 1.2, respectively.

Keywords: Breast cancer, Real Time-PCR, miR-9.

Introduction

Breast cancer (BC) is a cancer that affects a large number of women globally and is the primary cause of cancer-related mortality (1,2), with over 500,000 deaths per year worldwide, it is the leading cause of death for women (3). According to reports, 14% of women die from cancer-related causes, and the incidence of BC is continuing to rise (4).

MiRNAs are a class of endogenous, short, noncoding RNA that are widely distributed in eukaryotic cells, they have an approximate length of 22 nucleotides and regulate gene expression at the posttranscriptional level by interacting with target transcripts' 3′ untranslated regions (3′UTRs) (5, 6). There are already over 2500 mature *miRNAs* in humans that are known to be involved in apoptosis, differentiation, cell proliferation, and individual development (7).

The *miRNAs* linked to cancer are commonly divided into two groups, the first group comprises highly expressed oncogenic miRNAs, or oncomiRs, which play a key role in tumor growth and phenotypic maintenance (8). The second group includes tumor-suppressive miRNAs (miRsupps), which are often down-regulated in a variety of malignancies, they prevent carcinogenesis by

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regulating cell proliferation, death, immune-cell formation, and other cancer-associated processes (9).

Many cancer-associated miRNAs can behave in a tissue-specific way, they are often referred to as context-dependent miRNAs. In various malignancies, individual miRNAs can play either an oncogenic or tumor-suppressive role (10).

Due to their significant involvement in carcinogenesis and cancer, miRNAs have garnered a lot of attention in cancer research in recent decades, specifically, it has been found that MiR-9 contributes to the development, and advancement of many cancer types (11).

For example, miR-9 is known to influence metastasis, while most studies indicate an oncogenic role for miR-9 in breast cancer, some research suggests it may exhibit tumor-suppressive characteristics. Recently, miRNAs have been increasingly recognized for their role in regulating the initiation and progression of BC. Therefore, this study aims to evaluate the role of miR-9 in Iraqi women diagnosed with breast cancer.

Materials and Methods

Samples Collection from Iraqi women

Specimens were collected from women diagnosed with new BC from Alwiyah Hospital for Women's Care and the Oncology Teaching Hospital, with ages ranging from 25 to 50 years.

Blood samples were collected in 5 ml test tubes, totaling 50 serum samples, which included 25 from healthy women volunteers, and 25 from BC patients. The blood samples were allowed to sit for half an hour before being centrifuged for 5 minutes at 1600 rpm. The serum 100µl was then transferred into Eppendorf tubes containing 300µl of Trizol and stored at -20°C until analysis.

Real-Time PCR Amplification

- **RNA Extraction**

According to the manufacturer's recommendations, RNA was extracted utilizing the TransZol Up Plus RNA Kit.

- **Primers**

PCR was performed by using *miR-9*, F (5'-CTTTGGTTATCTAGCTGTATGAGTCGT -3'), and R (5'- ATCCAGTGCAGGGTCCGA -3'), and *U6* was used as housekeeping gene F (5'-GAGAAGATTAGCATGGCCCCT -3') and R (5'-ATATGGAACGCTTCACGAATTTGC -3') (12).

- **RT-PCR Amplification**

The conversion of RNA to cDNA is done according to EasyScript® First-Strand cDNA Synthesis SuperMix.

Thermo cycling conditions were as follows: initial denaturation at 10 min at 95 ° C, followed by 40 denaturation cycles at 95 ° C for 20sec, annealing at 55 ° C for 20sec, and extension at 72 ° C for 20sec. The values for the relative quantification were calculated utilizing the $2^{-\Delta\Delta Ct}$ expression formula (13).

Statistical analysis SPSS was used to analyze the results of miR-9 expression.

Results

Table (1), and Figure (1) illustrate the demographic characteristics of the study groups.

In this study, the expressions of miR-9 were investigated and shown to be up-regulated in the serum of Iraqi patients with BC women as shown in Table (2), and Figure (2). The expression from miR-9 in BC patients was highly significant as compared with serum from healthy with P= 0.0004.

