Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis

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A B S T R A C T

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder in which lymphocytic infiltration mediated mainly by pro-inflammatory cytokines. In this study, we examined the effect of combined exercise training on the levels of IFN-γ, IL-4 and IL-17 in the plasma and the supernatant of peripheral blood lymphocytes in women with multiple sclerosis. Expanded Disability Status Scale (EDSS), VO2max, muscle strength, and balance tests were obtained at baseline and post-treatment follow-up. Combined exercises training was designed for 24 sessions during 8 weeks. Each session was started with 5 min warm-up and was followed by 10 min stretch training, 20 min aerobic exercises and 20 min resistance–endurance training. The disability score was significantly decreased in test MS subjects after 8 weeks combined exercise training. Muscle strength and balance were increased significantly after the training program in test group. In this study, plasma, and peripheral blood mononuclear cell (PBMC) IL-17 and IFN-γ production was significantly decreased after 8 weeks combined exercise training. Our findings suggest that combined training has useful anti-inflammatory effects by decrease in PBMC and plasma IL-17 production.

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the brain and spinal cord in which lymphocytic infiltration leads to damage of myelin and axons [1]. MS is the most common neurological disorder in young adults, which has many implications for patients and society. It leads to substantial disability through deficits of sensation and of motor, autonomic, and neurocognitive function [1,2]. The cause of multiple sclerosis involves both genetic and environmental factors [3]. MS is mediated by auto-reactive T cells targeting myelin antigens in the central nervous system (CNS). MS is primarily an inflammatory disorder and this inflammatory process proposed to be mediated mainly by pro-inflammatory cytokines, chemokines and other mediators [4]. Understanding the mechanisms of cytokine-mediated CNS damage is necessary to develop new therapies that promote reversal of the MS pathology [5]. Clinical and animal studies have showed that MS is an autoimmune disorder caused by myelin-specific CD4 + T cells of the Th1 [1,6] and Th17 type [7–9]. Furthermore, anti-inflammatory cytokines of the Th2 type (e.g. IL-10, IL-4) have been associated with remissions and recovery from disease [1,10]. In contrast, Th1 and Th17 type cytokines (e.g. IL-1, IL-6, IL-12, IL-17, IFN-γ and TNF-β) are pro-inflammatory and play a key role in the pathogenesis of MS [11]. Several studies have indicated that exercise training can revert the chronic inflammation and its related pathologies. The anti-inflammatory effect of exercise training in chronic illnesses is mediated by decreased pro-inflammatory cytokine IL-6, IL-8, TNF-α, and IFN-γ [12,13] and an increase in anti-inflammatory IL-10 concentration [12–17]. The critical role of pro-inflammatory cytokines in the pathogenesis of MS make them a pivotal target for the therapeutic approach. In addition, aerobic exercise training can significantly reduce plasma inflammatory mediators such as CRP, IL-6, IL-18, and TNF-β [18]. Since studies on exercise and immunology highlight the potential of regular exercise as an anti-inflammatory therapy for patients with chronic inflammatory disease [19,20], exercise training may have therapeutic potential for the treatment of neuroinflammatory and neurodegenerative disease. Therefore, in this study we examined the effect of exercise training on the levels of cytokine IFN-γ, IL-4 and IL-17 in the plasma and the supernatant of peripheral blood lymphocytes.

2. Methods

2.1. Subjects

One hundred women from MS society of Iran and Sina clinic were introduced for this study. After explanation of the study to them, the volunteers recruited for selection according to inclusion/exclusion
criteria. Finally, 20 volunteers participated in this study and randomly divided to exercise group \( (n = 10) \) and control \( (n = 10) \) group.

2.2. Subject inclusion/exclusion criteria

Subjects were selected from patients in the age between 20 and 50, with clinically diagnosed relapsing remitting disease and expanded disability status scale (EDSS) of 0–4. Subjects who recently vaccinated and subjects with allergy, cancer, infection, or autoimmune disease other than MS excluded because this disease and related remediation affect immune parameters.

