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The Effect of IFN-β 1a on Biochemical Factors in Multiple Sclerosis Patients

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Abstract

Background: Multiple sclerosis (MS) is known as a progressive and demyelinating disease, which involves biochemical changes.
Objectives: To evaluate the effect of IFNβ-1a therapy on the biochemical factors in the MS patients.
Methods: In this descriptive study, 30 MS patients and 30 healthy controls were included. The study was conducted in the Neurology Center at the Shefa hospital, Kerman, Iran from September 2013 to July 2015. The patients' blood test was taken before and after six months of IFNβ-1a therapy and the biochemical factors (LDH, AST, ALT, Creatinine, Uric acid, Malondialdehyde (MDA) and nitric oxide (NO) were measured in all the samples. The participants were divided to three groups, namely main group (30 patients), females (22 patients) and males (8 patients). Before taking the medicine, the groups were compared to the control group. After six months of taking the medicine, each group was compared to its former state before taking the drug.
Results: In the patients group (30 patients), a significant difference was observed in their measured biochemical factors in comparison to the control group (P = 0.001), however, after six months of using IFNβ-1a, only MDA was shown in the main (P = 0.003) and female group (P = 0.003), and the ALT that was shown in the female group had a significant reduction in comparison to that before receiving IFNβ-1a.
Conclusions: This study showed that IFNβ-1a decreased oxidant impacts in MS patients, but had no influence in improving mitochondrial dysfunction.

Keywords: Multiple Sclerosis, Cinnovex, IFNβ-1a, Biochemical Parameters

1. Background

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS), identified with the destruction of myelin in brain and spinal cord. This chronic disease occurs often between the age of 20 and 40 years and is more common amongst females (1). Women are almost twice as likely to experience relapse-remitting MS (RRMS) than men (2). There are several reports regarding the prevalence of this disease in different regions of the world. In Europe and America, five and six cases per 100,000 people suffer from MS, respectively (3).

During the recent years, studies have also indicated the quite high prevalence of MS in the Middle East as well as Iran (1). The symptoms vary depending on the involvement area of CNS with sensory manifestation, motor, spinal cord, brain stem dysfunction, sphincter dysfunction, sensory disturbances, cognitive as well as behavioral dysfunction (1).

MS causes biochemical changes in the molecular level and the variation of neural cells (4, 5), which cause an imbalance in the ratio of oxidant to anti-oxidants. The increase of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as the reduction of uric acid (a natural antioxidant) has been reported in MS patients (6). Several studies have shown that ROS plays a significant role in creating pathologic symptoms of MS (6). Immunity system responses increase the amount of ROS and RNS (7). The ROS enhancement leads to (i) the interaction of monocytes with brain endothelium, (ii) inducing cytoskeleton rearrangements, (iii) the loss of the blood-brain barrier integrity, (iv) changes in the tight junctions and finally (v) the migration of leukocytes into CNS (8). Oxidative stress and inflammation are also alleged to promote tissue damage in MS patients (9). The CNS is also vulnerable to damages caused by oxidation. Lipids peroxidation may increase

blood-brain barrier permeability and lipid membrane peroxidation may cause the production of oxidized phospholipids. These phospholipids cause the monocytes to connect to endothelial cells (10). In MS, the balance of cell energy is disturbed due to mitochondrial dysfunction and leads to the reduction of the ATP amount in cells (11). As a result, the routes of purine metabolization increase due to the ATP decline, which, in turn, enhances nucleotides and oxy purines (hypoxanthine, xanthine and uric acid) in cerebral spinal fluid (CSF) (11). These combinations could be proper biomarkers for neurodegenerative (11). According to the literature, combinations derived from RNS and ROS in blood such as nitric oxide (NO) because ROS and RNS production to increase and also result in enhancement of lipid peroxidation in the blood sample of MS patients (6). The increase of NO production in MS patients damages myelin and oligodendrocytes. NO also increases cGMP, which consequently leads to the increase of the impacts of TNF-α and other cytokines (12).

