ORIGINAL ARTICLE – CLINICAL ONCOLOGY

High-intensity interval training can modulate the systemic inflammation and HSP70 in the breast cancer: a randomized control trial

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Abstract

Objective Exercise training is recently considered as a trend in adjuvant therapies for cancer patients, but its mechanisms need to be scrutinized further. This study is aimed to test the hypothesis that the patients who perform the high-intensity interval exercise training (HIIT) during hormone therapy would show improvements in low-grade inflammation and HSP70 compared to the controls receiving standard care.

Methods Fifty two non-metastatic and hormone-responsive breast cancer patients were randomly assigned to high-intensity interval exercise (HIIT) ($n = 26$) and usual care ($n = 26$) groups. The HIIT groups participated in a high-intensity interval training protocol on a treadmill 3 days/week for 12 weeks. The training intensity was determined according to the predicted maximal heart rate. Demographic characteristics and medical history were collected via an interviewer-administered questionnaire at the baseline visit. Body fat was estimated based on skinfold thickness measured with calipers on the participant's nonsurgery side at the triceps, suprailiac crest. VO_{2max} was estimated by 1-Mile Rockport Walk Test. Blood samples were collected 48 h before starting the exercise protocol and 48 h after the last exercise session. TNF-α, IL-6, IL-1β, IL-10, and HSP70 levels in serum were measured using the enzyme-linked immunosorbent assay (ELISA) method according to the manufacture's instruction. Supernatant cytokine concentrations were determined by ELISA for IL-4 and IFN-γ. The data were analyzed by ANCOVA test that the pretest values were considered as covariate at $P \leq 0.05$.

Results HIIT improved VO_{2max} in the HIIT group compared to the usual care group ($P = 0.002$). The serum levels of TNF-α ($P = 0.001$), IL-6 ($P = 0.007$), and IL-10 ($P = 0.001$) were lower in the HIIT group. The level of IL-4 ($P = 0.050$) in the stimulated peripheral blood mononuclear cells significantly increased in the HIIT group compared to the usual care group. Furthermore, the serum level of the HSP70 was significantly higher in the HIIT group in comparison to the usual care group ($P = 0.050$). The TNF-α/IL-10 ($P = 0.050$) and IL-6/IL-10 ($P = 0.042$) ratios were lower in the HIIT group.

Conclusion The results of this study indicated that HIIT has positive impacts on the cardiorespiratory fitness and inflammatory cytokines in the breast cancer patients undergoing hormone therapy.

Keywords Breast cancer · HIIT · Cytokine · IL-4 · IFN-γ · HSP70

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Introduction

Low-grade systemic inflammation is associated with breast cancer recurrence and may also reduce the survival rate (Friedenreich et al. 2012; McTiernan 2008). In particular, pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α may be secreted as part of the immune response to cancer cells or in response to tissue damage associated with cancer treatment (Herskind et al. 1998). Moreover, circulating inflammatory markers such as TNF-α, IL-6, and IL-1β may contribute to the formation of pre-tumor niche, which has an effective role in tumor metastasis and angiogenesis (Lu et al. 2006). The IL-6 serum levels correlate negatively with prognosis in breast cancer patients (Rao et al. 2006). In addition, a high level of circulatory pro-inflammatory cytokines is associated with cancer-related fatigue in breast cancer survivors (Bower et al. 2002). Interleukin-10 (IL-10) as cytokine synthesis inhibitory factor is mainly produced by Th2-like cells and activated monocytes. IL-10 has anti-inflammatory function through reducing the release of IFN-γ and IL-2 by activated Th1-like cells and inhibiting T cell activation and proliferation (Pestka et al. 2004). IL-10 is known to exhibit both pro- and anti-cancer. An anti-tumor effect of IL-10 is due to enhanced NK cell activity or CD8^{+} or CD4^{+} T cell function (Kundu 1996). In contrast, IL-10 may reduce immune response against cancer (Pinzon-Charry et al. 2005). Interferon-γ (IFN-γ) is a pro-inflammatory mediator the predominantly produced by T cells and NK cells. As we know, IFN-γ can play an important role in regulating an innate phagocytic response against metastatic breast cancer (Pulaski et al. 2002). Furthermore, Kamamura et al. demonstrated that the incubation of peripheral blood mononuclear cells (PBMC) from breast cancer patients with IFN-γ could increase the LAK activity (Kamamura et al. 1998). Breast cancer patients who have ER-positive tumors may be offered hormonal therapy with tamoxifen or aromatase inhibitors that can reduce the recurrence and mortality rates (Group EBCTC 2005). Tamoxifen can modulate the inflammatory markers such as IL-6 and TNF-α (Lamas et al. 2015).

