Short Communication

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The Association of miR-451 and miR-21 in Plasma with Lymph Node Metastases in Breast Cancer

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ABSTRACT

BACKGROUND AND OBJECTIVE: The expression of some circulating microRNAs (miRNAs) in biological fluids of healthy individuals is different from cancerous patients. circulating miRNAs are a new class of cancer biomarkers because of their high stability and sensitivity, ease of measurement and specificity due to their correlation with various cancer states. According to the miR-451 and miR-21 functions in the metastasis of some cancers, the aim of this study was to investigate the differences of expression levels of miR-451 and miR-21 in the plasma of breast cancer (BC) patients with and without lymph nodes metastasis (LNM).

METHODS: In this descriptive-analytical study, blood samples were collected from 47 women with BC and 24 healthy women with mammography confirmation. The presence/or absence of LNM was recognized from patients' medical records. The expression levels of miR-451 and miR-21 in the plasma, were investigated using Real-Time PCR.

FINDINGS: The median of expression of miR-451 in BC patients with LNM and without LNM was 1.739 and 3.187, respectively, and its expression in lymph node metastatic patients decreased 0.444 folds in comparison with non-metastatic patients (p=0.031). The median of expression of miR-21 in patients with LNM and in non-metastatic lymph nodes patients was 5.922 and 2.157, respectively, and its expression in metastatic status was 2 folds higher than non-metastatic (p=0.029).

CONCLUSION: The results of this study indicated that decreased miR-451 and increased miR-21 expression in plasma of BC patients was associated with LNM status.

KEY WORD: *Breast Cancer, Lymph Node Metastasis, miR-451, miR-21.*

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Introduction

Breast cancer is the most common type of cancer in women. Recurrence and metastasis are the main causes of death from this cancer (1). The status of metastasis to lymph nodes is one of the most important prognostic factors in breast cancer and it is important in clinical decision-making, proper surgical selection and treatment outcomes (2). This parameter is a measure of the survival status of patients (3). Most conventional diagnostic methods and biomarkers in breast cancer are not able to detect metastatic lymph nodes, and accepted methods such as lymphatic mapping and biopsy of lymph nodes can not be applied to all patients (4).

Therefore, the identification of noninvasive biomarkers is important for tracking lymph node metastases. miRNAs are a type of non-coding RNAs. Exposure profiles of microRNAs have high potential for specific detection of different cancers, as well as pathologic stages and pathologies different from one type of cancer (5). In breast cancer, miR-451 and miR-21 expressions are disregulated in tumor cells and some biological fluids such as plasma (7, 6). In many studies, the role of miR-21 as a key onco miR has been identified that increasing the expression of miR-21 in tumor tissue is associated with the onset, progression or metastasis of some cancers (8, 1).

On the other hand, miR-451 is a tumor suppressant. Consequently, the reduction of the expression of this miRNA plays a role in breast cancer (9). Considering the importance of the status of lymph node metastases for better managment of breast cancer in order to use the appropriate treatment protocol (2) and considering the role of miR-451 and miR-21 in metastasis (9), in the present study for the first time non-invasive analysis of the possible changes in the level of expression of these two miRNAs in the blood plasma of patients with breast cancer in relation to the status of metastatic lymph nodes compared to healthy subjects. By continuing this study in the future, it might be possible to use two miRNAs as noninvasive biomarkers to detect breast cancer metastasis to lymph nodes.

Methods

In this descriptive-analytical study, after approval by the ethics committee of Shahrekord University of Medical Sciences (Registration code: IR.SKUMS.REC.1394.178), peripheral blood of 47 women with breast cancer and 24 healthy women were collected. Women with all four stages of breast cancer and ductal carcinoma were included in the study. Healthy people were selected with a mean age similar to women with breast canser who had been mammographed during the sampling period and their health was confirmed. Confirmation of the health or illness of the women studied by examining or considering a mammogram report by an oncologist (10). Samples were collected from Isfahan and Shahr-e-Kord Hospitals, after obtaining written consent and during 18 months, from 2015 to 2016.