Increased level of oxidants in the blood may lead to a reduction in the natural antioxidant compounds such as uric acid (UA). Ascorbic acid, which has a similar tendency as UA toward the oxidants, is also reduced (6). Although some studies reported the increase of UA in MS patients (13), there exists no agreement in this regard. Several reports have indicated the decrease of UA in MS patients' blood (6). In MS patients, the amount of creatinine changes due to movement problems, myocyte involvement and mitochondrial dysfunction (14). Furthermore, most variations happen in malondialdehyde (MDA), which shows the peroxidation of unsaturated fatty acids in membrane phospholipids, known as an index of oxidative stress in the cells (15). Additionally, mitochondrial damages reduce energy production, which plays an important role in MS pathogens (11). Also, cytochrome C and mitochondrial complex IV lose activity in the areas damaged (16). Tissue damages are caused by defects in mitochondrial function due to the energy production drop (16). Mitochondrial dysfunction release cytochrome C, and as a result, apoptotic pathway is activated (17).

ALT and AST are other biochemical factors that change in MS patients (18). ALT and AST are proper indices for the liver function. ALT is a cytoplasmic enzyme and abounds in liver, while; AST is found in both cytoplasm and mitochondria and there for the amount of AST varies in mitochondrial dysfunction (18). Accordingly, improvement of the AST and ALT serum level can be a proper index for decreasing the progress of MS.

2. Objectives

This study determines the relationship between MS and biochemical factors such as MDA, UA, Creatinine, NO, LDH, AST and ALT. Additionally, it investigates the impact of MS patients IFN-β 1a therapy on serum levels of MDA, UA, Creatinine, NO, LDH, AST and ALT.

3. Methods

This descriptive study was conducted in the neurology center at the Shefa Hospital in Kerman, Iran from September 2013 to July 2015. During the study, 30 patients (22 females and 8 males) were enrolled. They were proved to suffer from MS and were given a neurologist diagnosis as well as radiology, laboratory, clinical and neurological evidences based on the McDonald criteria (19). The mean age of all participants was 29.8 $\pm$ 5.5, in women 28.7 $\pm$ 5.6 and 32.7 $\pm$ 4.3 years old in men. 59% of women and 75% men were married, .55% of women and 50 % of men had completed high school or equivalent, 45% of women and 25% of men had a college degree and 25% of men had a post graduate degree. The mean body mass index (BMI) of female and male patients was 22.1 $\pm$ 2.6 and 27.3 $\pm$ 4.6 Kg/m^2, respectively.

The patients were new diagnostic cases and had not received medication for treatment of MS. 30 additional individuals with the same demographic information, no disease and no medication, were chosen as the control group. Each participant in the patient group was assigned a counterpart in the control group. The participants with other autoimmunity disorders, infectious diseases, pregnancy and under treatment of immunoregulatory drugs have been excluded from the study. Accordingly, 30 MS out of 150 patients have been introduced to the study. All participants were required to fill out a questionnaire and also signed an informed consent form. The participants were non-smokers and suffered from no other inflammatory disease except MS (as per the patient group), they did not suffer from heart or kidney disease and did not use any anti-inflammatory drug.

The ethics committee of Kerman University of Medical Sciences, No k/92/587 issued the license for carrying out the research. Before receiving IFNβ-1a, pharmaceutical compositions of the company CinnaGen (Cinnovex, similar to the composition of Avonex), 5 mL of intravenous blood was collected in tubes containing anticoagulant EDTA. After being centrifuged at 2500 rpm for 5 minutes, 2.5 mL plasma was separated and the samples were immediately preserved at -70°C. Then, the patients were treated with standard doses of Cinnovex (a dose of 30 mcg injected intramuscularly once a week). The blood samples were

