ORIGINAL ARTICLE

Evaluation of miR-146a as a potential biomarker for diagnosis of cardiotoxicity induced by chemotherapy in patients with breast cancer

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Background: Cardiotoxicity from chemotherapy may result in cardiomyopathy and heart failure. Clinicians can use the evaluation of cardiotoxicity-specific biomarkers, such as microRNA, as a tool for the early detection of cardiotoxicity. The study's objective was to assess miR-146a levels as a potential biomarker for the detection of cardiotoxicity brought on by chemotherapy in patients with breast cancer. **Materials and Methods:** Using quantitative reverse transcription-polymerase chain reaction, the levels of miR-146a were assessed in the blood of 37 breast cancer patients receiving anthracyclines without cardiotoxicity and 33 breast cancer patients experiencing cardiotoxicity brought on by chemotherapy after chemotherapy. Left ventricular ejection fraction (LVEF) ≥50% was used to define heart failure by echocardiography. **Results:** MiR-146a did not show any significant difference in expression between these two study groups (P = 0.48, t-test). The expression level of miR-146a was not significantly associated with LVEF, age, and body mass index (P > 0.05), according to Pearson correlation. **Conclusion:** MiR-146a may be a diagnostic or prognostic biomarker for cardiotoxicity brought on by chemotherapy, even though there was no discernible difference in the expression level of miR-146a between the control group and the breast cancer patients who were experiencing this side effect of chemotherapy. Therefore, miR-146a expression needs to be examined in a sizable cohort of breast cancer patients who are experiencing cardiotoxicity brought on by chemotherapy.

Key words: Breast cancer, cardiotoxicity, chemotherapy, microRNA 146a, microRNAs

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INTRODUCTION

Breast cancer is one of the leading causes of cancer-related death in women worldwide which is characterized by the growth of malignant cells in the mammary glands.[1] The standard treatment for most breast cancer patients is a combination of surgery and chemotherapy.[2] Despite advances in the development of chemotherapy agents, treatment-related complications including chemotherapy-induced cardiotoxicity will limit their use.[3]

The cardiotoxicity induced by chemotherapy causes cardiomyocyte death or cardiomyocyte dysfunction which may not be reversible.[4] Furthermore, a cohort study has recently indicated that cardiovascular disease (CVD)-related mortality in breast cancer survivors after 7 years is almost 2-fold higher than in noncancer women.[5]

The most common detection of cardiotoxicity is using clinical symptoms and decreases in the left ventricular ejection fraction (LVEF).[6] However, the prediction of chemotherapy-induced cardiotoxicity before occurrence is often not possible. Therefore, identifying promising

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diagnostic or prognostic biomarkers can be helpful to predict the onset of cancer therapy-related cardiac dysfunction.

Several recent investigations have demonstrated that microRNAs (miRNAs) can be used as diagnostic and prognostic biomarkers for cancer. A large class of short, noncoding RNAs called miRNA regulates the posttranscriptional expression of target genes.[7] It is well known that miRNAs are expressed in the cardiovascular system and that they play a role in the pathophysiology of cardiovascular and cardiac disease.[8] Several miRNAs have been introduced for the early detection of heart diseases such as miR-1,-146a, -133b, -208a, -208b, and -423-5p.[9,10]

Two genes, miR-146a and miR-146b, make up the miR-146 family of miRNAs, were the first miRNAs described in humans.[11] Studies have revealed a link between miR-146a expression level and coronary artery disease severity.[12] Decreased expression of miR-146 is accompanied by the progression and severity of atherosclerosis.[13] Furthermore, miR-146a is a key inhibitor of vascular inflammation and has a protective role against the formation of atherosclerosis.[14] This miRNA provides negative feedback regulation of inflammation in endothelial cells by affecting of toll-like receptor4/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway.[15,16] It can reduce vascular inflammation by targeting the monocyte–macrophage lineage.[17] These evidence shows that there is a relation between miR-146a expression level and the severity of heart disease.

The cardiotoxic side effects that are associated with chemotherapy not only restrict the amount of dosage that can be administered but also result in increased morbidity and mortality for cancer survivors.[18] Since there is a link between changes in amounts of miR-146a expression and CVD, miR-146a may be used as a diagnostic or prognostic biomarker for the detection of cardiotoxicity brought on by chemotherapy.

Therefore, we performed this study to evaluate the expression levels of miR-146a in patients with breast cancer experiencing chemotherapy-induced cardiotoxicity.

