

# The Effect of Eight Weeks of Concurrent Exercise on NT-Probnp and Ferritin Serum Levels of Beta Thalassemia Major Patients

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## Original Article

### Abstract

**Introduction:** Nowadays one of the most important problems of Thalassemia major patients is the additional load of iron and hemosiderosis, the most important consequence of which is deposited iron in myocardial tissue and the incidence of cardiomyopathy caused by hemochromatosis. The aim of this study was to determine the effect of concurrent (resistance-endurance) exercise on NT-pro BNP and ferritin serum levels of beta Thalassemia major patients.

**Methods:** Participants were 18 patients with Beta Thalassemia major (mean age 22.64±3.5) that voluntarily divided in two groups: experimental (n=9) and control group (n=9). The subjects of the experimental group attended the concurrent (Resistance-Endurance) exercise for eight weeks. Subjects' blood samples were taken before and after exercise protocol in order to measurement NT-proBNP and Ferritin Serum levels. The levels of NT-proBNP and Ferritin were measured by using an enzyme-linked Electro immuno assay (ELISA) kits. For data analysis the Repeated measures ANOVA, was used. All statistical analysis was performed by SPSS 19 software. The level of significance was considered pvalue<0.05.

**Results:** The results indicated the reduction of NT-pro BNP after eight weeks of concurrent (resistance-endurance) exercise compared to the control group (pvalue <0.05) and also the levels of Ferritin was significantly decreased comparing with the control group (pvalue <0.05).

**Conclusion:** The reduction of NT-proBNP serum level and its natural range in the exercise protocol can be a regulating response to hemodynamics cardiac pressure in patients under certain exercise conditions without any pathological importance and improved heart function. A significant reduction of ferritin serum indicates lower iron toxicity and it can be known as an effective factor in delayed cardiomyopathy that caused by excessive iron.

**Key words:** Exercise, Beta Thalassemia, NT-pro BNP, Ferritin

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### Introduction:

Thalassemia major is the most common genetic disease in the Middle East and Iran (1). Maintaining hemoglobin levels within the range recommended in these patients guarantees their survival and growth, but their body iron overload is one of the most important problem in thalassemia patients that leads

to iron overload or hemosiderosis (2) the results show that of the cardiovascular disease and endocrine disorders caused by iron overload which leads to the premature death in thalassemia patients before the age of twenty-two.

The sport science studies have shown that exercises, especially aerobic exercises significantly

reduce ferritin and iron levels in the blood (3). Iron accumulation has a significant impact on the structure and function of the heart and other vital organs of the Beta thalassemia major (BTM) patients (4). Despite the progress made in blood therapy, heart failure is still one of the most important causes of death in BTM patients (5). Haemochromatosis (6) is one of the main pathogenic mechanisms and the progress of heart failure in these patients which especially leads to iron deposits in the myocardial wall and diastolic and systolic dysfunction of the left ventricular (7).

Brain natriuretic peptide (BNP) is primarily released by muscle cells of left ventricular wall in response to increased tension or stress (8). BNP and NT-pro BNP are both sensitive biological indices used to identify problems and dysfunction of the left ventricle (LV) and the diagnosis of the mentioned patients (9).

The presence of a small amount of non-binding iron in the heart tissue can cause harmful reactive oxygen and toxic concentrations of metabolic materials, acute pulmonary hypertension and myocardial inflammation (10, 11) due to iron overload which accelerated the cardiac dysfunction in these patients (12). Various studies have addressed the interaction between changes in indicators of cardiovascular disease, thalassemia and cardiac function. Besides, few studies have analyzed the interaction between physical activity and cardiovascular function of the above mentioned patients. Balkan et al. (2012) have studied the correlation between NT-proBNP and iron overload in thalassemia major patients in the early stages of heart disease. Their findings indicate that there is a correlation between NT-proBNP and iron overload levels. Also there was no correlation between NT-proBNP and diastolic dysfunction in the third decade of these patients' lives. However the secretion of NT-proBNP in the early stages of the disease will lead to an increase in diastolic dysfunction (12). In another research Heidary et al. (2011), studied the effect of 8 weeks of aerobic training on iron and Hematological Indices in girls with thalassemia minor in Kermanshah. They concluded that ferritin was significantly decreased in experimental group and iron absorption capacity of the blood was increased significantly. Other hematological parameters were not changed in both groups (13).