Table (1): Age, family history, and smoking in subjects.

	BC patients		Control	
	No.	%	No.	%
Age(Years)				
25-35	**6**	**24**	**8**	**32**
35-45	**9**	**36**	**8**	**32**
45-55	**10**	**40**	**9**	**36**
Family history				
Yes	**16**	**64**	**10**	**40**
No	**9**	**36**	**15**	**60**
Smoking				
Yes	**11**	**44**	**12**	**48**
No	**14**	**56**	**13**	**52**

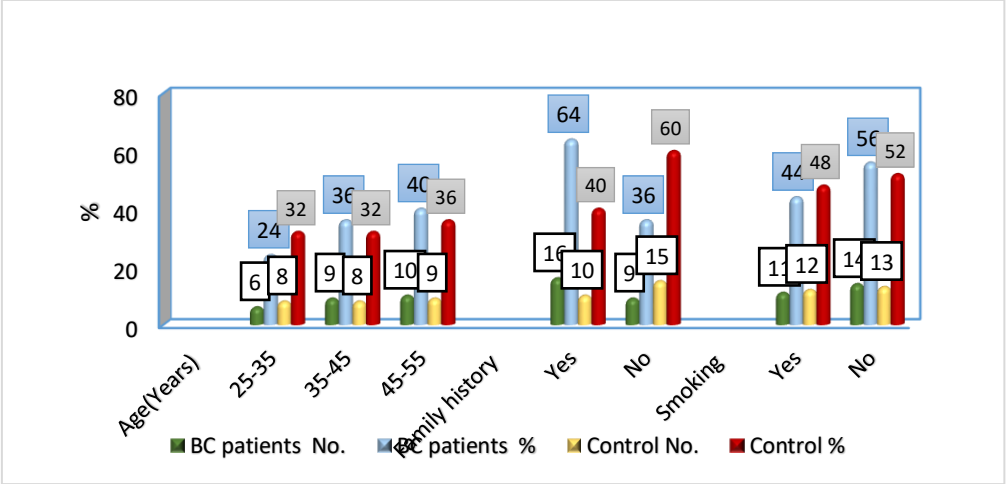

Figure (1): Demographic and clinical characteristics in Study groups.

Table (2): Fold change expressions by using RT-PCR.

$2^{-\Delta\Delta CT}$ (Fold change) miR-9 Mean ±SE	
Patients	**Control**
2.825±1.383	**1.264 ±0.844**
P= 0.0004	

