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Effect of Exercise, MitoQ, and Their Combination on Inflammatory and Gene Expression in Women with Multiple Sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system. Current treatments aim to manage symptoms and slow disease progression, but there is a need for effective interventions that target underlying disease mechanisms. In this study, we investigated the effects of exercise, MitoQ (a mitochondria-targeted antioxidant), and their combination on the gene expression of various biomarkers associated with MS in postmenopausal and premenopausal women.

We measured interleukin-6 (IL-6) and key molecular pathways involved in MS pathogenesis, including suppressor of mother against decapentaplegic 2 (SMAD2), signal transducer and activator of transcription 1 (STAT1), and transforming growth factor beta (TGF-β) using real-time polymerase chain reaction.

All interventions significantly lowered IL-6 levels and STAT1, especially in premenopausal women. Also, both exercise and MitoQ led to a significant increase in the SMAD2 and TGF-β expression, with a more pronounced effect on premenopausal women. Noteworthy, the effectiveness of the combination of exercise and MitoQ was considerably higher than each one alone.

These findings suggest that exercise and MitoQ, either alone or combined, can modulate various biological pathways implicated in MS pathogenesis.

Keywords: Exercise training; Inflammation; MitoQ; Menopause; Multiple sclerosis; Premenopause

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disease of the central nervous system that affects millions of people worldwide.[1] According to the National Multiple Sclerosis Society, approximately 2.8 million people globally are living with MS, with women being 2 to 3 times more likely to develop the disease than men.[2] Characterized by the immune system attacking the protective myelin sheath covering nerve fibers, MS leads to communication problems between the brain and the rest of the body.[3,4] The unpredictable nature of MS, coupled with its progressive course, poses significant challenges for patients, healthcare providers, and researchers alike.

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Inflammation and oxidative stress are pivotal in the pathogenesis of MS. The inflammatory response in MS is driven by autoreactive T cells that cross the blood-brain barrier, leading to demyelination and axonal damage.[5] Also, microglia and astrocytes contribute to the inflammatory milieu, perpetuating tissue damage.[6] In addition, there is solid evidence indicating that sex hormones, particularly estrogen and progesterone, play a significant role in modulating the immune response in women with MS. The hormonal fluctuations during menopause can also influence the inflammatory and oxidative stress pathways, thereby impacting the disease course.[7] Hence, understanding the interplay between inflammation and oxidative stress is crucial for developing therapeutic strategies aimed at mitigating the progression of MS.

The signal transducer and activator of the transcription 1 (STAT1) family plays a crucial role in relaying various cytokine-mediated signals, and disruptions in this signaling pathway are linked to the development of MS.[8] Likewise, interleukin-6 (IL-6) is a proinflammatory cytokine that plays a significant role in the immune response and is implicated in the pathogenesis of MS.[9] In MS, elevated levels of IL-6 are associated with increased inflammation and the activation of immune cells, which contribute to the demyelination and neurodegeneration characteristic of the disease.[10] On the other hand, transforming growth factor beta (TGF-β), a superfamily of cytokines and growth factors is integral to cell growth and differentiation within the immune system.[11,12] TGF-β signaling is particularly important for its anti-inflammatory properties.[13] Furthermore, TGF-β ligands transmit extracellular signals through SMAD2, which then activates downstream *TGF-β* gene transcription in the nucleus.[14,15] Studying these genes is vital, as understanding their roles in MS can provide insights into the underlying mechanisms of the disease and identify potential therapeutic targets.[16] Recently, the use of mitochondria-targeted antioxidant supplements, such as MitoQ and coenzyme Q10, which play essential roles in mitochondrial respiration, has shown promising results in patients with MS.[17,18] These compounds have been shown to reduce oxidative damage and inflammation by modulating immune responses.[19] Also, some studies suggest that MitoQ may enhance the function of immune cells, potentially improving their ability to respond to pathogens and reducing chronic inflammation. In addition, MitoQ may help protect neurons from oxidative damage, which is crucial in neurodegenerative diseases.[20] These properties of MitoQ can be particularly beneficial in conditions like MS, where inflammation and neurodegeneration play a significant role in disease progression. Moreover, aerobic exercise has been demonstrated to improve physical fitness, mobility, and overall quality of life in individuals with MS. It also exerts anti-inflammatory effects by modulating cytokine levels and enhancing antioxidant defenses.[21] Therefore, combining aerobic exercise with MitoQ supplementation may offer synergistic benefits in managing MS by addressing both inflammation and oxidative stress.[22]

