Research Article

Effects of Eight-Week Combined Resistance and Endurance Training on Salivary Interleukin-12, Tumor Necrosis Factor, Cortisol, and Testosterone Levels in Patients with Breast Cancer

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Abstract

Background: In recent years, several studies have shown the association between exercise and decreased risk of mortality in patients with breast cancer. However, the effects of combined resistance and endurance training on salivary Interleukin-12 (IL-12), tumor necrosis factor (TNF-α), Cortisol, and Testosterone levels in patients with breast cancer have not been investigated.

Objectives: This study aimed at determining the effect of 8 weeks of combined resistance and endurance training on salivary IL-12, TNF-α, Cortisol, and Testosterone levels in women with breast cancer.

Methods: Forty-two postmenopausal women with breast cancer were randomly selected and divided into training (intervention) and control groups. The training group performed resistance training with 2 to 3 sets, 10 to 18 repetitions, 50 to 70% 1 repetition maximum (1RM), and aerobic exercise with 50 to 70% maximum heart rate (maxHR) (12-14 degrees borg scale) for 20 to 40 minutes for 8 weeks. The salivary IL-12, TNF-α, cortisol, and testosterone levels were measured, using the enzyme-linked immunosorbent assay (ELISA) method. Two-way analysis of variance repeated measure was also used to analyze variance with the confidence interval of 95%.

Results: In the training group, there was a significant decrease in salivary TNF-α levels, cortisol, TNF-α/IL-12 ratio, and variables of weight, fat percentage, body mass index (BMI), and waist circumference ($P < 0.05$). Also, the results showed a significant increase in salivary testosterone and testosterone/cortisol ratio in the intervention group ($P < 0.05$). However, no significant changes were observed in the interaction between-group and time in IL-12 and waist–hip ratio (WHR) values ($P > 0.05$).

Conclusions: The results indicate that resistance and endurance training could be used as a useful method to improve salivary pro-inflammatory factors and hormonal levels in patients with breast cancer. Medical oncologists can underline a resistance and endurance training program for patients with breast cancer under their care.

Keywords: Breast Cancer, Cytokines, Exercise

1. Background

Breast cancer is the most common cancer and one of the leading causes of death in women (1, 2). Advancements in early detection and therapeutic options have led to a steady improvement in the survival of breast patients (3).

Exercise therapy/rehabilitation as an adjunctive strategy has been studied in various cancer populations to improve symptom control, inhibit tumor progression, and reduce the side effects of cancer treatments (4, 5). Reported data indicated that physical stress, such as endurance/resistance training, can influence various aspects of mitochondrial biology (6). Following physical stress, cortisol rises (7) and enhances the respiratory chain efficiency of mitochondria, which improves the prognosis of patients with cancer (8). Therefore, endurance exercise can lead to a decrease in tumor growth (9). Similarly, inflammation as a crucial player in cancer development significantly decreased after resistance training, and immune responses increased (10). In general, the combination of resistance and aerobic exercises is more effective than aerobic exercises in improving body composition,

cardiovascular fitness, physical fitness (3, 11). Also, the combination of resistance and aerobic exercises is associated with decreased risk of breast cancer via different biological processes, including regulation of the physiological and metabolic process, changing sex hormones, maintaining a healthy weight, reducing inflammation, and improving the immune response (12). Therefore, catabolic/anabolic and pro/anti-inflammatory biomarkers panel can be used to determine the improvement status and anti-tumor effects of exercise therapy. Cortisol/testosterone biomarkers ratio, as a valuable indicator for the anabolic-catabolic balance, can be used to assess the metabolic changes and the organism response caused by acute and chronic physical stressors such as exercise training (13).

Studies showed a negative correlation between breast hyperplasia and testosterone-free levels before and after menopause (14), while cortisol increases tumor size and accelerates metastasis (15). Cortisol, as the most well-known glucocorticoid, is released from the adrenal cortex into human serum or saliva in response to exercise and plays a critical role in metabolism and responses to physiological stress. Also, testosterone is considered a primary anabolic hormone in biological and physiological processes in the human (16).