2.3. Exercise training protocol

Combined exercises were designed for 24 sessions during 8 weeks. Each session was started with 5 min warm-up and was followed by 10 min stretch training and 20 min aerobic exercises. Endurance and resistance training was started from 10 min in first sessions and gradually was increased to 20 min in the last sessions. Relaxation methods were used as the last part for 10 min. Patients did the exercises under the control of coaches and medicines. Before and after the training program EDSS was assessed by a neurologist specialized in the care of MS patients. Maximal oxygen uptake \((\text{VO}_{2\text{max}})\); It is measured as milliliters of oxygen used in 1 min/kg of body weight), muscle strength, balance and flexibility was measured by digital instruments. Experimental design is shown in Fig. 1.

2.4. Blood collection and plasma preparation

Twenty milliliters of blood were collected in heparinized tubes from patients by venous puncture before the beginning of the training program and after the last part of the training program. Ten ml was stored for PBMC isolation and 10 ml was used for plasma preparation. For plasma collection, blood samples were centrifuged at 3000 rpm for 20 min. The supernatants were transferred to sterile microtubes and were stored at \(-70 \, ^\circ\text{C}\) until cytokine analysis.

2.5. Isolation and culture of peripheral blood mononuclear cell (PBMC)

For PBMC isolation, each heparin containing blood tube was inverted gently and diluted 1:1 with HANK solution. After mixing carefully, diluted blood was added to same volume of Ficoll–Paque and was centrifuged at 1200 rpm for 20 min. Middle opaque layer was separated and was stored at \(-70 \, ^\circ\text{C}\) for 20 min. The supernatants were transferred to sterile microtubes and were stored at \(-70 \, ^\circ\text{C}\) until cytokine analysis.

2.6. Cytokine production analysis

IFN-γ, IL-4 and IL-17 production in cell supernatants and collected plasmas were measured using ELISA kits (KOMABIOTECH). All samples were tested in triplicate and the amount of cytokines was determined according to standard samples.

2.7. Statistical analysis

Data analysis was performed using SPSS 13.0. Paired sample \( t \)-test was used to assess cytokine production before and after the combined training program. Effectiveness of the exercise training was tested using repeated measures analysis of variance (ANOVA) with time (pre vs. post). A value of \( P < 0.05 \) was considered significant. All values are expressed as means ± SD.

3. Results

3.1. Subjects

There were no significant differences in weight and \( \text{VO}_{2\text{max}} \) pre and post exercise in the test group \( (P \leq 0.05) \) (Table 1). The disability score was significantly decreased in test MS subjects after 8 weeks combined exercise training \( (P < 0.043) \). Muscle strength \( (P < 0.02) \) and balance \( (P < 0.004) \) were increased significantly after the training program in test group. As it is appeared in Table 1, all parameters were assessed in MS control subjects who had no exercise training. In control subjects, no significant differences were observed in age, weight, EDSS, \( \text{VO}_{2\text{max}} \), muscle strength and balance.

3.2. PBMC cytokine

Supernatants of PBMC culture of all subjects were collected after 72 h for IFN-γ, IL-4 and IL-17 measurement. Three dilutions were prepared from each supernatant and cytokine were detected by ELISA assay in triplicate for each dilution. The mean of cytokine production of the subjects is reported pre- and post-combined training program as scatter plots in the Fig. 2. Mean supernatant IFN-γ production was decreased significantly \( (P < 0.05) \) from 597 ± 128.26 pg/ml pre-exercise to 426 ± 74.60 pg/ml post exercise in the test group. In spite of the increase in the IL-4 concentration of some subjects, no significant changes were observed in mean concentration of IL-4 before \( (287.40 ± 67.88 \, \text{pg/ml}) \)
and after \((299.50 \pm 50 \text{ pg/ml})\) exercise in test group. Supernatant IL-17 production was significantly \((P < 0.05)\) decreased from \(519.50 \pm 108.94 \text{ pg/ml}\) to \(232 \pm 84.85 \text{ pg/ml}\) after 8 weeks of combined training. In control MS subject group, no significant changes \((P \leq 0.05)\) were detected in mean concentration of INF-\(\gamma\), IL-4 and IL-17 after 8 weeks.

