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Original article

Effect of a high-intensity interval training on serum microRNA levels in women with breast cancer undergoing hormone therapy. A single-blind randomized trial

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ABSTRACT

Background: The role of microRNAs (miRs) in hormone therapy (HT) is of keen interest in developing biomarkers and treatments for individuals with breast cancer. Although miRs are often moderate regulators under homeostatic conditions, their function is changed more in response to physical activity.
Objective: This single-blind randomized trial aimed to explore the effect of high-intensity interval training (HIIT) on serum levels of miRs in individuals with early-stage breast cancer undergoing HT.
Methods: Hormone receptor-positive women with breast cancer and healthy women were randomly assigned to a healthy control group ($n = 15$), healthy group with HIIT ($n = 15$), breast cancer group with HT (HT, $n = 26$), and breast cancer group with HT and HIIT (HT + HIIT, $n = 26$). The exercise groups underwent interval uphill walking training on a treadmill 3 times a week for 12 weeks. At the end of the study, we analyzed changes in levels of cancer-related miRs (oncomiRs) and tumour suppressor miRs (TSmiRs) in response to the HT and HIIT.
Results: In women with breast cancer versus healthy controls, the expression of some oncomiRs was significantly increased — miR-21 ($P < 0.001$), miR-155 ($P = 0.001$), miR-221 ($P = 0.008$), miR-27a ($P < 0.001$), and miR-10b ($P = 0.007$) — and that of some TSmiRs was significantly decreased — miR-206 ($P = 0.048$), miR-145 ($P = 0.011$), miR-143 ($P = 0.008$), miR-9 ($P = 0.020$), and let-7a ($P = 0.005$). Moreover, HT considerably downregulated oncomiRs and upregulated TSmiRs. HIIT for 12 weeks with HT significantly decreased the expression of the oncomiRs and significantly increased that of the TSmiRs as compared with HT alone.
Conclusions: HITT could amplify the decrease and/or increase in expression of miRs associated with HT in women with breast cancer. A prospective trial could determine whether the use of circulating miRs for monitoring treatment can be useful in therapy decisions.
Trial registration: Iranian Registry of Clinical Trials (No.: IRCT201202289171N1).

1. Introduction

Breast cancer is the most common diagnosed cancer among women and the second leading cause of gynecological cancer deaths in the United States. MicroRNAs (miRs) are a group of short non-coding RNAs that regulate protein-coding gene expression. Several studies have shown that the expression of miRs differs between normal tissue and tumour tissue [1]. MiRs can be cancer miRs (oncomiRs) and/or tumour suppressor miRs (TSmiRs). The expression of abnormal miRs results in a change in expression of the protein-encoding genes that can play a central role in tumorigenesis and/or tumour suppression [2]. The interaction of miRs with target genes can confirm their role in cell growth,

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apoptosis, and differentiation [3]. The circulatory levels of some miRs are higher in individuals with cancer than in healthy people [1]. Heneghan et al. demonstrated that the levels of miR-195 and Let-7a as oncomiRs could be significantly increased in the blood of individuals with breast cancer as compared with disease-free controls [1].

All over the world, more than 70% of breast cancer cases express a hormone receptor, either estrogen and/or progesterone receptor. Hormone therapy (HT) is a form of systemic treatment recommended for women with hormone receptor-positive breast cancer [4]. In this context, HT can lead to decreased expression of some miRs in individuals with breast cancer [2]. Likewise, miR-21, miR-155, and miR-10b may act as oncomiRs with consistent decrease in expression with HT [5]. Because of the easy identification and high stability of these miRs, they may be suitable as biomarkers [6]. The identification of circulating miRs and their regulation after exercise suggest that they may be useful biomarkers of health and adaptation to treatment interventions. There is some evidence of an association between cardiorespiratory and physical fitness with circulatory miRs [7,8]. Bye et al. showed that levels of miR-210, miR-21, and miR-222 were increased in healthy individuals with low maximal oxygen uptake ($VO2_{max}$) [7]. Regular physical activity can downregulate miR-21 expression in healthy people [9]. Exercise may also affect the levels of circulating anti-angiogenic miRs such as miR-20a, miR-210, miR-221, miR-222, and miR-328 [10]. Recently, in preclinical studies, our team showed that exercise could affect tumour growth via changes in levels of miRs such as miR21, miR206, and let-7a [11,12].