retaken after six months and if a patient had used anti-inflammatory agents (except Cinnovex) he/she would have been eliminated from the study. It is worth noting that the period of the experiments was chosen based on the work of Arababadi et al. (20). Similar to the previous stage, the plasma samples were immediately saved at -70°C. The plasma samples of the control group were also taken and preserved at -70°C to measure the biochemical factors. All experiments were performed in the besat-1 clinic laboratory, Kerman, Iran, and an expert neurologist has evaluated the clinical presentations of the patients. All the equipment that was used for clinical purpose in the Besat-1 clinic laboratory Kerman, Iran, was under the supervision of the reference laboratory and calibrated regularly by expert technicians. Commercial kits (all from Pars Azmoon, Iran) using a Selectra (XL) auto-analyzer determined the creatinine, UA, ALT, AST and LDH of the samples. The method of Rao et al. (21) was used to measure MDA. In this method, the MDA amount was investigated via the Thiobarbituric acid (TBA) method and Spectrophotometer Alpha-1860 (UV-4802). To measure NO (NO_2^-/ NO_3^-), ZellBiokit (GmbH, Deutschland) and Spectrophotometer Alpha-1860 (UV-4802) were utilized. The sensitivities of the kits have been considered as the excluding criteria and the results out of the kits sensitivity have been experimented again.

3.1. Statistical Analysis

Data is presented as mean ± SD (standard deviation) for numeric variables and are presented as numbers and percentages for categorical variables. Numeric variables are compared using the independent two-sample t-test across MS patients and normal people. Additionally, biochemical factors were compared using paired t-test in MS patients across the two time periods. Data was examined using the non-parametric Kolmogorov-Smirnov test for normal distribution and no statistically violations were detected from normality assumption ($P > 0.05$). Significance level was set at 0.05.

4. Results

4.1. A, The Comparison Between the Healthy Control Group and MS Patients That Did Not Received any Medication

As shown in Figures 1 and 2, the healthy control group and MS patients that did not receive any medication were analyzed comparatively.

As observed in Figure 1A, the amount of LDH enzyme was investigated in all MS groups (all individuals) including males and females and also in healthy controls. The level of the LDH in the all-patient groups was significantly higher than healthy groups ($P = 0.001$).

According to Figure 1B, regardless of gender, the amount of AST in the patient group was more than the control group. Although the AST quantity in male patients is more than the males in the control group, the interesting finding was that the AST amount was significantly lower in female patients compared to the females in the control group ($P = 0.001$).

An increase in the level of ALT enzyme in the male subgroup of MS patients that did not receive any medication were not significant in comparison to the control group ($P = 0.196$). However, with respect to other groups, ALT was significantly higher in the patient than in the control groups (Figure 1C). The creatinine concentration in the patients who had not received medication was significantly less than the healthy participants in all three groups (in all patients compared to the healthy participants regardless of gender, male and female groups) ($P = 0.001$) (Figure 1D).

From the comparison of uric acid (UA) in all patients, the male and female groups with that of the control group obtained interesting results. The UA in the all patients and female patients showed a decrease in comparison with the control group, however, in male patients, it was more than the control group ($P = 0.001$) (Figure 2A).

As observed in Figure 2B, MDA concentration in the patients who did not receive any medicine in all three groups (in all patients compared to the healthy participants regardless of gender, male and female groups) was significantly higher than healthy individuals ($P = 0.001$).

The findings showed that the nitric oxide metabolites (NO_2^-/NO_3^-) in all patients, females and males compared to the control group were highly augmented (Figure 2C).