MS is a complex disease influenced by various factors, including inflammation, oxidative stress, sex hormones, and gene expression. While previous research has explored the effects of exercise and MitoQ in animal models of MS,[23,24] the role of MitoQ in human subjects particularly in combination with exercise in both menopausal and premenopausal women has not been determined yet. This study aims to fill that gap by investigating the effects of exercise and MitoQ, alone and combined, in these 2 groups. To further elucidate the mechanisms underlying these interventions and their potential impact on the pathogenesis of MS, we measured a range of factors involved in inflammation, including IL-6, SMAD2, STAT1, and TGF-β in the participants.

MATERIALS AND METHODS

Study Population

The cross-sectional research was conducted at the MS Specialized Clinic in Shafa Hospital, located in Kerman, Iran, from June 5, 2023, to September 6, 2023. During this time, 108 patients who met the study's inclusion criteria and expressed interest in participating were chosen as participants. The criteria included the following: age between 18 and 65 years, an Expanded Disability Status Scale (EDSS) score of less than 6, being in the disease remission phase (at least 2 months after the last relapse), no acute attacks in the past 30 days, ongoing use of MS medications, and no consumption of illicit substances. The exclusion criteria for the study included individuals with liver and kidney diseases, cardiovascular diseases, diabetes, prominent visual impairments, significant musculoskeletal disorders or congenital abnormalities, specific diseases such as cancer, and mental disabilities.[24,25] The final

diagnosis of MS was made based on the reviewed McDonald criteria and confirmed by a neurologist.[26] The characteristics of the participants are briefly outlined in Table 1.

Table 1. Baseline demographics of the study participants

Parameter		Total (n=108)	Premenopause (n=60)	Menopause (n=48)	*p*
Individual indicators (mean ± SD)	Age (years)	44.6 ± 9.7	37.2 ± 5.8	53.8 ± 3.9	0.0001
	Weight (kg)	70.2 ± 10.5	69.2 ± 11.4	71.4 ± 7.9	0.310
	Height (cm)	163.6 ± 6.0	162.7 ± 6.1	164.7 ± 5.8	0.342
	BMI (kg/m^2)	25.1 ± 3.7	24.2 ± 4.1	26.3 ± 2.9	0.337
Marital status n (%)	Single	12 (11.1%)	12 (20.0%)	0 (0.0%)	0.063
	Married	83 (76.8%)	43 (71.7%)	40 (83.3%)	
	Divorced	3 (2.7%)	3 (5.0%)	0 (0.0%)	
	Unknown	10 (9.2%)	2 (3.3%)	8 (16.7%)	
Occupation n (%)	Housewife	57 (52.7%)	42 (70.0%)	15 (31.3%)	0.68
	Full-time	2 (1.8%)	2 (3.3%)	0 (0.0%)	
	Part-time	28 (25.9%)	14 (23.3%)	14 (29.2%)	
	Retired	18 (16.6%)	1 (1.7%)	17 (34.5%)	
	Unknown	3 (2.7%)	1 (1.7%)	2 (4.2%)	
EDSS (mean ± SD)		3.3 ± 0.8	2.8 ± 0.4	4.0 ± 0.6	0.068

SD: standard deviation; BMI: body mass index; EDSS: expanded disability status scale.

Study Design, Measurements, and Considerations

After initial measurements, 108 women diagnosed with relapsing-remitting (RRMS) and secondary progressive MS (SPMS) were selected. They were divided into 2 groups based on their menopausal status: menopausal (n=48) and premenopausal (n=60). Then, participants in each group were randomly assigned to 4 subgroups: control, MitoQ, exercise training, and a combination of MitoQ and exercise training. We followed the established guidelines and procedures for obtaining informed consent from the patients throughout this study. This included ensuring that participants clearly understood the study's purpose, risks, and benefits. We provided a detailed explanation of the study's methodology and objectives to the participants, and they completed and signed a written consent form afterward. Participants were asked to fill out a demographic information form.