A solid body of evidence links obesity and physical inactivity chronic inflammation in cancer prevention and control (Vucenik and Stains 2012; Lynch et al. 2010). On the other hand, regular physical activity can prevent and control breast cancer. In this regard, Thomas et al. (2017) showed that a combined resistance and aerobic exercise intervention improved body composition in breast cancer survivors taking aromatase inhibitors (Thomas et al. 2017). In this situation, the lifestyle interventions could potentially help to reduce the chronic inflammation in breast cancer survivors by lowering the adiposity and physical fitness (Pierce et al. 2009). As the evidence linking chronic inflammation to breast cancer progression grows, it becomes increasingly important to understand what can be done to ameliorate it. It has been proposed that the effect of exercise on inflammation in patients with breast cancer may be different from that of other people (Fairey et al. 2005). Moreover, the obesity and physical inactivity can drive the chronic inflammation. Physical activities with a longer duration and more intensity can cause a greater reduction in cancer risk (Adraskela et al. 2017). There is a growing body of evidence supporting the benefit of regular exercise in cancer patients (Thomas et al. 2017; Courneya et al. 2003; Schmitt et al. 2016). The low to moderate continued exercise has been known as a complementary therapy for cancer survivors (Dumitrescu and Cotarla 2005; Lee 2003; Azevedo et al. 2013; Rajarajeswaran and Vishnupriya 2009). Typically, these studies have examined the effects of light and moderate intensity exercise on physical and psychological indices in cancer patients. In contrast, the high-intensity exercise training (HIIT) has long been demonstrated to be helpful for improving the cardiorespiratory fitness and corresponding physiological variables in healthy individuals (Gillen and Gibala 2013). The high-intensity interval training (HIIT) employs repeated short to long bouts of relatively high-intensity exercise alternated with recovery periods of either low-intensity exercise or passive rest (Buchheit and Laursen 2013). So, the HIIT might further improve the physical capacity, the cardiorespiratory fitness, the metabolic profile and the low-grade systemic inflammation in varied populations including those with coronary artery disease, diabetes, and healthy obese individuals (Wisløff et al. 2007; Ahmadizad 2015). Therefore, considering the benefits of the HIIT, this useful training method has not been studied in patients with breast cancer so far. Our primary hypothesis was that the patients who perform the HIIT during hormone therapy would show improvements in the low-grade inflammation such as TNF-α, IL-6, IL-1β, and IL-10 as well as HSP70 compared to the individuals receiving the standard care. The second hypothesis was that the HIIT may affect the cytokines production such as IL-4 and IFN-γ in stimulated peripheral blood mononuclear cells.

Materials and methods

Research design

The present study is a single-center randomized controlled trial including two study arms

High-intensity interval aerobic exercise (HIIT) and the usual care (UC) control group. All participations have

Fig. 1 The trial profile in the present study
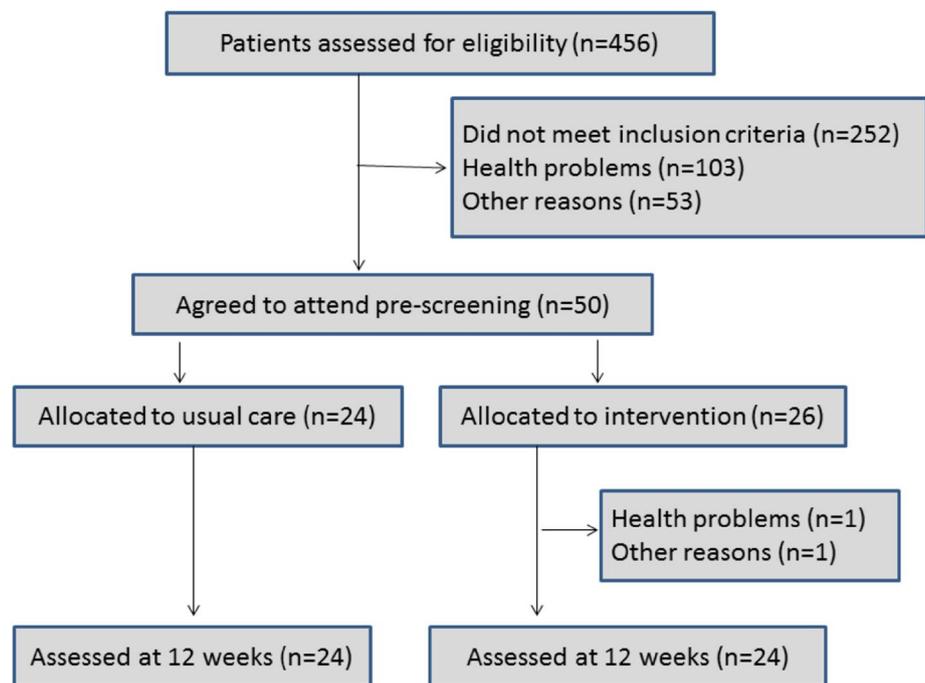
received the usual care, letrozole and tamoxifen as a hormone therapy regimen. Women assigned to exercise plan received the usual care and also attended a supervised interval exercise training program three times per week for 12 weeks. All study activities as described below were reviewed and approved by Medical Ethics Committee of Tehran University of Medical Sciences (NO: 58276). The trial was registered with the Iranian Registry of Clinical Trials as IRCT201202289171N1. Participants were recruited by their physicians from Cancer Institute of Iran and the person recruitment at one hospital-based oncology clinic. The eligible women consisted of non-metastatic (stages I, II and III), hormone-responsive breast cancer patients who had successfully completed either chemotherapy and/or radiotherapy sessions at least 1 month before recruitment for this study, and agreed to be randomized and also available for the duration of study intervention period. Participants were recruited from Cancer Institute of Iran (Hospital Imam Khomeini, Tehran, Iran), between January 2014 and January 2015. After the corresponding oncologist provided consent, the subjects were deemed eligible for the study if they met each of the following conditions: age ≥ 30 years, insufficient physical activity level (less than 150 min/week), hormone-responsive breast cancer; performing no strenuous exercise such as running, cycling, swimming or resistance training; completed chemotherapy and radiotherapy in the last month, taking aromatase inhibitors or tamoxifen (undergoing hormone therapy) and willing to be randomized. Non-inclusion criteria were as follows: current smoking; evidence of metastatic breast cancer; planning to receive any additional adjuvant chemotherapy or surgery; pregnant or breastfeeding; unable to baseline blood sample; cardiac conditions such as myocardial infarction or coroner artery diastase; liver conditions; lymphedema; uncontrolled hypertension, defined as systolic blood pressure ≥ 180 mm Hg or diastolic blood pressure ≥ 100 mm Hg, high-risk or uncontrolled heart arrhythmias, clinically significant heart valve disease, decompensated heart failure, or known aortic aneurysm; and any other condition which, in the opinion of the investigators, may impede testing of the study hypothesis or make it unsafe to engage in the exercise program (Fig. 1). The eligibility of patients to participate in high-intensity exercise program had been confirmed by a sport medicine specialist. Written informed consent was obtained from all patients prior to participation.