Typically, studies have examined the effect of light and moderate intensity exercise on physical and psychological indices in individuals with cancer [13]. High-intensity exercise training (HIIT) has long been demonstrated to help improve cardiorespiratory fitness and corresponding physiological variables in healthy individuals [14]. The training involves repeated short to long bouts of relatively high-intensity exercise alternating with recovery periods of low-intensity activity or passive rest [15]. HIIT may improve survival rate and reduce the risk of cancer recurrence [16]. However, considering the benefits of HIIT, this useful training method has not been studied in individuals with breast cancer.

In this study, we hypothesized that miRs secreted from muscle tissue or taken up by muscles or other organs can mediate transient and adaptive responses to the exercise. In this context, 2 main challenges for the successful treatment of breast cancer are the development of more specific biomarkers that predict therapeutic response to endocrine therapies and the identification of new therapeutic targets for endocrine-resistant disease. However, the identification of the expression pattern of the miRs characterizing exercise training may be useful for monitoring physical fatigue and recovery and even to evaluate physical performance capacity. Therefore, the present study aimed to investigate the effect of HIIT with or without HT on serum miR levels in women with early-stage breast cancer.

2. Methods

2.1. Study design

This single-blind randomized trial included 4 arms: healthy control and healthy control women with HIIT and women with breast cancer with HT or HT with HIIT (HT + HIIT) (Table 1). All women with breast cancer [estrogen receptor-positive (ER+) and progesterone receptor-positive (PR+) and/or only ER+] received letrozole or tamoxifen as an HT regimen. Participants were recruited from the Cancer Institute of Iran (Hospital Imam Khomeini, Tehran, Iran) from March 2013 to March 2014. The trial was registered with the Iranian Registry of Clinical Trials (IRCT201202289171N1).

Table 1
General characteristics of women with breast cancer in the study who received hormone therapy (HT) or HT plus high-intensity interval training (HIIT).

Variables	HT $n = 26$	HT + HIIT $n = 24$
Age, years, mean (SD)	48.42 (7.54)	49.2 (9.7)
Mean tumor size, mean (SD)	3.63 (2.07)	3.04 (1.7)
Tumor grade		
Well-differentiated	5	4
Moderately differentiated	17	16
Poorly differentiated	3	3
Tumor staging		
T1	9	6
T2	10	9
T3	6	8
T4	10	8
N0	13	14
N1	2	1

Participants who gave their signed written informed consent were deemed eligible for the study if they were between 30 and 60 years old; had insufficient physical activity level (< 150 min/week); had hormone receptor-positive breast cancer; performed no strenuous exercise such as running, cycling, swimming or resistance training; completed adjuvant chemotherapy and radiotherapy in the last month; and took letrozole or tamoxifen (HT). Non-inclusion criteria were current smoking; evidence of metastatic breast cancer; planning to receive any additional adjuvant chemotherapy or surgery; pregnant or breastfeeding; cardiovascular comorbidities such as myocardial infarction or coronary artery disease; uncontrolled hypertension defined as systolic blood pressure ≥ 180 mmHg or diastolic blood pressure ≥ 100 mmHg; high-risk or uncontrolled heart arrhythmias; decompensated heart failure; known aortic aneurysm; chronic obstructive pulmonary disease; or any other condition that may impede testing of the study hypothesis or make it unsafe to engage in the exercise program.

2.2. Randomization and blinding

The physical activity level of all participants was measured by International Physical Activity Questionnaire. Participants were randomized and stratified to the healthy control group ($n = 15$), healthy group with HIIT ($n = 15$), breast cancer group with HT (HT, $n = 26$), and breast cancer group with HT and HIIT (HT + HIIT, $n = 26$) according to a computerized random number generator (1998-2017 RANDOM.ORG). The randomization code was developed to select randomized permuted blocks. An investigator who was not involved in the assessment, treatment or statistical analysis performed the randomization.