3.3. Plasma cytokine

INF-\(\gamma\), IL-4 and IL-17 cytokine concentration were assessed on plasma dilutions in triplicate. Mean concentration of the cytokines for both test and control MS subjects are reported as scatter plots in Fig. 3. INF-\(\gamma\) and IL-17 concentrations were changed significantly \((P < 0.05)\) after 8 weeks combined training in MS patients. In these cases, INF-\(\gamma\) and IL-17 were decreased from \(884.50 \pm 265.02 \text{ pg/ml}\) and \(147.60 \pm 21.17 \text{ pg/ml}\) respectively. No significant change was detected in plasma IL-4 concentration of MS subjects after the exercise program. There was no significant difference in cytokine concentration of control MS subjects who had no exercise training between weeks 0 and 8 of the experiment.

4. Discussion

Multiple sclerosis is an inflammatory demyelinating disease of the CNS that created by the imbalance between pro-inflammatory and anti-inflammatory cytokines [21]. There are unknowns about the etiology and pathogenesis of MS patients who have a complex of physiologic problems like increased incidence of osteoporosis, depression, fatigue, decreased aerobic capacity, and cardiovascular diseases. It seems that most of these problems are consequences of inactivity that also influence the quality of life of the patients [22]. In recent years, many studies cleared the beneficial effect of exercise on impaired physiological function, fitness, and quality of life of the patients [23]. In addition to physiological and psychological improvements, physical activity can promote inflammatory cytokine changes that modulate MS progression. Functional improvements in the rehabilitation of patients will clarify the necessity of application of exercise programs [24]. Despite the controversial effect, literatures showed that various exercise programs that differ in duration and intensity is tolerated in MS patients. Dalgas et al. evaluated the effect of acute-chronic exercise and resistance-endurance programs on MS subjects [22,25,26], but there is still limited experiences on combined training effect in multiple sclerosis. There is also no report on cytokine changes after combined training programs in MS patients. Conraads et al. have showed that combined endurance/resistance training in patients with chronic heart failure has an anti-inflammatory effect through significantly reduction of plasma TNF-\(\alpha\) receptor. The patients had a four month exercise program (three times/week), each session was consisted of 30 min resistance and 20 min endurance training [27]. Here we designed an 8 weeks combined exercise program for MS patients to study the effect of this program on IL-17 changes as the most important inflammatory cytokine in MS.

**Table 1**

<table>
<thead>
<tr>
<th>Age [year]</th>
<th>Weight (kg)</th>
<th>EDSS</th>
<th>VO(_2)max (mL/kg/min)</th>
<th>Muscle strength (kg)</th>
<th>Balance [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>32.15 ± 7.57</td>
<td>57.97 ± 20.06</td>
<td>2.14 ± 1.06*</td>
<td>33.84 ± 13.08</td>
<td>76.69 ± 36.98*</td>
</tr>
<tr>
<td>Post</td>
<td>32.15 ± 7.57</td>
<td>61.78 ± 10.36</td>
<td>1.65 ± 1.12</td>
<td>32.07 ± 12.05</td>
<td>120.87 ± 121.6</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>33.75 ± 8.18</td>
<td>64.42 ± 14.42</td>
<td>1.95 ± 1.06</td>
<td>32.00 ± 4.99</td>
<td>87.00 ± 57.88</td>
</tr>
<tr>
<td>Post</td>
<td>33.75 ± 8.18</td>
<td>64.15 ± 11.76</td>
<td>2.12 ± 1.24</td>
<td>35.00 ± 15.78</td>
<td>100.50 ± 77.96</td>
</tr>
</tbody>
</table>

EDSS, Expanded Disability Status Scale; VO\(_2\)max, maximal oxygen uptake. Values are means ± SD, *Significant difference after 8 weeks of combined training program in test group \((P < 0.05)\).

