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Original Article

The effect of eight weeks of moderate-intensity endurance training on serum levels of troponin I and B-type natriuretic peptide in radiotherapy rats

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Abstract

Background and aims: One of the most important potential problems of radiotherapy is the heart problem caused by this treatment. Therefore, this research aimed to investigate the effect of 8 weeks of moderate-intensity endurance training on the serum levels of troponin I (TNI) and brain (B-type) natriuretic peptide (BNP) in rats undergoing radiation therapy.

Methods: In this experimental study, 32 male rats (4-6 months) were randomly divided into four groups of eight, including healthy control (C), aerobic training (AT), radiotherapy (RT), and AT+RT groups. First, rats were anesthetized with ketamine-xylazine solution (K: 60-90 kg/mg, Z: 6-10 kg/mg) and then located on a Plexiglas plate with a thickness of 1 cm. Photon beam RT was performed using X-rays with a dose of 11 Gy from an Elekta compact linear accelerator (Elekta Compact 6-MV China). AT program was performed for eight weeks, five days a week, and one session a day for 60 minutes (70-75% of maximal oxygen consumption). Finally, one-way ANOVA was run to examine the research variables.

Results: The results showed that there was no significant difference between the groups in terms of the TNI level ($P=0.23$). However, a significant difference was found in the amount of BNP between the RT and C groups ($P=0.009$). In addition, no significant difference was reported in terms of BNP between AT+RT with AT ($P=0.99$), RT ($P=0.32$), and C ($P=0.69$) groups, as well as between AT with RT ($P=0.1$) and C ($P=0.99$) groups.

Conclusion: Overall, radiation therapy caused a significant increase in BNP, but it had no significant effect on TNI. Aerobic training did not significantly affect TNI and BNP in healthy rats and those undergoing radiation therapy.

Keywords: Radiation therapy, B-type natriuretic peptide, Troponin I

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Introduction

The heart problem caused by radiotherapy is one of the most important potential problems of this treatment (1). The risk of heart complications is related to many factors, including the radiation dose, the dose of radiotherapy per session, and the volume of the heart that is undergoing radiotherapy. Accordingly, serum biomarkers play an important role in the diagnosis of heart damage after radiation therapy, and several important biomarkers have been studied to evaluate their changes in the diagnosis of heart disorders after radiation therapy (1).

Troponin T (TNT) and troponin I (TNI) are important and specific biomarkers of heart disease, and evidence exists regarding the role of TNI and TNI as essential biomarkers of heart damage after chemotherapy (2). In addition, brain (B-type) natriuretic peptide (BNP) and N-terminal prohormone of brain natriuretic peptide (NT pro-BNP) hormones released from the heart to the arteries can predict heart failure (3). Sheikhani Shahin et al showed that high levels of active or inactive BNP in plasma can be considered an indicator for predicting mortality in patients with cardiovascular failure (4). Some studies have examined the levels of this peptide after radiation therapy and shown that these proteins could be potential biomarkers for the early detection of radiation-induced cardiovascular disease. Specifically, Zhang et al analyzed BNP cardiac biomarkers before and after radiotherapy. Their findings represented high levels of both cardiac biomarkers after radiation therapy (5). However, D'Errico et al reported no significant changes in TNI levels in patients with left breast cancer (BC) 5-22 months after radiotherapy. However, they clearly observed an increase in NT-pro BNP levels (6). Erven et al found an increase in TNI levels in 51 patients with left BC after radiation therapy (7). Erven et al concluded that radiation therapy-induced left ventricular dysfunction was associated with increased TNI and BNP. Consistent with the above-mentioned results, other studies demonstrated a positive correlation between elevated TNI levels and high radiation dose to the left ventricle and the whole heart in patients with BC (7).

Cardiac TNI is a regulatory protein that forms part of the contractile system of heart cells (8). The results of the research revealed that exercise can be an effective factor in changing the levels of these heart damage markers. According to a study by Shi et al, regular aerobic exercise significantly reduces serum TNI levels in mice with ischemic-reperfusion injury (9). The results of a study by Conraads et al on heart patients indicated that the levels of NT-pro BNP and BNP decreased after a period of a combination of resistance and aerobic exercise in patients with heart failure. They also concluded that the decrease in NT-pro BNp in trained patients may be due to improved cardiac systolic function, cessation of sympathetic activity, and an improvement in tissue oxygenation (10). Yurtdaş et al reported that NT-pro BNP levels decreased after a period of endurance training in patients with heart failure (11). Despite the above-mentioned findings, no clear results have been found on the effect of aerobic exercise on TNI and BNP levels in radiotherapy specimens. Therefore, the aim of this research was to evaluate the effect of 8 weeks of moderate-intensity endurance training on the serum levels of TNI and BNP in rats undergoing radiation therapy.