2.3. Exercise intervention

Women with breast cancer who were assigned to HIIT received usual care (routine daily activity) and also attended a supervised high-intensity aerobic interval exercise program 3 times a week for 12 weeks. The HIIT groups participated in 3 familiarization sessions before starting the main exercise program.

We used the HIIT protocol that was previously reported as a safe training regimen for individuals with heart failure and coronary artery diseases [17]. The training intensity was determined according to the predicted maximal heart rate (HR), despite the long-standing limitation of this formula as compared with actual measurement of maximal HR (HR_{max}) by a maximum stress test

[18]. Each participant underwent the exercise session individually with supervision by an exercise physiologist. The main exercise intervals consisted of 4 × 4 min of uphill walking at 90% to 95% HR_{max} (exercise) and 4 × 3 min of uphill walking at 50% to 70% HR_{max} (active recovery) on a motorized treadmill (Impulse, USA) [17]. The overall time of each session was 38 min, consisting of 5 min warm-up, 5 min cool down, 16 min HIIT, and 12 min active recovery between intervals. The HR of participants was fully monitored during every training session [19]. All participants used a HR monitor (Polar Electro, Kempele, Finland) to obtain the assigned exercise intensity. The speed and inclination of the treadmill were continuously adjusted to ensure that every training session was carried out at the assigned HR throughout the training period. During the training sessions, participants were advised to respect their physical limitations. The moderators managed the participants' daily adherence to exercise courses. Women in the general care group were instructed to continue with their routine activities [17,19]. The general care was to maintain baseline physical activity levels of participants during 12 weeks of the intervention. The exercise trainers monitored adherence to the intervention.

2.4. Blood sampling

Blood samples were obtained from the antecubital vein in the morning after a 12-hr fast. The serum was collected and centrifuged at 12,000 g for 15 min to remove cell debris. Post-intervention blood collection was 3 days after the last training session to avoid the acute effect of the exercise. Samples were aliquoted and stored at −80 C until miR detection.

2.5. Identification of cancer-related breast miRs

The Gene Expression Omnibus database (GEO, http://www.ncbi.nlm.nih.gov/geo/) in the National Center for Biotechnology Information (NCBI) is the public gene expression resource and includes 214,268 samples and 4500 platforms [20,21]. The selected miRs were searched in the human miR database (HMDD; http://cmbi.bjmu.edu.cn/hmdd and http://202.38.126.151/hmdd/tools/hmdd2.html) to further select the differentially expressed miRs related to breast cancer. As a database for experimentally supported human miRs and disease associations, HMDD is a valuable resource for studying the roles of miRs in human diseases [22]. Furthermore, the target genes of the differentially expressed breast cancer-related miRs were predicted by five databases, namely MiRanda (http://microrna.sanger.ac.uk/) [23], MirTarget2 (http://nar.oxfordjournals.org/cgi/content/abstract/34/5/1646) [24], PicTar (http://pictar.bio.nyu.edu/) [25], PITA (http://genie.weizmann.ac.il/pubs/mir07) [26], and TargetScan (http://targetscan.org/) [27]. As well, the published oncomiRs and TSmiRs for breast cancer were selected from TSGene (http://bioinfo.mc.vanderbilt.edu/TSGene/) [28] and Tumor-Associated Gene (TAG; http://www.binfo.ncku.edu.tw/TAG/) databases [29]. In the present study, we used Kyoto Encyclopedia of Genes and Genomes (KEGG) and enrichment analyses of the genes for the identified target genes [30,31].

