Journal of Physical Activity and Hormones

(J Physic Act Horm)

Journal HomePage: *https://sanad.iau.ir/journal/jpah/*

The effect of six weeks of HIIT training on pain tolerance threshold in rats with Parkinson's disease (Running title: HIIT training and pain tolerance threshold)

Reza Zeinolebadi [1], Mohammad Amin Edalatmanesh[2]

[1] Ph.D. in sport physiology, Department of exercise physiology, Payame Noor University of Hormozgan, Iran.
[2] Associate professor in physiology, Department of biology, Shiraz branch, Islamic Azad University, Shiraz, Iran.

Keywords
HIIT training, swimming, Parkinson's disease, Pain tolerance threshold, MOR1.

Correspondence
E-mail address: tahaebadi@yahoo.com

Received: 15 Agu 2024; **Revised:** 20 Sept 2024; **Accepted:** 20 Oct 2024

DOI: https://doi.org/10.71878/jpah.2024.1127304

Abstract

Introduction: One of the common and liberating side effects of Parkinson's disease (PD) is pain and its related mechanisms. In this category of patients, the effect of high-intensity interval training (HIIT) on the amount of pain and the related mechanisms have not been determined correctly. Therefore, this study was conducted with the aim of investigating the effect of 6 weeks of HIT swimming training on the pain tolerance threshold and MOR1 gene expression in rats with PD.

Material & Methods: In this experimental study, 21 male Wistar rats aged 8-10 weeks with an average weight of 200 ± 10.2 grams were randomly divided into three groups: healthy control group, PD group, and training group. PD was induced by injecting 1 mg/kg body weight of reserpine for 5 days. The training group performed 20-30 second swimming bouts with 30 seconds rest between each bout for six weeks. The results were analyzed using a one-way analysis of variance and Tukey's post hoc test at a significance level of $P<0.05$ and SPSS-22 software.

Results: The results showed that the pain tolerance threshold ($P=0.001$) and MOR1 gene expression ($P=0.003$) in the PD group were significantly lower than the healthy control group and training group. No significant differences were observed between the healthy control group and the training group, between the pain tolerance threshold ($P=0.49$) and MOR1 gene expression ($P=0.16$).

Conclusion: According to the results, HIT swimming training is recommended to reduce the pain of PD patients.

1. Introduction

Neurodegenerative diseases are a set of disorders that disrupt the control of movements, learning, thinking, and memory by damaging nerve and brain tissues(1). Parkinson's disease (PD) is the most common neurodegenerative disease after Alzheimer's disease, whose prevalence is one to two percent in people over 50 years old, and epidemiological studies have shown that PD disease after Alzheimer's disease is one of the main causes of nervous system disorders, disability, and death (2). In addition, studies have shown that the risk of contracting this disease increases with age, in other words, 2% of men and 2.3% of women died of this disease in the past years, and statistics show that according to the aging of the world population, this number is increasing significantly (3). PD, depending on the degree of destruction of dopaminergic neurons of the substantia nigra dense area, with movement disorders, muscle stiffness, tremors of the limbs at rest, difficulty initiating movements, bradykinesia (bradykinesia of slow body movement or inability to move quickly), muscle weakness and muscle stiffness It is compatible (4). One of the most important and common complications of PD is pain, and increased pain in these

patients causes serious disability, reduced quality of life, paralysis, and ultimately death (5).

Two important factors that are seen in the progression of most neurodegenerative diseases include oxidative stress caused by the production of reactive oxygen species (ROS)(6) and nitrogen (RNS) and neuroinflammation caused by excessive activity of microglia and astroglial cells in the central nervous system (CNS). Pain is one of the complications of PD that 68-95% of these patients face and it severely affects their quality of life (7). The mechanisms of pain in PD are different, but it seems that one of the main causes of chronic pain in these patients is the increase in oxidative stress and inflammatory factors in the brain tissue, which leads to the disruption of pain-related receptors such as opioid receptors (8). Numerous studies have shown that opioids and cannabinoids have analgesic effects on the spinal cord and brain. The activation of the mentioned receptors in different areas of the pain processing pathways, including the gray matter, which is part of the descending system that regulates pain, causes analgesic effects (9).The opioid system has three main receptors, μ (mu), kappa (κ), and delta (δ), which activate the endogenous opioid peptides of these receptors. Opioid receptors, especially μ receptors, which are known as MOR receptors, and endogenous opioid peptides are distributed throughout the CNS and the peripheral nervous system (PNS) and play an important role in the reward system, motivation, and emotions (10). Also, these receptors play an essential role in controlling physiological responses such as analgesia (11) and stress regulation, and the results of recent studies have indicated a relationship between MOR receptors and stress and anxiety symptoms (10).