4.2. B, Comparison Before and After Taking IFN-β 1a in the MS Patients

Comparison before and after taking IFNβ-1a in the MS patients revealed the following results. The LDH enzyme amount had no significant change after taking the medicine ($P = 0.985$) (Figure 3A). AST decreased slightly after taking IFNβ-1a ($P = 0.136$) (Figure 3B). ALT enzyme reduction was trivial ($P = 0.123$) (Figure 3C). Creatinine (Figure 3D), UA (Figure 4A) and NO (Figure 4B) did not decrease after the consumption of the medicine and only MDA decreased significantly ($P = 0.003$) (Figure 4C). Henceforth, the patients were divided into male and female groups. The data analysis showed that in the females, only two factors i.e. ALT ($P = 0.024$) and MDA ($P = 0.003$) decreased significantly after taking IFNβ-1a. Other factors showed no remarkable decline. On the other hand, amongst the males, no significant change was observed in any of the factors.

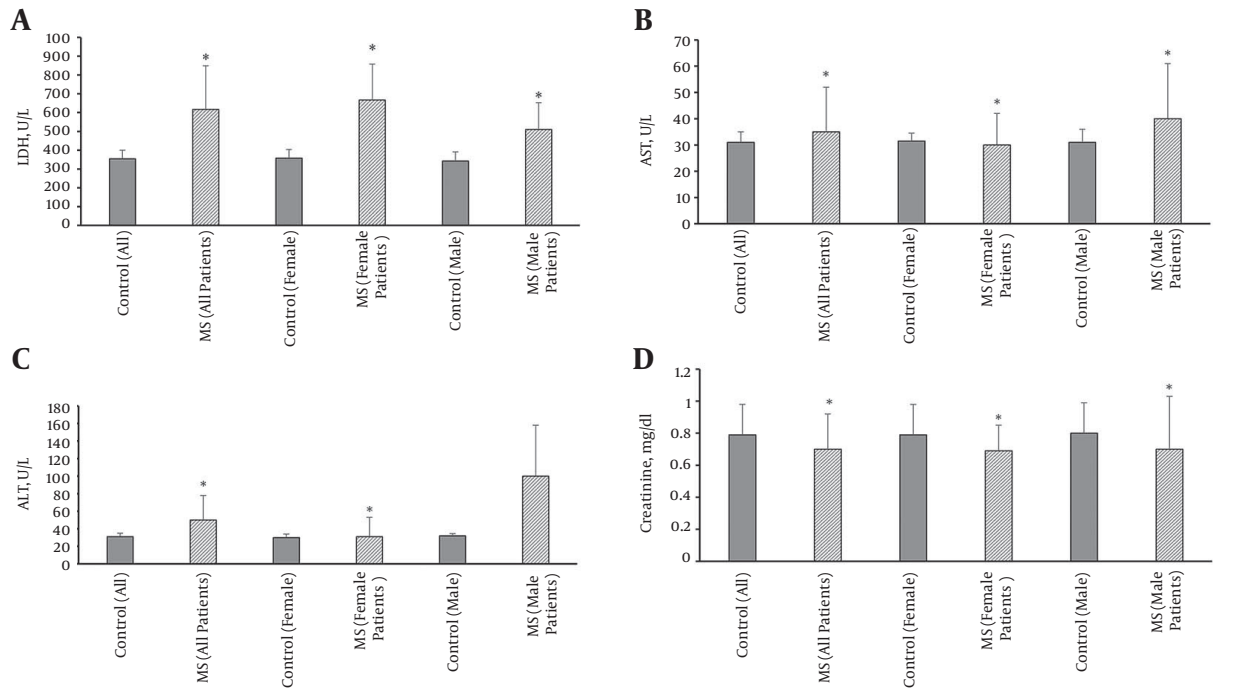

Figure 1. This figure shows the changes of the measured LDH (A), AST (B), ALT (C) and creatinine (D) in the healthy control group and the MS patients who did not receive any medicines. LDH was significantly different amongst all groups (A). AST significantly increases in the total and male MS patients, while it decreases in female MS patients in comparison to the females in the control group (B). ALT significantly increases in the total and female, but not in the male MS patients in comparison with healthy controls (C). Creatinine significantly decreases in total, the male and female MS patients in comparison with total, male and female in healthy control group, respectively (D).

