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Effect of Concurrent Resistance-Aerobic Training on Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio in Pediatric Type 1 Diabetes: A Randomized Trial

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Abstract

Objective: The role of post-exercise on the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) is not evident in pediatrics with type 1 diabetes (T1D) yet. This paper reports the results of a research study on how concurrent exercise training affects NLR and PLR in children with T1D.

Materials and Methods: In this randomized controlled trial, 40 children (boys and girls aged 11.11 ±2.29 years) were randomly divided into an experimental (n=20) and a control group (n=20). The training program included concurrent resistance-aerobic training, which was intermittently performed for 60 minutes at a rate of three times a week for 16 weeks. The participants were analyzed for blood glucose homeostasis, NLR and PLR before and after the program. Data were analyzed by SPSS 22 software with paired T-test and covariance analysis ($P< 0.05$).

Results: The results showed that PLR significantly ($P= 0.002$) decreased in the exercise group after 16 weeks of concurrent training. This significance was observed between the groups too ($P= 0.003$). HbA1c decreased both in the exercise group and between the groups ($P= 0.001$, $P= 0.003$). NLR exhibited a significant increase both in the exercise group ($P= 0.021$) and between the groups ($P= 0.012$.)

Conclusion: Concurrent exercise training reduces PLR and HbA1c in children with T1D, which may be related to the anti-inflammatory effects of exercise training.

Keywords: Diabetes mellitus, Neutrophil- lymphocyte ratio, Platelet-lymphocyte ratio, Pediatric

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Introduction

Type 1 diabetes (T1D) is a chronic inflammatory autoimmune disease that destroys beta cells and manifests in its involvement in insulin production and shortage (1). Chronic inflammation induces and aggravates T1D and its future side effects. In addition, there is a complicated relationship between inflammation and adaptive responses to preserve blood homeostasis (2). Evidence implies elevated inflammation induced by metabolic changes, e.g., elevated levels of insulin and glucose (3,4). Recently, the neutrophil- lymphocyte ratio (NLR), the count of white blood cells (WBC), and the platelet-lymphocyte ratio (PLR) have been identified as new inflammation markers in diabetes (5,6). NLR can be an excellent marker to assess the risk of the escalation of blood fat and diabetic neuropathy in pediatric T1D (5) .On the other hand, PLR is recognized as the chronic inflammation marker in the following stages of diabetes (7), and also can be an alternative for NLR in calculating cell immunity inflammation markers (8). Evidence reveals that higher NLR and PLR are related to inflammation in T1D. In this respect, a research study reported that children suffering from T1D had significantly higher NLR and lower PLR than the control group, and this lowered PLR had a negative relationship with diabetes duration, HbA1C, and leukocyte count (5).

Despite advances in the control of this disease over time, T1D is still a difficult disease to be controlled (9). So, exercise is necessary for children, especially those suffering from T1D in which inflammatory factors play a role, to prevent the future complications of the disease. Interestingly, concurrent exercise training as an efficient alternative training method can contribute to improving the safety and stability of blood glucose content during exercise and reducing inflammation indices (10,11), which are ascribed to the anti-inflammation activity of exercise (12).This can be related to the increased level of anti-inflammation cytokines induced by muscle contraction (myokines) (13) and the reduced expression of Toll-like receptors (TLRs) on monocytes and macrophages (14). In this respect, it has been established that basic inflammation markers are reduced by regular exercise training (12,15). For example, a study revealed that NLR decreased in women suffering from metabolic syndrome after three weeks of exercise training (16). Similarly, another study showed that NLR decreased in overweight adolescents after 4-week exercise (17). However, some studies have reported that exercise increases these indices (18) or has no effect on them (16,19) in patients and healthy people.

Despite the research on the benefits of exercise training among children, there is little data on the effect of concurrent exercise training on prepubescent children's performance (20,21). Furthermore, there is no clear recommendation for the type of physical activity that T1D patients should engage in (22). In addition to disagreements in this type of exercise training, the role of NLR and PLR during exercise is not clear in T1D children and research has paid inadequate attention to the role of PLR in the exercise (8). So, this research aimed to check the variations in NLR and PLR in T1D children during exercise training.