Dellaporta et al. (2013) studied the correlation between NT-pro BNP and cardiac iron accumulation

in patients with thalassemia major who were subject to blood transfusion. They found a significant relationship between NT-pro BNP values and iron overload in patients with thalassemia (14). ZareZadeh et al (2008) analyzed the effect of aerobic exercise on body iron indices in healthy people and patients with thalassemia major. The results show that the variables under study changes significantly immediately after and 48 hours after exercise compared to pre-exercise but there was no difference in this respect between the genders. Concluded that Iron and ferritin serum of both groups had a significant reduction after aerobic exercise compared to pre-exercise and iron capacity (TIBC) levels were significantly increased (15). In another study Serrano-Ostariz (2011) studied the effect of duration and intensity of exercise on cardiac parameters release. The increased duration of exercise had a significant influence on the secretion of NT-pro BNP and increased its value while the intensity of training had no significant effect on it (16).

Since in the last two decades the interactive effects of resistance-endurance exercise to on response, adaptation and transformation of the main indicators have been focused by mentioned researches, the effect of combined resistance - endurance exercise on important Heart cardiac indicators (NT-pro BNP) on patients with thalassemia major is a new field. This question is, thus, raised that do eight weeks of concurrent resistance-endurance exercises change the serum levels of NT-pro BNP? Or does this kind of exercises have any effect on the serum levels of ferritin in BTM patients?

## Methods:

Research subjects included 18 beta Thalassemia major patients in Abureyhan specific diseases center of Bandar Abbas, which voluntarily participated in the study. Finally, after obtaining informed consent they were voluntarily grouped into experimental (n=9) and control (n=9) groups.

Obtaining the history, physical cardiology examination, and echocardiography were performed on all patients. Due to the potential impact of hematocrit changes on the results, blood sampling and echo of all patients were taken at least 72 hours after blood transfusion (17,18). One week before the concurrent exercise program, the subjects were familiarized with research steps and training program. Anthropometric characteristics of subjects,

including height, weight, body mass index and body surface area and the maximum aerobic capacity and hemoglobin levels were measured one week before the exercise program (Table 1). It should be mentioned that the subjects' diet, medication and blood transfusions were considered. One week after the familiarization stage and teaching the implementation techniques, the workout program including 8 weeks of concurrent resistance-endurance exercise was performed as three sessions a week for a total of 24 sessions. The subjects performed both concurrent resistance-endurance exercises at the same session. Resistance exercise was always performed before endurance exercise to prevent premature fatigue caused by endurance exercise (19). For the implementation of this resistance protocol the 100 percent power of the subjects was determined using the formula  $\text{Max Power} = \text{Load}/1-0.02$  (reap). In order to control the intensity of the exercise to perform endurance protocol, the maximum heart frequency of subjects (based on maximum heart rate equation  $(220 - \text{age}) \pm 10$ ) was calculated. Also, to keep the heart rate during the endurance exercise, PM45 heart rate monitor was used. The subjects had a 10 minute warm up before each session and had a 5 minute recovery after the session. Exercise protocols have been shown in table 1. All exercises were performed in Azad University of Bandar Abbas gym. The subjects' diet was based on their regular diet and they were asked not to use drugs other than their usual drugs. Blood samples (10cc for each time) were collected from the antecubital vein in the stages of pre and post-tests in order to measure the serum levels of NT-pro BNP and Ferritin. The collected

blood samples were put in pre-cooled serum tubes and allowed to be clotted within one hour at the room temperature. Samples were then centrifuged in 4000 g within 15 minutes at 4°C. The obtained serum was discharged into Eppendorf tubes and stored at -70°C to be analyzed. NT-pro BNP was measured by ELISA using kits specified to human samples based on the instruction of manufacturer (Elecys and cobas e analyzer, Germany) pg/ml. Ferritin was also measured by ELISA using kits specified to human samples based on the instruction of manufacturer (Abbot-i2000SR, Germany) pg/ml. As data distribution was natural based on Kolmogorov-Smirnov test. To analyze the data, Repeated measures ANOVA were used.

### Results:

Table 1 presents mean values and standard deviation of the age, height, and BMI, BSA, HG and  $\text{VO}_2\text{max}$  among research various groups. Results showed that prior to intervention, all variables were homogeneous (Table 2). The Repeated measures ANOVA showed that the 8 weeks of Concurrent exercises have effect on the serum level of NT-pro BNP and Ferritin ( $p\text{value} < 0.05$ ) Table 3.