Randomization and blinding

The physical activity level of all participants was measured by an international physical activity questioner or questionnaire. According to the results of questioner, all participants had insufficient physical activity (less than 600 MET/min/week). The allocation sequence and group assignment were generated by a research assistant and then enclosed in sequentially numbered and sealed envelopes. The contents of the envelopes were concealed from the project director who assigned participants to groups. Investigators and participants had no knowledge of group assignment until the completion of baseline assessments.

Table 1 General characteristics of the study participants

Variables	Levels	Usual care group[a] (N=26)	HIT group[b] (N=24)
Age	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	5	4
	Moderate	17	16
	Poor	3	3
Chemotherapy		16	17
Radiotherapy		13	11
Tumor staging	T1	9	6
	T2	10	9
	T3	6	8
	T4	10	8
	N0	13	14
	N1	2	1

[a]Hormone therapy

[b]Hormone therapy plus exercise

Then, the participations were randomly assigned to the HIIT or usual care groups. Laboratory staff and those who assessed the trial endpoints were blinded to the treatment assignment.

Measurements

After obtaining written approval of the physician, the participants completed a clinic visit. The physician approval form included a brief description of the study and a request that the physician reviewed this information and provided approval that they believe their patient is medically fit to participate in a high-intensity aerobic exercise study. All data were collected after obtaining patient consent to participate in the study. All below described measures were completed at the baseline and the end of 12 weeks. All measures were conducted by the staff blinded to the study group.

Anthropometric and medical history measurements

Demographic characteristics and medical history were collected via an interviewer-administered questionnaire at the baseline visit (Table 1). The body composition was indirectly assessed through changes in the body weight, body mass index (BMI), and the subcutaneous sum of skinfolds. Body weight was assessed using an electronic scale (SECA, Japan). Standing height was also determined by the standard method. BMI was calculated as weight in kilograms divided by height in meters squared. The waist-to-hip ratio (WHR) was determined by measuring the waist circumference at the narrowest region between the costal margin and iliac crest and then dividing this measurement by the hip circumference measured at its greatest gluteal protuberance. Body fat was estimated from skinfold thickness measured with calipers on the participants' nonsurgery side at the triceps, suprailiac crest, and quadriceps using Jackson–Pollack equations to determine lean body mass and body fat percent. All measurements were taken twice in succession by the same technician and averaged for data entry.

Rockport 1-mile walk test

Participants were asked to complete a validated test of fitness. V_{O2max} was estimated by 1-Mile Rockport Walk Test (RWT) as a valid predictive submaximal test. Kline et al. (1987) have reported a 0.88 coefficient correlation value between VO_{2max} estimated based on performances during the RWT and a direct measure of VO_{2max} during an increment test. In this test, an individual walked 1 mile (1.6 km) as fast as possible on a track surface. Total time was recorded and the heart rate was measured for 60 s immediately after the RWT (Kline et al. 1987). To measure the VO_{2max}, the formula used to calculate VO_{2max} was as follows:

$$VO_{2max} = 132.853 - (0.0769 \times \text{body weight}) - 0.3877 \times \text{age} + (6.315 \times \text{sex score}) - (3.2649 \times \text{time}) - (0.1565 \times \text{heart rate at the end of the walk}).$$

Exercise intervention

The assigned patients to the exercise plan received usual cares (routine daily activity) and also attended a supervised high-intensity aerobic interval exercise program three times per week for 12 weeks. The high-intensity aerobic interval exercise (HIIT) group participated in three familiarization session prior to starting the main exercise training program. The participants assigned to the control group were not prescribed personalized exercise program and were not asked to initiate any structured exercise over the course of the intervention.

We used the high-intensity interval training protocol that has previously been reported as a safe exercise training regimen for heart failure and coronary artery diseases (Rognmo et al. 2004). The training intensity was determined according to the predicted maximal heart rate, despite the long-standing limit of this formula compared to the actual measurement of maximal heart rate by a maximum stress test. The exercise training session was individually conducted for each patient under supervision of an exercise physiologist. The main exercise intervals consist of 4×4 min of uphill walking at 90–95% HR_{max} (exercise) and 4×3 min of uphill

walking at 50–70% HR_{max} (active recovery) on motorized treadmill (impulse, USA) (Rognmo et al. 2004). Overall time of each session duration was 38 min, consisting of 5 min of warm-up, 5 min of cool down, 16 min of high-intensity interval and 12 min of active recovery between intervals. To fully manage the process of continuous aerobic exercise and intervals, the targeted heart rates of the participants were fully monitored during every training session (Wisløff et al. 2007). All subjects used a heart rate monitor (Polar Electro, Kempele, Finland) to obtain the assigned exercise intensity. The speed and inclination of the treadmill were adjusted continuously to ensure that every training session was carried out at the assigned heart rate throughout the training period. During the training sessions, the participants were advised to respect their own physical limitations. The patients' adherence to exercise sessions was managed by daily contacts through the moderators. Women in the usual care group were instructed to continue with their usual activities. The adherence to each session was 85%, yet tow patients in the HIT group were reluctant to continue their exercise training for personal and motivational reasons; they were excluded from the study. Therefore, 24 participations in the exercise group were included in the final analyses. The usual care participants were asked to maintain their baseline physical activity levels during 12 weeks of intervention. The exercise trainers monitored adherence to the intervention.

