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Combined Training Improves the Expression Profile of Inflammation-associated Antimicrobial Peptides, MicroRNAs, and TLR-4 in Patients with Multiple Sclerosis

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ABSTRACT

Some antimicrobial peptides (AMPs), microRNAs (miRs), and Toll-like receptor 4 (TLR-4) are involved in autoimmune diseases, which may be affected by exercise training. The purpose of this study was to investigate the effect of an eight-week combined exercise training (aerobic and resistance) on the expression of inflammatory factors, including, human beta-defensin-2 (hBD-2), cathelicidin (LL-37), TLR-4, miR-23b, miR-155, and miR-326 in women with relapsing and remitting multiple sclerosis (RRMS), which has not been investigated yet.

Twenty-three women (20-40 years) with RRMS were randomized into the combined training (CT) and control (CON) groups. The CT group subjects completed eight weeks of supervised CT using a treadmill and stationary bicycle for aerobic exercise and weight machines for resistance exercise. The expression levels of hBD-2, LL-37, TLR-4, miR-23b, miR-155, and miR-326 were measured by real-time polymerase chain reaction (RT-PCR) at the baseline and end of the study.

Although the expression of hBD-2 and miR-23b decreased in both CT and CON groups, the reduction was lower in the CT group than in the CON group (p=0.001). The expression of LL-37 in the CT group remained unchanged, but that of the CON group increased; thus, the between-group difference was significant. Although the TLR-4, miR-155, and miR-326 expression increased in both groups compared to the baseline, the increase in the CT group was lower than the CON group.

Our results showed that the combined training might improve inflammatory symptoms by affecting the expression of some AMPs, miRs, and TLR-4 in patients with relapsing and remitting multiple sclerosis.

Keywords: Antimicrobial cationic peptides; Circuit-based exercise; Inflammation; Multiple sclerosis; MicroRNAs

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INTRODUCTION

Multiple sclerosis (MS) is associated with the

inflammatory demyelination of the central nervous system.[1] Some genetic and immunological factors contribute to the development and progression of MS. Moreover, the physical inactivity that is common among people with MS, as an environmental factor may have a significant role in the disease process.[2,3] Exercise training can have beneficial effects for patients with MS, and it may improve MS-related inflammation through the favorable changes in the antimicrobial peptides (AMPs), microRNAs (miRs), and Toll-like receptor 4 (TLR-4). In this regard, recently, it has been reported that the combined training (CT; aerobic, and resistance) has positive effects on muscle strength, endurance, some inflammatory factors, and fatty tissue in MS patients.[4-8]

Antimicrobial peptides have a significant role in the innate immune system and may contribute to neurodegenerative diseases.[9] Cathelicidin (LL-37) and human beta-defensin-2 (hBD-2) are two AMPs that serve as innate immunity components, they are considered next-generation antibiotics,[10,11] and their overexpression can be associated with autoimmune diseases.[12,13] Besides their antimicrobial activity, also these peptides are known as immune modulators.[10] hBD-2 acts as a primary immune factor within the central nervous system (CNS), and it is induced by pro-inflammatory stimuli or microorganisms.[9,14] Expression of the hBD-2 can be triggered by bacteria-derived molecules, immune system, cytokines such as interleukin (IL)-17, and damaged cells.[15] Excessive expression level of IL-17 has been detected in the brain in MS patients.[16] LL-37 may interact with the innate immune response.[17] LL-37 may trigger pro-inflammatory signals, and exposure to it can recruit inflammatory cells.[13] Considering the LL-37 sequence and lipopolysaccharides (LPS) exposure, the effects of LL-37 on TLR-4 can be pro-inflammatory.[18] TLR-4 plays a key role in pathogen recognition and activation of various inflammatory signaling pathways.[19] TLR-4 regulates the autoimmune responses, and its overexpression can result in the development of neurological diseases.[20]