METHODS

Patient population

Two groups of breast cancer patients who were referred to the cancer referral centers of the hospital in Isfahan in 2020–2021 participated in the cross-sectional study, which was designed for that purpose. Patients in the control group (n = 37) were recovered from chemotherapy with anthracyclines and/or paclitaxel without cardiotoxicity. Thirty-three patients with breast cancer recovering from cardiotoxic chemotherapy made up the experimental group.

Earlier going into the study, all patients were examined by oncology and cardiologists and entered into the study according to the inclusion criteria.

Following the end of the primary treatment,[19] they were chosen by a cardiologist with a focus on heart failure based on results from the Minnesota Living with Heart Failure Questionnaire, and they had a LVEF ≥50%. The results are confirmed by echocardiography.

Preparing RNA samples and synthesis of complementary DNA

RNA was extracted from blood and RNA concentrations were quantified using the Epoch microplate spectrophotometer (USA). The transcriptor first-strand complementary DNA (cDNA) synthesis kit (AnaCell, Tehran, Iran) was used to perform the reverse transcription step on 500 ng of RNA.

With the aid of the reverse transcription (RT) stem-loop primer, cDNA was created from total RNA. The thermal cycling conditions were as follows: incubation for 60 min at 37°C and then stopped by heating at 70°C for 5 min. Before using, the cDNA was kept at 20°C.

Quantitative reverse transcription-polymerase chain reaction

Real-time RT-polymerase chain reaction (RT-PCR) was performed according to two steps. In the first step in this assay, RT stem loop captured specifically the miRNA, and the RT was done.

Then RT product was quantified by RT-quantitative PCR with predesigned specific primers for *hsa-miR-146a* and also the universal reverse primer (UniLoop) (AnaCell, Tehran, Iran). The 2X RT-PCR master mixes were used to run all real-time PCR processes (BioFACT™, High ROX, Korea). Real-time PCR reactions were performed in 20 µL volumes using a Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia). The following thermal cycling conditions were used: one hold at 95°C for 15 min, and 45 cycles at 95°C for 30 s and 60°C for 1 min. Melt curves were obtained after the reaction cycles by heating the reactions from 55°C to 95°C. The relative expression of *hsa-miR-146a* was determined using the $2^{-\Delta Ct}$ method.

Statistical analysis

For comparison of the difference between groups, an independent Student's t-test was used. P <0.05 was considered statistically significant. Bivariate analysis was conducted to assess the strength of the relationship between

two variables and their correlation. SPSS 20 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis.

Ethics approval and consent to participate

The Isfahan University of Medical Sciences ethics committee, located in Isfahan, Iran, gave its approval to this study (IR.MUI.MED.REC.1399.096). Before being asked to sign a consent form to participate in the study, participants received a brief explanation of the study's protocol.

RESULTS

Patient characteristics

We performed our tests on blood samples of breast cancer patients treated with the anthracycline-containing regimen. After the anthracycline-based regimen, these patients have typically received paclitaxel (67.4%) or docetaxel (26.1%). As materials and methods have mentioned, 33 of the 70 patients with breast cancer had experienced chemotherapy-induced cardiotoxicity. The median age of the patients was 40 years in the control group and 47 years in the case group. In addition, the median body mass index (BMI) was 0.002 in each group. Our result did not show any significant difference in age ($P > 0.05$) and BMI ($P > 0.05$) between the two groups. Patients who experienced chemotherapy-induced cardiotoxicity had a baseline left LVEF below normal (<50, LVEF <50).

MiR-146a expression in blood samples

The expression level of whole blood miR-146a was not significant between two groups of breast cancer patients with or without heart failure ($P = 0.48$, independent *t*-test). Furthermore, the relative expression software tool (REST 2009) measured the level of miR-146a in patients with heart failure in comparison to the control group ($P > 0.05$). Figure 1 presents the data.