Blood sampling and analysis

Participants were not instructed to exercise for at least 48 h before blood collection to eliminate immune modulation from an acute bout of exercise. Blood was collected between 07.00 and 10.00 am after a 12-h water only fasting period from the antecubital vein, and then coagulated at room temperature for 0.5–2 h. Following centrifugation at $2000 \times g$ for 5 min, the serum was collected and centrifuged again at 12,000 (RPM) for 15 min for removing cell debris. The post-intervention blood collection was done 3 days after the last training session to avoid the acute effects of the exercise. It was then aliquoted and stored at -80 °C until further experiments.

Isolation of peripheral blood mononuclear cell and PHA mitogen stimulation

Peripheral blood mononuclear cells (PBMCs) were isolated from ethylenediaminetetraacetic acid (EDTA) (Sigma, USA) anticoagulated whole blood of women bearing breast cancer. The low-density PBMCs were subsequently separated by ficoll-hypaque ($\rho = 1.077$ g/mL) density gradient medium (Biosera, UK). Next, carefully 5 mL of the whole blood too slowly poured over 3 mL of ficoll-hypaque in a 15 mL conical tube (SPL, Korea). The conical tube was centrifuged at $400g$ for 25 min at 4 °C in a swinging bucket rotor without brake and accelerator (Eppendorf, Germany). The interphase layer, containing PBMCs, was aspirated using a sterile pipette pasteur (Brand, Germany) and transferred to a new 50 mL conical tube (SPL, Korea). The conical tube with cold PBS was filled and centrifuged at $350g$ for 10 min at 4 °C. Supernatant was removed and the cell pellet was resuspended in complete RPMI medium supplemented by 10% fetal bovine serum (FBS) with 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (All provided of Gibco, USA), and was centrifuged at $350g$ for 10 min at 4 °C. Supernatant was disposed of and the cell pellets were resuspended in complete RPMI medium and adjusted 10^6 cells per mL. The cell suspension in a triplicate manner was seeded 2×10^6 cell per well on 24-well cell culture plates (Greiner, Germany). The PBMCs were stimulated with 1% v/v phytohemagglutinin (PHA)-M (Invitrogen, USA) as a T cell mitogen, and 48 h were incubated at 37 °C in a humidified 5% CO_2 incubator (Memmert, Germany). After 48 h, each well was separately harvested and centrifuged at 2000 rpm for 5 min at 4 °C. Then, the supernatant was aspirated and the cell pellet was disposed of. Finally, the supernatant was collected and stored at -70 °C for the subsequent cytokine analysis.

Serum inflammatory markers

TNF-α (Human TNF-α, Catalog Numbers: DY210-05/DY210/DY217B), IL-6 (Human IL-6, Catalog Numbers: DY206-05/DY206), IL-1B (Human IL-1β, Catalog Numbers: DY201-05/DY201), IL-10 (Human IL-10, Catalog Numbers: DY217B-05) and HSP70 (Human HSP70/HSPA1A, Catalog Number: DY1663-05) levels were measured using the enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instruction.

Cytokine assays in PBMC supernatant

Culture supernatants were collected after 48 h to measure the IL-4 and IFN- γ contents. Supernatant cytokine concentrations were determined by ELISA for IL-4 (Human IL-4, Catalog Numbers: DY204-05/DY204), and IFN-γ (Human IFN-γ, Catalog Numbers: DY285-05/DY285).

Statistical analysis

Sample size calculation was based on the effects of exercise on the cardiorespiratory fitness in breast cancer survivors in the literature (Courneya et al. 2003). Twenty-five

Table 2 The baseline levels of body composition, blood pressure, resting heart rate and VO_{2max} in samples

Variable	Baseline	P value*
Body weight (kg)		
Exercise group	70.2 ± 9.1	0.432
Control group	71.4 ± 11.24	
BMI (kg m^2)		
Exercise group	27.85 ± 4.01	0.933
Control group	27.98 ± 3.90	
WHR		
Exercise group	0.86 ± 0.07	0.860
Control group	0.85 ± 0.06	
Body fat (%)		
Exercise group	35.63 ± 2.33	0.606
Control group	35.31 ± 6.53	
Waist circumference (cm)		
Exercise group	89.26 ± 6.61	0.980
Control group	89.5 ± 9.23	
Resting heart rate (b m)		
Exercise group	74.5 ± 9.6	0.712
Control group	75.8 ± 10.4	
Systolic blood pressure (mmHg)		
Exercise group	120.5 ± 18.5	0.560
Control group	113.9 ± 34.7	
Diastolic blood pressure (mmHg)		
Exercise group	77.1 ± 11.7	0.485
Control group	80.1 ± 10.4	
VO_{2max} (ml kg min)		
Exercise group	28.9 ± 10.1	0.574
Control group	33.38 ± 25.3	

Data are presented as the mean $\pm$ standard deviation

*P value for difference between the groups at baseline

participants are needed for each group with a power of 0.80, two-tailed $\alpha < 0.05$ and large effect size $(d) = 0.80$. The baseline measures were compared by independent t tests ($P \leq 0.05$). For detecting effects of the HIIT on the measured variables, the data were analyzed using analysis of covariance (ANCOVA) after checking and confirmation of all the assumptions of this test ($P \leq 0.05$). The baseline values were considered as a covariate variable.