Several miRNAs regulate Toll-like receptor (TLR) signaling by targeting MyD88,[21] and some autoimmune diseases are associated with the change in miRs.[22,23] MS patients have a dysregulated miRs, and even minor changes in the miRs expression may lead to significant alterations in gene expression.[24] The severity of MS is directly related to the expression levels of miR-155, miR-326, and miR-23b.[25-27] The up-regulation of miR-155 and miR-326 as pro-inflammatory miRs, and the down-regulation of miR-23b as anti-inflammatory miRs can lead to inflammation.[24] The miR-23b expression is remarkably low in MS-associated inflammatory lesions.[27,28] The miR-155 expression is up-regulated in the brain lesions in patients with MS and is involved in inflammation.[29-31] The miR-326 expression correlates with autoimmune diseases, and its expression level in peripheral blood mononuclear cells is significantly higher in the MS patients compared to healthy people and MS patients at the recovery stage.[25,32] TLR-4 expression is directly related to the miR-155 expression[33] and inversely related to the miR-23b expression.[21] Moreover, it has been reported that the expression levels of IL-17 are inversely associated with miR-23b expression and directly with miR-326.[25]

Although several studies reported that acute exercise (a single session) increases hBD-2[34,35] and LL-37,[34,36,37] no chronic (long-term) exercise training studies have been conducted regarding hBD-2 and LL-37. However, a study has shown that TLR-4 expression increases after a 2-week high-intensity interval training,[38] some studies have reported that the expression of TLR-4 decreases after several weeks of exercise training.[39-41] Moreover, a few studies have examined the changes of miRs in response to exercise training in patients and none in MS patients. Therefore, to the best of our knowledge, the effects of exercise training on the expression of antimicrobial peptides, microRNAs, and TLR-4 have not been studied on MS. Thus, the purpose of this study was to determine if an eight-week combined training would attenuate inflammation in women with relapsing-remitting multiple sclerosis (RRMS) by affecting the hBD-2, LL-37, TLR-4, miR-23b, miR-155, and miR-326.

MATERIALS AND METHODS

Participants

Ninety women patients with RRMS expressed an interest in participating in this study. Forty-eight volunteers did not meet the inclusion criteria, and 18 volunteers withdrew from the study due to personal reasons. After baseline testing, the subjects were stratified for Expanded Disability Status Scale (EDSS) score and age, and then randomly assigned to either a combined training (CT) group (n=12) or a control (CON) group (n=12). One subject of the CON group

dropped out due to personal reasons, and so 23 patients remained for the final analysis (CT group, n=12 and CON group, n=11) (Figure 1). The sample size was calculated based on the results of the effect of eight weeks of combined exercise training on women with multiple sclerosis.[7] An alpha error of 5%, a beta error of 90%, and an effect size of 0.40 were considered for study groups. According to these parameters and using G*Power software (Version 3.1.9.2), a total sample size of 20 subjects was calculated. The sample size was taken into account by 24 participants (12 subjects in each group) to accord with an anticipated 20% dropout rate.

The main inclusion criteria were: (a) confirmed diagnosis of RRMS, (b) an EDSS of 1-4, (c) no corticosteroid therapy within the last three months, (d) ability to participate in the exercise, (e) no exercise training within the last six months, and (f) the age range of 20-40 years. The exclusion main criteria were: (a) exacerbation of MS and (b) corticosteroid therapy during the study period.

All participants gave their informed consent before their inclusion in the study. They were informed of the benefits and risks of the investigation before signing consent. This study was approved by the research ethics committee of the Sport Sciences Research Institute (IR.SSRC.REC.1398.071). Furthermore, we have registered this study in the Iranian Registry of Clinical Trials (IRCT20191006045004N1).

Combined Training Protocol

The CT group completed four supervised exercise sessions per week (three aerobic and one-resistance sessions) for eight weeks (Table 1). The CON group did not perform any exercise during the study period. All training sessions were conducted in the morning at 22 to 24°C and relative humidity of 55% to 65%.[5] Exercise intensity was monitored according to the exercise protocol using ratings of perceived exertion (very light=9 to moderate=13).

Aerobic Exercise

Participants walked/ran on a treadmill and/or pedaled on a stationary bicycle at an intensity level of 40 (first week) to 70 (eighth week) percent of maximum heart rate (HR_{max})[6,7] (Table 1). The HR_{max} was estimated using the equation: HR_{max}=220–age and was applied to determine the intensity of aerobic exercise for each session. Heart rate was monitored; using a Polar heart rate monitor (RCX5, Kempele, Finland).