Clinical information such as the stage of cancer, the presence or absence of metastasis to the lymph nodes after surgery, and the sending of mass samples to the laboratory were determined by the pathologist. Blood plasma was isolated from other parts by blood centrifuge in 7 minutes and with 2500 rpm. The expression of miR-451 and miR-21 variables in two groups of breast cancer patients (eligible/without metastasis of lymph nodes) were evaluated using Real-Time PCR.

From 250 µl of plasma samples, miRNAs were extracted using a kit (exiqon, 300112). Subsequently, cDNA synthesis was performed using kits (00101005, Pars Genome) and based on the company protocol. Real-Time PCR reaction was performed using SYBRGreen (RRS20Q, Takara) and miR-451, miR-21 specific primers and internal control gene U6snRNA (00101007, Pars Genome).

The Real-Time PCR reaction was performed with three repetitions for each sample. Expression ratios for miR-21 and miR-451 in plasma of patients were calculated by the $\Delta\Delta Ct$-2 formula in ΔCt = Ctinterested miRNA - CtU6snRNA and $\Delta\Delta Ct$ = ΔCtsample (patient) - ΔCtcontrol. Data analysis was performed using SPSS software version 18 (SPSS Inc., Chicago, Ill., USA) and the analysis of variance and Fisher's exact test were used for comparison between the two groups and the non-parametric Mann-Whitney U test was used for comparison of expression changes and $p<0.05$ was considered significant.

Results

Variables: Clinopathological profile: The mean age of healthy and ill women were 45.6±14.3 and 49.6±10 years, respectively. Out of 47 women with breast cancer, 29 had metastasis to lymph nodes (Table 1). The association of miR-451 expression in plasma with

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metastasis of lymph nodes: The mean ratio of expression of miR-451 in breast cancer patients with lymph node metastases and no lymph node metastasis was 1.73 and 3.871, respectively. Therefore, the expression of miR-451 in the plasma of metastatic patients decreased by 0.444 (p=0.031) compared to non-metastatic patients (Fig.1A). The association of miR-451 expression in plasma with other clinical features: changes in the expression of miR-451 in relation to the status of ER, PR, Her2 and the stage of breast cancer were not significant (p=0.682, p=0.973, p=0.823 and p=0.344). The association of miR-21 expression in plasma with metastasis in the lymph nodes: The mean ratio of miR-21 expression in patients with metastatic lymph nodes and in patients without metastasis were 5.92 and 5.157, respectively. The expression of miR-21 in metastatic state was 2 times higher than non-metastasis (p=0.029) (Fig. 1B).

Table 1. Clinopathological profile of patients: The number of each patient is shown in terms of the variables studied

Variable	Level of variable	Number of patients
Stage	one	2
	Two	17
	three	4
	four	3
ER Receptor	positive	18
	negative	5
PR Receptor	positive	16
	negative	7
Her2/nu Receptor	positive	5
	negative	18
Lymph node metastases	positive	29
	negative	18