2.6. Quantitative RT-PCR

Total RNA was extracted from 100 μL plasma samples by using 1 ml Trizol reagent according to the manufacturer's instructions (Sinagene, Tehran, Iran). Qualitative and quantitative assessments of isolated RNA involved electrophoresis and spectrometry methods [31,32]. The RNA was stored at −80 °C. For quantification of miRs by RT-PCR in all samples, 10 μL total RNA was reverse-transcribed in a 20-μL reaction mix by using the BONmiR1st-strand cDNA synthesis kit (Bonyakhteh, Tehran, Iran) following the manufacturer's recommendations. Then, cDNA was used in the RT-PCR assays with the BONmiR qPCR Kit (Bonyakhteh, Tehran, Iran) based on the manufacturer's instructions. RT-PCR analyses of miRs were performed in triplicate. The miR levels were normalized to SNORD RNA level (an internal control). MiRgene expression was analyzed by using the Step-One system (ABI, Massachusetts, USA). Relative expression of miRs was calculated by the $2^{(-\Delta\Delta ct)}$ method. ΔCT was calculated by subtracting the CT values for SNORD from those of the target miRs [31]. ΔΔCT was then determined by subtracting the mean ΔCT of the control sample from that of the case samples. The fold change in levels of candidate miRs was calculated by the equation $2^{(-\Delta\Delta ct)}$ [31,33]. Sequences of the forward primers are as follows: MiR-21 forward primer: ACGTGTTAGCTTATCAGACTG; MiR-155 forward primer: CCGTTAATGCTAATCGTG; MiR-10b forward primer: GGTTAATAAAGCCGCCATCC; Let-7a forward primer: GGCTGAGGTAGTAGGTTGTATAG; Mir-9 forward primer: AGGCATCTTTGGTTATCTAG; Mir-27a forward primer: CCGTTCACAGTGGCTAAG; Mir-143 forward primer: CTGTTGAGATGAAGCACTGT; Mir-145 forward primer: GTCCAGTTTGCCCAGGA; Mir-221 forward primer: AGCCGAGCTACATTGTCT; Mir-206 Forward primer: GGAATGTAAGGAAGTGTGTG; Snord forward primer: ATCACTGTAAAACCGTTCCA. Universal Reverse Primers were obtained from Bonyakhteh Co. (Tehran, Iran).

2.7. Statistical analysis

The primary and secondary outcomes were physical activity and miR level, respectively, at baseline and after the intervention. Sample size calculation was based on the effects of exercise on cardiorespiratory fitness in breast cancer survivors in the literature [34]. With a power of 0.80, two-tailed alpha < 0.05, and large effect size (d) = 0.80, we needed 26 participants in each cancer group. All data are presented as mean (SD). All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL). Kolmogorov–Smirnov test was used to evaluate the distribution of data. Univariate ANOVA was used to compare means, and Tukey test was used to determine pairwise differences. $P < 0.05$ was considered statistically significant.

2.8. Ethics

The study was approved by the Medical Ethics Committee of Tehran University of Medical Sciences (No.: 113825) and the Iranian Randomized Control Trial (IRCT) ethical board (No.: IRCT2014081018745N1).

3. Results

Fig. 1 reflects the participants' distribution in the study. We included 52 non-metastatic and hormone receptor-positive women with breast cancer aged 31 to 69 years and healthy women aged 30 to 60 years. Two women with breast cancer dropped out of the study for personal reasons. Therefore, data for 50 women with breast cancer (26 each group) were analyzed. The physical activity amount of participants was < 600 MET/minute/week: healthy controls (mean [SD] 400 [125]), HIIT (426 [96]), HT (325 [113]), and HT + HIIT (345 [116]). In the post-test, mean (SD) physical activity amount did not significantly change in healthy controls (450 [165], $P = 0.097$) and HT (330 [130], $P = 0.086$) groups. We observed a significant increase in mean (SD) physical activity level in HIIT (705 [99], $P = 0.032$) and HT + HIIT (650 [48], $P = 0.023$) groups after exercise training.