Resistance Exercise

Participants performed resistance exercise (knee flexion and extension) at an intensity level of 50 (first week) to 70 (eighth week) percent of one repetition maximum using an exercise machine[6] (Table 1).

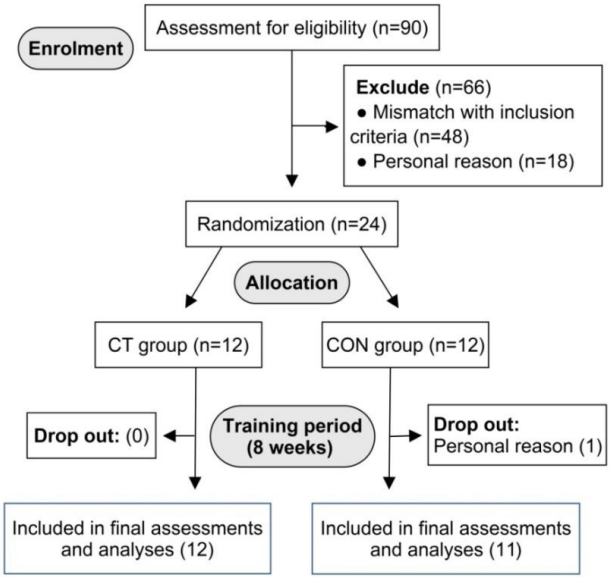

Figure 1. Flow chart of the study. CT: combined training; CON: control.

Table 1. Combined training protocol

Weeks	Aerobic exercise			Resistance exercise		
	Cycling†	Rest (min)	Walking on treadmill†	Knee extension‡	Rest (min)	Knee flexion
1	10 min at 40%	10	10 min at 40%	3×10, 50%	5	3×10, 50%
2	10 min at 50%	10	10 min at 50%	3×10, 55%	5	3×10, 55%
3	15 min at 50%	10	15 min at 50%	3×10, 60%	5	3×10, 60%
4	15 min at 55%	10	15 min at 55%	3×10, 60%	5	3×10, 60%
5	15 min at 55%	10	15 min at 55%	3×10, 65%	5	3×10, 65%
6	20 min at 55%	10	20 min at 55%	3×10, 65%	5	3×10, 65%
7	20 min at 60%	10	20 min at 60%	3×10, 70%	5	3×10, 70%
8	20 min at 70%	10	20 min at 70%	3×10, 70%	5	3×10, 70%

†Intensity is expressed as a percentage of maximal heart rate (HR_{max}). ‡Intensity is expressed as a percentage of one-repetition maximum (1RM).

Anthropometric Measurements, Blood Analysis, and EDSS Evaluation

Subjects completed a standard anthropometric assessment of height, weight, and BMI. Blood samples (3 mL) were obtained from the antecubital vein at baseline and 48 hours after the last exercise session using standard procedures. EDSS was scored by our colleague (Dr. Nahid Jivad) as a neurologist with experience in MS, before and after the eight-week exercise program.

Evaluation of mRNA and MicroRNA Expression Levels

The expression levels of mRNAs and miRs were measured; using the real-time polymerase chain reaction (RT-PCR) technique following RNA extraction and cDNA synthesis. Total RNA extraction was performed using Iraizol extraction kits (RNA Biotech, Co, Isfahan, Iran) according to the manufacturer's manual. Briefly, 1 mL of RNA extraction buffer (Iraizol, RNA Biotech, Co, Isfahan, Iran) was added to 700 µL of the blood sample. The mixture then incubated at room temperature for 5 minutes, followed by the addition of 200 µL chloroform (Merck, Germany) before being vigorously shaken for 10 to 15 seconds. The mixture then was incubated at room temperature for 5 minutes, followed by 2 minutes centrifugation at 1000 rpm at 4°C, which resulted in the formation of two separate layers. The top clear layer which contained RNA was carefully separated and transferred to a nuclease-free tube followed by the addition of 1000 µL of 100% ice-cold ethanol (Merck, Germany) and incubation at -20°C for 15-20 minutes. Finally, the tube was removed from the freezer and centrifuged for 8 minutes at 1000 rpm at 4°C. The supernatant was then discarded and 50 µL of RNase-free water was added to the column followed by 1-minute centrifugation at 10,000 rpm. The obtained solution contained extracted RNA. The RNA concentration and purity were assessed by measuring 260/280 nm absorbance on a nano spectrophotometer (Epoc, Biotech, USA). The absorbance ratios were between 1.6 and 1.9 for all RNA samples, which then were stored immediately at -80˚C until further use.

