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Effect of concurrent training on telomere length in patients with myocardial infarction: Randomised clinical trial of cardiac rehabilitation

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ABSTRACT

Telomeres are structures at the ends of DNA and protect chromosome ends. The length of DNA telomeres is an important parameter of the proliferative potential of tissues. Shorter telomeres have been seen in myocardial infarction patients. TRF1 (Telomeric repeat binding factor 1) and TRF2 (Telomeric repeat binding factor 2) are the most important Shelterin complex proteins. The purpose of this study was to investigate the effect of concurrent training on telomere length in Patients with myocardial infarction. In this Quasi-experimental pre-post intervention study, twenty male patients from Taleghani Hospital selected and randomly assigned to the training and control group. Training protocol was eight weeks of concurrent training, 3 times per week. Blood samples were taken a half hour before the first training session and 24 h after last training session. The findings of the present study revealed that concurrent training prevents telomere shortening ($p = 0.001$) and increases telomerase activity ($p = 0.002$). In addition, there was a significant increase in TFR1 and TFR2 levels after training in patients with myocardial infarction ($p = 0.05$ and $p = 0.001$, respectively). Therefore, it seems that Eight weeks of concurrent training favorably affected telomere biology in Patients with myocardial infarction. Hence, concurrent training exercise is suggested during rehabilitation for myocardial infracted patients.

1. Introduction

The myocardial infarction causes sudden loss of contractile cells of the heart muscle or cardiomyocytes and is replaced by scar tissue. The survived cardiomyocytes undergo hypertrophy and as a result, the heart suffers remodeling. These adaptive mechanisms are dangerous in the long run and ultimately lead to a heart failure. Therefore, it is imperative that contractile tissue of the heart be reconstructed after myocardial infarction and chronic heart failure (Barile et al., 2013).

Telomeres are DNA special protein structures that are found at the ends of chromosomes, protect the genome against nucleolytic degradation, unnecessary recombinant, inter-chromosomal restoration and fusion; thus, telomeres play an important role in the preservation of genome information. It has been seen that telomere is shorter in patients with myocardial infarction. Telomerase and Shelterin complex have a vital role in regulating telomere length. TRF1 (Telomeric repeat binding factor 1) and TRF2 (Telomeric repeat binding factor 2) are two of the most important Shelterin complex proteins (Ludlow et al., 2013). Telomere length is affected by many internal and external factors. Therefore, the factors which have a positive effect on increasing or maintaining telomere length can have a role in improving many diseases, including myocardial infarction. Some studies have reported that exercise training has an effect on telomere biology (Puterman et al., 2010; Osthus et al., 2012). Hence, in the current study, we tested the hypothesis that concurrent training affect telomere length in patients with myocardial infarction.

Abbreviations: TRF1, Telomeric repeat binding factor 1; TRF2, Telomeric repeat binding factor 2; ELISA, Enzyme Linked Immunosorbent Assay; QTD, Quantitative Telomerase Detection; qPCR, quantitative polymerase chain reaction; SCG, single-copy gene.

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2. Materials and methods

2.1. Participants

This study was a Quasi-experimental clinical trial that uses a control group and pre-test that Conducted at The Sports Medicine Center of Tehran's Taleghani Hospital. Out of the patients with myocardial infarction admitted to Tehran's Taleghani Hospital from November 2014 to January 2015, 20 Volunteer patients having inclusion criteria were selected and by simple random, divided into experimental ($n = 10$) and control ($n = 10$) groups (Table 1). After familiarizing the subjects with work method, personal information forms, medical history and written consent were filled out. Then, their height and weight were measured. The exclusion criteria were: unstable pectoral angina, decompensated heart failure, ventricular arrhythmia, orthopedic

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Table 1
Sporting characteristics and demographic data of subjects in training and control groups.

Variable	Training group	Control group
Sex	Male	Male
Age (year)	57.3 ± 5.56	58.4 ± 5.44
Height (cm)	172.2 ± 5.20	173.4 ± 5.57
Weight (kg)	76.9 ± 8.10	78.1 ± 7.20
BMI (kg/m^2)	25.9 ± 1.40	26.1 ± 1.60
TG (mg/dL)	142.5 ± 10.8	144.1 ± 12.4
LDL-C (mg/dL)	121.4 ± 8.4	120.6 ± 11.5
HDL-C (mg/dL)	36.8 ± 4.1	37.1 ± 2.7
SBP (mmHg)	132.5 ± 12.8	130.2 ± 10.2
DBP (mmHg)	78.6 ± 4.6	79.2 ± 5.1
WHR	94.3 ± 6.2	95.5 ± 2.1

TG: Triglycerides, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, WHR: waist-to-hip ratio.

problems. Inclusion criteria were: passing four to eight weeks since the occurrence of myocardial infarction, stable of patient's condition, quitting smoking and alcohol during the study. The patients were informed about the possible dangers of the current study. The study was carried out under the auspices of Ethics Committee of Tehran's Taleghani Hospital.