In the path of using non-invasive methods for the prevention and treatment of diseases, sports capacities and the use of various exercises have always been the subject of interest to most researchers (12, 13). High-intensity interval training (HIIT) are exercises that are performed with high intensity and in short-term intervals, and because of the different effects they may have compared to other exercise methods, they are welcomed today (14). It has been stated that by doing this type of exercise, new adaptations are created, including environmental, neurological, and cardiovascular adaptations (12). Also, in the meantime, high-intensity interval training creates new adaptations, including environmental, neural, cardiac, and vascular adaptations (15). Now, if this type of exercise is performed in water, unlike the exercises that are performed on land, due to the hydrodynamic properties, the characteristic of fluid movements, and the creation of weightless conditions, it can reduce the injuries caused by the exercise(16).

Researchers have investigated the effect of various HIIT performances on the rapid improvement of exercise capacity and skeletal muscle energy metabolism. Different types of activity on a cadence bike or repetitions on a treadmill have been used to investigate the effects of HIIT on physiological adaptations(17). Intense interval training is used as an effective approach to improve fitness in a short period. Currently, there is no comprehensive definition of HIIT, but generally, HIIT is attributed to repeated bouts with relatively short intermittent activities with full intensity or an intensity close to the intensity that VO2peak obtained. According to the intensity of the exercises, a HIIT effort may last from a few seconds to a few minutes, and the various intervals are separated by a few minutes of rest or low-intensity activity (18).

Changes in pain sensitivity appear to be associated with increases in plasma beta-endorphin levels that occur following exercise activity. Specifically, it has been suggested that an increase in beta-endorphin produces exercise-induced euphoria, which raises the pain threshold (19). On the other hand, it has been shown that exercise and physical activity increase the release of endogenous pseudo-opioid peptides, especially beta-endorphins, resulting in the effects of morphine and other pseudo-opioid receptor agonists, and thus training can increase the pain threshold. Receptor disorders related to opioid receptors and the occurrence of pain in PD (20) and lack of response to exercise especially HIIT, led to the present study to determine the effect of 6 weeks of HIT swimming training on pain tolerance threshold and MOR1 gene expression in rats with.

2. Methodology

2.1. Materials and methods

The present experimental study was conducted with a post-test design with a control group. According to the background of the studies and also using the resource equation method, 7 rats were selected for each group (21). Thus, the number of 21 Wistar male rats with an age range of 8-10 weeks and an average weight of 200 ± 10.2 grams were purchased from the laboratory animal breeding and reproduction center of Islamic Azad University, Shiraz branch, and were transferred to the animal laboratory of this university. Before the experiment, the rats were housed in pathogen-free conditions, maintained a 12 hr: 12 hr light/dark cycle with water and food, with ad libitum access to water and food, and then transferred to the institution's sports physiology laboratory.

During the implementation of the research, the Helsinki Treaty was observed and the research process was approved by the Ethics Committee of the Research Vice-Chancellor, Shiraz branch, Islamic Azad University, and the code of ethics was issued by this committee with the number IR.IAU.SHIRAZ.REC.1402.49.

2.2. Participants

In this study, PD was induced through intraperitoneal injection of reserpine. Induction was done one week after keeping the mice in laboratory conditions so that the animals get familiar with adapting to the environment. After the adaptation period, reserpine (manufactured by Sigma Aldrich, India) was injected at the rate of 1 mg/kg of body weight into 14 rats for five consecutive days with a regular schedule around the clock (22). For this purpose, the desired amount of reserpine was dissolved in 0.03 ml of glacial acetic acid solution and then the solution was brought to volume using distilled water. After the end of the induction period, and to confirm the induction of the disease, the rotation test was used. In this test, about 2 cm above the point where the tail joins the mouse's body was taken and the mouse was raised so that the animal's nose was placed 2 cm above the support surface. If the animal could not maintain its balance and started turning to both sides, it was considered a sign of induction of PD (23). After ensuring the induction of the disease, 14 Rats were divided into two groups training group and PD group. 7 rats

without disease induction were considered as the healthy control group.