As mentioned above, endurance/resistance training after cancer therapy improves the immune response that could be mediated by a protective effect against increased inflammatory activity (17). Therefore, the evaluation of pro/anti-inflammatory biomarkers can help to understand the efficacy of exercise training as effective adjuvant therapy and combination with anti-cancer treatment (18). Interleukin-12 (IL-12), a known potent pro-inflammatory type 1 cytokine, is considered potential immunotherapy for cancer (19). IL-12 inhibits angiogenesis, acts as the host of natural killer (NK) and T cells and cellular immunity, and induces the expression of different cytokines (20). Also, exercise training can decrease systemic inflammation through the reduced production of macrophage or adipocyte pro-inflammatory cytokines (17). Tumor necrosis factor (TNF-α) is considered the main arm of the immune system and the main marker of inflammation. TNF-α is one of the essential cytokines that can cause tumor growth and metastasis (21).

These biomarkers can be evaluated in biological samples such as blood, serum, and saliva. A blood sample is commonly used as the best biological fluid to measure biomarkers. However, blood collection involves risks to subjects such as transient discomfort, bruising, and infection (22). Checking biomarkers in saliva has acquired great interest because salivary sample collection is an easily accessible, non-invasive, and low-cost that have economic and logistic advantages over venipuncture (22, 23). Therefore, many studies have focused on assessing the inflammatory and hormonal biomarkers during and after exercise training (4, 10, 14, 24). However, few studies on the effect of endurance/resistance training on saliva cytokines and hormone levels affect inflammation in patients with cancer.

2. Objectives

In this research, we studied endurance/resistance training on the levels of salivary IL12, TNF-α, cortisol, and testosterone in women with breast cancer.

3. Methods

We followed an experimental pretest-posttest research design. In this study, 42 postmenopausal women with breast cancer were randomly divided into control (age = 46.21 ± 8.71, BMI = 28.26 ± 0.89) and intervention (exercise training) (age = 46.08 ± 8.93, BMI = 28.42 ± 0.55) groups. Training and control groups were matched for body mass index (BMI), age, and menopause status. All breast patients were from Kermanshah Province of Iran with Kurdish ethnic backgrounds and had been admitted to the Kermanshah University of Medical Sciences Hospital. The diagnosis of breast cancer was according to standard clinical, radiological, and histological parameters (25, 26). An oncology specialist determined entry requirements for subjects. All of the patients with breast cancer were at stages I (n = 18, 43%) and II (n = 24, 57%), and the tumor size was between 1.2cm and 5 cm (median: 1.7). Patients with breast cancer were diagnosed postmenopausal by a gynecologist. The national comprehensive cancer network (NCCN) defines menopause as no menses for 1 year in the absence of prior chemotherapy or tamoxifen use or no menses after surgical removal of all ovarian tissue (27). Patients participating in the study were eligible if they were at least 2 years after adjuvant tamoxifen or letrozole therapy, not engaged in any formal exercise programs, without musculoskeletal disturbances for at least 1 year, and without menstruation in the last 1 year. Also, subjects were excluded if they have a history of oral and dental inflammatory, alcohol drinking, or smoking. Before starting the main training, the purpose of the research and its method, as well as the risks and benefits that were involved, were explained to the subjects. Besides, all participants signed the informed consent form in line with the principles of the Helsinki II declarations. This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Iran (IR.KUMS.REC.1397.708).

3.1. Training Protocol

Before starting the main training, 1 week before the start of the main training, anthropometric measurements such as age, weight, BMI, waist-hip ratio (WHR), fat percentage, and waist circumference were performed (Table 1).

To reduce the learning effects, all subjects participated in a meeting 1 week before starting the main program to learn the main movements of the gym. Also, 48 hours before the start of the exercise program, the subjects performed a one-repetition maximum (1RM) test by using the indirect Brzysky method (Equation 1; indirect estimation formula for one repeated major).

$$1RM = \frac{Weight\ of\ displaced}{[1.0278\ of\ displaced.m\ (1RM)\ n\ prog]}$$

The training included 8-week endurance and resistance training, 3 days per week, including 10 minutes warm-up and cool-down with a 40% maximum heart rate. The main part of the training was divided into resistance and aerobic exercise training. The main movements of resistance training included a barbell chest press, barbell curl, lying barbell triceps, pull-down of posterior cervical muscles, and lower body movements, including smith machine squat, leg extensions, lying leg curls, and standing calf raises. The rest period of each exercise for the subjects was 1 to 3 minutes and was set according to the number of weeks of the training program. Also, aerobic training included walking and running. In aerobic training, every 10 to 15 minutes, the subjects' heart rates were evaluated by counting the subjects' pulse within 15 seconds and calculating 1 minute. In addition to this method, the Borg RPE scale was used to control the intensity of aerobic training. The intensity of the borg rating of perceived exertion (RPE) was between 12 and 14. The intensity of resistance and aerobic exercises and the duration of each are shown in Table 2.

