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Research Article

The Effect of Swimming Exercise on Interferon-Gamma Gene Expression in Mice with Multiple Sclerosis Exposed to Air Pollution

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Abstract

Introduction: Interferon-gamma (IFN-γ) is recognized as a key pro-inflammatory cytokine in the pathogenesis of multiple sclerosis (MS). This study aimed to investigate the effect of swimming exercise on IFN-γ levels in mice with experimental autoimmune encephalomyelitis (EAE) exposed to air pollution.

Methods: In this study, 24 male C57BL/6 mice with an average weight of 24±2 grams were randomly selected and, after an acclimation period, divided into four groups of six: 1) healthy control, 2) EAE-induced, 3) EAE-induced exposed to air pollution, and 4) EAE-induced exposed to air pollution combined with moderate-intensity swimming exercise. EAE was induced by injection of myelin oligodendrocyte glycoprotein (MOG). The swimming protocol was performed five days a week for six weeks. At the end of the study, serum IFN-γ levels were measured using an ELISA kit. Inter-group comparisons were conducted using one-way ANOVA and Tukey's post-hoc test.

Results: IFN-γ levels in the EAE group exposed to air pollution (76.98 ± 2.40 pg/mL) were significantly higher than those in the EAE group (31.20 ± 1.48 pg/mL) and the healthy control group (15.30 ± 1.21 pg/mL) ($P<0.05$). IFN-γ levels in the exercise group (43.18 ± 3.46 pg/mL) showed a significant decrease compared to the EAE group exposed to air pollution ($P<0.05$).

Conclusion: Moderate-intensity swimming exercise can significantly modulate the increased IFN-γ levels resulting from simultaneous exposure to an MS model and air pollution.

Keywords:
Air Pollution, Multiple Sclerosis, Interferon-Gamma, Swimming Exercise, Animal Model.8

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1. Introduction

Multiple sclerosis (MS) is considered one of the most common disabling neurological diseases in young adults, posing a major challenge to global health systems (1). The disease is characterized by chronic inflammatory processes, axonal demyelination, and ultimately progressive neural degeneration (2). According to the latest reports from the International Federation of Multiple Sclerosis, the prevalence of the disease has shown a significant upward trend over the past decade, with over 2.8 million people currently living with MS worldwide (3). The complex pathogenesis of MS involves genetic and environmental factors leading to abnormal activation of the immune system and attack on the central nervous system (4). Among these, cytokines, as key mediators of the immune response, play a decisive role in initiating and sustaining inflammatory processes (5). Interferon-gamma (IFN-γ), a potent pro-inflammatory cytokine primarily produced by T helper type 1 (Th1) cells and NK cells, plays a pivotal role in the pathogenesis of MS (6).

Numerous studies have shown that IFN-γ exacerbates inflammatory processes and demyelination by inducing the expression of MHC class II molecules on antigen-presenting cells, activating macrophages, and increasing blood-brain barrier permeability (7). Furthermore, this cytokine contributes directly to disease pathology by stimulating the production of anti-myelin antibodies and activating extracellular matrix-degrading enzymes (8).

From an epidemiological perspective, the role of environmental factors in modulating the course of MS has increasingly gained attention (9). Among these, air pollution is recognized as a serious threat to nervous system health (10). Studies conducted in urban areas with high levels of pollution have shown a significant association between exposure to particulate matter ($PM_{2.5}$) and increased incidence and severity of MS (11).

$PM_{2.5}$ particles, due to their small size and ability to penetrate deep into the lungs and enter the bloodstream, can cross the blood-brain barrier and directly affect the central nervous system (12). These particles create an unfavorable inflammatory environment in the CNS by inducing oxidative stress, activating microglia, and stimulating the production of pro-inflammatory cytokines (13).

Alongside these risk factors, immune-modulating strategies, including physical exercise, have gained attention as complementary approaches to MS management (14). Scientific evidence indicates that regular physical activity can influence the course of the disease through multiple mechanisms (15). However, exercise intensity is a known determinant in directing immune responses (16). Moderate-intensity exercise exerts protective effects on the central nervous system by modulating cytokine secretion, reducing oxidative stress, and increasing neurotrophic factors (17). In contrast, high-intensity exercise may have adverse effects on disease progression by inducing inflammatory responses (18). Among various exercises, swimming, due to its unique characteristics such as reduced mechanical stress on joints and simultaneous activation of multiple body systems, has been suggested as a suitable intervention for MS patients (19).

Given the complex interaction between environmental factors like air pollution and non-pharmacological interventions like exercise, this study was designed to investigate the effect of moderate-intensity swimming exercise on IFN-γ levels in an animal model of MS exposed to air pollution. A better understanding of these interactions could pave the way for designing more effective treatment protocols for MS patients living in urban areas with high levels of air pollution.

2. Materials and Methods

The animals used in this research were male C57BL/6 mice weighing 20-25 grams. The animals were maintained under standard conditions (controlled temperature, humidity, and light cycle) with free access to food and water. All procedures involving animals were conducted in accordance with the ethical principles for working with laboratory animals approved by the Shiraz University of Medical Sciences.