5. Discussion

In this study, the control group was compared with the patient group, which did not receive any medication, and the difference was significant in all of the measured factors, which had been also approved in previous studies (22-24). Our goal was to investigate the impact of IFN-β 1a on biochemical factors in MS patients. It was found that IFNβ- 1a only significantly decreases MDA after six months of medication and has no influences on the other factors. The decline of ALT in the female group was noteworthy, but no major impact was observed with respect to the other factors.

In this study, the increase of LDH in the MS patients who had not received any medication was considerable in comparison to healthy controls. This shows a mitochondrial defect in MS patients and was in agreement with previous studies (11). This outcome may reflect the activity of the immune system and the consequent of neuronal-cell damage as well as demyelinization. The ascending amounts of LDH might originate from immune cells passing the blood-brain barrier in damaged tissue, or from the multiple sclerosis plaques, as speculated by other researchers (11). The amount of LDH in this study had no significant decrease after six months of taking IFN-β 1a (Figure 3A). Therefore, it can be concluded that IFN-β 1a has no impact on metabolic adjustment of MS during the clinically active disease process, although further research is needed in this scenario. AST and ALT are other biochemical factors that change due to mitochondrial dysfunction or drug consumption (25). Based on the fact that IFN-β 1a medicines reduce the activity of cytochrome P450 and other drug-metabolizing enzymes and also has toxic impacts on the liver cells, ALT and AST increase in MS patients, treated with IFN-β 1a medicine. Therefore, improvement of the serum level of AST and ALT can be a proper index for deterioration of the MS progress (18). Regardless of the gender, the amount of AST in the patient group was more than the control group so that in the male patients it was more than and in the females less than the control group. AST augments in all the patients, regardless of their gender, because of its great increase in male MS patients. To further explain this, it can be stated that AST exists both in cytoplasm and mitochondrial cells. When minor damages are made to tissues, a great quantity of AST is cytoplasmic and a little portion is mitochondrial. Severe damages to tissues, however, release a great quantity of mitochondrial AST. Since men have more muscle mass than women, dur-