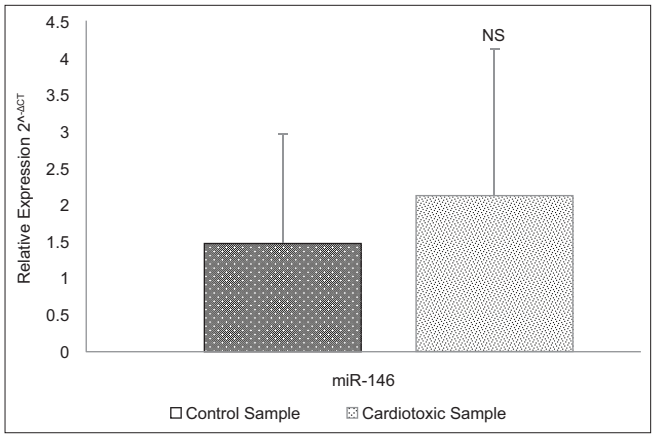

Figure 1: The comparison of miR-146a expression level between patients with chemotherapy-induced cardiotoxicity and patients without experienced cardiotoxicity. There was no significant difference between groups ($P = 0.48$, *t*-test). Each bar presents the mean of ($2^{-\Delta CT}$) ± standard deviation. NS = Nonsignificant

Furthermore, the correlation between expression levels of miR-146a with LVEF was examined. The value of miR-146a expression was not significantly associated with LVEF ($r = -0.06$ and $P = 0.70$). Although the expression level of miR-146a showed no significant association with age and BMI ($P > 0.05$, Pearson correlation), a significant correlation was indicated between BMI and ejection fraction ($r = 0.83$ and $P = 0.00$). Some data of patients are showed in Table 1.

DISCUSSION

miRNAs are key regulators of gene expression which are passively released into extracellular fluids by death or apoptotic cells.[20,21] MiRNAs in circulation are remarkably stable. Therefore, they could be applied as putative biomarkers for the diagnosis or prognosis and follow-up of various diseases.[22,23]

In the present study, we examined miR-146a in blood samples from patients with breast cancer who were experiencing cardiotoxicity brought on by chemotherapy. However, there was not any significant difference between miR-146a expression levels of patients that experienced chemotherapy-induced cardiotoxicity in comparison with the control group.[24]

The protective and harmful functions of miR-146a in the heart have been the subject of conflicting reports. Some findings indicated that the overexpression of miR-146a could prevent cardiotoxicity induced by doxorubicin. Indeed, miR-146a plays a protective role in DOX-induced cardiotoxicity using a reduction of the stability of P53. Therefore, miR-146a could attenuate the apoptosis of cardiomyocytes and improve autophagic disorder and reverse the DOX-induced cardiotoxicity.[25] MiR-146a can delay inflammatory responses oxidized low-density lipoprotein accumulation, apoptosis, or angiogenesis in atherosclerotic plaques.[26] MiR-146a expression is induced in the heart upon activation of the transcription factor NF-κB. The expression of miR-146a could negatively regulate inflammation induced through the innate immune response. Therefore, since heart failure is associated with enhanced inflammation in the heart, overexpression of

Table 1: Characteristics of the patients with/without heart failure

Variables	Patients with HF (*n*=33)	Patients without HF (*n*=37)
Age (median)	47	40
BMI (kg/m^2)	0.0027±0.0006	0.0026±0.0004
LVEF	<50	50
Relative expression of miR-146 (-ΔCt) (median)	5.4	5.01

BMI=Body mass index; LVEF=Left ventricular ejection fraction; HF=Heart failure

miR-146a could attenuate the inflammation and protect cardiomyocytes against oxidant stress.[27-29]

While another study has shown that the overexpression of miR-146a induced cell death in cardiomyocytes by the reduction of the Erb-B2 Receptor Tyrosine Kinase4 expression in the hearts of mice after doxorubicin treatment.[30] He *et al*. indicated that the improvement of hemodynamics and cardiac function and decrease of cardiac remodeling could be done using inhibition of miR-146a in heart failure rats.[24] Some studies demonstrated that the expression of miR-146a was downregulated 24 to 48 h after the initiation of doxorubicin treatment.[31,32] Beg *et al*.'s study revealed that patients with heart failure had significantly higher levels of miR-146a in their exosomes but not in their plasma.[33] Evaluation of the miR-144a levels in exosomes rather than blood might be a reason why we could not make a significant difference. In addition, our study used a small sample size and might not have had enough statistical power. Note that retrospective case–control studies are often assumed to have a selection bias; our study seems to be subject to this bias. Similar to a previous study, the current study has shown that there was no correlation between miR-146a and LVEF.[32]

CONCLUSION

According to the previous studies, the roles of mir-146a remain controversial in CVD. We could not find any significant differences in miR-146a expression level. Therefore, further studies, including the evaluation of miR-146a expression by a large cohort of breast cancer patients experiencing chemotherapy-induced cardiotoxicity, are needed to clarify the role of miR-146a as a diagnostic or prognostic biomarker.

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Conflicts of interest

There are no conflicts of interest.

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