2.2. Training protocol

The training group performed an 8-week exercise training protocol in three sessions per week. Overall training protocol took from January 2015 to Mars 2015. The training program was in a way that initially subjects began to warm up for 5 min through doing a slow walk. Then, in order to improve individuals' muscular endurance, resistance training was carried out using free weight and machine with intensity equivalent to 13 RPE (<30% of one maximum repetition, 5 to 10 reps, and 1 to 3 sets). In this section, muscles of the hands, shoulder girdle and legs were exercised using combinational and multiple joints movements. It was tried, the intensity of resistance training gradually reach to 15 ≥ RPE (50 to 60% of one maximum repetition, 8 to 15 repetitions, 1 to 3 sets). Then, aerobic training were carried out for 25 to 35 min, such that subjects' VO_{2peak} was determined using the modified Bruce protocol. The subjects exercised with an intensity equal to 50–60% of VO_{2peak} (12 to 13 RPE, 60% of maximum heart rate) by an ergometer bicycle and treadmill afterwards. An ergometer bicycle was used early in the training program, due to the prevention of further orthopedic injuries and treadmill was rather used at the end of the program. But in general, exercise by bicycle or treadmill ratio depended on the individual's sporting health. It was tried, the intensity of aerobic training gradually reach to 80% of VO_{2peak} (15 to 16 RPE, 90% of maximum heart rate). Then, the subjects performed cool down for 5 min (Fletcher et al., 2013; Pescatello et al., 2014). From the moment starting the exercise until the end of the exercise session, the subjects were closely monitored by the cardiac monitoring. The subjects in the control group continued their daily lives during this period.

2.3. Blood sampling

The first blood samples were taken half an hour before the start of the training and the second blood samples were taken 24 h after the last training session. In every blood collection, 10 mL of blood from the antecubital vein of the subjects' arm were taken into the test tube containing anticoagulant (EDTA).

2.4. PBMC isolation

Prior to isolation of the PBMC via Ficoll gradient (density 1.077), the blood samples diluted 1:1 with room-temperature normal saline (0.9% w/v). A half equal volume of Ficoll added directly to the bottom of the tube and the diluted blood gently, on top of the Ficoll via pipette. Then the samples Centrifuged at 800 RCF for 20 min. The PBMCs formed a distinct layer above the Ficoll and under plasma.

2.5. TRF-1 and TRF-2 determination

After cell lysis and supernatant preparation, TRF-1 and TRF-2 levels were measured using Enzyme Linked Immunosorbent Assay (ELISA) method. Human TRF-1 ELISA kit and TRF-2 ELISA kits prepared from Cusabio Biotech, Wuhan, China. The assay sensitivity and precision for TRF-1 and TRF-2 were 9.7 pg/mL, 14 pg/mL, 7.1% and 6.6% respectively. In addition, total protein of the samples were assayed too (Bradford total protein assay kit, ZellBio GmbH, Ulm, Germany), and the content of TRF-1 and TRF-2 expressed based on pg/mg protein.

2.6. Telomerase activity

In this study, telomerase activity was determined using a real-time polymerase chain reaction (qPCR) assay (Quantitative Telomerase Detection [QTD] kit, US Biomax) according to the manufacturer's instructions. The blood sample PBMC was isolated and lysed, the lysate was centrifuged for 30 min at 12,000*g* and 4 °C, and the supernatant kept at −80 °C for sensitive protein content determination (Bradford method). The extracted sample used as a template for the qPCR assay. The increase in fluorescence caused by the SYBR Green I dye binding to the double-stranded DNA monitored for a direct detection of the PCR product. Briefly, the qPCR master mix comprised 12.5 μL of the QTD premix containing telomere primers, 1 μL of PBMC extract, and heat-inactivated extracts (negative control) and with water for a final volume of 25 μL. Forty cycles with 20 min at 25 °C, 10 min at 95 °C, 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, was the qPCR program. Data collections were performed at three steps: during annealing, extension, and during melt curve analysis. For estimation of telomerase activity, a positive control used to produce a standard curve, consisting of six serial dilutions. The analysis of samples consisted of extracted sample with and without heat-treated.

2.7. Telomere length

To measure the telomere length, peripheral blood mononuclear cells (PBMC) were used. The DNA from these cells was extracted using standard salting out- proteinase K method. The concentration and quality of the extracted DNA examined by Nanodrop (NanoDrop-2000) at wavelengths of 260 and 280 nm, and the ratio of the two wavelengths were used. Telomere length assessed by quantitative polymerase chain reaction (qPCR). Two PCR reactions were performed for each sample, the first reaction for telomeric DNA fragment and the second for its control gene, acid ribosomal phosphoprotein. The primers for the telomere PCR were *forward* [5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′] and *reverse* [5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′]. The primers for the single-copy gene (acid ribosomal phosphoprotein) PCR were 36B41 [5′CAGCAAGTGGGAAGGTGTAATCC-3′] and 36B42 [5′-CCCATTCTATCATCAACGGGTACAA-3′]. DNA telomeric length was calculated based on the ratio of telomere to control gene. Real Time PCR kit was SYBR® Green PCR Master Mix manufactured by Applied Biosystems (USA). Real Time PCR using specific primers for telomere and primer for 36B4 (acidic ribosomal protein-coding) as a single-copy gene (SCG). Briefly, the primers concentration was 100 nM and DNA concentration was 20 ng of DNA in the reaction. Temperature Cycle for the reaction was as follows: a 95 °C for 10 min, which followed by 40 cycles consisting of 95 °C for a minute and 60 °C for 15 s. After determining of CT related to telomere and 36B4, for telomere length, T/S calculated.