MitoQ Supplementation

Participants in the intervention groups followed the guidelines provided by the MitoQ company (MitoQ, New Zealand) and took a single 20-mg capsule of MitoQ supplement on an empty stomach every day for 8 weeks.[27] The control groups received a placebo instead of the supplement. Both groups were provided with the designated number of MitoQ and placebo capsules weekly and were sent regular SMS reminders to ensure consistent consumption.

Exercise Training Protocol

The exercise program included 8 weeks of training, with each session lasting 30 minutes, 3 times a week. The training was conducted using a stationary bike ergometer (Leg ergometer; Monark, Sweden). The exercise intensity was set at 65% to 75% of the peak oxygen uptake (VO_2Peak), which was measured using the Åstrand 6-minute test. Each exercise session

consisted of a 5-minute warm-up, 20 minutes of main exercise training at the target exercise intensity and heart rate, and a 5-minute cool-down to reach 40% of the maximum heart rate. To design and adjust the exercise training intensity, we took VO_2Peak measurements before starting the exercise training and every 2 weeks during the exercise sessions. Finally, we conducted another VO_2Peak measurement after completing the exercise training program. We instructed participants to refrain from engaging in any other exercise training programs. Additionally, individuals continued their routine medical care throughout the exercise training regimen.[28,29] The study timeline is shown in Figure 1.

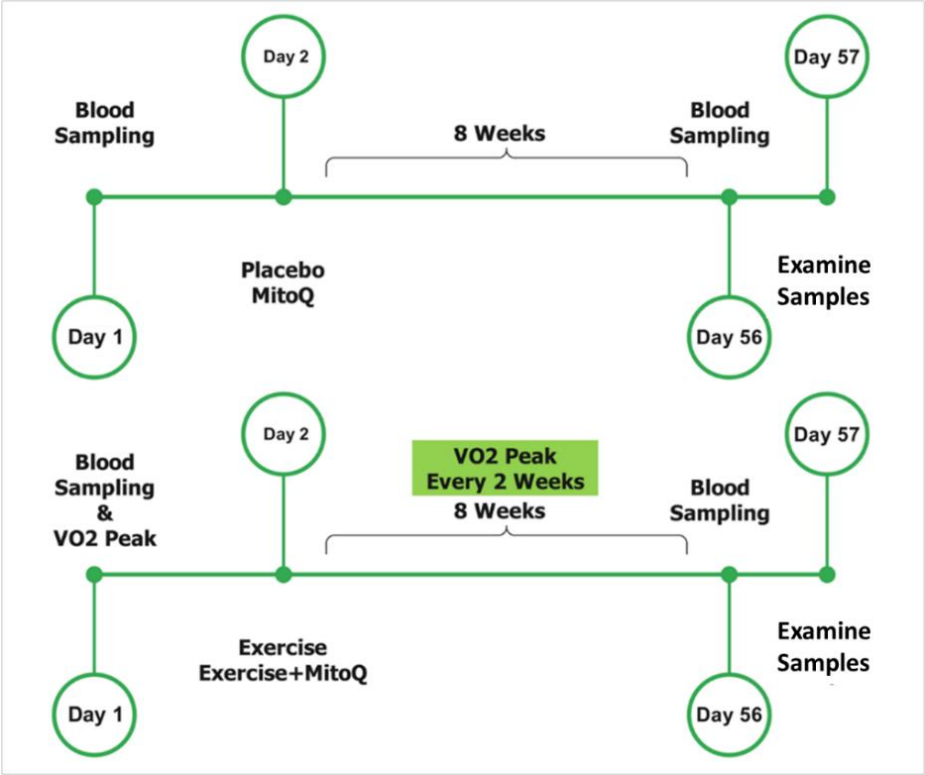

Figure 1. The study timeline illustrates the process and outlines the participation in interventions, with or without exercise. VO2 Peak: peak oxygen uptake