**Fig. 2.** IL-17, INF-\(\gamma\), and IL-4 production by PBMC at pre (week 0) and post (week 8) combined training program in MS patients (A–C). IL-17, INF-\(\gamma\), and IL-4 production by PBMC in control MS patients at weeks 0 and 8 of the experiment (D–E). * \(P < 0.05\) within groups.
progression [9,28]. The role of IL17 in pathogenesis of experimental allergic encephalomyelitis (EAE) has been detected in some studies [9,28,29], and the recent study indicated that IL-17 participates in the development of EAE by impairing the integrity of the blood-brain barrier [30]. Moreover, there is some evidence suggesting the role of IL-17 in MS patients. The expression of IL-17 mRNA and protein in perivascular lymphocytes as well as in astrocytes and oligodendrocytes has been detected in the active areas of MS lesions [31]. In addition, increased IL-17 expression in the peripheral blood mononuclear cells of patients has been detected during disease exacerbation [32]. Kebir et al. have showed that the endothelial cells express high levels of IL-17 receptors and are more permeable in response to IL-17, and promote CNS inflammation in MS patients [33]. A recent report indicated that the frequency of Th17 cells is significantly higher in the cerebrospinal fluid of patients with relapsing-remitting multiple sclerosis during relapse [34]. Graber et al. have showed that IL-17 and IL-6 production increased in PBMCs of transverse myelitis and MS patients [35]. In this study, we observed the significant decrease in plasma and PBMC IL-17 production after 8 weeks combined training. Our findings suggest that combined training has useful anti-inflammatory effects by decrease in PBMC and plasma IL-17 production and may affect astrocyte and CSF inflammatory cytokines. Gamma interferon as an important cytokine product of Th1 type response, can induce an inflammatory response and increase prior to relapse in MS patients [36]. Many studies showed that increase in IL-12 activate Th1 cells in MS subjects. IL-12 secretion correlate with clinical measures of disease activity (EDSS) that induce Th1 type inflammatory response [37]. IFN-γ therapy helps the reduction of inflammation by decrease in the number of IFN-γ secreting cell and production of IFN-γ by PBMCs [38]. Acute and chronic exercise has different effect on cytokine production. Castellano et al. have showed that after acute exercise (30 min, three times a week) plasma IL-6 and IFN-γ was decreased from the baseline during 3 h post-exercise, but after chronic exercise (8 weeks aerobic exercise) plasma IL-6 was decreased and plasma IFN-γ was increased significantly in MS subjects [39]. Our data present a significant decrease of IFN-γ concentration in plasma and PBMC of MS patients after 8 weeks combined training. According to inflammatory effect of IFN-γ, this exercise program has beneficial effect on MS subjects, especially if the IFN-γ cytokine change remains for a long time. Multiple sclerosis is an inflammatory disease that progress by Th17 and Th1 immune responses. Many studies have showed that boosting of Th2 response by IFN-γ acetate or other alternatives may oppose to Th1 and inflammatory response [40,41]. White et al. found that concentration of IL-4 as a Th2 cytokine was decreased in plasma after 8 weeks of resistance training [42]. Steensberg et al. showed that after prolonged strenuous exercise Th1 lymphocyte number was decreased while Th2 lymphocytes remained relatively unaltered [43]. Our data showed no changes in plasma and PBMC IL-4 cytokine changes in spite of significant decrease in IFN-γ. It seems that this exercise program duration and strength affected Th17 and Th1 responses more efficient than Th2 response. Suraka et al. have showed the beneficial effect of combined training on fitness of MS patients. This randomized controlled trial study demonstrated that after six month aerobic and strength exercises, motor fatigue decreased significantly in women patients [44]. In another randomized study walking speed of MS patients improved after 6 months combine training [45]. In the present study, we found the beneficial effect of combined training on the muscle strength and balance of MS patients. This change will improve their fitness and quality of life. Beside reduction in the inflammatory cytokines, reduction of clinical disability of MS subjects, EDSS, demonstrate the performance of combined training as an exercise program.

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References