Results

The participants consisted of the 52 non-metastatic and hormone receptor-positive breast cancer patients between 31 and 69 years old. Nevertheless, two individuals were dropped from the study because of personal reasons.

Therefore, the data of 50 patients were analyzed. Table 1 also depicts the general characteristics of the individuals with breast cancer in both usual care and HIIT groups.

The results of t test did not show differences between baseline of measures in the HIIT and usual care groups (Table 2). The HIIT and usual care groups had low levels of physical activity at baseline. The pre-test data obtained from the international physical activity questioner, and the physical activity amount of participants were less than 600 MET/min/week: the breast cancer patients with hormone therapy (325 ± 113) and the breast cancer patients with HT together with the HIIT (345 ± 116) groups. In post-test, the physical activity amount of usual care group groups did not change in comparison with pretest (330 ± 130, $P = 0.086$). We observed the significant increase in physical activity level in the HIIT group in comparison with pretest (650 ± 48, $P = 0.023$). In addition, the results of ANCOVA showed that the physical activity level of HIIT group increased significantly as compared with usual care group (P = 0.025).

The t test analysis between baseline and post-intervention differences showed that the HIIT significantly improved body composition measures including body weight ($P = 0.002$) and BMI ($P = 0.016$) (Table 2). The percent of body fat and WHR, resting heart rate and resting blood pressure did not show any significant changes (Table 2). The HIIT participants showed a 21.65% increase in VO_{2max} ($P = 0.002$) compared to the usual care group (Table 2).

Analyses of covariance with the baseline value as the covariate indicated the significant differences between fitness of the groups at the post-test on the serum levels of TNF-α ($P = 0.001$), IL-6 ($P = 0.007$), IL-10 ($P = 0.001$) (Fig. 2a–c), and HSP70 ($P = 0.050$) (Fig. 3) and non-significant differences of IL-1β (Fig. 2d) ($P = 0.093$). The IL-4 level was significantly attenuated in the stimulated PBMCs by PHA in the HIIT group ($P = 0.050$) (Fig. 4b). However, the production of IFN-γ did not show any significant changes in the PHA-positive culture by the HIIT ($P = 0.660$) (Fig. 4a). Moreover, we observed a significant decrease in the TNF-α/IL-10 ratio ($P = 0.050$) (Fig. 5a) and IL-6/IL-10 in the HIIT group ($P = 0.042$) (Fig. 5b).

Discussion

The main finding of the present study was that high-intensity interval exercise (HIIT) can improve the cardiorespiratory fitness and the body weight of breast cancer patients undergoing hormone therapy. In addition to physical parameters, the serum levels of the inflammatory cytokines such as IL-6, TNF-α, TNF-α/IL-10 and IL-6/IL-10 ratios were decreased in the HIIT group compared to the usual care group. Furthermore, the serum level of HSP70 increased in the HIIT group compared with the usual care group. It is widely known that
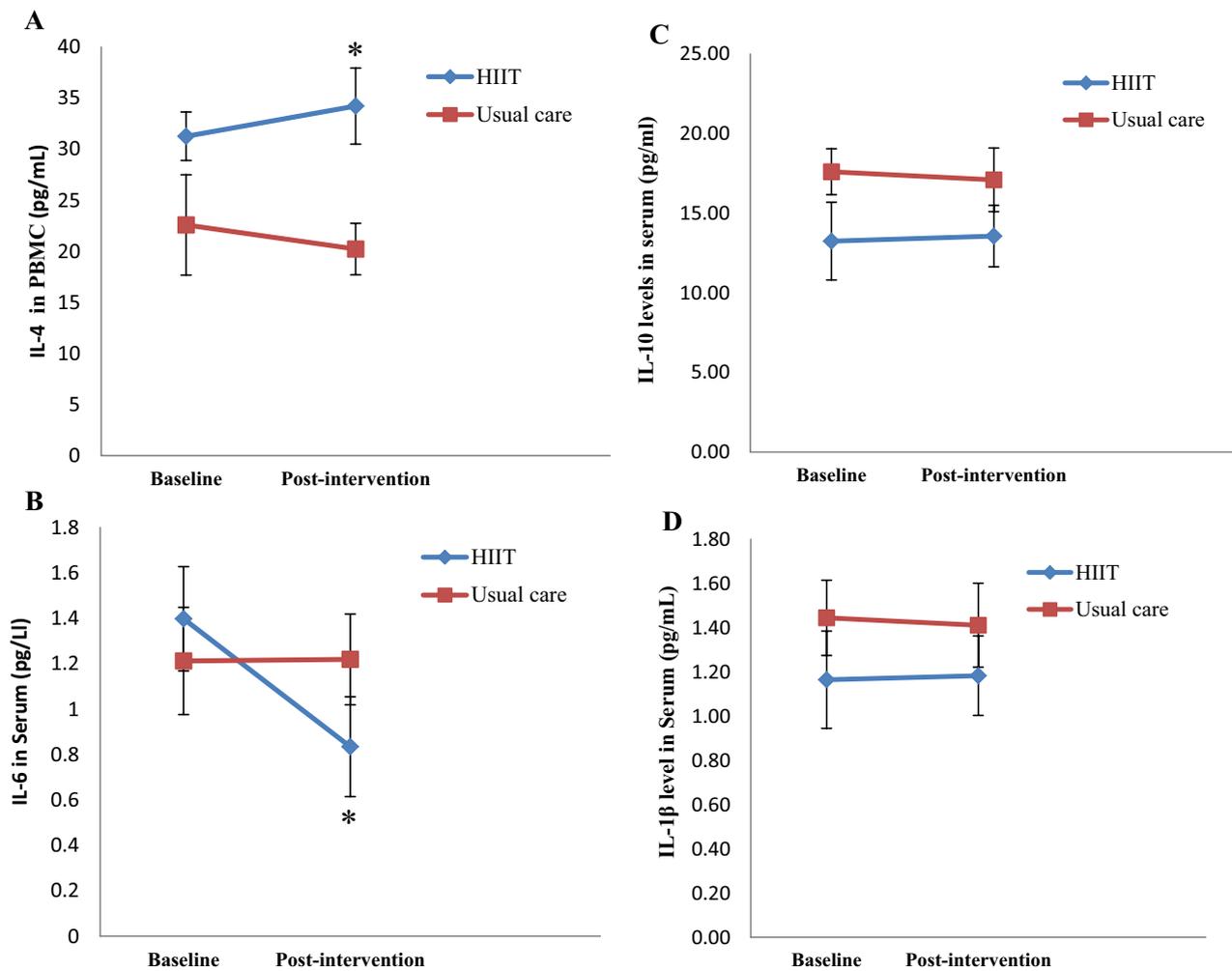
Fig. 2 The effect of 12 weeks of the HIIT on the serum level of the TNF-α (**a**), IL-6 (**b**), IL-10 (**c**) and IL-1β in the breast cancer patients undergoing hormone therapy. *$P \leq 0.05$ compared to usual care
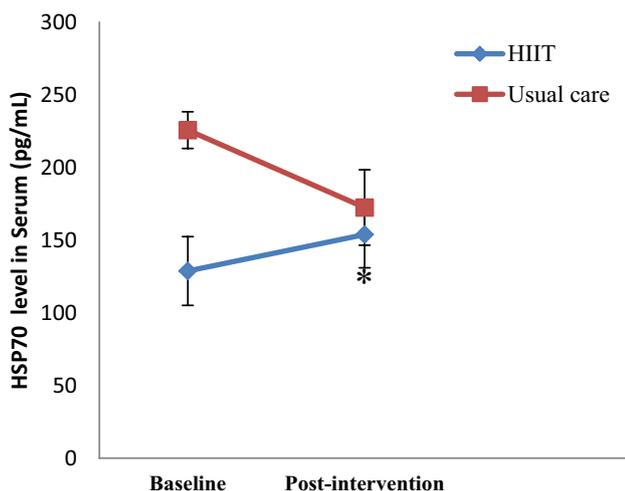