Collection of Blood Samples

Before and after the interventions, a blood sample (5 mL) was taken from the middle cubital vein. The collected samples were first allowed to clot for 30 minutes at room temperature. After that, they were centrifuged at 2200 rpm for 10 minutes to separate the serum. Finally, the serum was carefully stored at a temperature of −80°C.[30]

Gene Expression Assay

Gene expression levels were determined using quantitative polymerase chain reaction (qPCR). We followed the manufacturer's instructions to extract total RNA from the samples using TRIzol reagent (Sigma, MI, USA) and measured it using a NanoDrop spectrophotometer (Thermo Scientific, MA, USA). We used the Takara cDNA Kit and performed qPCR using the SYBR Green in the Corbett Rotorgene 3000 cycler system. Also, the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was applied as a housekeeping gene in this study. The primer and control gene sequences can be found in Supplementary Table 1. The expression of target genes was evaluated using the $2^{-\Delta\Delta CT}$ method, and we calculated ΔCT using the following formula:

$$\Delta CT = CT(target) - CT(control)$$

Statistical Analysis

Data analyses were conducted using SPSS version 22.00 and GraphPad Prism version 8.0. One-way analysis of variance (ANOVA) was employed to compare values within control and treatment groups. Two-way ANOVA was used to examine differences between menopause and premenopausal groups. Tukey's post-hoc tests were applied for pairwise comparisons between groups. A significance level of $p<0.05$ was adopted.

RESULTS

The impact of each treatment on the expression level of IL-6 in different study groups is shown in Figure 2. After undergoing treatment, all interventions resulted in a significant decrease in the IL-6 levels in both premenopausal and menopausal women ($p<0.001$). Additionally, there were significant differences in the effectiveness of exercise + MitoQ when compared to each treatment alone, in both study groups ($p<0.01$). Furthermore, our results indicated a markedly lower level of IL-6 in premenopausal women compared to menopausal women ($p<0.001$), indicating the higher effect of the interventions on premenopausal patients.

Changes in SMAD2 Levels

Figure 3 illustrates the impact of the different treatments on the expression level of *SMAD2* in various study groups. Following the interventions, the SMAD2 levels significantly increased in premenopausal and menopausal groups ($p<0.001$). It is worth mentioning that, in both groups, the combination of exercise and MitoQ was significantly more effective than each treatment alone ($p<0.05$). Additionally, our findings indicated a higher effect of the interventions on premenopausal participants than menopausal ones. The expression of SMAD2 in premenopausal women was significantly higher than in menopausal women in treated groups ($p<0.001$).

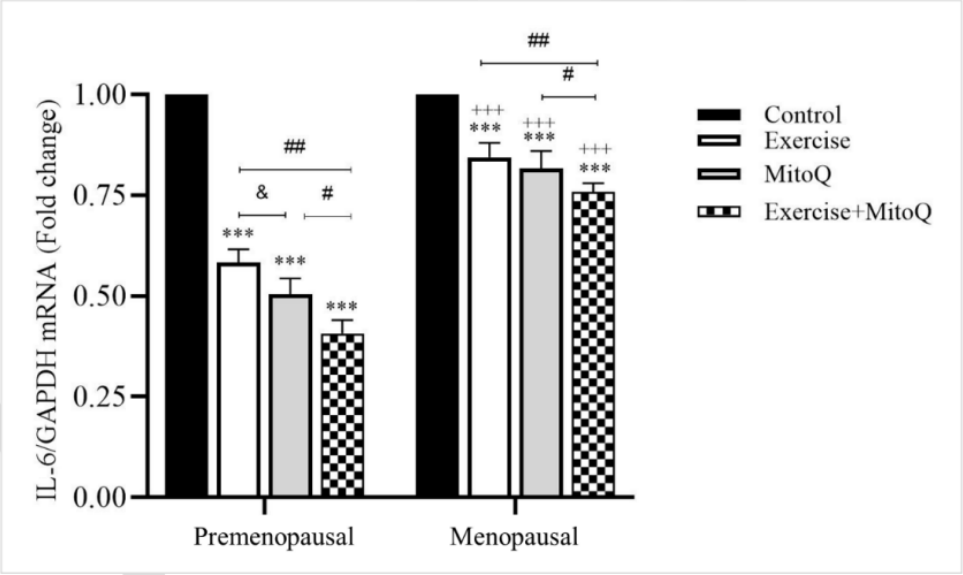

Figure 2. Changes in levels of *IL-6* (coding interleukin-6) gene expression in premenopausal and menopausal individuals in different treatment groups. Data are expressed as mean ± SD.