cDNA Synthesis

cDNA was synthesized using the RB MMLV Reverse Transcriptase Kit (RNA biotech, CO, Isfahan, Iran) according to the manufacturer's instructions. This consisted of mixing 0.5µg RNA and 2 µM Oligo (dT) Primer in a 0.25 ml nuclease-free Eppendorf tube. Then, DEPC-Treated water was used to bring the volume up to 10 µL. The mixture was then gently mixed and heated at +65˚C for 10 minutes in a Biorad technology thermocycler (USA). Eppendorf tubes were placed rapidly on ice for 8 to 10 minutes. Followingly, 4 µL RT buffer (5X) and 1 µL (200 unit) reverse transcriptase enzyme were added to each tube and mixed gently by pipetting up and down. Samples were shortly spun and finally incubated at +50˚C for 50 min followed by a 15-minute incubation at +72˚C to stop the cDNA synthesis reaction. The obtained cDNAs then were stored at -20˚C until further use.

Primer Design

The primers targeting hBD-2, LL-37, TLR-4, mir-155, miR-326, and miR-23b were designed using Oligo software. A BLAST search was carried out for each primer (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to check for sequence specificity. Moreover, the cDNAs encoding GAPDH and miR-U6 were used as the internal control for the normalization of mRNAs and miRs expression in RT-PCR, respectively (Table 2).

All experiments were performed in biological triplicate and technical duplicate. For each sample, 12.5 µl RB s3p SYBR Green Master Mix (2X) (RNA biotech, Co, Isfahan, Iran) was added to 10 pmol of each forward and reverse primer (Table 2) and 100 ng cDNA, and the final volume was increased up to 25 µL by the addition of ddH_2O. Finally, nuclease-free water was used to reach the desired volume. This mixed in sterile 0.25 mL microtubes (Applied Biosystems, USA) and then vortexed and gently centrifuged for 1 minute in a bench centrifuge. For each sample, 25 µL of the above mixture was transferred in triplicate into 0.1 mL RT-PCR microtubes (Applied Biosystems, USA), which then centrifuged before being loaded into a Rotor-Gene 6000 RT-PCR machine (Corbett, USA). The RT-PCR conditions were as follows: 95°C for 10 minutes (Hot start) followed by 45 cycles of 95°C for 10 seconds (Denaturation), 60°C for 15 seconds (Annealing/ extension) 72°C for 20 seconds.

Table 2. The primer sequences of LL-37, hBD-2, TLR-4, miR-155, miR-23b, miR-326, and internal controls (GAPDH and miR-U6)

Name	Forward	Reverse	Data Base
LL-37	GTGACTTCAAGAAGGACGGG-3	5-GGGTAGGGCACACACTAGGA-3	NCBI
hBD-2	GGTGAAGCTCCCAGCCATCA	TATCTTTGGACACCATAGTT	NCBI
TLR-4	CAGAGTTGCTTTCAATGGCATC	AGACTGTAATCAAGAACCTGGAGG	NCBI
GAPDH	TGAAGGTCGGAGTCAACGGATTTGGT	CATGTGGGCCATGAGGTCCACCAC	NCBI
miR-155	UUAAUGCUAAUCGUGAUAGGGGUU	-	miRBase
miR-23b	AUCACAUUGCCAGGGAUUACCAC	-	miRBase
miR-326	CCUCUGGGCCCUUCCUCCAG	-	miRBase
miR-U6	GCGCGTCGTGAAGCGTTC	GTGCAGGGTCCGAGGT	miRBase

LL-37: cathelicidin; hBD-2: human beta-defensin-2; TLR-4: Toll-like receptor 4; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; NCBI: National Center for Biotechnology Information.

Statistical Analysis

To evaluate the expression levels of cDNAs, the RT-PCR results were analyzed; using LinRegPCR software. The Ct values of cDNAs were obtained followed by calculation of ΔΔCt and $2^{-\Delta\Delta ct}$ for all of the samples.