Materials and Methods

In the current experimental study, 32 male rats (4-6 months old) were used, which were kept at the Animal Breeding Center of Arak University of Medical Sciences in the summer of 2021. The animals were kept at 23˚C for 12 hours in the dark and 12 hours in the light with free access to food and water. This study was conducted keeping in mind all the ethical principles of working with laboratory animals approved by the Ministry of Health, Treatment, and Medical Education.

Then, 32 rats were randomly assigned to 4 groups of 8, including the control (C) group, which did not exercise during the study period and received no RT. The other groups were the aerobic training (AT) group, which performed AT for 8 weeks, the radiotherapy (RT) group, which underwent RT according to the protocol mentioned below, and the AT + RT group, which performed AT for 8 weeks and underwent RT (Figure 1).

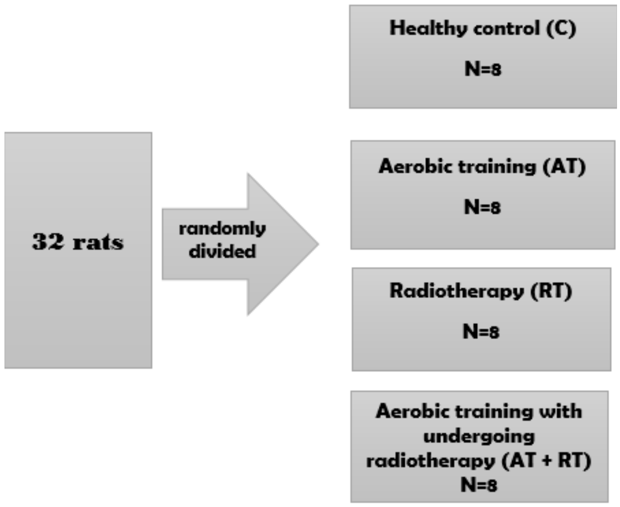

Figure 1. Schematic diagram of different groups

Radiation therapy

This plate was placed on a plate of similar material with a thickness of about 2 centimeters placed under the device. A plate with a thickness of 1 centimeter was placed at a distance of 2 centimeters from the surface of the rat's chest. Therefore, mpp = 3 cm was calculated. The distance between the fountain and the top plexiglass was considered to be 100 cm. The dose of 11 Gy was chosen according to previous studies in this field. X-ray photon radiation with a dose of 11 Gy was performed with a linear accelerator (Elekta Compact 6-MV China) in the Radiotherapy Department of Khansari Arak Hospital (12).

Exercise Protocol

An aerobic exercise program with moderate intensity was performed for eight weeks on a rodent treadmill. Exercises were performed five days a week, and each session lasted 60 minutes between 8:00 AM and 12:00 AM at different speeds. Once the adaptation was complete, the animals (to learn how to follow the aerobic exercise protocol) were placed on a treadmill for the first week.

The rats walked at a speed of 15 m/min with a zero-degree incline for 15 minutes. In the second and third weeks, the speed and duration of training on the treadmill gradually increased to 28 m/min, equivalent to 70%-75% of the maximum consumption. In total, the amount of training was average (8.4 km/wk). At the end of the exercise, the speed was reversed to zero speed to cool down the machine (13).

Twenty-four hours after the last training session, blood samples were taken from the rats. After blood collection (5 cc) and coagulation, the samples were centrifuged and their serum was extracted at a speed of 3500 rpm for 10 minutes and stored at -70 ˚C for measurement. Serum TNI and BNP levels were measured by mouse ELISA kit (Eastbiopharm) for mice (made in China and licensed in the United States) according to the manufacturer's instructions.

In this research, the Shapiro-Wilk test was used to check the normality of data distribution. Further, the analysis of variance (ANOVA) and Tukey's post hoc test were employed to examine the research variables. All studies were conducted at the level of $P \leq 0.05$ using SPSS software, version 21.

Results

Based on the results of one-way ANOVA, no significant change was observed in the weight of mice before and after the test ($P \leq 0.05$, Tables 1 and 2).