The saliva samples were examined for checking IL-12, TNF-α, testosterone, and cortisol by the German ZELLBIO human-specific saliva kit and enzyme-linked immunosorbent assay (ELISA) method. Also, BMI, WHR, and weight were measured by the body composition ZEUS 9.9. To analyze the salivary levels of IL-12, TNF-α, testosterone, and cortisol, the first salivary was sampled between 8 and 10 A.M. with fasting from 2 hours before with no tooth brushing. The second sampling was performed 2 days after the last session of training under all initial sampling conditions.

3.2. Statistical Method

Descriptive statistics were used to describe the characteristics of the subjects. To compare the differences between the variables, two-way ANOVA repeated measures were used. Bonferroni's post hoc test was used to examine significant differences. The significance level of all tests was set as $P < 0.05$. SPSS version X9 was used for data analysis.

4. Results

The results of the study showed that after 8 weeks of resistance and aerobic training for patients with breast cancer, the level of il-12 in the salivary of the training group increased from 17.96 ± 1.52 pg/mL to 21.17 ± 1.72 pg/mL, but this increase was not significant ($P = 0.105$). However, the TNF-α in the training group significantly decreased from 53.84 ± 2.48 pg/mL to 47.64 ± 2.63 pg/mL, and the effects of time and interaction between the groups were also significant ($P < 0.001$); however, the TNF-α/IL-12 ratio decreased from 2.815 ± 0.74 to 2.323 ± 0.93 ($P = 0.002$). Also, in the training group, salivary testosterone significantly increased from 28.46 ± 0.70 pg/mL to 30.53 ± 0.67 pg/mL ($P = 0.001$). Besides, in the training group, cortisol significantly decreased from 132.12 ± 11.03 pg/mL to 124.49 ± 12.14 pg/mL ($P = 0.001$). The testosterone/ cortisol ratio significantly increased from 0.252 ± 0.11 to 0.361 ± 0.17 ($P = 0.003$) (Table 3). Weight in overweight patients with breast cancer significantly decreased from 70.13 ± 1.71 kg to 68.72 ± 1.70 kg in the training group ($P = 0.001$). BMI also decreased from 28.42 ± 0.55 kg/m^2 to 26.78 ± 0.55 kg/m^2 ($P = 0.001$). The effect of time and group interaction was also significant ($P = 0.001$). There was no significant change in WHR, and its level changed from 0.86 ± 0.01 to 0.87 ± 0.01 ($P = 0.93$). Also, after 8 weeks of training, the fat percentage in the training group significantly decreased from $37.23 \pm 0.89\%$ to $35.99 \pm 0.90\%$ ($P = 0.001$). Besides, the waist circumference decreased from 93.39 ± 1.89 cm to 92.04 ± 1.90 cm, which was significant ($P = 0.033$). These changes are shown in Table 1.

5. Discussion

This study aimed at determining the influences of 8 weeks of resistance and endurance training on salivary levels of IL-12, TNF-α, TNF-α/IL-12, testosterone, cortisol, testosterone/cortisol ratio, and anthropometric characteristics in women with breast cancer. The main purpose of this study, which distinguished it from previous studies, was to measure IL-12 and TNF-α in saliva. Saliva has different components that act as mirror health of the human body and help to provide biomarkers for disease. Also, there were few studies on the effects of resistance and endurance training in these patients. In this study, we

Table 1. Anthropometric Characteristics of Subjects [a]

| Variable | Mean and Standard Deviation | | | | | |
| | Control (Age = 46.21 ± 8.71) | | | Training (Age = 46.08 ± 8.93) | | |
	Pre	Post	P-Value	Pre	Post	P-Value
Weight	70.59 ± 2.21	70.86 ± 2.14	0.099	70.13 ± 1.71	68.72 ± 1.70*↓†	0.001
BMI	28.26 ± 0.89	43.28 ± 0.88	0.084	28.42 ± 0.55	26.78 ± 0.55*↓†	0.001
WHR	0.87 ± 0.01	0.88 ± 0.01	0.07	0.86 ± 0.01	0.87 ± 0.01↑	0.93
Fat percent	37.07 ± 1.31	37.53 ± 1.30	0.007	37.23 ± 0.89	35.99 ± 0.90*↓†	0.001
Waist	94.82 ± 2.25	94.21 ± 2.28	0.86	93.39 ± 1.89	92.04 ± 1.90*↓†	0.033

[a] * Significant difference with pre-test, † Significant difference with the control group by interaction, ↑ Increase, ↓ decrease, - Unchanged.