The serum levels of ferritin though significantly decreased after 8 weeks of Concurrent exercises in comparison with the control group ( $p\text{value} = 0.001$ ). Also in exercise group the levels of NT-pro BNP in post-test were significantly decreased from pre-test ( $p\text{value} = 0.003$ ).

Figure 1 depicts NT-pro BNP changes among various groups. Ferritin changes have been shown in figure 2.

Table 1-Concurrent Exercise protocol

	Time	First(4 weeks)	Second(4 weeks)
Resistance Exercise	Leg press	$\frac{60-65\%}{12} \times 2$	$\frac{65-70\%}{8} \times 2$
	Lat	$\frac{60-65\%}{12} \times 2$	$\frac{65-70\%}{8} \times 2$
	Leg extension	$\frac{60-65\%}{12} \times 2$	$\frac{65-70\%}{8} \times 2$
	Dumbbell Curl	$\frac{60-65\%}{12} \times 2$	$\frac{65-70\%}{8} \times 2$
	Dead Lift	$\frac{60-65\%}{12} \times 2$	$\frac{65-70\%}{8} \times 2$
	Endurance Exercise	Treadmil	$\frac{60-65\%}{10\text{min}} \times 2$

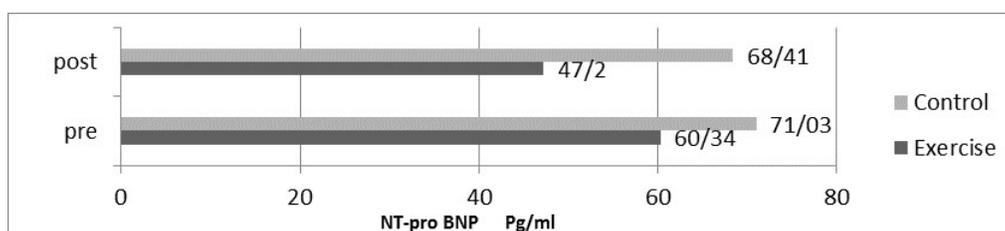


Figure1- Variation of NT-pro BNP concentration among different groups

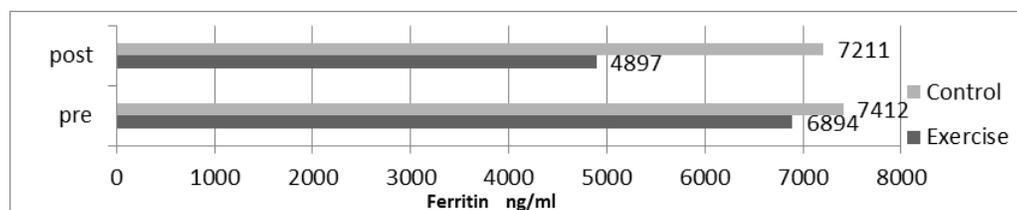


Figure2- Variation of Ferritin concentration among different groups

Table 2- Anthropometric and Biochemical characteristics of subjects at Baseline

Group/characteristics	Control(n=9)	Exercise(n=9)	p value
Age (years)	26.67±3.6	22.62±3.5	0.655*
Weight (kg)	46.27±1.47	48.59±1.05	0.842*
Height (cm)	149±1.26	152±1.42	0.543*
BMI (kg.m <sup>-2</sup> )	20.31±2.66	19.6±2.14	0.667*
VO <sub>2max</sub> (ml.kg <sup>-1</sup> .min <sup>-1</sup> )	47.3±3.34	49.1±4.86	0.506*
BSA	1.48±0.07	1.44±0.06	0.617
HGB (g. dl <sup>-1</sup> )	10.9±0.01	10.8±0.9	0.558*
NT-pro BNP( pg. ml <sup>-1</sup> )	71.03±1.5	60.34±15.21	0.73*
Ferritin (ng. ml <sup>-1</sup> )	7412±2327	6894±2741	0.54*

Table 3- Compares difference between groups after 8 weeks of Concurrent Exercise protocol

Group Characteristics	F	Post test		p-value between groups	
		Control	Exercise	Control	Exercise
NT-pro BNP (pg.ml <sup>-1</sup> )	3.44	68.41±1.2	47.2±12.36	0.73	0.003*
Ferritin (ng.ml <sup>-1</sup> )	2.19	7211±1845	4897±1861	0.54	0.000*

### Conclusion:

This study examined the effect of concurrent resistance-endurance exercise on serum levels of NT-pro BNP and Ferritin in patients with thalassemia major. According to the analysis of the researcher there is no released information in terms of the effect of concurrent resistance-endurance exercise on serum levels of NT-pro BNP.