The results of ANOVA demonstrated that there was a significant difference between the groups in terms of BNP ($P = 0.01$). Moreover, the results of Tukey's post hoc test represented a significant difference only between the C group of radiation therapy and the healthy C group ($P = 0.009$). However, no significant difference was reported between the other groups (Tables 2 and 3; Figure 2).

According to the results of ANOVA, there was no significant difference between the groups in terms of the TNI level ($P = 0.23$; Table 3 and Figure 3).

Discussion

This study sought to evaluate the effect of eight weeks of moderate intensity aerobic training on the serum levels of TNI and BNP in rats undergoing radiotherapy. The results showed that there was no significant difference in terms of TNI between the groups. In the study of D'Errico et al, no significant changes were observed in TNI levels in patients with left BC 5-22 months after radiotherapy (6). Similarly, Dueland et al reported no significant changes in TNI levels in patients with left BC 5-22 months after radiotherapy (14). Conversely, Xu et al demonstrated high levels of TNI after radiation therapy (14). It seems that serum TNI is not a suitable indicator for heart damage in samples undergoing radiotherapy. In our study, aerobic exercise had no significant effect on TNI in the healthy control and under radiotherapy groups. Regarding the effect of aerobic exercise on TNI in samples under radiotherapy, we could not find consistent or inconsistent research, but in non-radiotherapy samples, Shi et al found that regular aerobic exercise significantly reduces serum TNI levels in mice with ischemic-reperfusion injury (9). Probably, the training performed in our research was not of sufficient intensity or duration.

Cardiac troponins (cTn) are released after damage to the heart due to various mechanisms such as oxidative stress, ischemia, apoptosis, and inflammation. This biomarker is the best indicator for assessing anthracycline-induced cardiac toxicity. An increase in cTnI has been reported in one-third of patients treated with high-dose anthracycline blood or BC and is associated with a degree of ventricular dysfunction (5). Some research represented that *interleukin*-6 levels can be a good alternative if troponin changes do not change significantly after radiation therapy (15). Other studies demonstrated that BNP is an important indicator of heart damage (5).

In this regard, the results of this study indicated that there is a significant difference between the radiation therapy group and the healthy group in terms of the BNP level, while no significant difference was reported between the other groups. In a meta-analysis study, Zhang et al also reported that radiation therapy increases BNP in BC specimens (5). Portaluri et al observed similar results in this regard (16). Sheikhani Shahin et al concluded that high levels of active or inactive BNP in plasma can be considered an indicator for predicting mortality in patients with cardiovascular failure (4).

BNP is an endogenous peptide that is initially produced by ventricular cardiomyocytes as a 134-aa propeptide (4). It is secreted as a result of left ventricular dysfunction, and in heart failure and acute syndrome. The coronary artery, which includes acute myocardial infarction and unstable angina, represents an enlargement. Instead of being stored in normal myocardial tissue, BNP protein secretion occurs in bursts, and after myocardial synthesis, it is rapidly released in the surrounding tissues. BNP as an accepted marker of heart failure is widely used in clinical trials, especially in the diagnosis of heart failure (17).

Although our results demonstrated no significant change in BNP due to exercise in the radiation therapy and healthy groups, some studies showed a significant reduction in this respect. Based on the results of a study by Malandish et al on the role of exercise training on cardiovascular peptides in patients with heart failure: A systematic review and meta-analysis (18).

They also concluded that exercise training, especially aerobic exercise can be improved the NT-proBNP concentrations in patients with HF (irrespective of overweight/obesity status), which may be a sign of positive physiological adaptations to aerobic exercise. Lipsett

Table 1. Weight in all groups

Markers	C	AT	RT	AT + RT
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Weight pre	228.83 ± 30.68	268.83 ± 19.32	265.16 ± 44.72	248.66 ± 23.11
Weight post	275.33 ± 22.8	306.66 ± 38.95	281.33 ± 37.56	278.66 ± 20.1

Note. C: Control; AT: Aerobic training; RT: Radiotherapy; SD: Standard deviation; BNP: B-type natriuretic peptide.

Table 2. Results of the ANOVA test

Markers	Sum of squares	*df*	Average of squares	F	*P* value
Weight pre	6008.79	3	2002.93	2.08	0.13
Weight post	3692.66	3	1230.88	1.27	0.3
Troponin I	18141.240	3	6047.080	1.54	0.23
BNP	333.085	3	111.028	4.79	0.01

Note. ANOVA: Analysis of variance; df: Degrees of freedom; BNP: B-type natriuretic peptide.