Table 2. Resistance and Aerobic Exercise Protocols

Weeks	1	2	3	4	5	6	7	8
Resistance intensity	2 (50/18)	2 (55/16)	2 (60/14)	2 (60/14)	3 (65/12)	3 (65/12)	3 (70/10)	3 (70/10)
Resistance time (min)	20	25	30	30	35	35	40	40
Aerobic intensity (% max HR)	50	55	60	60	65	65	70	70
Borg scale	12	12	12	13	13	13	14	14
Aerobic time (min)	20	25	30	30	35	35	40	40

Table 3. Changes in Pre-test and Post-test Variables in the Control and Training Groups [a]

| | Measurement Steps (Mean and Standard Deviation) | | | | | |
| | Control | | | Training | | |
	Pre	Post	P Value	Pre	Post	P Value
IL-12	18.95 ± 1.02	19.50 ± 0.97	0.607	17.96 ± 1.52	21.17 ± 1.72*↑	0.006
TNF-α	50.70 ± 2.72	53.16 ± 2.60	0.341	53.84 ± 2.48	47.64 ± 2.63*↓†	0.001
TNF-α/IL-12	2.642 ± 0.70	2.838 ± 0.84	0.189	2.815 ± 0.74	2.323 ± 0.93*↓†	0.001
Cortisol (C)	128.70 ± 8.62	123.82 ± 10.62	0.996	132.12 ± 11.03	124.49 ± 12.14*↓†	0.001
Testosterone (TS)	28.64 ± 0.68	27.86 ± 0.38	0.133	28.46 ± 0.70	30.53 ± 0.67*↓†	0.001
TS/C	0.262 ± 0.09	0.264 ± 0.11	0.97	0.252 ± 0.11	0.361 ± 0.17*↑†#	0.001

[a] * Significant difference with pre-test, # Significant differences with the control group, † Significant difference with the control group by interaction, ↑ Increase, ↓ decrease, - Unchanged.

tested endurance/resistance training on salivary levels of IL-12, TNF-α, TNF-α/IL-12 ratio, testosterone, cortisol, testosterone/cortisol ratio in patients with breast cancer. The results of this study showed that after 8 weeks of resistance and aerobic training in patients with breast cancer in the training group, salivary IL-12 increased, but this increase was not significant.

The results of the current study are inconsistent with the findings of Lester et al. (2010) that resulted in the association between stressful gross anatomy tests with salivary levels of IL-12 (28). Slavish et al. (2015), in a systematic review study, showed that several inflammatory markers reliably determined from saliva and have increased signif-

icantly in response to stress across multiple studies with different effect sizes ranging (29). The different results of these studies can be attributed to differences in training protocol, and population studied, or various methods used to measure the level of inflammatory and hormones. Therefore, this increase in IL-12 may be due to the low intensity of exercise, which is consistent with the results of Deckx et al. (2016) and Goh et al. (2014). The findings of Deckx et al. revealed that 12-week simultaneous training did not change the serum IL-12 levels in subjects with Multiple sclerosis (MS), the production of inflammatory mediators and TNF-α decreased, and 12-week exercise program reduced the secretion of inflammatory mediators upon Toll-

like receptor stimulation and promoted the immune regulatory function of circulating plasmacytoid dendritic cells (30). The findings of this research were also consistent with the results of Goh et al. (2014). They found that 5 weeks of aerobic training, as well as 2 months of simultaneous training, did not change the serum levels of TNF-α and IL-12 in patients with breast cancer (31). IL-12 inhibits angiogenesis (32), while TNF-α causes tumor growth (21). The findings showed that salivary levels of TNF-α decreased significantly in the training group. The findings of this study were consistent with the study of Hagstrom et al. (2016) that investigated the effect of 16-week resistance training on TNF-α in a woman with breast cancer.

