http://pmjournal.ir Original Article

Winter 2020, Volume **5**, Issue 16 (15-16)

Evaluation of miRNA-21 expression in plasma samples of breast cancer patients

kazhaleh Mohammadi[1*], Mehrdad Tavakoli[2], Nafise Poorhasan[3], Maryam forouhi[4]

[1] Researcher and lecture assistant at the Medical Laboratory Science Department, Knowledge University, Kurdistan Region, Erbil, Iraq.

[2] Department of biology, Zanjan, Islamic azad university, Zanjan, Iran.

[3] Personalized Medicine Research Center of AmitisGen, Tehran, Iran.

[4] National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

**Corresponding author: kazhaleh Mohammadi, Researcher and lecture assistant at the Medical Laboratory Science Department, Knowledge University, Kurdistan Region, Erbil, Iraq*

E- mail: Kazhaleh.mohammadi@Knu.edu.iq

DOI: 10.22034/pmj.2020.40429

Submitted: 2020/01/21
Accepted: 2020/03/01

Keywords:
breast cancer
blood biomarker
MicroRNA-21
gene expression

Abstract

According to the World Health Organization, one in eight to ten women will develop breast cancer. Therefore, the development of new blood / serum markers is important as an alternative to traditional diagnosis methods. Early detection of breast cancer plays an important role in choosing the right treatment approach and treating the patient. In this study, the expression of miRNA21 gene in the plasma sample of people with breast cancer compared to healthy people was evaluated. The results of qPCR showed that the expression levels of miRNA-21 in the plasma samples of breast cancer patients were significantly increased compared to those of the healthy controls. These results suggest that Mir 21 could be a very good blood biomarker for the early detection of breast cancer.

INTRODUCTION

Breast cancer is a serious public health problem, and its incidence is on the rise around the world. According to the World Health Organization, one in eight to ten women will develop breast cancer (1). According to Iranian statistics, one in every 10 to 15 women is more likely to develop breast cancer, but the incidence of breast cancer in Iranian women is at least ten times lower than in women in developed countries (2). Therefore, the development of new blood / serum markers is important as an alternative to traditional methods of diagnosing cancer. The average age at diagnosis of breast cancer is 56 years in Western countries and 45 years in Iran (3). Recently, miRNAs in the bloodstream have been reported to be stable against RNase enzyme activity and can act as blood biomarkers to diagnose cancer. MiRNAs include a large family of small, non-conducting single-stranded RNA molecules that regulate various modes of gene expression through the inhibition of translation or mRNA degradation (4). These molecules play an important role in a wide range of cellular physiological and pathological processes (5). In addition, miRNAs are involved in almost all aspects of cancer biology, such as cell proliferation, cell cycle regulation, apoptosis, and metastasis or invasion (6). The miRNA expression profile is for each specific tissue or cell that directly reflects the diverse pathophysiological processes and therefore represents the different stages of the cancer (7). High levels of miRNA in body fluids can be used as a blood-based diagnostic tool for the early detection of cancer. It has recently been established that the plasma and serum of cancer patients have tumor-associated miRNAs (8). These molecules included (miR-15b, miR-16, miR-24 and miR-141) in the plasma and (miR-155, miR-210, miR-21) in the serum of breast cancer patients. For example, the ratio of miR-92a / miR-638 in plasma has recently been reported as a sensitive indicator in patients with acute leukemia (9). Moreover, serum levels of miR-141 have been shown to be increased in patients with prostate cancer compared to healthy individuals. Therefore, circulating miRNAs are thought to be biological markers for various diseases, including cancer. In this study, the expression of the miRNA21 gene in plasma samples of breast cancer patients was evaluated and compared with that of healthy people.

METHODS AND MATERIALS

Samples of 5 ml of blood were taken from 15 women with breast cancer and 10 healthy individuals with their consent in tubes containing K2-EDTA anticoagulant and immediately placed on ice to prevent degenerate RNA. To separate the plasma, the samples were centrifuged at 5000 rpm for 10 min. Plasma was separated from whole blood and transferred to 1.5-ml microtubes. Samples were stored at -40 °C until the miRNA was extracted. The SanPrep

Column microRNA extraction Miniprep BioBasic kit was used to extract the miRNA, and the extraction steps were performed according to the manufacturer's protocol. The cDNA was synthesized from the total miRNA by BONmiR high sensitivity MicroRNA1st Strand cDNA Synthesis kit according to the manufacturer's instructions. In this kit, cDNA was synthesized using a polyA polymerase enzyme for all miRNAs. The miRNA-21 expression was evaluated using the Realtime PCR relative method (2^Dct), and the miRNA-16 gene was used as a control. The qPCR process was performed using the BON microRNA QPCR master mix, which contained the reverse general primer. The expression of miRNA-21 was measured using specific primers (Table 1). Statistical analyses were performed using SPSS software and a level of $p < 0.05$ was considered significant.