After a one-week acclimation period, the mice were randomly divided into four groups of six:

- Group 1: Healthy control

- Group 2: EAE-induced

- Group 3: EAE-induced exposed to air pollution

- Group 4: EAE-induced exposed to air pollution + moderate-intensity swimming exercise

EAE Induction: To induce experimental autoimmune encephalomyelitis (EAE) as an MS model, myelin oligodendrocyte glycoprotein peptide (MOG35-55) was used along with complete Freund's adjuvant (CFA) and pertussis toxin (PT) (12).

Air Pollution Exposure: Groups 3 and 4 were exposed to a specified concentration of PM2.5 particulate matter in a controlled exposure chamber for six weeks (2 hours per day, 5 days a week) (13).

Swimming Exercise Protocol: Group 4 underwent a moderate-intensity swimming exercise program. Exercises were performed for six weeks, five sessions per week, in a pool with controlled temperature (31±1°C). The exercise duration was gradually increased, reaching 50 minutes per day in the final weeks (14).

IFN-γ Level Measurement: At the end of the intervention period, following anesthesia, cardiac blood collection was performed and serum samples were separated. Serum IFN-γ levels were measured using a mouse-specific ELISA kit (R&D Systems) according to the manufacturer's instructions. Statistical Analysis: Data were analyzed using SPSS software version 26. One-way analysis of variance (ANOVA) was used to compare mean IFN-γ levels between groups. In case of significant differences, Tukey's post-hoc test was employed for pairwise comparisons. Data are presented as mean ± standard deviation, and a significance level of $P<0.05$ was considered.

3. Results

The results of serum IFN-γ level measurements in the different groups are presented in Table 1.

Table 1: Serum IFN-γ levels (pg/mL) in the study groups

Group	Mean	Standard Deviation	Number (n)
Healthy Control	15.30	0.96	6
EAE	31.20	1.25	6
EAE + Air Pollution	76.98	2.08	6
EAE + Air Pollution + Moderate Exercise	43.18	3.22	6

Figure 1: Mean Interferon-Gamma Levels Across Study Groups

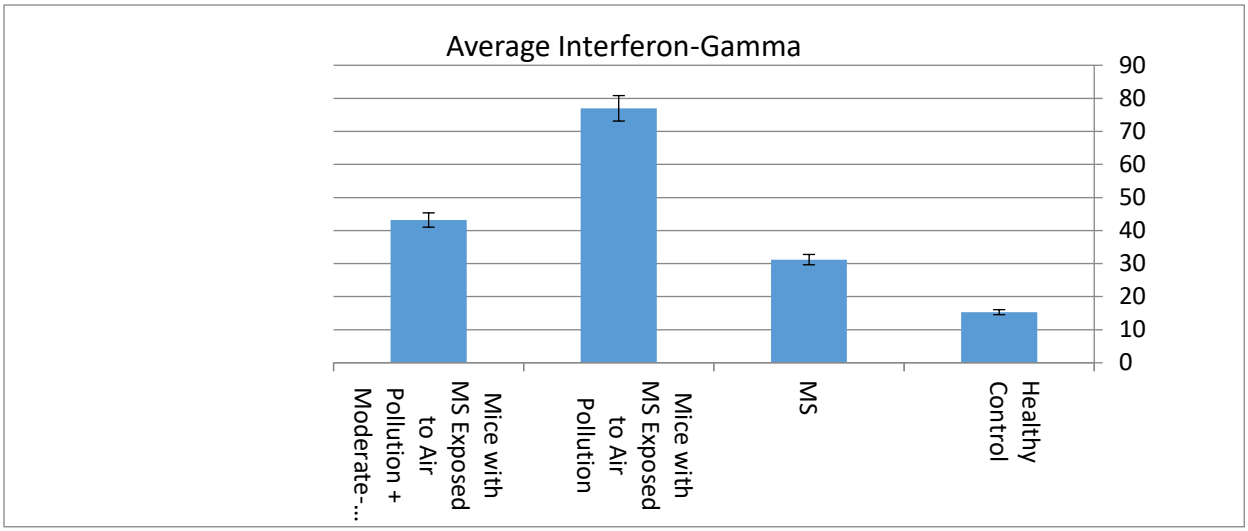

Table 2: Results of One-Way Analysis of Variance (ANOVA)

The results of the one-way ANOVA showed a significant difference between the mean IFN-γ levels in the four groups ($F=726.47$, $P<0.0001$). The ANOVA results are presented in Table 2.

4. Discussion

The findings of this study demonstrated that induction of the experimental MS model (EAE) leads to a significant increase in serum interferon-gamma levels compared to the healthy control group. This result aligns with the established role of IFN-γ as a pivotal pro-inflammatory cytokine in the pathogenesis of MS (6). A notable finding was the strong exacerbating effect of air pollution on this inflammatory response. Mice in the EAE group exposed to PM2.5 showed a marked increase in serum IFN-γ levels, which was also significantly different from the EAE group without pollution exposure. This observation experimentally confirms epidemiological evidence linking air pollution to increased disease activity in MS patients (11). The mechanism is likely mediated by the penetration of fine particles into the central nervous system, induction of oxidative stress, activation of microglia, and stimulation of inflammatory pathways such as nuclear factor kappa-B (NF-κB), all of which can increase the production of pro-inflammatory cytokines including IFN-γ (13).