The mechanism of decreased TNF-α expression may be related to lactate. Lactate increases response to acute resistance training, and the cyclic adenosine monophosphate (cAMP) can be adjusted by adjusting lactate. The TNF-α expression is suppressed by cAMP (10). Also, the TNF-α/IL-12 ratio, which consists of 2 different factors, can be considered an effect of training on the tumor. This ratio decreased, and this decrease indicates that these patients have improved inflammatory factors.

Reducing salivary cortisol is another possible mechanism for increasing T-cells in peripheral blood circulation after training since cortisol has a significant effect on lymphopenia (reduced white blood cell count) (33). In this study, cortisol significantly decreased in the training group. Therefore, exercise training decreases cortisol as a stress hormone and improves the treatment of patients with breast cancer. The findings of the present research were consistent with the results of Vadiraja et al. (2009) that investigated the effect of yoga training on patients with breast cancer. Changes in stress response in patterns and their signaling pathway of hypothalamic–pituitary–adrenal (H-P-A) can reduce cortisol and, subsequently, improve the immune function and increase the number of NK cells following the training (4). Deckx et al. (2016) studied the effect of 12-week aerobic and resistance combination training on cortisol levels in MS patients and found that exercise training increases cortisol levels (30). This was inconsistent with the results of our research.

Meanwhile, based on the research of Dimitrakakis et al. (2010), the serum level of testosterone in patients with breast cancer was lower than that in the control group (14). However, our findings showed that salivary levels of testosterone significantly increased in the training group (P = 0.001). In previous studies, acute and short-term training with high intensity increased serum levels of testosterone and moderate physical activity increased the concentration of testosterone in the blood (34). These results are consistent with our findings. However, our findings

showed that salivary levels of testosterone significantly increased in the training group. Testosterone is one of the most important anabolic steroid hormones in men and women that are responsible for different physical characteristics, while cortisol plays a catabolic role. Menopause is also accompanied by a reduction in the proportion of anabolic hormones to the catabolic ratio. In this regard, Majumdar et al. found that 8 weeks of resistance training on the adaptive responses of androgens and serum cortisol in postmenopausal women was associated with a 71% increase in serum testosterone and a 125% testosterone/cortisol ratio. The testosterone response to cortisol, especially the ratio of these two hormones, indicates the anabolic-catabolic state. When this ratio is high, it indicates an anabolic state, but when it is reduced to 30% or higher, it indicates a catabolic state (7). These results were consistent with our findings, which showed that the testosterone/cortisol ratio significantly increased. Friedenreich et al. reported that the alteration in the cortisol: corticosterone ratio in breast women suggests the possibility of an alteration in the adrenal steroid biosynthesis pathway as a result of an exercise intervention (12).

The findings of this study showed that 8-week endurance/resistance training in patients with breast cancer significantly decreased weight from 70.13 $\pm$ 1.71 kg to 68.72 $\pm$ 1.70 kg, decreased BMI from 28.42 $\pm$ 0.55 kg/m^2 to 26.78 $\pm$ 0.55 kg/m^2, decreased fat percentage from 37.23 $\pm$ 0.89% to 35.99 $\pm$ 90% in the training group, and decreased waist circumference from 93.39 $\pm$ 1.89 cm to 92.04 $\pm$ 1.09 cm (P < 0.001). There was no significant change in WHR, whose level changed. This may be due to our limitation in this study that the control of the subject's nutrition was not possible during the training period. Studies show that exercise training improves body composition and fitness (35), while the findings of Matthews et al. (2007) are different. They did not see any significant changes in weight, BMI, and body composition due to exercise training in women with breast cancer (36). This difference seems to be following the type, intensity, duration, number of weeks of training, the gender and age of the subjects, as well as the methods for measuring these indicators. Kim et al. (2015) examined the effect of 12 weeks of walking on body composition and immune system in patients with breast cancer. Results are consistent with our findings regarding weight, fat percent, and BMI (37). Herrore et al. (2005) investigated the effects of short-term resistance and short-term aerobic exercise programs on the body composition of women breast cancer survivors. The results showed that the fat in the training group decreased, and the muscle mass increased, and our findings were consistent with the results of Herrore et al. 's study about the

fat percentage (35).

5.1. Conclusions

In general, it can be said that resistance and endurance training can play an essential role in improving inflammatory factors and modifying hormonal factors. Resistance and endurance training could be used as a useful method to improve salivary pro-inflammatory factors and hormonal levels in patients with breast cancer. Medical oncologists can underline a resistance and endurance training program for patients with breast cancer under their care.

Acknowledgments

Footnotes

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