Materials and Methods

Participants

Of the 484 T1D patients who visited the 17-Shahrivar Children's Hospital in Rasht and were between the ages of 8 and 14 years, 40 were voluntarily chosen for involvement in the clinical trial study after a public call in February. All experiments on the subjects were in accordance with the Declaration of Helsinki.

The inclusion criteria of the participants were as follows: (1) pediatric type 1 diabetes, (2) HbA1c≥ 7, (3) age 8-14 years, (4) duration

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of diabetes for at least one year. The exclusion criteria included not completing the research plan based on the research objectives and absence for two consecutive or three non-consecutive sessions.

Study design

After filling out a consent form and a health status form, eligible participants were randomly divided into an experimental (n=20) and a control group (n=20) to participate in a 16-week trial.

The red and blue balls that the subjects randomly selected from the box served as the basis for the randomization process (Red Orb and the Blue Orb of the experimental and control group). An impartial researcher was in charge of overseeing this procedure. The group in the control did not exercise. Throughout the course of the trial, the individuals were asked to follow the dietary advice. Absence for more than three sessions would lead their exclusion. A 3-day food diary (two weekdays and one weekend) was used to measure dietary consumption in both groups at the beginning and end of the trial.

It should be noted that to find out the tolerance of the exercise method by the subjects, the training protocol was first applied in a pilot study of five male and female patients with T1D for two weeks. These patients were not included in the final sample. The results revealed that the training protocol can be executed on diabetic patients aged 8-14 years.

Sample size calculation

Using G Power software (ver. 3.1.9.2) and T-test at the α= 0.05 error level and with an 80% power, the sample size was calculated to be 20 per group.

Measurements

Measurements were recorded a week before the exercise and 48 hours after the intervention. Inbody 3.1 (South Korea) was used to measure body composition. Given the hormonal effects on puberty, the Tanner scale was employed to determine relative homogenization. The subjects' maturity stage was found using the self-reported five-step Tanner scale image (23). They were in stages 1 to 5 of puberty based on the Tanner scale.

The subjects and parents were asked to adhere to a pre-blood sampling instruction composed of, 12 hours fasting, comfortable sleep, and no exercise.

For the blood test of both experimental and control groups, a 5 ml sample was collected from the arm vein in a seated position at rest. Fasting blood glucose and HbA1c were measured by kits from the Pars Azmun Company (Iran) and Biosystem (Spain) with a sensitivity of 5 and 1 mg dL^{-1} by biochemical method, respectively.

Count NLR and PLR were analyzed with the automated hematology cell counter XP-100 (Sysmex, Kobe, Japan). NLR was determined as the ratio between the accurate neutrophil and lymphocyte count that both had been obtained from the same automated blood sample. The PLR was estimated as the simple ratio between the absolute platelet and lymphocyte count. NLR and PLR were computed for each subject.

Design of exercise program

The training program included 16 weeks of interval concurrent resistance-aerobic exercises for 60 minutes at a rate of three times weekly. First the resistance training (20 minutes of Pilates exercises + 20 minutes of body weight-bearing exercises) was performed by the subjects. Then, they performed the aerobic exercises, and marched at 50-75% of maximum heart rate for 20 minutes (10 minutes + 10 minutes with two minutes of break between them).

A 2-minute break was considered between the training models (24-26) (Table 1).

Statistical analysis

Data were analyzed by SPSS ver. 22 software at the $P< 0.05$ level of significance. The Kolmogorov-Smirnov test was used to check their natural distribution, the parametric

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paired t-test was used to check the variations of the groups, and the analysis of covariance (ANCOVA) was used to compare the groups.

Ethical considerations

The study was approved by the Ethics Committee of Islamic Azad University of Rasht, Iran under the Ethics approval cod of IR.IAU.RASHT.REC.1398.011. It was also registered in the Iranian Registry of Clinical Trials under the code of IRCT 20150531022498N30 in June 2019.