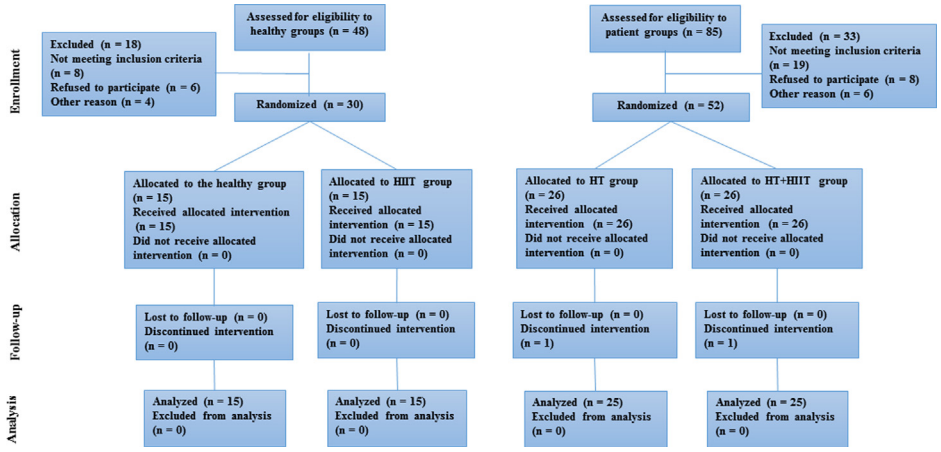

Fig. 1. Flow diagram for distribution of participants. HT: hormone therapy; HIIT: high-intensity interval training.

3.1. Expression of oncomiRs

Mean (SD) levels of some oncomiRs were higher for women with breast cancer than healthy controls: miR-21 (5.5 [0.6] vs. 0.47 [0.01], $P < 0.001$), miR-155 (5.2 [0.4] vs. 0.33 [0.07]; $P = 0.001$), miR-221 (4.8 [0.5] vs. 0.33 [0.01], $P = 0.008$), miR-27a (5.2 [0.06] vs. 0.13 [0.01], $P < 0.001$), and miR-10b (4.2 [0.8] vs. 0.15 [0.01], $P = 0.007$) (Fig. 2). HT considerably downregulated the expression of the oncomiRs (4.2 [0.4]; $P = 0.004$, 3.2 [0.3]; $P = 0.01$, 3.1 [0.6]; $P = 0.035$, 3.1 [0.1]; $P = 0.002$, and 3.05 [0.12]; $P = 0.008$, respectively) (Fig. 2). HIIT for 12 weeks with HT conferred a greater reduction in expression of the oncomiRs (2.5 [0.5]; $P = 0.018$, 1.3 [0.4]; $P = 0.005$, 1.5 [0.02]; $P = 0.037$, and 1.95 [0.6]; $P = 0.031$, respectively) as compared with HT alone (Fig. 2). However, miR-221 was not further downregulated as with HIIT (2.8 [0.4]; $P = 0.137$) was not significant (Fig. 2C). Of note, the expression of the oncomiRs before and after training in healthy controls did not change as compared with healthy controls with HIIT (Fig. 2).

3.2. Expression of TSmiRs

Mean (SD) levels of some TSmiRs were lower in women with breast cancer than healthy controls: miR-206 (1.1 [0.8] vs. 2.8 [0.02], $P = 0.048$), miR-145 (1.8 [0.12] vs. 5.8 [0.07],