Fig. 3 The effect of 12 weeks of the HIIT on the serum level of the HSP70 in the breast cancer patients undergoing hormone therapy. *$P \leq 0.05$ compared to usual care

cancer treatments such as chemotherapy or radiotherapy may cause unfavorable changes in aerobic and functional capacity (Perez et al. 2004) and body composition (Freedman et al. 2004) of breast cancer patients. In recent years, physical activity and exercise intervention programs have been suggested as an effective way to compensate for these complications. These studies predominantly apply the low to moderate continuous aerobic exercise protocols (Azevedo et al. 2013). In this trial, we found that high-intensity exercise protocol is also safe and feasible for breast cancer patients. In addition, this type of exercise can improve body composition and aerobic capacity of participants undergoing hormone therapy. There is little knowledge about the effects of HIIT in cancer survivors. However, some studies have proposed that vigorous exercise may reduce the risk of cancer and also improve the survival of cancer patients (Hayes et al. 2009). Dolan et al. (2016) have observed that aerobic interval training similar to continued moderate training can safely
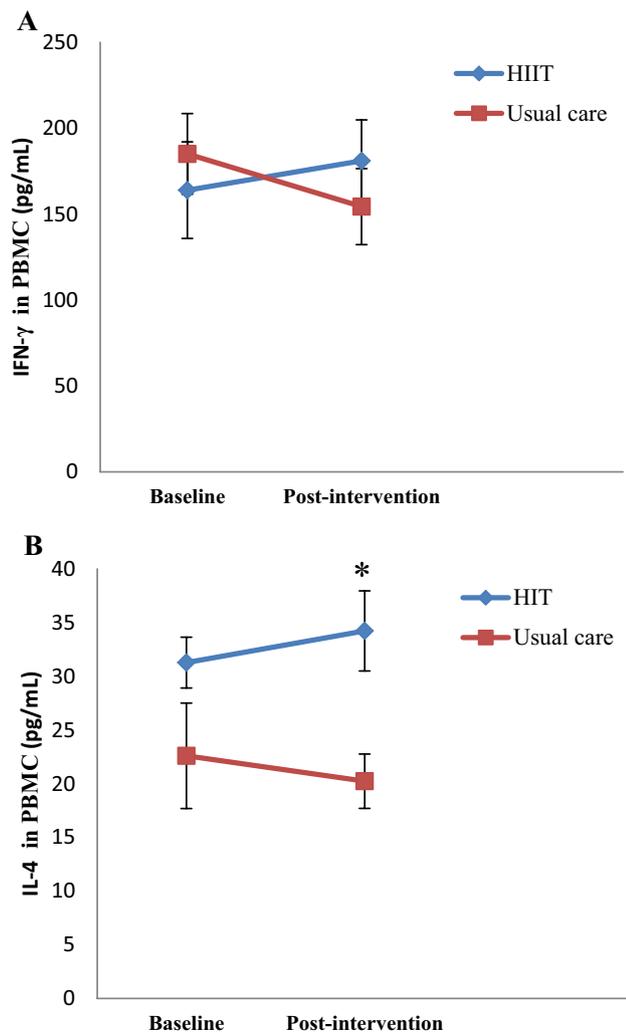
Fig. 4 The effect of 12 weeks of the HIIT on the production of the IFN-γ (**a**) and IL-4 (**b**) by the PHA-stimulated PBMC in the breast cancer patients undergoing hormone therapy. *$P \leq 0.05$