The Shapiro–Wilk test was used to determine the normality of the data. A two-way analysis of variance (ANOVA) with repeated measures [group (CON group and CT group) × time (before and after 8 weeks)] was used to analyze the data. When a significant main effect was found, paired t-tests were used for post-hoc comparisons. Mann–Whitney U and Wilcoxon tests were used when the data didn't have a normal distribution (BMI and EDSS). The body weight data were analyzed by independent t-tests. Data were analyzed; using IBM SPSS Statistics 25 and are expressed as mean± standard deviation (SD). Statistical significance was set at $p<0.05$. The graphs were plotted using Microsoft excel.

RESULTS

Bodyweight significantly decreased ($p=0.005$) in the CT group (from 62.21±9.15 to 61.28±9.07 kg), and significantly increased ($p=0.001$) in the CON group (from 66.07±8.69 to 67.09±9.25 kg), so there was a significant between-group difference ($p=0.001$) (Table

3). No significant between-group differences were observed in BMI (p=0.215), after eight weeks of CT (Table 3). EDSS significantly decreased (p=0.023) in the CT group (from 2.25±1.22 to 1.83±1.09 score), but did not change in the CON group significantly (p=0.414), and there was a significant difference (p=0.015) between groups (Table 3).

Table 3. The effects of eight weeks of combined training on weight, BMI and EDSS

	CON group		CT group	
	Pre (n=11)	**Post (n=11)**	**Pre (n=12)**	**Post (n=12)**
Age (years)	33.18±4.67	-	33.00±5.99	-
Height (cm)	164.09±5.03	-	163.00±3.33	-
Weight (kg)[#]	66.07±8.69	67.09±9.25[*]	62.21±9.15	61.28±9.07[*]
BMI (kg/m^2)	24.60±3.60	24.98±3.80	23.40±3.33	23.06±3.32
EDSS (score)[#]	2±0.95	2.09±1.02	2.25±1.22	1.83±1.09[*]

Values are presented as mean ± SD. CT: combined training; CON: control; Pre: before training intervention; Post: after training intervention; BMI: body mass index. EDSS: expanded disability status scale. [#]Significant difference between CT and CON groups (p<0.05). [*]Significant within-group differences (p<0.05).

hBD-2

The relative expression level of hBD-2 significantly decreased in both groups (CON group: p=0.001, CT group: p=0.001) compared to the base (from 7.64±1.35 to 4.38±1.18 in the CON group and from 9.73±1.58 to 6.48±1.11 in the CT group), but the reduction was lower (p=0.001) in the CT group (33.40%) than in the CON group (42.67%) after exercise intervention (Table 4 and Figure 2).

LL-37

The relative expression level of LL-37 in the CT group did not significantly change (p=0.982, 0.14% reduction) following eight weeks of CT; but significantly increased in the CON group compared to the baseline (p=0.001) (from 7.51±0.73 to 12.17±1.88). The between-group difference in LL-37 levels was significant (p=0.001), which attributed to a relative increase (62.05%) in LL-37 expression in the CON group (Table 4 and Figure 2).

TLR-4

The relative expression level of TLR-4 significantly increased in both groups (CON group: p=0.001, CT group: p=0.001) compared to the base (from 4.51±1.01 to 10.38±0.81 in the CON group and from 3.76±0.76 to 6.07±0.98 in the CT group), but the increase in the CT group (61.44%) was significantly (p=0.001) lower than in the CON group (130.15%), after eight weeks of CT (Table 4 and Figure 2).

MiR-23b

The relative expression level of miR-23b significantly decreased in both groups (CON group: p=0.001, CT group: p=0.001) compared to the base (from 8.38±2.42 to 2.66±1.11 in CON group and from 9.06±1.88 to 5.99±1.52 in the CT group), but the reduction was significantly (p=0.001) lower in the CT group (33.88%) than in the CON group (68.26%), after exercise intervention (Table 4 and Figure 2).

MiR-155

The relative expression level of miR-155 significantly increased in both groups (CON group: p=0.001, CT group: p=0.012) compared to the base (from 5.14±1.30 to 11.21±1.34 in the CON group and from 6.01±1.14 to 7.29±0.85 in the CT group), but the increase in the CT group (21.30%) was significantly (p=0.001) lower than in the CON group (118.09%), after eight weeks of CT (Table 4 and Figure 2).