The most significant finding of this research was the efficacy of moderate-intensity swimming exercise in modulating this exacerbated inflammatory response. The six-week swimming exercise program successfully and significantly reduced IFN-γ levels in the exercise group compared to the EAE group exposed to pollution (without exercise). This observation is consistent with a wide body of previous studies emphasizing the anti-inflammatory effects of moderate exercise. Bernardes et al. (2018) showed that moderate-intensity exercise can slow disease progression in the EAE model by modulating the cytokine response (20). Furthermore, research by Rossi et al. (2021) confirmed that moderate aerobic exercise can reduce the expression of inflammation-related genes in the central nervous system (21).

Several mechanisms may explain the beneficial effects of moderate-intensity swimming exercise on IFN-γ levels. The first notable mechanism is the reduction of oxidative stress. Moderate exercise increases the expression of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, neutralizing reactive oxygen species and reducing oxidative stress (22). This effect is particularly important in the context of air pollution exposure, which itself is a strong stimulator of free radical production.

The second mechanism is the modulation of anti-inflammatory cytokine secretion. Studies have shown that moderate-intensity exercise can increase the production of anti-inflammatory cytokines such as IL-10 and TGF-β (23). These cytokines help establish immune balance by inhibiting the differentiation of Th1 cells and reducing IFN-γ production. Additionally, IL-10 reduces the recruitment of immune cells to the central nervous system by inhibiting the expression of inflammatory chemokines.

The third mechanism involves the effect of exercise on the regulatory T cell (Treg) population. Research indicates that moderate-intensity exercise can increase the number and function of Treg cells (24). These cells play a fundamental role in controlling immune responses by producing anti-inflammatory cytokines and inhibiting the activity of autoreactive T cells. An increased Treg to Th1 cell ratio may explain the reduction in IFN-γ levels in the exercise groups.

The fourth mechanism is the inhibition of the NF-κB signaling pathway. This transcription factor plays a key role in regulating the expression of inflammatory genes, including IFN-γ (25). Studies suggest that moderate exercise can reduce the expression of pro-inflammatory cytokines by inhibiting NF-κB activation, likely through increased expression of inhibitory proteins such as IκB. The fifth mechanism is the increase in neurotrophic factors.

Exercise elevates levels of neurotrophic factors such as BDNF and NGF, which not only exert protective effects on neurons but can also reduce the production of inflammatory cytokines by modulating microglial activity (26).

Regarding the effects of air pollution, the finding that $PM_{2.5}$ exposure significantly increases IFN-γ levels is consistent with numerous reports. A study by Calderón-Garcidueñas et al. (2020) showed that chronic exposure to air pollution can lead to an inflammatory environment in the central nervous system by activating microglia and astrocytes (27). Furthermore, research by Heydarpour et al. (2014) confirmed that high levels of air pollution are associated with increased disease activity in MS patients (28).

A crucial point in this study is the interaction between exercise and air pollution. It appears that moderate-intensity swimming exercise can largely neutralize the inflammatory effects caused by air pollution. This finding is clinically significant, as many MS patients live in urban areas with high pollution levels and may benefit from exercise.

Limitations of this study include the relatively short intervention period, the use of a specific mouse strain, and the measurement of only one inflammatory marker. Additionally, investigating the effects of exercise on other inflammatory and anti-inflammatory cytokines could provide a more complete picture of the mechanisms of action. Future studies are suggested to consider more factors, including a complete cytokine profile, oxidative stress status, and histopathological changes, to examine these effects more precisely.

5. Conclusion

The results of this study indicate that while air pollution can exacerbate the inflammatory response (by increasing IFN-γ) in the experimental MS model, intervention with regular moderate-intensity swimming exercise can significantly modulate this pollution-induced inflammatory increase. This finding emphasizes the importance of considering exercise as a complementary, non-pharmacological strategy in managing patients with MS, especially for residents of areas with high levels of air pollution. Integrating regular, appropriately intense physical activity alongside standard pharmacological treatments may help better control inflammation and improve clinical outcomes in these patients.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: MR.BA, Kh.J,B.M, F.T; Methodology: MR.BA, Kh.J, B.M, F.T; Software: Kh.J, MR.BA, F.T; Validation: MR.BA, Kh.J, B.M, F.T; Formal analysis: MR.BA, Kh.J, B.M, F.T; Investigation: MR.BA, Kh.J, B.M, F.T Resources: Kh.J, MR.BA; Data curation: MR.BA, Kh.J, B.M, F.T; Writing - original draft: MR.BA, Kh.J, B.M, F.T; Writing - review & editing: MR.BA, Kh.J, B.M, F.T; Visualization: MR.BA, Kh.J, B.M, F.T Supervision: MR.BA, Kh.J, B.M, F.T; Project administration: MR.BA, Kh.J, B.M, F.T Funding acquisition: : MR.BA.

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