Fig. 5 The effect of 12 weeks of the HIIT on the TNF-α/IL-10 (**a**) and IL-6/IL-10 ratios(**b**) in the breast cancer patients undergoing hormone therapy. *$P \leq 0.05$ compared to usual care

improve the aerobic fitness of breast cancer survivors. The results of the present study confirm that the HIIT protocol is beneficial to breast cancer patients through the physiological improvements such as aerobic capacity and body composition. According to the results, body weight and BMI significantly reduced in the HIIT group in this study. Moreover, body fat decreased by about 1.5% in the HIIT group. This finding is consistent with the previous studies (Ahmadizad 2015; Fairey et al. 2005) that the HIIT can reduce body weight, BMI and subcutaneous fat in obese women. In contrast, Pinto et al. (2005) have observed no significant changes in body composition after 12 weeks of home-based light to the moderate intensity walking in the breast cancer survivors. Therefore, the intensity and the mode of training might play the pivotal roles in training adaptation in cancer patients on hormone therapy.
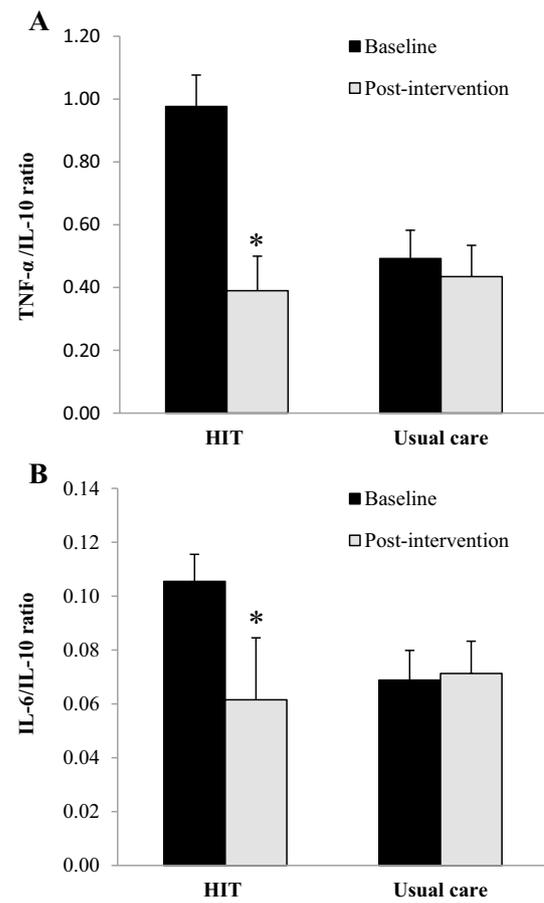
Improving inflammatory status by regular physical activity is known as one of the pathways involved in reducing the risk of breast cancer (Friedenreich et al. 2010). It seems that exercising can also improve systemic levels of inflammatory markers in women with breast cancer patients undergoing hormone therapy. Overall, our results indicate the improved systemic inflammation of patients due to HIIT program. In this regard, we found lower levels of IL-6 and TNF-α as the main marker of low-grade systemic inflammation by HIIT. IL-6 is an inflammatory cytokine with multiple effects on the immune system and hemostasis control (Kurebayashi 2000). The high level of IL-6 has been found to be associated with the response to chemo-endocrine therapy (Pierce et al. 2009) and aromatase activity in breast tissues (Purohit et al. 1995). Thus, lower circulatory levels of IL-6 may help endocrine therapy in breast cancer patients. In line with our results, Jones et al. (2013) demonstrated that IL-6 is related to the dose of exercise in breast cancer survivors. They also indicated that body fat is related to CRP among trained breast cancer survivors. Meneses-Echavez et al. (2016) also found

that exercise has positive effects on the control of low-grade inflammation in breast cancer survivors. The improvement of $\dot{V}O_{2max}$ may be one of the reasons for the lower levels of IL-6 and TNF-α in the HIIT group. In this way, Kullo et al. (2007) observed the inverse relation between inversely related to $\dot{V}O_{2max}$ and IL-6 in asymptomatic men (Kullo et al. 2007). Interestingly, we fund lower levels of IL-6 and TNF-α independent of reduction in adiposity that is in accordance with Kullo et al. (2007). This reduction may be explained by improvement of antioxidant capacity (Leeuwenburgh and Heinecke 2001) and reduction in oxidation of low-density lipoprotein (LDL) cholesterol by exercise training (Shern-Brewer et al. 1998). Increasing exercise capacity by high-intensity exercise is likely to have significant health benefits, even without reduction in body fat in breast cancer patients. Therefore, cancer patients should be encouraged to achieve and maintain a high level of physical fitness.