MiR-326

The relative expression level of miR-326 significantly increased in both groups (CON group: p=0.001, CT group: p=0.001) compared to the base (from 3.21±0.76 to 11.64±2.87 in the CON group and from 3.75±1.04 to 6.03±1.35 in the CT group), but the increase in the CT group (60.80%) was significantly (p=0.001) lower than in the CON group (262.62%), after eight weeks of CT (Table 4 and Figure 2).

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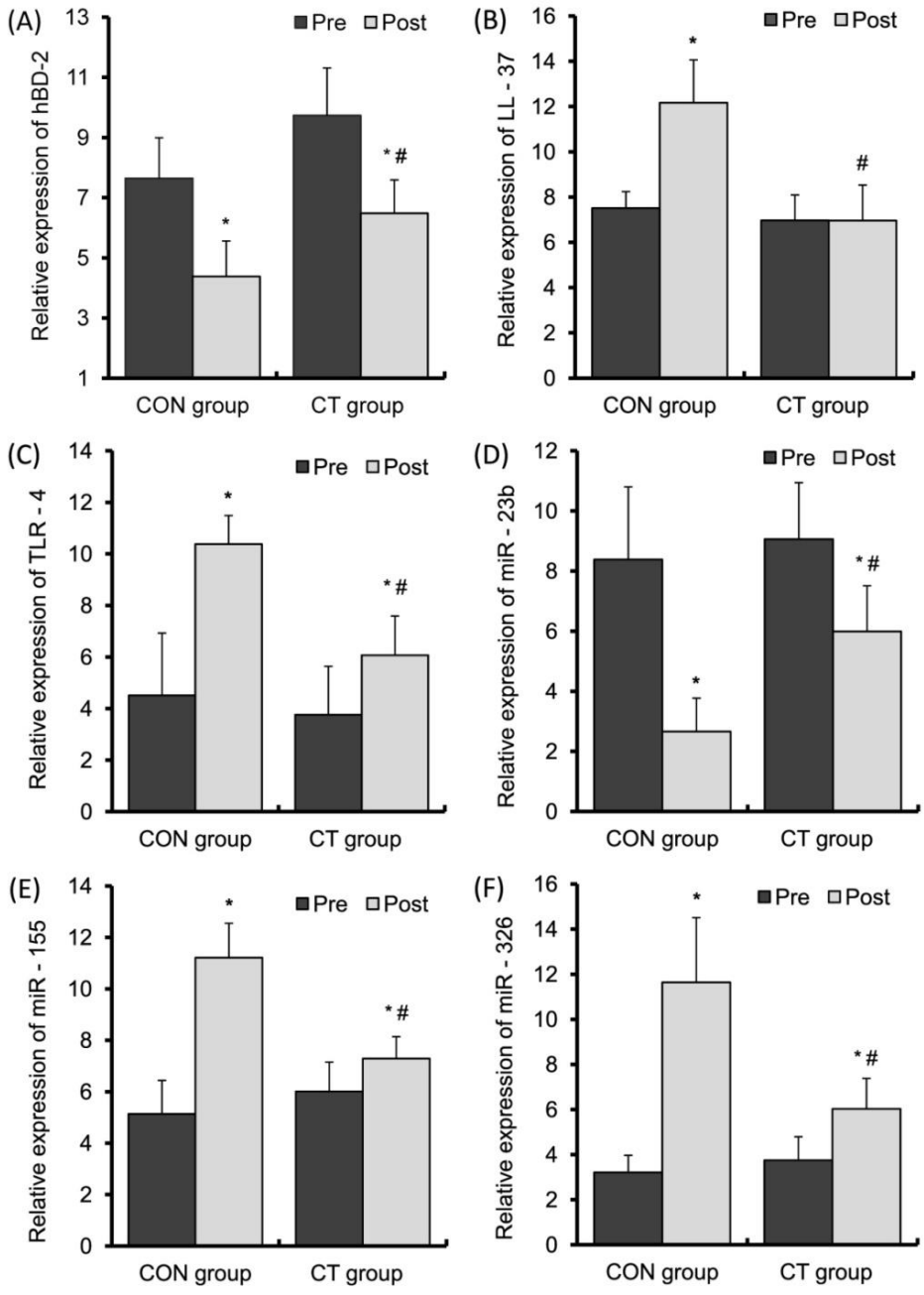