$^{***}p<0.001$ vs. control; $^{\#}p<0.05$ and $^{\#\#}p<0.01$ MitoQ or Exercise vs. Exercise + MitoQ; & $p<0.05$ Exercise vs. MitoQ; $^{+++}p<0.001$ premenopausal vs. menopausal; *GAPDH*: glyceraldehyde 3-phosphate dehydrogenase.

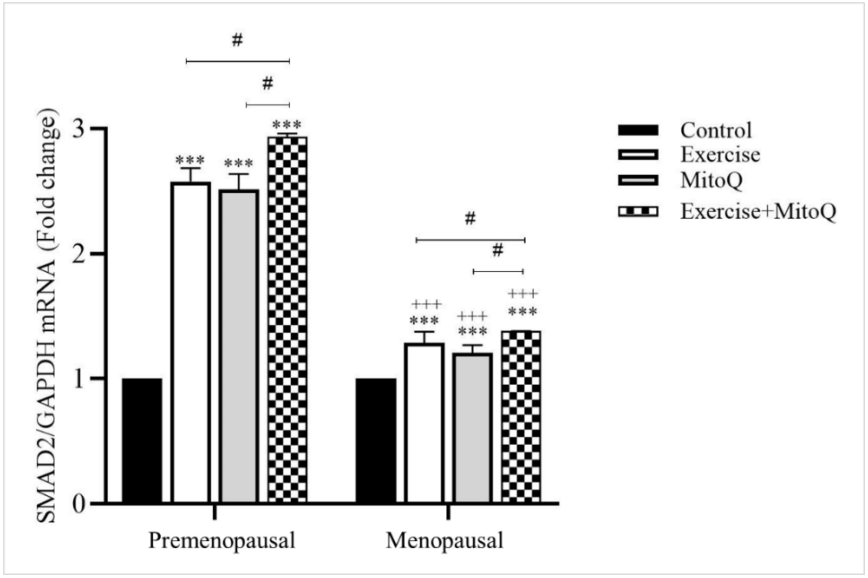

Figure 3. Changes in levels of *SMAD2* (small mothers against decapentaplegic family member 2) gene expression in premenopausal and menopausal individuals in different treatment groups. Data are expressed as mean ± SD.
$^{***}p<0.001$ *vs.* control; $^{\#}p<0.05$ MitoQ or Exercise vs. Exercise + MitoQ; $^{+++}p<0.001$ premenopausal vs. menopausal; *GAPDH*: glyceraldehyde 3-phosphate dehydrogenase.

Changes in STAT1 Levels

The effects of the different treatments on the expression level of STAT1 in various study groups are shown in Figure 4. Following the interventions, the *STAT1* levels significantly decreased in both groups ($p<0.001$). Noteworthy, in both groups, the combination of exercise and MitoQ was significantly more effective than each treatment alone ($p<0.01$). Moreover, our findings revealed a considerably higher effect of the interventions on premenopausal women than on menopausal ones. In treated groups, the expression of *STAT1* in premenopausal individuals was significantly lower than in menopausal women ($p<0.001$).