Results

All subjects completed the trial, except for four who withdrew due to family reasons. The subjects were divided into an experimental group (20 subjects) and a control group with 20 subjects.

Table 2 presents the descriptive statistics of the subjects. As shown in Table3, there are significant decreases in the PLR rate (*P*= 0.002) and HbA1c (*P*= 0.001) in the experimental group versus the pre-exercise stage after 16 weeks of concurrent training. NLR exhibited a significant increase in the experimental group (*P*= 0.021).

By using one-way analysis of variance test (ANOVA), significant difference was observed between the groups, both in NLR (*P*= 0.012), PLR and HbA1c (*P*= 0.003) (Table 3).

Discussion

This study was conducted on the effect of exercise training on NLR and PLR in pediatric T1D. The most interesting finding is that in children with T1D, the PLR inflammation index decreased in the exercise group even at moderate training intensity. These variations in children signify its importance as a critical component in children's adaptation to exercise training, whose potential mechanism can be ascribed to the relationship between the PLR index and diabetes so that it has been shown that low PLR is negatively related to diabetes duration, HbA1C, and leukocyte count (5).

Furthermore, most studies have reported the lack of any changes in term of PLR during acute training (27) and an increase during intense exercises (18). However, it has been shown that low NLR during exercise training at a moderate level had a positive impact on the life quality of Type 2 diabetes patients (15).

Table 1. Concurrent training protocol

Exercise	Week	Rest	Sets/repetitions	Duration	Intensity
Pilates (5 exercise)	1-8	30 sec	1-2 s 8-10 r	10 min	------
	9-16	30 sec	2-3 s 10-12 r	20 min	------
Body weight bearing (5 exercise)	1-8	30 sec	1-2 s 8-10 r	10 min	------
	9-16	30 sec	2-3 s 10-12 r	20 min	------
Aerobic training (5 exercise)	1-8	1 min between sets	2 set (5 min+5 min)	10 min	50-60% HR
	9-16	2 min between sets	2 set (10 min+10 min)	20 min	65-75% HR

Table 2. Participants' characteristics

Variable	Exercise (n=20)	Control (n=20)	*P* (pre-test)
Age (year)	11.22 (±1.90)	11.00 (±2.67)	0.267
Height (cm)	145.15 (±11.75)	141.22 (±19.27)	0.101
BMI(Kg/m^2)	18.96 (±4.11)	17.28 (±1.87)	0.316
Tanner stage	2.45 (±0.99)	2.35 (±1.13)	0.297
Diabetes duration (years)	3.04 (±1.83)	3.07 (±1.87)	0.123
HbA1c (%)	7.98 (±1.02)	7.26 (±1.51)	0.421
PLR	120.63 (±27.10)	128.75 (±5332)	0.321
NLR	1.50 (±0.135)	1.54 (±0.12)	0.221

BMI, Body mass index. NLR, Neutrophil–lymphocyte ratio; PLR, Platet–lymphocyte ratio

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Table 3. Changes in blood indices of the study subjects in the experimental and control groups

Variable	Group	Baseline	Post -test	Paired-test	ANCOVA
HbA1c (%)	Exercise	7.98 (±1.02)	7.83 (±1.00)	0.001*	0.003*
	Control	7.26 (±1.51)	8.15 (±1.12)	0.117	
Inflammatory factors					
WBC	Exercise	5340.05 (±1193.56)	7385.0 (±2042.25)	0.001*	0.001*
	Control	4565.0 (±1427.64)	4755.60 (±1666.31)	0.275	
Accurate neutrophil ($x10^6$/ml)	Exercise	3113.82 (±705.18)	4544.05 (±1320.13)	0.001*	0.001*
	Control	2680.65 (±841.25)	2787.74 (±962.84)	0.297	
Accurate lymphocyte ($x10^6$/ml)	Exercise	2080.62 (±481.97)	2677.95 (±860.07)	0.001*	0.010*
	Control	1746.15 (±558.23)	1840.39 (±664.67)	0. 175	
Accurate Monocite ($x10^6$/ml)	Exercise	72.60 (±29.62)	87.30 (±35.66)	0.068	0.109
	Control	80.40 (±39.57)	77.60 (±46.09)	0.655	
Accurate Eosinophil ($x10^6$/ml)	Exercise	73.0 (±32.54)	73.85 (±20.42)	0.916	0.038*
	Control	63.70 (±36.69)	56.45 (±26.07)	0.275	
Platelet	Exercise	244350.0 (±57617.86)	225700.0 (±7647.03)	0.237	0.493
	Control	203150.0 (±4693.3888)	20500.0 (±45157.61)	0.353	
PLR	Exercise	120.63 (±27.10)	87.89 (±33.00)	0.002*	0.003*
	Control	128.75 (±5332)	123.92 (±50.45)	0.269	
NLR	Exercise	1.50 (±0.135)	1.77 (±0.51)	0.021*	0.012*
	Control	1.54 (±0.12)	1.52 (±0.13)	0.104	