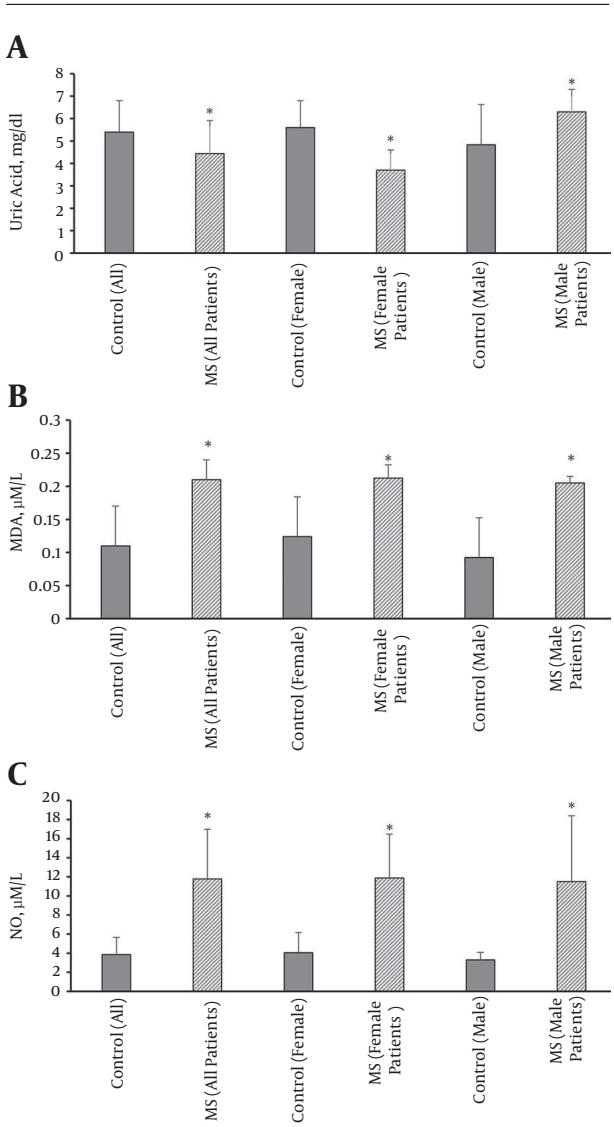

Figure 2. The graph shows the changes of uric acid (A), MDA (B) and nitric acid ($NO_2 + NO_3$) (C) in the healthy participants and the MS patients who did not receive any medication. The figure illustrates that uric acid significantly decreased in the total and the female patients when compared to healthy controls (A), while uric acid in the female patients (A) and MDA (B) and nitric acid (C) in all MS groups significantly increased in comparison with healthy controls.

ing MS disease, myocytes are damaged and more AST is released, which leads to the AST amount growth in the blood.

The comparison of the ALT enzyme in the healthy and MS patients who did not receive any medicine showed that the ALT had no significant increase in the male patient samples (P = 0.196). However, in other groups, the increase of ALT in the patient groups samples were considerable compared to the control group (p=0.001). ALT enzyme is found more in liver and kidney cells. When the liver is damaged, ALT increases in the blood and since the liver in females is more vulnerable than in males, its increase in females is more likely.

The change of the AST enzyme before and after taking IFN-β 1a was reduced in all groups; however it was not statistically substantial.

ALT amount descended in all MS patients after six months of taking IFN-β 1a. It can be hypothesized that although IFN-β 1a is a harmful agent for hepatocytes, it may reduce serum levels of ALT in the patients' samples after six months of treatments due to a decrease in inflammation, which has a negative relation with liver enzymes growth. Nevertheless, it appears that more studies can shed light on the roles of IFN-β 1a on the hepatocytes to release ALT.

Previous studies showed that in MS, the balance of metabolism, oxidative/nitrosative and antioxidant status is disturbed (6). A study demonstrated that UA in MS patients decreased because of its antioxidant effects and scavenging activity featured with respect to nitrates (6). However, several studies showed that UA increases in MS because of phosphorylated purine catabolism (ATP, GTP) and the destruction of nucleic acids. The reason for these problems in MS is the decrease of ATP. ATP is not enough to fulfill the needs of cells on such occasions. In MS patients, the need for ATP increases, yet it is not fulfilled because of mitochondrial dysfunction (12). According to our study, UA in the MS patients decreased significantly in comparison with the control group, which was because of the increase in the female in the control group. However, in the male group, a significant increase was observed compared to the control group, which is quite important. The discrepancies in the reports on changes of UA in MS patients are because of overlooking the gender. According to our best knowledge, this is the first research that studies the changes of biochemical factors in MS patients based on their gender. IFNβ-1 had an ascending impact on the amount of UA of plasma in MS patients after six months of medication. To further explain this, it can be stated that the duration of the study was not long enough and a study in longer term is needed to answer the questions.

Creatinine in MS patients is increased in plasma because of the lack of metabolic balance of the myocytes and consequently moderate disability in walking (24). In these patients, those combinations that influence nitrogen metabolism such as creatinine increase (22). In this study, creatinine in the MS patients who did not receive medication increased significantly in comparison with the healthy control group, which is in agreement with other relevant studies (10, 14).