2.3. Measurements

In this study, the hot plate test was used to evaluate the pain tolerance threshold, the animals of each group were placed on the hot plate machine, which was set at a temperature of 52 degrees Celsius, and the response time to the thermal stimulus following the change in behavior (licking) soles, jumping, or vigorous paw shaking) were recorded. It should be mentioned that 30 seconds was considered as the cut-off point of this test, and the mice that stayed longer than this time without reaction on the hot plate were not included in the study (25).

2.3.1. Tissue collection

In order to investigate the effect of the training program on the possible changes of the dependent variables according to the predetermined program, 48 hours after the last training session, all the rats were injected intraperitoneally with a combination of ketamine (50 mg per kilogram of body weight) and xylazine (3 mg per kilogram of body weight) were anesthetized. Finally, the hippocampal tissue was extracted and immediately transferred to the laboratory for further measurements and frozen at -80.

2.3.2. Real-time PCR

The mRNA of the brains was isolated by RNA iso plus (Takara, Japan), as previously described (26). Isolated mRNA was dissolved in DEPC water, and 1µg of mRNA was converted to complementary DNA sequence by reversed transcription using a cDNA synthesis kit according to the manufacturer's protocol (Takara, Japan). cDNA expression of MOR1 was assessed by real-time PCR with the following primers (Table 1).

Table 1. *The primer sequence used in the study*

Gene	Primer sequence
MOR1	F: 5′ CAAAATACAGGCAGGGGTCCA 3′ R: 5′ TAGCATGCGAACGCTCTTGA 3′
TBP	F: 5′ GCGGGGTCATGAAATCCAGT 3′ R: 5′ AGTGATGTGGGGACAAAACGA 3′

2.3.3. Exercise protocol

In this research, HIT swimming training was performed, and the rats a week familiarizing themselves with the animal pool (diameter 160 cm and height 80 cm) before starting the main training period. To acquaint the rats with swimming training, on the first day, the rats were carefully and calmly placed in the animal pool and swam at the desired speed for five minutes. In the next sessions, when the rats were familiar enough to get to know the type of interval training, after swimming for one minute, they were taken out of the water by the resting plate and then put back in the water. The intense interval swimming training program was carried out for six weeks (three days a week, one day every other day). It should be noted that in this training method, the load applied in the first week was a weight equal to seven percent of the body weight of each rat, which was tied to their tail and added to its weight by one percent every week; So, in the last week (sixth week), the rats practiced swimming with a weight of 12% of their body. It is worth mentioning that the exercises were performed in the evening (the best time of exercise in the natural cycle of rats) (24). After each training session in water, the rats were dried with a towel and transferred to the storage place. During the study period, the samples of the healthy control group did not have any exercise program.

2.4. Statistical Methods

In this research, the Shapiro-Wilk test was used to check the normal distribution of data. Since the data had a normal distribution, a one-way analysis of variance with an LSD post hoc test was used in SPSS-22 software to investigate the changes in the studied variables. The minimum significance level was considered to be $P<0.05$.

3. Results

The results of the one-way analysis of variance test to evaluate the pain tolerance threshold in different groups are presented in Figure 1. The results of this test showed that there is a significant difference in the pain tolerance threshold between the research groups (P=0.001 and F=96.22). To determine the difference between the groups, the results of Tukey's post hoc test are presented. The post hoc results showed that the pain tolerance threshold in the PD group was significantly lower than the healthy control group (P=0.001) and training group (P=0.01) and no significant differences were observed between the healthy control group and training group (P=0.4).

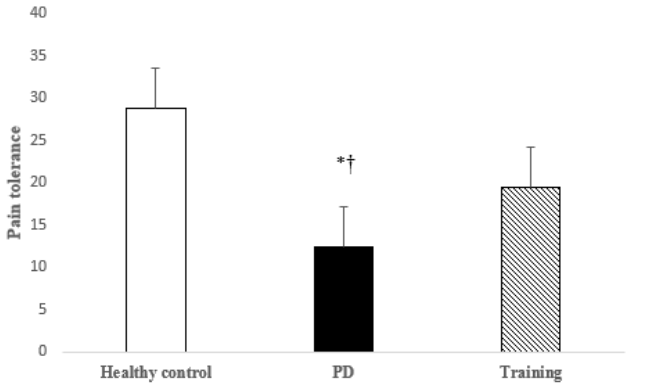

Figure 1. *Pain tolerance threshold in different groups*
* Significant difference with the control group, p<0.05
† Significant difference with the training group, p<0.05

The results of the one-way analysis of variance test to evaluate the MOR1 gene expression in different groups are presented in Figure 2. The results of this test showed that there is a significant difference in the MOR1 gene expression between the research groups (P=0.003 and F=11.53). To determine the difference between the groups, the results of Tukey's post hoc test are presented. The post hoc results showed that the MOR1 in the PD group was significantly lower than the healthy control group (P=0.001) and training group (P=0.03) and no significant differences were observed between the healthy control group and training group (P=0.054).

