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miR-155 Expression in the Serum of Patients with Ductal Carcinoma in Situ (DCIS) of the Breast and Patients with Invasive Ductal Carcinoma (IDC) of the Breast

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Abstract

Background: Breast cancer (BC) is an illness affecting millions of women across the world. The transition from ductal carcinoma in situ to invasive ductal breast cancer is a crucial event in the progress that is still not well understood. microRNAs (miRNAs) have recently been documented to play an important role in cancer development. miRNAs have been discovered to control this critical transition. The miR-155 plays an essential role in the pathogenesis of breast cancer. miR-155 has been implicated in developing breast cancer.
Objectives: This study aimed to investigate the expression of miR-155 in the serum of patients with breast cancer, according to clinical characteristics (DCIS and IDC) of breast cancer.
Methods: 60 patients referring to hospitals in Ahvaz during 2012 and 2015 were divided into 2 groups according to clinical characteristics (DCIS and IDC). miRNA was extracted, and complementary DNA (cDNA) was synthesized in line with the guidelines of the Kit manufacturer . A real-time PCR method was performed as the expression assay.
Results: The mean expression level of miR-155 in DCIS group was 6.45 ± 0.545. In addition, the mean expression level of miR-155 in serum of DCI group was 40.42 ± 0.742; the difference was statistically significant ($P < 0.0001$).
Conclusions: Based on the results of this study, the serum level of miR-155 evidenced a statistically significant difference in invasive breast cancer (IDC) patients. The study results showed that checking the serum level of miR-155 expression in patients with invasive breast cancer (IDC) might be helpful.

Keywords: miR-155, Breast Cancer, MicroRNA, Ductal Carcinoma in situ, Invasive Ductal Carcinoma

1. Background

Breast cancer (BC) is a malignant proliferation of epithelial cells lining the breast ducts or breast lobules. The most important origin of epithelial cancer in women is BC (1). Ductal carcinoma in situ (DCIS) is the most common type of noninvasive BC in women (2). Evidence supports a stepwise pattern for progression of BC from DCIS to invasive ductal carcinoma (IDC) (3).

Unlike similar patterns of gene expression in IDC and DCIS, Micro RNAs (miRNA) expression profiles are significantly different (4). miRNAs are small endogenous noncoding RNAs that act as post-transcriptional regulators of gene expression. miRNAs play a significant role in cell proliferation, apoptosis, and differentiation. Different studies show that miRNA is expressed in normal cells of tumor tissues as well as in many types of tumors (5). miRNA is released in vesicles of circulating tumor exosomes and transported apoptosis bodies (6).

A recent study conducted on patients with breast cancer showed the expression of miR-155 in the serum of breast cancer patients (7). miR-155 initially acts as a gene copy; thereof by the insertion of a promoter in an area that for retrovirus in lymphocyte's B activated in common. The cancer lymphoma is detected in its BIC (B-cell Integration Cluster). BIC has a 3-exon gene on chromosome 21q21.3, which has a length of about 13 Kb and miR-155 BIC gene is located within exon 3 (8). Cancer symptoms include maintaining the signal replication and loss of growth inhibitors. Resistance to programmed cell death, angiogenesis, invasion, and metastasis activity has been determined. As a miRNA, miR-155 has oncogenic properties, almost all signs of cancer in the breast cancer affected different routes (9).

2. Objectives

The current study aimed to assess serum expression levels of miR-155 in the serum of patients with breast cancer, according to noninvasive BC (DCIS) and invasive BC (IDC).

3. Methods

3.1. Study Population

In a case-control study, 60 female BC patients who had been diagnosed by pathological examinations in Ahvaz between 2012 and 2015 were recruited. 50 healthy subjects were selected as controls.

3.2. Ethics

The research protocol of this project was approved by a suitably constituted ethics committee of Ahvaz Jundishapur University of Medical Sciences.

3.3. Sample Preparation

5 cc of blood samples was collected in SST tube (for serum). Blood samples were centrifuged at 2500 rpm for 5 minutes at room temperature. Cruor was then transferred to a brand new tube and centrifuged at 3000 rpm for 5 minutes at room temperature. Samples were aliquoted and then kept at -80°C for subsequent use.

3.4. miRNA Isolation

miRNAs from serum samples were extracted using miRNeasy Serum/ Plasma Kit (Qiagen, Germany). miRNA extraction was performed in accordance with the manufacturer's instructions at -80°C.