However, these findings suggest that the intensity and duration of exercise program used in this study has decreased NT-pro BNP levels. Although the exact mechanisms of the secretion of NT-pro BNP and the factors affecting the level of secretion during exercise is unknown, it is recently assumed that NT-pro BNP changes due to exercise can be caused by the secretion of inflammatory cytokines. This assumption is rooted in the observations that indicate

Pro inflammatory cytokines modify BNP gene expression and secretion. Vidotto et al (2005) and Middleton et al (2006) have reported the increased level of NT-proBNP level after severe endurance exercise (20,21). In a study Thomas et al. (2006) have reported increased plasma levels of NT-proBNP related to heart dysfunction in young athletes. While Middleton et al (2006) showed an increase in plasma levels of NT-proBNP in athletes could not be attributed to heart dysfunction in young athletes (19).

Scharhag et al (2008) reported the increase in NT-proBNP plasma level caused by the increased heart rate caused by cardiac myocytes stretch during endurance activity (22) and it is possible to report a positive relationship between endurance physical activity and plasma concentration of NT-proBN. Mechanical and neural-hormonal stimulation along with ventricular myocytes stretching in response to the ventricular expansion can lead to molecular increase in BNP and NT-proBNP plasma (23-25). Therefore, reducing the serum levels of NT-proBNP in patients with thalassemia major can indicate a lack of disease or heart dysfunction, and lowered NT-proBNP plasma levels can be related to the imposed reduced hemodynamic pressure and the physiological response of the heart to decrease in heart pressure. Therefore, the evaluation of NT-proBNP plasma concentration levels can be used as an indicator to distinguish between physiological and pathological changes patients with thalassemia major. With a closer look at some of the above studies, the reason of this inconsistency can be related to the differences in the measure variable of BNP instead of NT-proBNP plasma in one hand and the differences in the type and intensity of sports activities, blood sampling and subjects on the other hand.

Considering that initial clearance and destruction of BNP and NT-proBNP plasma is done by Neutral Endo peptidase and glomerular purification, the extent of BNP and NT-proBNP refinement is reported differently (26). Given the high molecular weight of NT-proBNP plasma and its long half-life (60 minutes) to BNP (22 minutes), it can be assumed that the reduction in plasma concentration of NT-proBNP after an eight-week of concurrent resistance-endurance exercise can be response to increased rate of initial purification release by the kidneys. On the other hand, this increase may be due to changes in the purification can be cause by

changes in permeability of kidney cells, their increased rate of uptake mechanism or enough time for purification that affects kidney extraction pace (27).

This study showed that concurrent resistance-endurance exercise can decrease significantly ferritin serum in patients with thalassemia major. Few studies have analyzed the effect of aerobic exercise on iron index in thalassemia major patients and often have reported similar results. The results of the present study were consistent with Vashtani et al. (2009) who reported that the submaximal aerobic efficient program can be set for thalassemia major patients according to medical examination and tests because in addition to the acquisition of a happy mood, concentration of ferritin and iron is reduced significantly. The reason of similarity between the results of the present study and that of Vashtani et al. (2009) is related to the duration of the eight-week training program and similar subjects (27). In addition, Zarezadeh et al. (2008) examined the effect of aerobic exercise on body iron indices in healthy subjects and patients with thalassemia major. There was no difference in this respect between both genders. Similar to this study Iron and ferritin serum of both groups had a significant reduction after aerobic exercise compared to pre-exercise and iron capacity (TIBC) levels were significantly increased. In addition they reported that iron serum, ferritin and TIBC had a significant increase 48 hours after sports activities compared to pre-exercise (15).

In thalassemia patients with heart failure and weak ventricular function, training capacity is limited for various reasons. Cardiac output in increasing blood flow for muscle contraction and blood distribution is ineffective and therefore aerobic metabolism occurs earlier. Heart failure is caused by a deficiency in iron would cause in mitochondrial respiration and affects cellular metabolism. A level of anemia causes reduced oxygen and muscle content due to reduced arterial oxygen and affects exercise (28,29).

Assuming exercise-induced immune response has a role in changes in NT-proBNP serum levels of patients with thalassemia major; it is believed that low plasma concentration of NT-proBNP is related to low levels of ferritin.