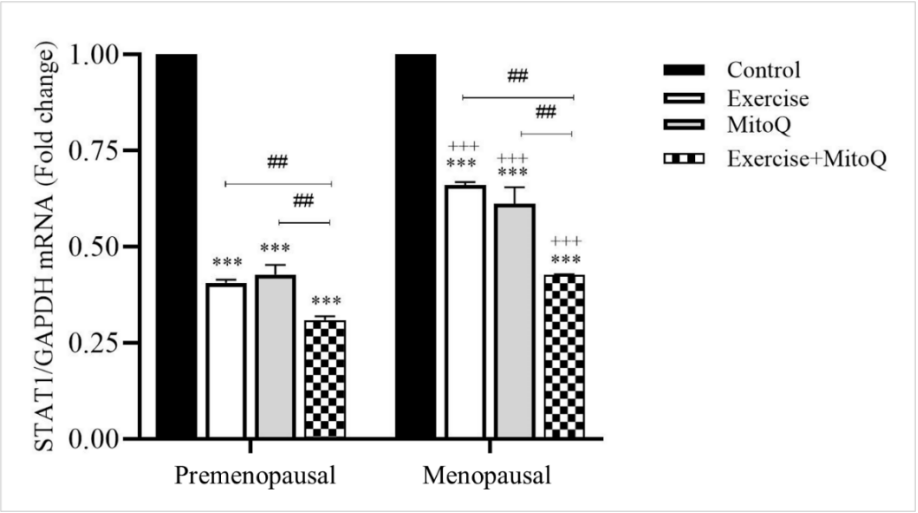

Figure 4. Changes in levels of *STAT1* (signal transducer and activator of transcription 1) gene expression in premenopausal and menopausal individuals in different treatment groups. Data are expressed as mean ± SD.
$^{***}p<0.001$ vs. control; $^{\#\#}p<0.01$ MitoQ or Exercise vs. Exercise + MitoQ; $^{+++}p<0.001$ premenopausal vs. menopausal; *GAPDH*: glyceraldehyde 3-phosphate dehydrogenase.

Changes in TGF-β Levels

Figure 5 illustrates the impact of the different treatments on the expression level of *TGF-β* gene in various study groups. After undergoing treatment, the TGF-β levels significantly increased in premenopausal and menopausal groups ($p<0.001$). It is worth mentioning that, in both groups, the combination of exercise and MitoQ was significantly more effective than each treatment alone ($p<0.05$). Importantly, our findings indicated a higher effect of the interventions on premenopausal participants than menopausal ones. In treated groups, the expression of *TGF-β* in premenopausal women was significantly higher than in menopausal women ($p<0.001$).

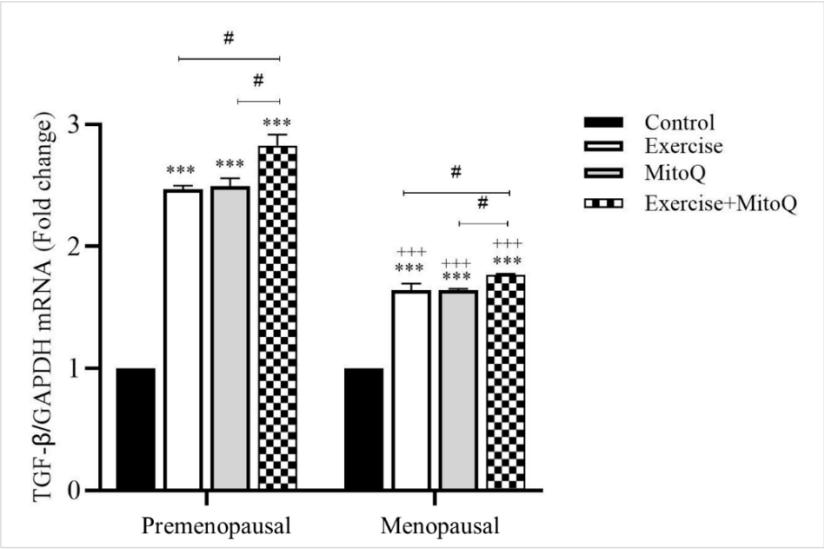

Figure 5. Changes in levels of *TGF-β* (transforming growth factor-beta) gene expression in premenopausal and menopausal individuals in different treatment groups. Data are expressed as mean ± SD.
*** $p<0.001$ vs. control; $^{\#}$ $p<0.05$ MitoQ or Exercise vs. Exercise + MitoQ; $^{+++}$ $p<0.001$ premenopausal vs. menopausal; *GAPDH*: glyceraldehyde 3-phosphate dehydrogenase.