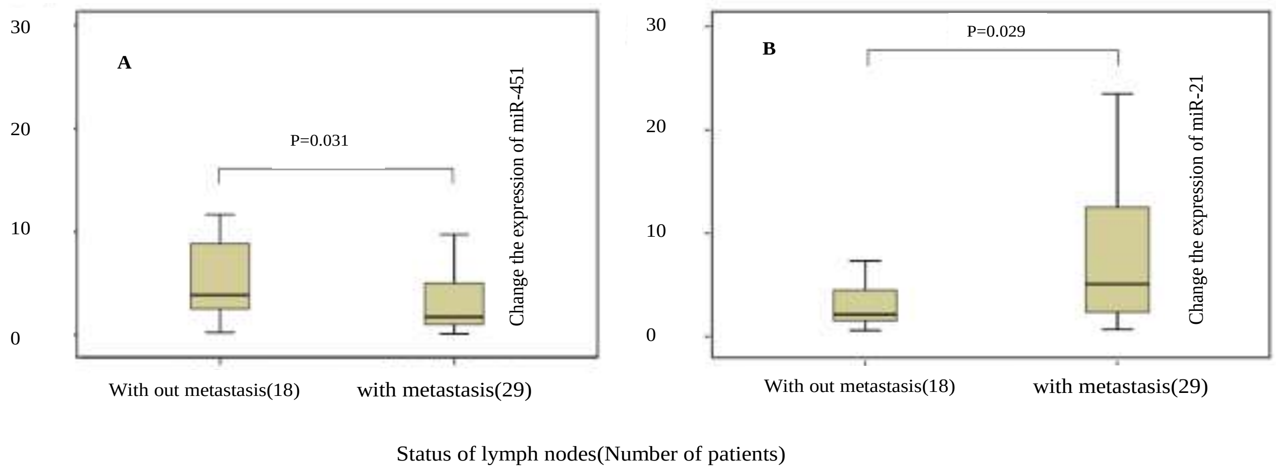

Figure 1. MiR-451 and miR-21 expression patterns associated with metastasis of lymph nodes in breast cancer: (a) related to changes in the expression of miR-451; and (b) miR-21 expression changes. The level of expression of these two microRNAs in comparison with the absence of metastasis was decraesed 0.442 fold (p = 0.031) and increased 2 times, respectively (p=0.029).

Discussion

In this study, changes in plasma miR-451 and miR-21 plasma expression in patients with breast cancer, with regard to the status of metastasis to lymph nodes, were significantly altered in these patients. Identification of lymph node metastases plays a very important role in the treatment of breast cancer and plays a role in the choice of surgical procedure and subsequent therapeutic treatment (4). Previous studies have shown that miR-451 plays a role in tumor suppression in various cancers. Pan et al. showed that most of the target genes of the miRNA, such as c-Myc,

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RAB14 and others, are oncogene and play a role in tumorogenesis and metastatic pathways. Obviously, the results of many studies on various cancers indicate a reduction in the expression of miR-451 in tumor cells (11). The results showed that the plasma expression of miR-451 is reduced in relation to breast cancer metastasis. Bandres et al showed that a lower expression of miR-451 is associated with a lower survival of gastric cancer patients (12).

Another study by Wang et al. on lung cancer revealed a reduction in the expression of miR-451 with more advanced stages, metastasis to lymph nodes, and tumor differentiation (13). Plasma miR-21 plasma concentrations were higher in patients with metastatic lymph nodes than in patients without lymph node metastasis. This finding is also consistent with some similar studies in this area. Yan and colleagues compared the breast tumor tissue with healthy surrounding tissues and it was found that increased and elevated expression of miR-21 was associated with more advanced stages of cancer and metastases to lymph nodes (14).

In a study by Ota et al. on bone marrow tissue in breast cancer patients, individuals with metastatic lymph nodes had a higher expression of miR-21 than non-metastatic patients (15). Due to the higher value of noninvasive studies than biopsy, in the present study, the use of plasma versus tissue samples has been used in previous studies. The results suggest that expression of miR-21 increases when disease progresses, and especially during metastases. Melnik et al showed that miR-21 is a key onco miR, and in many cancers, the level of its expression is elevated. Maintaining the prolifration signal, escape from growth inhibitors, activating invasion and metastasis, inducing angiogenesis and oxidative stress, as well as genetic instability are some of its roles (16). Zhu et al. showed that PDCD4 (programmed cell death protein) and Maspin are miR-21 targets that reduce the potential for invasion and metastasis in breast cancer. Thus, miR-21 is important for tumor cell invasion by targeting metastasis inhibitors (17). In general, these studies can explain the link between increased expression of miR-21 and a greater reduction in the expression of miR-451 in parallel with the presence of metastasis to lymph nodes. It can be concluded that with significant changes in the level of expression of miR-451 and miR-21 in relation to metastasis of lymph nodes in breast cancer patients, and on the other hand, due to changes in expression in plasma of patients that compared with tissue samples in the previous studies is less invasive, so it may be possible to use these two miRs in determining the prognosis of the metastasis and, consequently, the determination of therapeutic approaches.

Suggestions: In order to confirm the correlation between the clinical features of breast cancer with changes in the expression of miR-451 and miR-21 in the plasma of patients and their use as prognostic biomarkers in breast cancer, it is suggested a study with more sample size in this regard.

Conflict of Interest: No conflicts of interest.

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