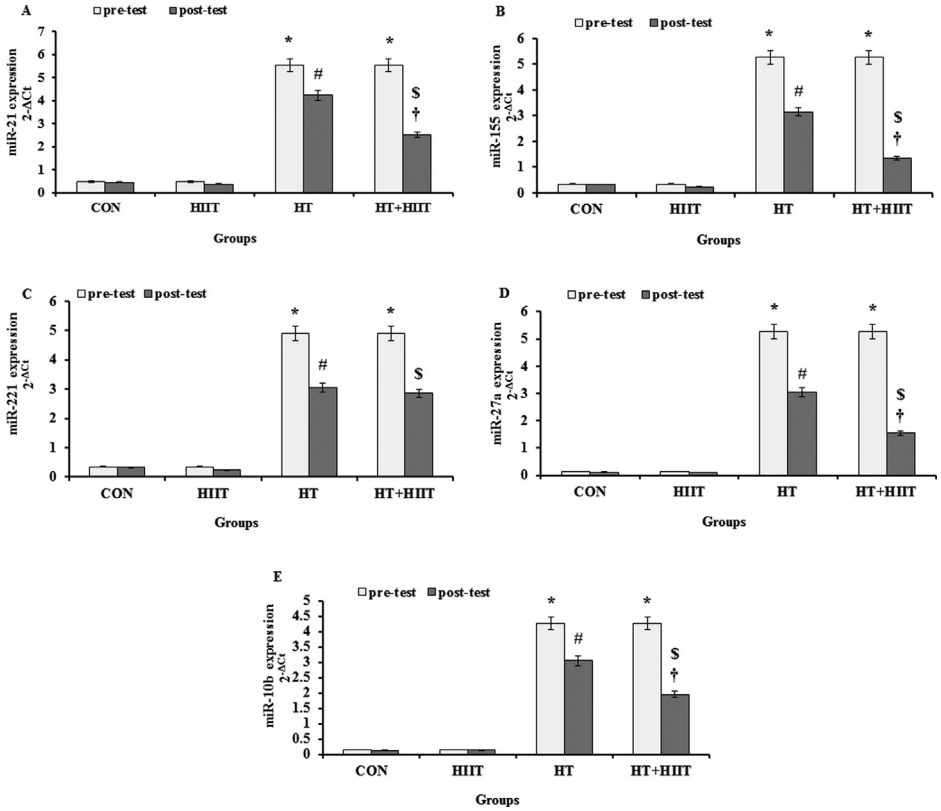

Fig. 2. Effect of HIIT on the expression of miR-21 (A), miR-155 (B), miR-221 (C), miR-27a (D), and miR-10b (E) in women with breast cancer undergoing HT. Data are mean (SD). $^{*}P < 0.05$ compared to control, $^{\#}P < 0.05$ compared to pre-HT, $^{\$}P < 0.05$ compared to pre-HT + HIIT, $^{\dagger}P < 0.05$ compared to post-HT. CON: control; HIIT: high-intensity interval training; HT: hormone therapy. Values are relative to SNORD as an internal control.

$P = 0.011$), miR-143 (2.08 [0.5] vs. 5.08 [0.02], $P = 0.008$), let-7a (0.90 [0.5] vs. 4.6 [0.01], $P = 0.005$), and miR-9 (2.2 [0.27] vs. 2.14 [0.01], $P = 0.0207$) (Fig. 3). The TSmiRs were upregulated by HT (2.33 [0.05]; $P = 0.009$, 5.1 [0.01]; $P = 0.001$, 3.8 [0.1]; $P = 0.012$, 1.8 [0.81]; $P = 0.005$, and 4.04 [0.3]; $P = 0.001$, respectively). Moreover, HIIT for 12 weeks with HT significantly upregulated the levels (3.01 [0.5]; $P = 0.008$, 6.9 [0.2]; $P = 0.001$, 6.1 [0.5]; $P = 0.023$, and 2.6 [0.25]; $P = 0.036$, respectively) as compared with HT alone (Fig. 3). However, miR-9 was not further upregulated with HIIT (4.3 [0.5]; $P = 0.566$) (Fig. 3D). As well, the expression of the TSmiRs did not differ before and after the exercise protocol in healthy controls as compared with health controls with HIIT (Fig. 3).

4. Discussion

Our study showed a change in expression of several oncomiRs and TSmiRs in response to 12 weeks of HIIT and HT in women with breast cancer. The expression of the miRs did not change in healthy women after 12 weeks of HIIT. HIIT together with HT had a remarkable effect on the expression of some measured miRs.

Recently, Di Leva et al. showed that overexpression of oncomiRs in ER-positive individuals with breast cancer might account for the development of a more invasive and deadly tumour phenotype [35]. In this context, altered levels of some circulating miRs may reflect the dysregulation of cell growth and the impact of the therapy [36]. A recent study showed upregulation of oncomiRs such as miR-221 in therapy-resistant breast cancer cells [37]. MiR-221 is overexpressed in ER-α-associated (ER-α) breast cancer cells and tumours [38,39]. It has also been identified as an independent predictor of the response to tamoxifen [40], which has been shown to have a role in increasing progression-free survival [41]. MiR-221 is a negative regulator of p27kip1, a cell cycle inhibitor and tumour suppressor [42,43], and the upregulation of miR-221 and significant reduction in p27kip1 level have been reported in tamoxifen-resistant breast cancer cells. Therefore, miR-221 might regulate tamoxifen sensitivity via the direct targeting of p27kip1 [38,44].