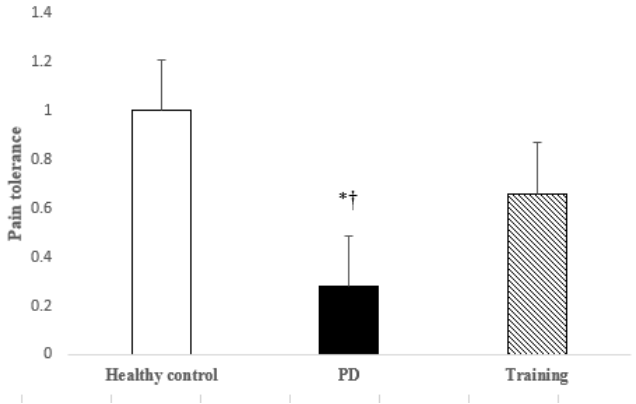

Figure 2. *MOR1 gene expression in different groups*
* Significant difference with the control group, p<0.05
† Significant difference with the training group, p<0.05

4. Discussion

The present study aimed to investigate the effect of HIT swimming training on the pain tolerance threshold and MOR1 gene expression in rats with PD. The results of the present study showed that in the PD group, the pain tolerance threshold was significantly lower than in the healthy control group. The study results showed that there is a significant difference in the MOR1 gene expression between the researchers. The post hoc results showed that the MOR1 in the PD group was significantly lower than the healthy control group and training group and no significant differences were observed between the healthy control group and training group.

Data show that the increase of inflammatory factors and oxidative stress cause the destruction of μ-opioid receptors especially MOR1 in PD(27). The available evidence shows that the activity of microglia is associated with the excessive production of free radicals, and in this way, it causes an increase in hydrogen peroxide, hydroxyl radicals, and cytokines, which in the end causes the production of TNF-α, IL-1β, and in Finally, they can damage the neurons and cause some side effects such as inflammation and pain for patients (28). Data show that the increase of inflammatory factors and oxidative stress cause disruption of pain-related mechanisms and destruction of opioid receptors (29). This is further reduced by the increase of prostaglandins and the stimulation of pain receptors. In line with the results of the present study, it has been observed that the gene expression and plasma levels of IL-1β and TNF-α increase in Parkinson's patients 7 (30). However, Eidson et al observed a decrease in TNF-α levels in PD compared to the healthy control group, and the authors attributed this difference to the difference in blood sampling time (31).

Previous research has shown that the activity of microglia is controlled by transforming growth factor-beta 1 (TGF-β1), which is an anti-inflammatory cytokine . In addition, TGF-β1 has the property of protecting neurons of the central nervous system against damage and inflammation (32). It has been observed that this factor modulates the activity of microglia in animal samples with Parkinson's disease, thereby reducing the production of inflammatory cytokines such as TNF-α, IL-1β, nitric oxide gas, and reactive oxygen species (33). In their study, Zimura et al. (34) observed that the level of TGF-β1 increased and the level of TNF-α in the blood of Parkinson's patients decreased significantly. Although the amount of TGF-β1 was not measured in the present study, it seems that the decrease observed in the gene expression of inflammatory factors is partly due to the increase of TGF-β1 and the decrease of microglia activity. However, the results of the present study showed that in the HIT swimming training group, the pain tolerance threshold was significantly higher than in the patient control group. Based on the results of previous studies, various sports activities have been a practical way to improve various aspects of PD, including improving the quality of life, improving disability, improving oxidative stress and antioxidant defense of the body, as well as increasing factors for improving neuron function in the central nervous system(35). However, the number of sports studies, especially HIIT, on blood levels or gene expression of inflammatory factors in Parkinson's patients is small due to the limitations of the current study, including the lack of measurement of pain, lack of measurement of inflammatory and pro-inflammatory factors in the brain, and lack of activity evaluation. Microglia and the amount of alpha-synuclein, it seems necessary to conduct other studies to investigate the mechanisms of pain reduction in Parkinsonian samples by doing sports activities. In general, it seems that HIT swimming training is effective in reducing the pain in PD by increasing opioid receptors (painkillers) due to the reduction of some inflammatory factors.

