

circulating MicroRNAs as Biomarkers for Breast Cancer Detection

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Abstract

Breast cancer (BC) is the second most common cause of cancer deaths among females. It caused by many predisposing factors. Early diagnosis of breast cancer favors the outcome of the disease. Recently, many studies demonstrated that microRNAs have emerging role in diagnosis and prognosis of breast cancer. microRNAs are small uncoding RNAs composed of about 23 nucleotides. miRNAs can affect breast cancer progression by regulation of cell growth and apoptosis. Our study aimed to assess the expression pattern of microRNA-329 (miRNA-329) in BC females and correlate it with the clinical stage of the disease also the potential of miRNA-329 in the diagnosis of BC.

Introduction:

Breast cancer (BC) is one of the most frequently diagnosed malignancies and the leading cause of death from cancer in women worldwide. For the year 2016, it was estimated that in the US approximately 246,660 female patients would be diagnosed with BC and 40,450 would die from it (1). Efficient early detection is critical for optimizing management strategies to this disease, but despite improvements in cancer screening techniques, major challenges remain (2).

In Egypt, BC is estimated to be the most common cancer among females accounting for 38.8% of total cancer cases and is responsible for 29.1% of cancer deaths (3).

Various approaches, ranging from self-examination of the breast to mammography screening, are aimed at detecting BC as early as possible. However, the precision of all the methods currently used is limited, and particularly in younger women, mammography is often difficult to interpret due to high-density breast tissue. Reliable and early-stage blood-based biomarkers to support imaging methods of BC detection have been explored extensively in recent years (4). Potential molecular marker candidates include gene expressions, proteins, as well as miRNA expression signatures (4). SO, it is very important and urgent to find a novel biomarker-based assay for breast cancer diagnosis with high sensitivity and specificity.

MiRNAs are small, non-coding RNAs about 23 nucleotides in length. They regulate functions of multiple genes by initiating gene silencing by degradation of its mRNA targets. Significant evidence has accumulated in the last few years, showing a fundamental role of miRNAs in the development of many diseases (5). Particularly in cancer, aberrations in miRNA expression levels have been linked to the onset and progression of various types of cancer (6).

Extensive research has demonstrated that miRNAs are dysregulated at all stages of BC and could have a potential role as prognostic and predictive biomarkers (7).

MiR-329 located on 14q32.31(8). MiR-329 is known to function as a tumor suppressor in multiple malignancies with its aberrant expression in some types of tumor tissues indicating its pivotal role in both the cancer pathogenesis and progression (9).

In this study will assess the expression pattern of miRNA-329 in breast cancer females and the ability to use it as a diagnostic and predictive marker for BC.

Subject and methods

This study was carried out between December 2020 and October 2021 after approval of the study scheme by the research ethical committee of Benha Faculty of Medicine and obtaining informed consent from the included subjects. This study included 60 subjects of females which were selected from Department of General Surgery, Faculty of Medicine, Benha University Hospital. BC group gathered 30 female patients and the control group had 30 healthy females.

Blood was gathered on ethylene diamine tetra-acetic acid (EDTA) tube. The blood sample was aliquoted in 2 Eppendorf tubes and kept at -20°C.

Statistical analysis

The collected data were summarized in terms of mean \pm Standard Deviation (SD) and range for quantitative data; and frequency and percentage for qualitative data. Comparisons between cases and control were carried out using student-T test, to compare quantitative data between two groups and Chi-squared (χ^2) test, to compare proportions of two or more groups. Pearson correlation was used to estimate the correlation between miRNA-329 and age of the studied group. The corresponding test statistics were calculated, and the corresponding P-values were obtained. P-value 0.05 was considered statistically significant, while P-value $>$ 0.05 was considered statistically non-significant. Analysis is performed using the Statistics Program for Social Sciences (SPSS) and Microsoft Office Excel is used for the data processing and data analysis.

Results

The baseline characteristics of the study population are presented in Table-1. There was insignificant difference between cases and control groups regarding age & age at menarche.

Table (1) Demographic data of the studied groups

| | | Cases group | Control group | p- value |
|------------|---------------|--------------------|----------------------|-----------------|
| Age | mean \pm SD | .88 | .45 | 0.39 |

| | | | | |
|------------------------|---------|-----|-----|------|
| Age at menarche | mean±SD | .83 | .55 | 0.87 |
|------------------------|---------|-----|-----|------|

Expression level of miRNA 329 of studied groups

microRNA 329, were studied and their expression was analyzed in blood samples. The expression of miR-329 was significantly lower ($p < 0.001$) in breast cancer tissues as compared to controls (table 2).

Table (2): Difference between breast cancer group and control group regarding miR 329 relative expression.

| Study Group MiR-329 (Log) | Control group | breast cancer group | T- Test | P-value |
|------------------------------|---------------|---------------------|---------|---------|
| Mean ± SD | 5.9 ± 0043 | 5.7 ± .179 | 6.3 | < 0.001 |

SD: Standard deviation, $P \leq 0.05$ significant, $P > 0.05$ non-significant, analysis done by independent samples Student T test.

DISCUSSION:

Breast cancer (BC) is one of the most common malignant diseases in the world. BC has a highest mortality worldwide among women, accounting for 522,000 deaths in 2012(11). Breast cancer is a metastatic cancer and can commonly transfer to distant organs such as the bone, liver, lung and brain, which mainly accounts for its incurability. Early diagnosis of the disease can lead to a good prognosis and a high survival rate (12). Mammography is used as screening approach in the detecting of breast cancer and help to reduce the mortality effectively (12). In patients who have been identified with breast tumor, different strategies of management are used such as targeted therapy, hormonal therapy, radiation therapy, surgery, and chemotherapy (13).

MiRNAs play the role as either tumor suppressors or oncogenes in regulating the expression of immigration and angiogenesis-related genes (14). The involvement of miRNAs in gene

regulatory processes and their implication in several diseases, including cancer, makes them very attractive for diagnosis, prognosis, and treatment in clinical application (15).

Many miRNAs have been observed to be significantly dysregulated in breast cancer and play suppressive or oncogenic roles (16). For example, the tumor suppressor role of microRNA-205 has been observed in renal cell cancer tissues, and it was shown to suppress tumor cell progression (17). MicroRNA-584 was found to correlate with cell invasion and migration in thyroid carcinoma (18).

MiR-329, an extensively studied miRNA, has been investigated in some cancers and its clinical value was highlighted in previous studies (19).

In this study expression levels of miR-329 were measured by qRT-PCR. We found that the circulatory level of miR-329 expression was decreased in breast cancer females comparing to those of healthy females. According to these data, miR-329 might function as a potential tumor suppressor in breast cancer.

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