Table1. specific primer for mir-21 amplification and annealing temperature

Gene	Specific forward primer sequence	Tm
miRNA-21	UAGCUUAUCAGACUGAUGUUGA	60c
miRNA-16	UAGCAGCACGUAAAUAUUGGCG	60c

RESULTS

The expression of the miRNA-21 gene in breast cancer patients and healthy controls was measured by Realtime PCR (qPCR), and the data was normalized for the expression of the miRNA-16 control gene. The qPCR results showed that the expression level of miRNA-21 in the plasma samples of breast cancer patients was significantly increased compared to the healthy controls (Fig. 1).

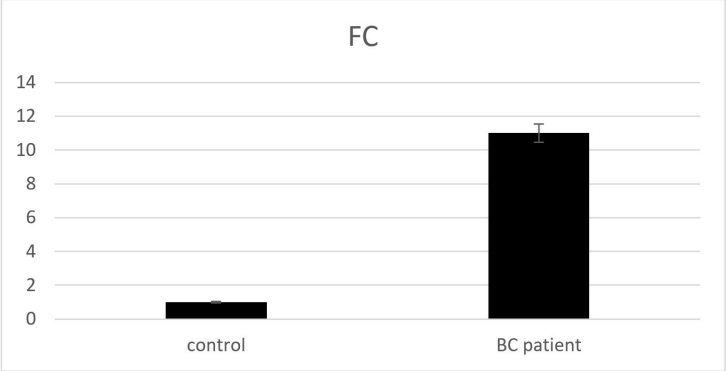

Fig 1. Comparison of mir-21 fold change in two groups of patients and healthy group, in this study, it was observed that the expression of Mir-21 in the plasma samples of people with breast cancer increased 11 times compared to healthy people.

DISCUSSION

Breast cancer is a serious public health problem, and its incidence is on the rise around the world. Early detection of breast cancer plays an important role in choosing the right treatment approach and treating the patient (10). Blood biomarkers can be one of the best ways to diagnose cancer early. The circulating miRNAs are relatively stable, very accessible, easily testable, and stage specific biomarkers for non-invasive diagnosis in various tumors (11). Studies have shown that the expression of miRNA21 increases in breast cancer tumors. Tissue-based studies have shown that miRNA-21 is an oncomiR and has a significantly increased expression in tumors compared to healthy tissue (12). It is also involved in various cancer-related processes, such as invasion, migration, and metastasis. In this study, the expression of Mir 21 in breast cancer plasma samples was evaluated. The results showed a 10-fold increase in the expression of this gene in patients compared to healthy individuals. These results suggest that Mir 21 could be a very good blood biomarker for the early detection of breast cancer, although more studies with a larger number of samples and different stages of cancer in the future are recommended.

REFERENCES

1. Saad E D (2011) *Indian J Med Res* 134, 413-418
2. Shetty P (2012) *Lancet* 379, 992-993
3. Bagchi S (2008) *CMAJ* 179, 27-27- a
4. Chin L J & Slack F J (2008) Cell Res 8, 983-984
5. Mitchell P S, Parkin R K, Kroh E M, Fritz B R, Wyman S K & Pogosova-Agadjanyan E L (2008) *PNAS* 105, 10513-10518
6. Chen X, Ba Y, Ma L, Cai X, Yin Y & Wang K (2008) *Cell Res* 18, 997-1006
7. Nilsen T W (2007) *Trends Genet* 23, 243-249
8. Bortolin-Cavaille M L, Dance M, Weber M & Cavaille J (2009) *Nucleic Acids Res* 37, 3464-3473
9. Xia M & Hu M (2010) J Cancer Mol 5, 33-39
10. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W & Tuschl T (2002) *Curr Biol* 12, 735-39
11. Xiao Y, Xu C, Guan J, Ping Y, Fan H & Li Y(2012) *PLoS* One 7, 1-11
12. Cortez M A, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood A K & Calin G A (2011) Nat Rev *Clin Oncol* 8, 467-477