Figure 2. Relative expression changes in the hBD-2 (A), LL-37 (B), TLR-4 (C), miR-23b (D), miR-155 (E), and miR-326 (F) in RRMS patients after eight weeks of CT intervention. #A significant difference between the CT group and CON group (P<0.05). *Within-group significant differences (P<0.05). CT: combined training; CON: control; Pre: before training intervention; Post: after training intervention; hBD-2: human beta-defensin-2; LL-37: cathelicidin; TLR-4: Toll-like receptor 4; RRMS: relapsing and remitting multiple sclerosis.

Table 4. Relative expression levels of antimicrobial peptides and miRs in multiple sclerosis patients before and after 8 weeks of combined training

	CON group		CT group		Eta-squared
	Pre (n=11)	Post (n=11)	Pre (n=12)	Post (n=12)	(η2)
hBD – 2[#]	7.64±1.35	4.38±1.18[*]	9.73±1.58	6.48±1.11[*]	0.594
LL – 37[#]	7.51±0.73	12.17±1.88[*]	6.97±1.12	6.96±1.57	0.668
TLR – 4[#]	4.51±1.01	10.38±0.81[*]	3.76±0.76	6.07±0.98[*]	0772
miR – 23b[#]	8.38±2.42	2.66±1.11[*]	9.06±1.88	5.99±1.52[*]	0.430
miR – 155[#]	5.14±1.30	11.21±1.34[*]	6.01±1.14	7.29±0.85[*]	0.496
miR – 326[#]	3.21±0.76	11.64±2.87[*]	3.75±1.04	6.03±1.35[*]	0.523

Values are presented as mean ± SD. [#]Significant difference between CT and CON groups (p<0.05). [*]Significant within-group differences (p<0.05). CT: combined training; CON: control; Pre: before training intervention; Post: after training intervention; hBD-2: human beta-defensin-2; LL-37: cathelicidin; TLR-4: Toll-like receptor 4.

DISCUSSION

The present study investigated the effects of an eight-week CT on some antimicrobial peptides, microRNAs, and Toll-like receptor 4 in patients with RRMS. We found that the reduced expression of hBD-2 and miR-23b in the CT group was lower than the CON group; the LL-37 expression remained unchanged in the CT group (almost constant), but increased in the CON group; and the increased expression of TLR-4, miR-155, and miR-326 in the CT group was lower than the CON group. The unchanged expression of LL-37 and the low levels of hBD-2, miR-23b, TLR-4, miR-155, and miR-326 in the CT group, can be attributed to the effects of CT; however further studies are necessary to confirm these results.

Some studies have demonstrated that exercise training reduces systemic inflammation.[42,43] Moreover, acute exercise up-regulates pro-inflammatory genes, while anti-inflammatory genes balance the inflammatory response, and any disruption in this pathway may lead to chronic inflammation and induce various inflammatory diseases, including MS.[44,45]

Several studies have reported that hBD-2[34,35] and LL-37[34,36,37] have increased in response to acute exercise. However, no study has investigated the effects of chronic exercise training on hBD-2. Nevertheless, two studies have reported that the excessive expression of hBD-2 and LL-37 may be associated with autoimmune diseases.[12,13]

As far as we know, there is no report on the chronic effect of exercise on the LL-37. Only in a study, LL-37 expression acutely was up-regulated after an exercise session in the experienced cyclists.[46] It has been reported that 10 μg/mL of LL-37 during the differentiation of monocytes into macrophages induces a pro-inflammatory response.[13] Moreover, neutralizing the TLR-4 activation through lipopolysaccharide, down-modulating the responses of inflammatory cytokines, and preventing the invasion and inflammatory responses to pathogenic bacteria, are among the LL-37 strong anti-inflammatory effects.[13] Accordingly, the unchanged LL-37 expression levels in the present study (in the CT group), may be considered as an anti-inflammatory adaptation. It is worth noting that LL-37 affects the expression of TLR-4.[47] Therefore, the lower increase of the TLR-4 expression can correlate with the constant levels of LL-37 in the CT group.