In this study, we have demonstrated that HIIT could decrease miR-221 level in women with breast cancer. A differential expression pattern of circulatory miR-221 may be involved in angiogenesis depending on type and duration of the exercise. In this regard, Baggish et al. observed that miR-21 and miR-221 were immediately upregulated after peak acute exercise in trained rowers. Conversely, their levels were significantly decreased after 1 h of rest following acute exercise, which suggests that these dynamic changes may reflect a real response to exercise [45]. Moreover, Wardle et al. observed a differing pattern of miRs by type of exercise: upregulation and downregulation of miR-21 and miR-221 in endurance and strength training athletes, respectively [46]. Thus, levels of particular miRs may change in opposite directions after endurance versus resistance exercise. However, other studies have reported conflicting results. Some reported lower circulating levels of miR-21 and miR-222 in individuals with high versus low $VO2_{max}$ [7] or a reduction in miR-21 level in trained men after 12 weeks of endurance exercise [9] in athletes or healthy people. However, in the present study, for the first time, we showed a lower level of miR-221 after 12 weeks of HIIT in women with breast cancer undergoing HT.

Likewise, Jung et al. [47] suggested that the circulating miR-21 level is useful for predicting and/or monitoring the therapeutic response to treatment. Upregulation of miR-21 expression has been found associated with resistance to trastuzumab in human

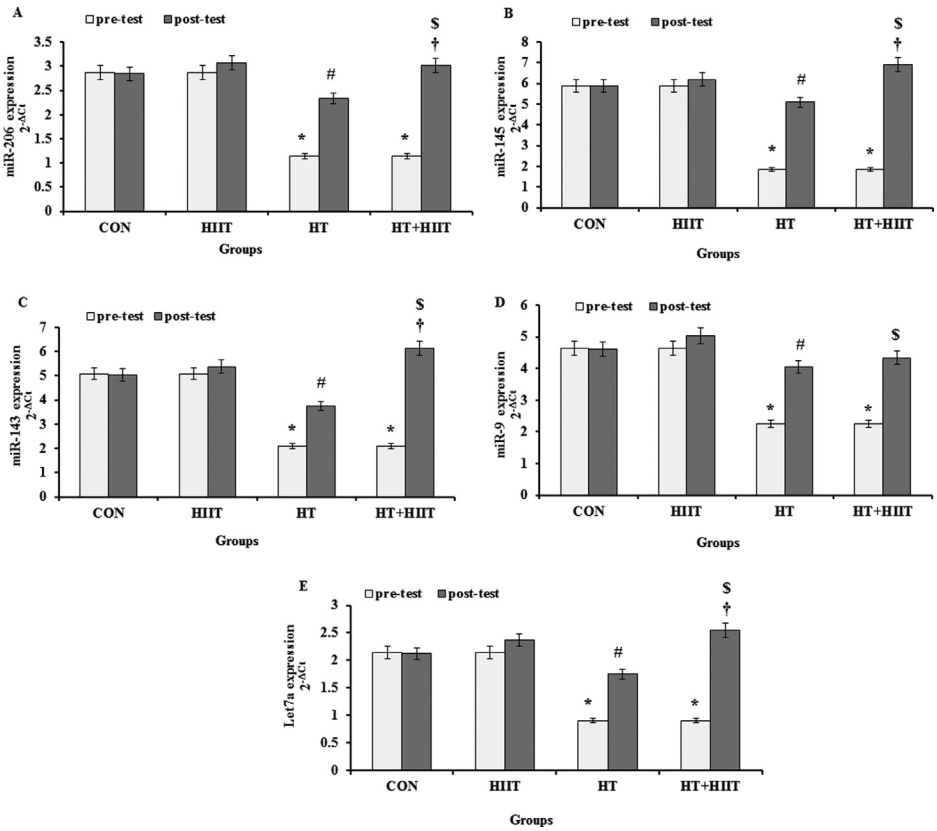

Fig. 3. Effect of HIIT on the expression of miR-206a (A), miR-145 (B), miR-143 (C), miR-9 (D), and let-7a (E) in women with breast cancer undergoing HT. Data are mean (SD). $^{*}P < 0.05$ compared to control, $^{\#}P < 0.05$ compared to pre-HT, $^{\$}P < 0.05$ compared to pre-HT + HIIT, $^{\dagger}P < 0.05$ compared to post-HT. CON: control; HIIT: high-intensity interval training; HT: hormone therapy. Values are relative to SNORD as an internal control.