In our research, IFNβ-1α has a descending impact on the plasma creatinine of the MS patients. This might be attributed to the fact that IFN-β 1a has no effect on the adjustment of bioenergetical system and mitochondrial

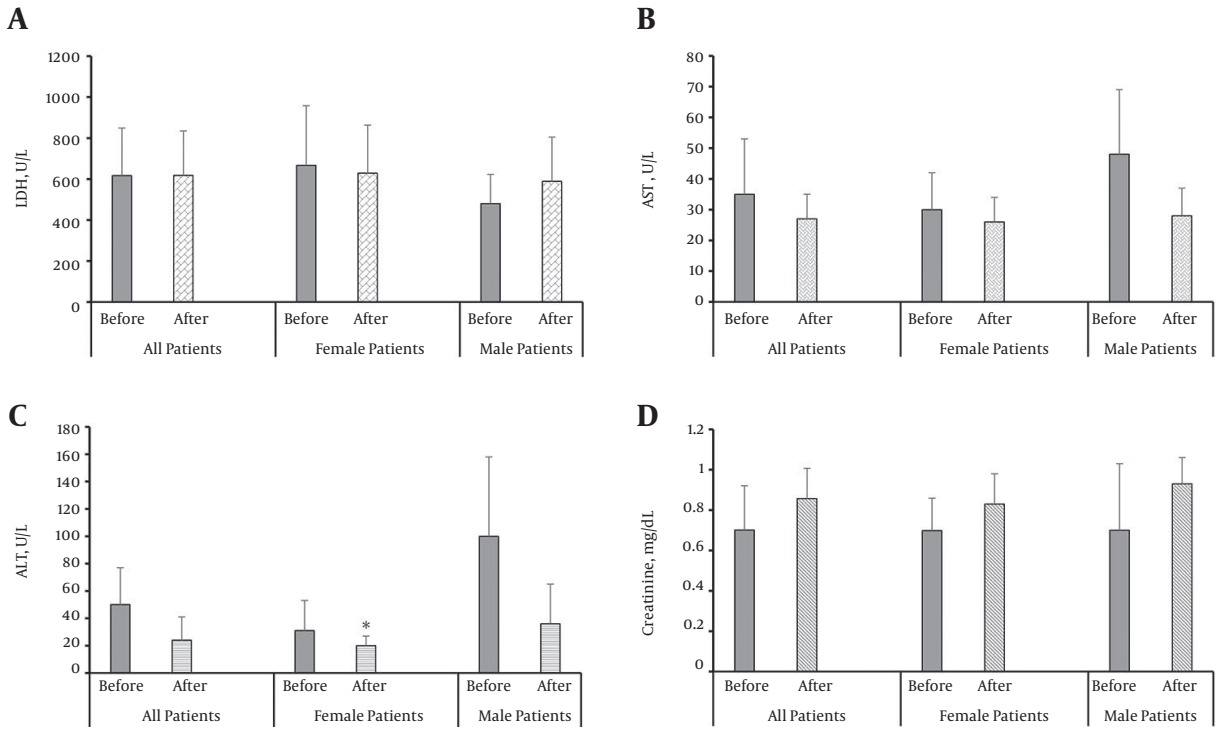

Figure 3. This figure shows the change of LDH (A), AST (B), ALT (C) and creatinine (D) concentration before and after taking IFN-β 1a. The figure shows that IFN-β 1a therapy led to a reduction in ALT and did not affect LDH, AST and creatinine in MS patients.

function. The use of medications that improve the performance of mitochondria oxidative and phosphorylation system may decrease the complications and recurrence of relapse-remitting MS.

Our results demonstrated that NO (NO_2^-/NO_3^-) increased in MS patients when compared with healthy controls; this shows oxidative/nitrosative stress, which leads to the growth in the production of NO ($NO_2^-/NO3^-$). As a result, the risk of radical production of nitrate peroxide oxidizer (NO_3^-) augments, which may impose many threats to brain tissues (7). According to the results, IFN-β 1a could not decrease NO in the plasma (Figure 4B). However, it increased after taking the medication, yet the increase was trivial. To explain this, one could say that the great increase of ROS and RNS is observed in MS patients and thus the use of a supplement with IFN-β 1a, which can play an antioxidant role, may decrease ROS and RNS, which seems to be quite useful.