A review article reported that regular exercise could reduce the expression level of TLR-4 in patients with MS.[1] In the present study, the TLR-4 expression in the CT group increased lower than that of the CON group. This result is consistent with some previously reported results, although their subjects and their exercise types differ from our study. In a study, the expression of TLR-4 decreased in inactive adults who were at high risk for type two diabetes (age, 52 years) after an 8-week (10 sessions) of either high-intensity interval training or moderate-intensity continuous training;[40] and also in two other studies, the monocyte TLR-4 decreased following the exercise training.[39,41] Contrary to our research, the TLR-4 increased after two weeks of high-intensity interval training.[38] TLRs act through the myeloid differentiation factor 88 (MyD88) or the Toll/IL-1R domain-containing IFN-inducing adapter

(TRIF) pathway that leads to the activation of downstream signaling pathways, and consequently, TLR activation results in the production of pro-inflammatory and IFN inducible genes.[1]

In a recent study, serum levels of miR-155 decreased after a 6-month Tai Chi intervention in patients with coronary heart disease (age, 63.61 years).[48] In another recent study, the serum miR-155 levels decreased following 12 weeks of high-intensity interval training in women with breast cancer (age, 49.2 years) who have elevated miR-155 levels.[49] These findings are consistent with our study. Recently, skeletal muscle miR-23b expression increased after four weeks of resistance training in recreationally active men (Age, 22.5 years), which, regardless of the type of subject, age, sex, and exercise training, are consistent with our results.[50] Dysregulation of miR-326 and miR-155 can lead to inflammation.[24] The injection of anti-miR-326 and anti-miR-155 can decrease the severity of experimental autoimmune encephalomyelitis (EAE), and overexpression of miR-23b reduces the disease severity.[24]

Interestingly, different TLRs, including TLR-4, up-regulate the expression levels of miR-155 in innate immune cells, especially in macrophages and dendritic cells.[22,26] Higher expression of TLR-4 in the CON group, compared with that of the CT group, could be associated with increased expression of miR-155 and decreased expression of miR-23b in the CON group; because TLR-4 expression is directly related to the miR-155 expression[33] and inversely related to the miR-23b expression.[21]

It has been reported that miR-23b plays a key role in the downstream signaling pathway of MyD88[21]. Increased expression of miR-23b leads to the inhibition of TAB2, TAB3, and IKK-α in this pathway, which ultimately leads to decreased levels of some inflammatory factors such as IL-17.[21,33]

Although we did not test the effect of CT on the IL-17 levels, it has been reported that the expression level of miR-23b inversely correlates with the expression of IL-17. In a study, the expression levels of IL-17 down-regulated after eight weeks of CT resulted in an anti-inflammatory effect.[7] The previous reports indicated that IL-17A inhibits the excessive expression of miR-23b by activating the NF-kB pathway.[27]

We found no studies about the potential effects of exercise training on miR-326. The up-regulated expression of miR-326 in EAE mice leads to more severe EAE, as well as the increased Th17 differentiation.[25] Moreover, a previous report has suggested that the expression of miR-326, in T cells, induces Th17 differentiation by targeting the Ets1 transcription factor (a factor involved in the negative regulation of differentiation into Th17).[25] It seems that exercise training may reduce the inhibition of Ets1 via down-regulation of the expression of miR-326 in RRMS patients, which consequently may lead to the tendency of Th17 to differentiation.

We found that the EDSS was significantly decreased in the CT group compared with the control group. Our result is consistent with a previous study by Golzari et al.[7] The within-group improvement in EDSS, in the experimental group, may have been due to weight loss and improved lower body strength in participants, although it seems that further research needs to be done in this area.

The effects of exercise training on the expression patterns of AMPs-associated miRs have not been previously investigated in patients with MS. Therefore, further studies are needed to be conducted to explore the mechanisms underlying the expression regulation of AMPs-associated miRs in patients with MS.

Although the sample size was calculated based on the results of a previous study,[7] the relatively larger sample size and longer duration of the intervention period can guarantee results in future studies.

Our results showed that the combined training might improve inflammatory symptoms by affecting the expression of some AMPs, microRNAs, and TLR-4 in patients with RRMS. Moreover, it seems that combined training may be used as a complementary therapeutic strategy to attenuate inflammation in MS patients with mild to moderate disability.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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