Table 3. Tukey's post hoc test results for BNP

	Group	Group	Mean difference	SD	*P* value
BNP	AT+RT	AT	1.48	2.77	0.99
		RT	5.7	2.77	0.32
		C	4.56	2.77	0.69
	AT	RT	7.18	2.77	0.1
		C	3.08	2.77	0.99
	RT	C	10.26	2.77	*0.009

Note. SD: Standard deviation; BNP: B-type natriuretic peptide; C: Control; AT: Aerobic training; RT: Radiotherapy. * Significant by Tukey's post hoc test.

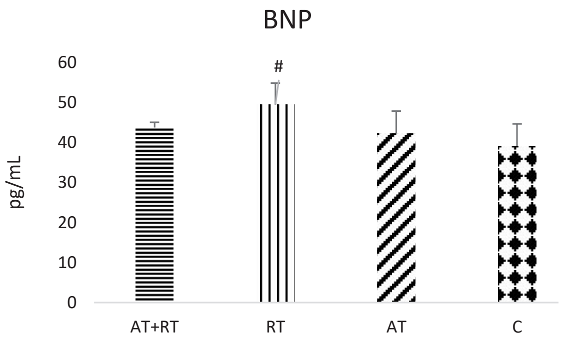

Figure 2. BNP Values in Different Groups. *Note*. BNP: B-type natriuretic peptide; C: control; AT: aerobic training; RT: radiotherapy. # Significant difference compared to the healthy control group

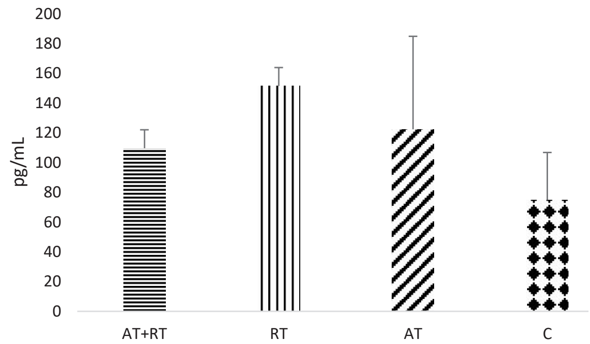

Figure 3. Troponin I in Different Groups. *Note*. SD: C: Control; AT: Aerobic training; RT: Radiotherapy

et al, in a meta-analysis study, found that combined training (endurance-resistance) significantly reduces BNP in cancer patients (19). Bordbar et al also reported a decrease in BNP after 8 weeks of aerobic exercise in sedentary individuals. It seems that insufficient training intensity in our study was one of the reasons for the lack of a significant change in BNP (20). In this regard, Benda et al concluded that exercise-induced changes in BNP and cardiac troponin were similar between a period of high-intensity interval training and endurance training in healthy controls and patients with heart failure (21). Even if the exercise is intense, excessive cardiac stress can be prevented if the duration is short (22,23). The intensity, frequency, and optimal duration of exercise for health in people with and without heart disease are not fully understood, but high-intensity exercise that depends on fitness may be beneficial to health.

Conclusion

According to the results of this study, radiation therapy caused a significant increase in BNP, while it exerted no significant effect on TNI. Compared to TNI, BNP seems to be a suitable serum index for diagnosing heart damage in samples undergoing radiotherapy. The findings further revealed that 8-week aerobic training had no significant effect on TNI and BNP in healthy animals and undergoing radiation therapy rats. The time or intensity of training should probably be increased for this purpose. Additionally, we have not induced cancer in this research. Only the samples have been subjected to radiotherapy and training. Different results would be obtained if the samples were infected with cancer.

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Authors' Contribution

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Supervision: Khalid Mohamadzadeh Salamat, Mohammad Parastesh.
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Writing–review & editing: Khalid Mohamadzadeh Salamat, Mohammad Parastesh, Kamal Azizbeigi, Mohammad Reza Bayatiani.

Competing Interests

The authors of this article have no conflict of interests.

Ethical Approval

This article is an excerpt from the doctoral dissertation in the field of sports physiology of Islamic Azad University, Sanandaj Branch, with the code of ethics IR.ARAKMU.REC.1398.162.

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