3.5. Reverse Transcription (RT) and Quantitative PCR

Using a High Capacity cDNA kit (Life Technologies, America) for miRNA isolated from peripheral blood serum, cDNA was prepared to continue to the next step at product temperature of -20°C. Proliferative responses were performed in a final volume of 20 μL and a double in 96-well plates and devices using Real-Time PCR (Steponeplus ABI, America) were used. To prepare mixed reaction, 10 μL SYBR-Green PCR (Master Mix Takara), 1 mL primer (Forward) and reverse (Reverse) specific for each gene, and 5 μL of cDNA were mixed and finally by adding distilled water reached to the final volume of 20 μL. The primers for miR-155 were used as follows: forward: 5'-GATACTCATAAGGCACGCGG-3' and reverse: 5'-GTGCAGGGTCCGAGGT-3' to compare expression levels of miR-155 in the serum of patients with breast cancer. The relative amount of miR-155 was normalized against SNORD 47. In this study, we calculated the fold-change between cancerous patients and normal controls for miR-155 using the $2^{\Delta\Delta Ct}$ method.

3.6. Statistical Analysis

Laboratory data were analyzed by SPSS®ver 20.0 for Windows®. In the descriptive study, data were expressed as percentage and analyzed by analysis of variance (ANOVA). Statistical significance was assessed at 0.05 probability level in all analyses.

4. Results

Among patients with BC (N = 60) with a mean age of 51 ± 10.15, 33 (55%) cases had DCIS and 27 (45%) had invasive BC. The control group consisted of healthy subjects (N = 50) with mean age of 50 ± 10.56.

According to Table 1, there was a statistically significant difference (P = 0.146) so that the mean expression level of miR-155 in DCIS group was higher than that of the healthy subjects. In addition, the mean expression level of miR-155 was higher in DCI group than both healthy subjects and DCIS group. Results showed upregulation (about 10 fold) in DCI group.

Table 1. The Mean Change in Serum miR-155 Expression in Two Groups of DCIS and IDS

Group	No.	Mean Expression of miR-155	95% Confidence Interval
Control	50	4.727 ± 0.545	3.646 to 5.809
DCIS	33	6. 45 ± 0.545	5.121 to 7.783
IDS	27	40.42 ± 0.742	38.949 to 41.891

To compare the miR-155 expression levels in BC patients, real-time analysis was performed. In this regard, expression of miR-155 was compared between DCIS patients and healthy subjects that showed no significant difference (P = 0. 146), while miR-155 expression showed a significant difference between IDC patients and healthy subjects (P < 0.0001). In addition, the comparison of miR-155 expression between DCIS and IDC groups showed significant differences (Table 2).

Table 2. Comparison of DCIS and IDS Breast Cancer Patients in Terms of miR-155 Expression and Corresponding 95% Confidence Intervals

Pairwise Comparisons	Mean Difference	P Value[a]	95% CI[a]
Control- DCIS	1.724 ± 0.864	0.146	3.378 to 3.828
Control- IDS	35.692 ± 0.921	< 0.0001	33.4529 to 37.932
DCIS- IDS	33.968 ± 1.007	< 0.0001	31.534 to36.402

[a]Bonferroni corrected.

5. Discussion

Benefits of molecular research may increase the management of patients (10). To date, a large number of genes have been considered to involve in breast cancer. Research has shown that miRNA is directly related to the progression of the disease, having an inverse relationship in cancer patients (11). Oncogenic miR-155 is a well-established miRNA in many tumors, including breast cancer (12) where it can function as an oncogene to affect cancer development (13).

The obtained results showed that serum levels of miR-155 in breast cancer patients were higher than that of healthy subjects. Studies conducted by Roth et al. (14), Wang et al. (15), and Sun et al. (16) showed that high levels of miR-155 were associated with breast cancer. The current experiment was consistent with previous studies indicating that high level of miR-155 was associated with breast cancer. This study was conducted for the first time in the serum of patients with DCIS and IDC. We further performed expression circulating miR-155 in patients with noninvasive breast cancer (DCIS) and invasive breast cancer (IDC). This analysis showed very high expression level of mir-155 in invasive breast cancer (IDC).

Oncogenic miR-155 is well known as an oncogenic miRNA, which plays its role in different pathways (17). Currently, a sizable number of gene history is regarded to involve in breast cancer (18). It is reported that miR-155 is regulated via the effect of SOCS1, JAK2/STAT3 (19), and by transforming growth factor-β- induced epithelial-mesenchyme transition, RhoA (20) and thus activates the inflammatory cascade rather than the relationship between miR-155, inflammation, and cancer (21). Another target of miR-155 is the tumor suppressors such as FOXO3a (22) and TP53INP1 (23) in multiple breast cancer cell lines and tumors (24).

In the current study, there was a difference between the mean expression levels of miR-155 in serum of patients with breast cancer, according to noninvasive BC (DCIS) and invasive BC (IDC).

5.1. Conclusion

In general, the results for this study showed that the serum levels of miR-155 evidence a statistically significant difference in invasive breast cancer (IDC) patients. The study results also indicated that checking the serum levels of miR-155 expression in patients with invasive breast cancer (IDC) might be helpful. It is therefore recommended for future similar studies to include other populations and larger sample sizes.

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