5. Conclusion

In general, the results of the present study showed that HIT swimming training due to the delicate state of the fluid and the hydrodynamic capability, can be an effective and useful solution in increasing the pain tolerance threshold in PD and conducting studies with the aim of applying this type exercises in human subjects seem necessary to generalize to human societies. Therefore, HIT swimming training can be effective in reducing the pain of PD patients by increasing opioid receptors by improving MOR1 gene expression.

6. Acknowledgment

We are grateful to all the loved ones who have cooperated in this study.

Conflict of interests: There was no conflict of interest between the authors.

References

[1] Pajares M, I. Rojo A, Manda G, Boscá L, Cuadrado A. Inflammation in Parkinson's disease: mechanisms and therapeutic implications. Cells. 2020;9(7):1687.
[2] Haghdoost-Yazdi H, Sarookhani M, Faraj A, Fraidouni N, Dargahi T, Yaghoubidoust MH, et al. Evaluation of the association between blood homocysteine concentration and the degree of behavioral symptoms in the 6-hydroxydopamine-induced Parkinsonism in rat. Pharmacology Biochemistry and Behavior. 2014;124:297-304.
[3] Brakedal B, Toker L, Haugarvoll K, Tzoulis C. A nationwide study of the incidence, prevalence and mortality of Parkinson's disease in the Norwegian population. npj Parkinson's Disease. 2022;8(1):19.
[4] Vazifehkhah S, Karimzadeh F. Parkinson Disease: from Pathophysiology to the Animal Models. The Neuroscience Journal of Shefaye Khatam. 2016;4(3):91-102.
[5] Ryu D-W, Han K, Cho A-H. Mortality and causes of death in patients with Parkinson's disease: a nationwide population-based cohort study. Frontiers in Neurology. 2023;14:1236296.
[6] Shamsi M, Elmieh A, Shabani R. Strength-endurance training with olive oil consumption on motor performance and oxidative stress level in the brain of male parkinsonian rats. Journal of Jiroft University of Medical Sciences. 2022;9(2):926-37.