NLR= neutrophil–lymphocyte ratio; PLR = platet–lymphocyte ratio; WBC= withe blood cell
* Significant difference of $P < 0.05$

Also, this effect seems to be related to the anti-inflammatory effects of exercise training. The role of hormones, myokines, and cytokines has been established in the anti-inflammation effects of exercise, varying with the intensity, duration, and type of the training (12). The mechanisms of the anti-inflammatory effects include reducing the number of pre-inflammatory monocytes (28), increasing the count of T cells (TReg cells) (29), increasing catecholamine and cortisol during exercise (30), and increasing the production and release of IL-6 and other myokines from skeleton muscles during the activity (12). Considering these mechanisms, it seems that due to the moderate intensity and longer duration (16 weeks) of the training in the present study, myokines may take a more important role in long-term exercise training. On the other hand, it can be said that intermittent training can reduce inflammatory factors by stabilizing and balancing the exudation of growth factors.

Inconsistent with our study, Joisten et al. examined women and men suffering from multiple sclerosis (MS) and found that after three weeks of HIIT training, PLR did not change but NLR decreased versus MCT training (16). Others have also reported no change (27) or an increase in PLR (31) after acute training in healthy people. The inconsistency of the results to the type of training protocol (HIIT and acute training versus concurrent training at a moderate intensity) and the type of participants (T1D children versus MS patients and healthy people) were find. Furthermore, the activity in stressful conditions and acute training may increase inflammatory factors (22).

However, these exercises could reduce PLR, increase NLR in these patients. This result may be related to the increase in blood glucose during physical activity and this stress increases and activates inflammatory cytokines. Nonetheless, this stress can be compensated by the activation of an inflammatory response and daily exercise training at a moderate rate (32).

This increase is consistent with Korkmaz et al. (2018), Wei et al. (2017), and Svendsen et al. (2016) conducted on adult patients and healthy people after acute and chronic exercise training (33). In contrast, some research has reported a decline in NLR after acute training in obese adults (17) and MS patients (16).

Decreases in NLR and PLR have been shown to be helpful predictors of diabetes. As in previous studies, NLR and PLR have been

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shown to indicate chronic inflammation in diabetes (34).

The current study has several strengths. First, given the hormonal effects of puberty, the Tanner scale was used and participants in the control and exercise group were evenly distributed by tanner stages. Second, the study used Pilates trainings, which are low-cost, healthy, and safe, especially for children. They also need no specific instrument and are readily available everywhere.

The most important limitation was that the voluntary sampling technique was applied. This may limit the reliability of the results. Also, we described only inflammatory biomarkers in the present study. Future research should examine other inflammatory biomarkers among participants with type 1 diabetes.

Future research needs to use random sampling with bigger sample sizes. Nonetheless, the participants of both groups were selected homogeneously. The participants enrolled in the study already had seemingly very good glucose control. Furthermore, to improve effectiveness, exercise training should be longer.

Conclusions

In general, it can be said that given the significance of controlling T1D in children and preventing its future consequences, concurrent training at a moderate rate three days a week is an effective strategy to improve NLR and PLR and reduce the future complications of the disease. In addition, it is recommended that these patients run exercise training at a higher intensity and duration to further improve its effectiveness.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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