Moreover, IL-10 as anti-inflammatory cytokine has anti-tumor effects in different ways such as angiogenesis (Bando and Toi 2000). The IL10 is also related to better prognosis and surveillance in breast cancer survivors (Li 2014). We observed lower serum level of IL-10 in the HIIT patients. In accordance with the present finding, Gómez et al. (2011) have demonstrated a non-significant reduction of the IL-10 serum level in breast cancer survivors following a period of concurrent exercise training. The released IL-6 from the skeletal muscle during exercise can cause a subsequent rise on the plasma level of IL-10 (Petersen and Pedersen 2006). The improvement in the serum IL10 concentrations is one of the positive impacts of the physical exercise on the lowering of inflammation in breast cancer survivors (Meneses-Echavez et al. 2016). We present the ration of IL-6/10 and TNF-α/IL-10 ratio to show deep understanding of the effects of exercise on inflammatory markers. We observed a reduction in the IL-6/IL-10 ration and also TNF-α/IL-10 ratio in the HIIT group. The lower IL-6/IL-10 ratio and also TNF-α/IL-10 ratio might indicate lower inflammation milieu. Rogers et al. (2013) have shown the negative effect of size for IL-6/IL-10 and TNF-α/IL-10 ratios in active women with breast cancer (Rogers et al. 2013). These observations suggest that HIIT could suppress inflammatory cytokines such as IL-6 and TNF-α. Moreover, it may reduce inflammation through a regulation of IL-6/IL-10 ratio. We also showed a decrease in the level of TNF-α after the HIIT. In this respect, Huffman et al. (2008) concluded that the exercise training may reduce the expressions of the TNF-α and IL1-β by reducing adipose tissues. However, we detected no significant decrease in the IL-1β serum level. In this respect, the IL-10/TNF-α ratio significantly increased in the HIIT group. It has recently been exhibited that the IL-10/TNF-α ratio can be demonstrated as a marker of the inflammation intensity, which is associated with the survival. In this regard, Lira et al. (2009) observed an increased IL-10/TNF-α ratio in the

adipose tissue following the exercise, so it may be seen as an anti-inflammatory mechanism of regular exercise (Lira et al. 2009). In contrast, Gómez et al. (2011) did not find any significant changes in TNF-α, IL-10, or IL-6 in breast cancer survivors by concurrent training. However, they observed a higher IL-10/TNF-α ratio in trained patients.

Our third main finding was the effects of HIIT on the IL-4 and IFN-γ production by the PBMCs which are known to be the cytokine effectors in the humoral and cell-mediated immune responses. IFN-γ has the prominent effects on activating macrophages and may affect the immune surveillance against tumor cells (Viallard et al. 1999). It has been previously suggested that the IFN-γ and IL-4 produced by PBMCs were lower and slightly higher, respectively, in breast cancer patients than their healthy pairs (Sato et al. 1998). On the contrary with IL-4 levels, the present results did not show a significant increase of the IFN-γ in the HIIT group. In contrast to our results, Conroy et al. (2016) indicated that one-year exercise training did not change the circulatory level of the IL-4 in post-menopause breast cancer survivors (Conroy et al. 2016). Fairey et al. (2005) also did not observe any effects of the exercise training on the IFN-γ and IL-4 produced by PBMCs in breast cancer survivors. However, they have found an increased cytotoxic activity of NK cells in trained subjects. Furthermore, it has been shown that IFN-γ production by PBMCs was decreased in the ischemic heart disease after the exercise training, while the production of the IL-4 was increased by 36% (Smith et al. 1999). This may be explained by the difference in the exercise mode as they have used moderate aerobic exercise protocols, but our intervention was high-intensity interval training.

We also observed a significant increase of HSP70 level in the HIIT group. Heat shock proteins have an essential function as molecular chaperones in cells with multiple roles in cell signaling (Walsh et al. 2001). Extracellular HSP70 has been accepted as immune-regulatory molecule (Ortega 2010), and as local "danger signals" can trigger the immune response (Calderwood et al. 2007). In this context, Walsh et al. have demonstrated that exercise training can induce the release of HSP70 into the peripheral circulation (Walsh et al. 2001). The impact of exercise on the HSP70 is associated with exercise intensity rather than its duration (Liu et al. 2004). Considering the increased level of the IFN-γ in the PBMCs, higher levels of the circulatory HSP70 can play an important role in the breast cancer patients on the hormone therapy.

Limitations

Limitations of the present study include not assessing nutritional status and the absence of an active control group to compare with the HIIT intervention. As it is widely acknowledged, nutritional status can affect the level of inflammatory

factors in all individuals, especially cancer patients. Therefore, it seems that simultaneous examination of exercise intervention and nutritional status of breast cancer patients can provide us with precise information about the relationship between exercise and inflammation. Also, comparing an active control group with the HIIT intervention group can provide a better understanding of the effect of the type of exercise. In the present study, due to the limited number of eligible patients, we could not have this group. Therefore, we suggest that effects of HIIT intervention to compare with an active control group in breast cancer patients undergoing hormone therapy be investigated in more detail.

Conclusion

In summary, the results of the present trial indicated the positive effects of HIIT on inflammatory cytokines in breast cancer patients undergoing hormone therapy. Consequently, we observed the HIIT protocol as safe and feasible exercise methods for cancer patients. These data can help us develop new adjuvant therapies including high-intensity exercise training, which can reduce the effects of inflammation in breast cancer patients.

Author contributions AMA study conception and design, and manuscript preparation. AI: study conception and design, and manuscript preparation. SS: clinical annotation. MM: data analysis and sample processing. BK clinical annotation. ZMH study conception.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest.

Ethical approval All the processes of this study were ethically approved and supervised by the Institutional Review Board of Tehran University of Medical Sciences. The study was performed in accordance with the Declaration of Helsinki.

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