DISCUSSION

In the context of women with MS, several interconnected factors have been identified that contribute to the disease process. These factors include IL-6,[9] SMAD2 and STAT1 transcription factors, and TGF-β signaling.[31] This study investigated the effects of exercise, MitoQ, and their combination on the above-mentioned factors in premenopausal and menopausal women with MS.

In our study, we observed that both exercise and MitoQ significantly reduced IL-6 levels in patients with MS, with a more pronounced decrease noted in premenopausal women compared to their menopausal counterparts. Elevated levels of IL-6 have been documented in both serum and cerebrospinal fluid of MS patients.[10] Supporting our findings, Mao and colleagues[32] demonstrated that treatment with MitoQ in experimental autoimmune encephalomyelitis (EAE) mice led to reductions in IL-6 and oxidative stress. Additionally, MitoQ has been shown to decrease the activation of microglia and astrocytes in traumatic brain injury models, potentially mitigating the release of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6.[33] In MS, oxidative stress and inflammation can damage myelin and neurons and aggravate this disease, so controlling these effectors is very important in the management of MS.[34]

In addition to MS, research has highlighted the potential benefits of MitoQ in various other disorders associated with the nervous system. For instance, McManus et al (2011)[35] reported that MitoQ effectively prevented the onset of cognitive deficits in a female mouse model of Alzheimer's disease. Additionally, oral administration of MitoQ in mice with amyotrophic lateral sclerosis resulted in modifications to the disease

symptoms.[36] In experimental models of Parkinson's disease, MitoQ was shown to inhibit neuronal loss.[37] Furthermore, this compound reduced neuronal damage in mice with Huntington's disease.[38]

Also in line with our findings, it has been revealed that exercise can suppress IL-6 production in the central nervous system, thereby inhibiting microglial activation.[39] Similarly, a study found that an 8-week regimen of combined exercise in women aged 25 to 40 with MS resulted in reduced serum levels of IL-6.[40] Another research indicated that both MitoQ and moderate-intensity exercise, whether administered separately or in combination, can lower IL-6 levels in individuals with hypertension.41 Also, the progressive adaptations of exercise cause regular endurance exercise to reduce the production of free radicals[42] and increase the antioxidant defense in skeletal muscle.[43]

In addition, we found that the combination of exercise and MitoQ treatment was more effective in reducing the IL-6 expression. The combination therapy has recently been shown to decrease TNF-α expression in the aged rats' hippocampus.[44] Moreover, the intake of Q10 alongside regular exercise has been associated with reduced serum levels of IL-6 and TNF-α in football players.[45] However, a study found that a 9-week aerobic exercise did not impact IL-6 levels in individuals with MS, which contrasts with our findings.[46] The differences in results may be due to the complex effects of exercise on MS, influenced by various molecular pathways and immune functions. Additionally, our study focused solely on female participants, and the timing of cytokine measurements varied from other studies, which could also explain the discrepancies in outcomes.

Our results also demonstrated that all interventions increased the expression of *TGF-β*, with a more pronounced effect observed in premenopausal women. Consistent with our findings, it has been reported that swimming exercise enhances TGF-β levels in the spinal cord of EAE mouse models.[47] Furthermore, exercise has been shown to regulate T-cell differentiation in favor of reducing proinflammatory cytokines, such as IFN-γ and IL-17, and increasing the anti-inflammatory cytokines, like IL-10, thereby providing neuroprotection through immune modulation.[5,48] In addition, similar research indicates that exercise alters the expression levels of numerous genes involved in inflammatory processes via TGF-β signaling.[49] The effects of physical exercise are often linked to gene expression changes that can occur through various mechanisms, including the generation of second messengers and the modulation of myogenic regulatory and epigenetic factors.[50,51]