epidermal growth factor receptor 2-positive breast cancer [48]. Besides, there is some evidence that exercise can affect miR-21 expression in in vitro and in vivo studies. For example, Fernandes et al. observed decreased expression of miR-21 associated with improved micro-vascularization in animals with aerobic swimming training [49]. In this context, we have previously shown downregulated miR-21 in exercise-trained animals, an ER-positive breast cancer model [12]. Similarly, Bye et al. reported an inverse association between cardiopulmonary fitness ($VO2_{max}$) and miR-21 expression [7]. Exercise could decrease the miR-21 level in blood circulation in healthy individuals [9]. Therefore, ER-α-associated tumours may be associated with increased miR-21 expression [50]. According to our results, HT as an ER pathway suppressor can decrease the expression of miR-21. Therefore, for the first time, we showed a low expression of miR-21 in individuals with breast cancer after HIIT.

However, Adams et al. showed miR-206 expression strongly inhibited by ER-α agonists [2]. Isanejad et al. recently found high expression of miR-206 after the HIIT protocol with HT in a mouse model of breast tumour [12]. The authors concluded that miR-206 might have anti-angiogenic effects in breast tumours and thus decreased tumour size in trained animals. Yan et al. [51] also provided insights into the role of the miR-143/145 cluster as a tumour suppressor in breast tumours. MiRs including miR-221/222, miR-342-3p [52], miR-873 [53], and Let7b/Let-7i [54] can downregulate ER-α protein expression, which agrees with our data. However, studies concerning the direct effects of specific circulating miRs on exercise performance and physiological responses are few. In this context, it seems that the altered levels of the above-mentioned miRs are related to the adaptability of the HIIT to endurance/aerobic capacity and modulation of angiogenesis, inflammation, muscle damage, skeletal muscle and heart functions in response to HIIT. Thus, by increasing or decreasing the expression of specific circulating miRs, the interval exercise protocol may modulate the gene expression profile in many cells and tissues, inducing related physiological adaptations by different mechanisms [55]. In this context, Nielsen et al. [56] demonstrated that muscle-specific miRs such as miR-1, miR-133a, miR-133b, and miR-206 are negatively modulated in human muscles after 12 weeks of endurance exercise. Several of these miRs are associated with oxygen tension, angiogenesis and a developmental tissue network for adaptation to aerobic training [57]. Moreover, the exercise can reveal the mechanism of benefits by having an impact on different immune cells, including neutrophils, macrophages, and lymphocytes. In this context, miR-155 seems essential to the balance between pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages during skeletal muscle regeneration and could be a target molecule for degenerative muscle diseases [58].

Although the HIIT in our study could protect women with breast cancer, future research is needed to define the ideal rate, duration, and intensity of the physical activity that can attenuate cancer progress. Furthermore, these findings may support further evaluation of the predictive potential of the HIIT for HT in cancer and they can suggest that these miRs may also mark the potential therapeutic aims in clinical applications. Moreover, the results may inform decisions on HT combined with the exercise.

5. Conclusion

Our results indicate that a decrease or increase in expression of some miRs could play a vital role in estrogen-dependent functions in women with breast cancer. Altered patterns of the expression of the circulating miRs can indicate the impact of treatment on cancer and thus could provide an early decision for treatment continuation. A prospective randomized controlled trial is needed to clarify the effects of the HIIT in this condition. Although finding new miRs intervening in HT is imperative, future research is needed to define the mechanisms of the identified miRs with unknown roles in HT and develop "targeted" therapeutics to miR dysregulation and enhance hormonal sensitivity.

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Authors' contributions

SA: study conception and design. AI: sample collection, sample processing, and article revision. SK: data analysis and sample processing. SS: clinical annotation. AMA: study conception and design and manuscript preparation.

Ethical approval

All procedures performed in studies involving human participants followed the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Disclosure of interest

The authors declare that they have no competing interest.

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