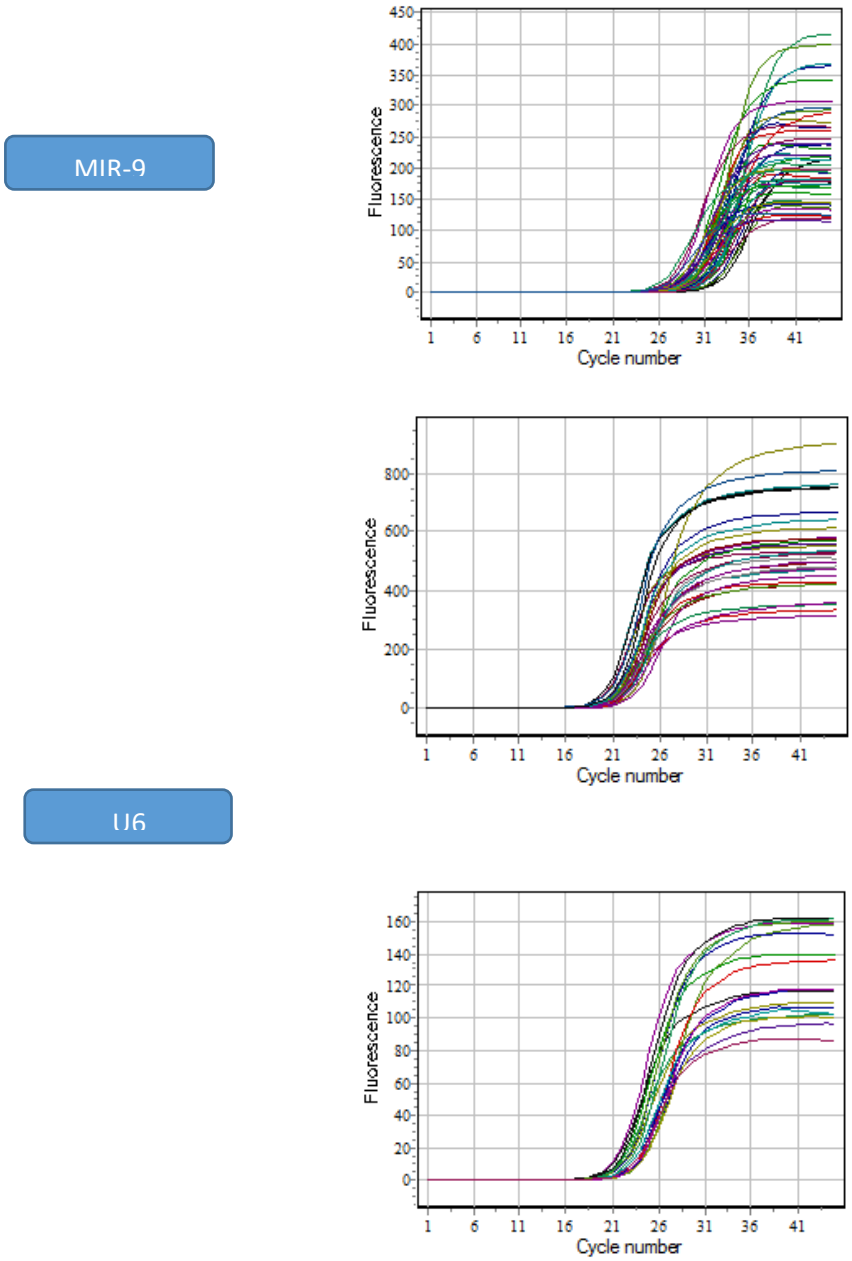

Figure (2): Amplification of MIR-9 cDNA product and U6 genes were submitted to the RT-PCR

Discussion

Breast cancer development and progression have been linked to microRNA-9 (miR-9) in some malignancies, studies on the expression levels of miR-9 in the setting of BC have produced a variety of results, indicating that both high and low levels may be connected to distinct clinical outcomes (14).

A different study found that higher miR-9 levels were linked to more aggressive tumor behavior, by focusing on the genes that prevent cell division and invasion, miR-9 can also increase levels miR-9 may augment the metastatic capacity of breast cancer cells, facilitating their capacity to infiltrate neighboring tissues and disperse to remote locations (15).

This attribute may result in advanced disease at the time of diagnosis, adversely influencing treatment results, this agreement with our study (16). Additionally, sensitivity to some treatments,

including chemotherapy, may be associated with high expression of miR-9, making the disease more challenging to treat (17).

While reduced miR-9 expression levels could indicate a decline in the protein's tumor-suppressive properties, in some contexts, miR-9 functions to impede certain processes, such as the epithelial-mesenchyme transition (EMT), which is essential for the spread of cancer, reduction of miR-9 may result in heightened vulnerability to invasion and metastasis (18).

In contrast to our study, the findings of the Tavakolian *et al*. study showed that although the mRNA expression level of miR-9 was markedly decreased in BC tissues, in serum samples, there was no discernible change in this miRNAs expression level (19).

The contradictory roles that miR-9 plays in breast cancer serve as a reminder of how intricately microRNA acts in oncogenes, the microenvironment, interactions with other regulatory pathways, and tumor subtype, are some of the variables that may affect how miR-9 expression affects a patient's prognosis (20).

Moreover, the molecular heterogeneity of breast cancer may be reflected in the variable expression from miR-9, the precise function of miR-9 in breast cancer, and its potential as a biomarker for diagnosis, prognosis, or therapeutic targeting requires more investigation (21).

Ethical approval

The project received approval from the local ethical commission at Ibn Sina University, Baghdad, Iraq.

Conflicts of interest: None.

Funding: None.

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