We observed that MitoQ alone was effective in altering expression levels of tested genes, though a thorough literature review revealed a lack of studies directly comparable to our findings. Based on the existing studies, the administration of mitochondrion-targeted antioxidant SkQ1 significantly elevates mRNA levels of the transcription factor Nrf2 and genes encoding superoxide dismutase 1 (SOD1) and SOD2, catalase, and glutathione peroxidase 4.[52] Additionally, a study indicated that MitoQ effectively regulates immune imbalances by promoting the differentiation of anti-inflammatory regulatory T cells while inhibiting the differentiation of dendritic cells and helper T (Th)1, Th2, and Th17 cells in the livers of arsenic-exposed mice.[53]

In another aspect of this study, our findings revealed that both interventions led to an increase in SMAD2 levels in both premenopausal and postmenopausal groups, while STAT1 levels decreased. It has previously been reported that the expression of the *STAT1* gene is significantly upregulated in MS patients compared to healthy individuals.[8] Likewise, differential expression of SMAD genes has been observed in blood samples of patients with MS.[54] Although there is a scarcity of specific studies directly linking exercise to the expression of *SMAD2* and *STAT1* in MS patients, a study indicated that exercise reduces the expression of several interferon-related genes, including *STAT1* and *STAT3*, which are known to trigger widespread pro-inflammatory responses in the host.[55] The regulation of *STAT1* expression by exercise may occur through various mechanisms. For instance, research indicates that interferon-gamma (IFN-γ) can modulate STAT1 transcription levels and potentially influence the NLRP1 and NLRP3 inflammasomes.[56] Also, a recent study found that just 2 weeks of exercise can lead to a significant increase in *SMAD2* gene expression, as well as enhance the rate of adult hippocampal neurogenesis.[57] Additionally, it has been demonstrated that exercise-induced alterations in cytokines, such as IL-6, may also impact SMAD2 activity.[58] Also, exercise can modulate the expression of *SMAD2* through epigenetic mechanisms like DNA methylation.[57]

Regarding the more profound effect of the interventions on premenopausal participants, it could be argued that this can be related to the different levels of sex hormones in these 2 groups. Estradiol and

progesterone play crucial roles in neuroprotection and inflammation modulation, making them valuable in the management of MS.[59] Estradiol, in particular, has been shown to influence immune responses and mitigate inflammatory processes.[60] Research indicates that the exacerbation of MS symptoms is associated with low estrogen levels during menstruation and menopause,[61] as well as in the first 3 months postpartum.[62] Conversely, elevated estrogen and progesterone levels during late pregnancy have been linked to a reduction in symptom severity.[63] The prolonged decline in estradiol levels during menopause may disrupt brain regeneration mechanisms, potentially accelerating neurodegeneration and the progression of MS.[64] This highlights the importance of considering hormonal status when evaluating treatment responses in MS patients.

This study highlights that both exercise and MitoQ, whether used individually or in combination, exert beneficial effects on various biomarkers implicated in MS pathogenesis in postmenopausal and premenopausal women. We found that all interventions significantly decreased IL-6 levels and STAT1 while increasing *SMAD2* and *TGF-β* expression. Noteworthy, the interventions showed a more pronounced effect on premenopausal women. Also, the effectiveness of the combination treatment was considerably higher than each alone, suggesting a synergistic effect that could be leveraged for therapeutic strategies in managing MS.

While exercise and MitoQ show promise as therapeutic strategies for MS, further research is essential to assess their long-term effects on disease progression and clinical outcomes. Future studies should focus on optimal exercise parameters and MitoQ delivery methods, as well as the mechanisms behind their impact on biomarkers. Additionally, personalized interventions considering factors like menopausal status may enhance treatment effectiveness for women with MS.

Overall, this study provides preliminary evidence supporting the potential of exercise and MitoQ to modulate various biological pathways implicated in MS pathogenesis, offering promising avenues for future therapeutic interventions in women with this debilitating disease.

STATEMENT OF ETHICS

The research protocol was fully reviewed and approved by the Ethics Committee of Kerman University of Medical Sciences with the ethics code IR.KMU.AH.REC.1401.159. The study register code is IRCT20221120056558N1.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Data Availability

Upon reasonable request from Mohammad Khaksari (email: mkhaksari@kmu.ac.ir).

AI Assistance Disclosure

Not applicable.

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