Previous investigations revealed that MDA increases in MS patients (6). MDA is made by the peroxidation of unsaturated fatty acids in membrane phospholipids, which shows oxidative stress. Tavazzi et al. (6) found that plasma MDA in MS patients increased 210 times more than the control group. In this study, MDA considerably augmented in MS patients who did not receive any medicine compared to the control group. IFN-β 1a could significantly decrease MDA in plasma after six months of taking the medicine. This shows that IFN-β 1a decreases the peroxidation of the lipid membrane. The decrease was also important in the female group; however it was not major for the males, which might be related to the small number of males in this study.

The novelty of our study is regarding the commercial IFN-β 1a formula, which is used for treatment of MS patients. As mentioned in the material and method section it was purchased from the CinnaGen Company (Cinovex). Additionally, the effects of the drug have been evaluated after six months, for the first time in this investigation.

The limitation of the present research included the limited number of participants who could fulfill the inclusion criteria and participate in the experiments during the study. Despite many of the attempts we made, only a few patients, 30, agreed to participate in the experiments during the six months.

5.1. Conclusion

This study showed that IFN-β 1a decreases oxidant effects in MS patients, but it does not improve mitochon-

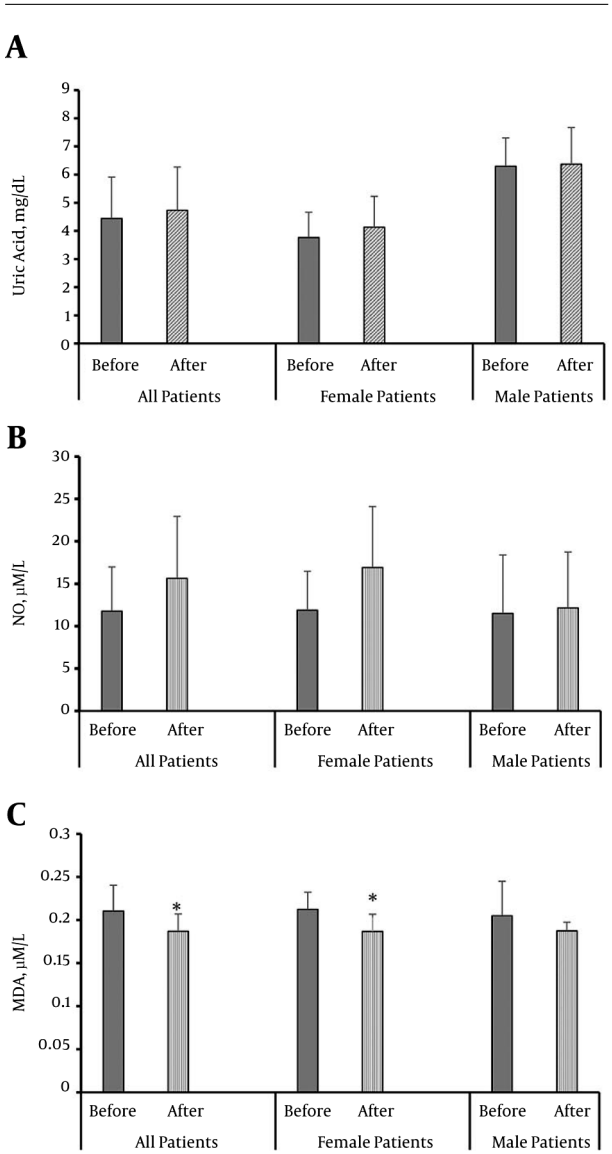

Figure 4. This curve shows the change of uric acid (A), nitric oxide ($NO_2 + NO_3$) (B) and MDA (C) concentration before and after taking IFN-β 1a. According to the figure, although IFN-β 1a therapy was unable to affect uric acid and nitric oxide, it led to MDA reduction in the total and the females and not in the male patients.

drial dysfunction. To decrease the clinical effects in patients with MS, supplements with an antioxidant role are suggested to improve the mitochondrial function.

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