[7] Buhmann C, Wrobel N, Grashorn W, Fruendt O, Wesemann K, Diedrich S, et al. Pain in Parkinson disease: a cross-sectional survey of its prevalence, specifics, and therapy. Journal of neurology. 2017;264(4):758-69.
[8] Zhou J, Ma R, Jin Y, Fang J, Du J, Shao X, et al. Molecular mechanisms of opioid tolerance: From opioid receptors to inflammatory mediators. Experimental and therapeutic medicine. 2021;22(3):1-8.
[9] McMahon SB, Koltzenburg M, Tracey I, Turk D. Wall & Melzack's Textbook of Pain E-Book: Expert Consult-Online and Print: Elsevier Health Sciences; 2013.
[10] Nummenmaa L, Tuominen L. Opioid system and human emotions. British journal of pharmacology. 2018;175(14):2737-49.
[11] Corder G, Castro DC, Bruchas MR, Scherrer G. Endogenous and exogenous opioids in pain. Annual review of neuroscience. 2018;41(1):453-73.
[12] Bolhagh SS, Shabani R, Sajedi D. The Effect of Aerobic and Resistance Exercise with Olive Extract on VO2 max, PTEN, and AKT in Rats with Parkinson's disease. Journal of Physical Activity and Hormones. 2023;5(1).
[13] Shamsi-Soosahab M, AR E, Shabani R. The effect of exercise training and olive oil consumption on the spatial learning, genes expression of the AKT and caspase3 of rats with parkinson's disease. Feyz, Journal of Kashan University of Medical Sciences. 2023;27(2):176-85.
[14] Zahraei H, Mogharnasi M, Afzalpour ME, Fanaei H. The effect of 8 weeks of continuous and high intensity interval swimming on chemerin levels in liver and visceral fat tissues and insulin resistance in male rats with metabolic syndrome. Journal of Sport and Exercise Physiology. 2022;15(1):33-44.
[15] Bayati M, Gharakhanloo R, Farzad B. Adaptations of physiological performance following high-intensity interval training. Sport Physiology. 2015;7(26):15-32.
[16] Nagle EF, Sanders ME, Franklin BA. Aquatic High Intensity Interval Training for Cardiometabolic Health: Benefits and Training Design. American journal of lifestyle medicine. 2017;11(1):64-76.
[17] Pirani H, Salehi O, Kiansab M. The effect of four weeks of intense interval training (HIT) on some aerobic and anaerobic indicators of the women of the Iranian national basketball team. Researches in Sport Sciences and Medical Plants. 2023;3(10).
[18] Anami A, Nazari M, Shabani R. Comparison of the effect of high-intensity interval training and futsal training on some of the factors of physical fitness, quality of life and negative symptoms in patients with paranoid schizophrenia: A clinical trial study. Journal of Gorgan University of Medical Sciences. 2019;21(4):29-38.
[19] Jafari H, Daryanoosh F, Zarifkar A, Ghiasi e, Mohammadi M. Effect of Exercise on Pain and Morphine-Induced Analgesia in the Formalin Test in Male Mice. Journal of Rafsanjan University of Medical Sciences. 2014;13(1):97-108.
[20] Hoffman MD, Lee J, Zhao H, Tsodikov A. Pain perception after running a 100-mile ultramarathon. Archives of physical medicine and rehabilitation. 2007;88(8):1042-8.
[21] Yazdian MR, Khalaj A, Kalhor N. The effect of caloric restriction and treadmill exercise on reserpine-induced catalepsy in a rat model of Parkinson's disease. The Neuroscience Journal of Shefaye Khatam. 2018;6(4):45-52.
[22] Hubrecht RC, Kirkwood J. The UFAW handbook on the care and management of laboratory and other research animals: John Wiley & Sons; 2010.
[23] Shafiee A, Gaeini A, Soleimani M, Nekouei A, Hadidi V. The effect of eight week of high intensity interval training on expression of mir-210 and ephrinA3 mRNA in soleus muscle healthy male rats. Journal of Arak University of Medical Sciences. 2014;17(3):26-34.
[24] Abbasi M, Kordi M, Daryanoosh F. The effect of eight weeks of high-intensity interval swimming training on the expression of PGC-1α and IL-6 proteins and memory function in brain hippocampus in rats with non-alcoholic steatohepatitis induced by high fat diet. Journal of Applied Health Studies in Sport Physiology. 2023.
[25] Paudel KR, Das BP, Rauniar G, Sangraula H, Deo S, Bhattacharya S. Antinociceptive effect of amitriptyline in mice of acute pain models. 2007.
[26] Huang C, Chu JM-T, Liu Y, Chang RC-C, Wong GT-C. Varenicline reduces DNA damage, tau mislocalization and post surgical cognitive impairment in aged mice. Neuropharmacology. 2018;143:217-27.
[27] Wang H-Y, Burns LH. Naloxone's pentapeptide binding site on filamin A blocks mu opioid receptor–Gs coupling and CREB activation of acute morphine. PloS one. 2009;4(1):e4282.
[28] Salim S, Chugh G, Asghar M. Inflammation in anxiety. Advances in protein chemistry and structural biology. 2012;88:1-25.
[29] Halim S, Mohamad N, Toriman ME, Bakar NHA, Hashim SN, Adnan LHM, et al. Role of Zamzam water as a vital mineral supplement in the treatment of opioid dependence and tolerance: A Review. Research Journal of Pharmacy and Technology. 2016;9(7):957-63.
[30] Usenko T, Nikolaev M, Miliukhina I, Bezrukova A, Senkevich K, Gomzyakova N, et al. Plasma cytokine profile in synucleinophaties with dementia. Journal of Clinical Neuroscience. 2020;78:323-6.
[31] Eidson LN, Kannarkat GT, Barnum CJ, Chang J, Chung J, Caspell-Garcia C, et al. Candidate inflammatory biomarkers display unique relationships with alpha-synuclein and correlate with measures of disease severity in subjects with Parkinson's disease. Journal of neuroinflammation. 2017;14:1-16.
[32] Zhou X, Spittau B, Krieglstein K. TGFβ signalling plays an important role in IL4-induced alternative activation of microglia. Journal of neuroinflammation. 2012;9:1-14.
[33] Chen X, Liu Z, Cao B-B, Qiu Y-H, Peng Y-P. TGF-β1 neuroprotection via inhibition of microglial activation in a rat model of Parkinson's disease. Journal of Neuroimmune Pharmacology. 2017;12:433-46.
[34] Szymura J, Kubica J, Wiecek M, Pera J. The immunomodulary effects of systematic exercise in older adults and people with Parkinson's disease. Journal of Clinical Medicine. 2020;9(1):184.
[35] Hoseini SM, Falah Mohammadi Z, Talebi V. Preventive Effect six Weeks of Swimming Training on the Levels of Cytokines and Brain-Derived Neurotrophic Rat Model of EAE. Journal of Sport Biosciences. 2020;12(1):53-65.