The results of our current study showed that the reduced level of NT-proBNP serum and its natural range in this training protocol can be a regulatory response to cardiac hemodynamics pressure in

patients under special circumstances without pathological importance and only related to their physical activity. However the reduction of NT-proBNP serum caused by training in the in patients without heart disease is different from its reduction in cardiovascular patients with systemic inflammation. A significant decrease in ferritin serum indicates the reduced iron toxicity and it can be used as a factor of cardiomyopathy caused by the excessive iron and it can be known as an effective factor in delay in the incidence of cardiomyopathy caused by excessive iron. Bandeira et al. (2013) analyzing the chronic inflammation in sickle cell patients reported that inflammatory pattern in these patients seems to be related to genetic polymorphism and finally suggest the genetic evaluation to make this issue clear (29). Although in this study, diet was controlled, but factors such as stress and environmental conditions as well as the specific conditions of patients and limited samples size could have affected the results.

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## اثر هشت هفته تمرین مقاومتی - استقامتی همزمان بر مقادیر سرمی NT-pro BNP و فریتین بیماران بتا تالاسمی ماژور

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### چکیده

**مقدمه:** امروزه یکی مهم‌ترین مشکلات بیماران تالاسمی ماژور، اضافه بار آهن و هموسیدروز می‌باشد، که پیامد آن رسوب آهن در بافت میوکارد و وقوع کاردیومیوپاتی ناشی از همراکمروماتیز است. هدف از انجام پژوهش حاضر، تعیین تأثیر هشت هفته تمرین مقاومتی - استقامتی همزمان بر مقادیر سرمی NT-pro BNP و فریتین، بیماران بتا تالاسمی ماژور بود.

**روش کار:** بدین منظور ۱۸ نفر از بیماران بتا تالاسمی ماژور مرکز بیماری‌های خاص ابوریحان شهرستان بندرعباس با دامنه سنی  $22/64 \pm 3/5$  سال به عنوان نمونه پژوهش انتخاب و به صورت داوطلبانه به دو گروه تمرینی (n=۸) و کنترل (n=۸) تقسیم شدند. آزمودنی‌های گروه تمرینی، به مدت ۸ هفته در برنامه تمرینی مقاومتی - استقامتی همزمان شرکت کردند. از گروه کنترل درخواست شد در مدت اجرای پژوهش از انجام هر گونه فعالیت ورزشی هدفمند خودداری کنند. پس از دو مرحله خونگیری در پیش آزمون و پس آزمون، شاخص NT-pro BNP و فریتین سنجیده شد.

**نتایج:** برای آنالیز داده‌ها، آنالیز واریانس اندازه‌گیری‌های مکرر استفاده شد. کلیه محاسبات آماری با استفاده از نرم‌افزار SPSS 19 انجام شد. آزمون‌ها کاهش مقادیر NT-pro BNP (پیکوگرم بر میلی لیتر) پس از ۸ هفته تمرین مقاومتی - استقامتی همزمان (pvalue<۰/۰۵) و همچنین کاهش مقادیر فریتین پس از ۸ هفته تمرین مقاومتی - استقامتی همزمان نسبت به گروه کنترل را نشان دادند (pvalue<۰/۰۱).

**نتیجه‌گیری:** کاهش میزان سرمی NT-proBNP و محدوده طبیعی آن در این پروتکل تمرینی می‌تواند پاسخ تنظیمی به فشار همدینامیکی قلب بیماران تحت شرایط ویژه تمرینی و بدون اهمیت پاتولوژیکی و بهبود عملکرد قلب در آن‌ها باشد. کاهش معنی‌دار میزان فریتین سرمی، بیانگر کاهش اثر سمیت آهن بوده و از آن می‌توان به عنوان عاملی مؤثر در تعویق وقوع کاردیومیوپاتی ناشی از آهن مازاد، نام برد.

**کلیدواژه‌ها:** تمرین، بتا تالاسمی، NT-pro BNP، فریتین.

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**ارجاع:** ارباب گلناز، نیک‌پرور مرضیه، گایینی عباسعلی، سبحانی علیرضا. اثر هشت هفته تمرین مقاومتی - استقامتی همزمان بر مقادیر سرمی NT-pro BNP و فریتین بیماران بتا تالاسمی ماژور. مجله پزشکی هرمزگان ۱۳۹۵؛ ۲۰(۱): ۱۰-۱۷.