Original Article

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Internal Medicine Section

The Effect of Interferon Beta-1b and Methylprednisolone Treatment on the Serum Trace Elements in Iraqi Patients with Multiple Sclerosis

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ABSTRACT

Objective: To study the trace element levels and the effect of methylprednisolone and interferon beta-1b on these levels in the serum of patients with multiple sclerosis.

Methods: This study was conducted on 32 patients who received methylprednisolone, 32 patients who received interferon beta-1b and on 32 patients who received no treatment at the Multiple Sclerosis Center, Baghdad Teaching hospital, Baghdad, Iraq, from December 2010 to September 2011. In addition to these, 32 age and gender matched healthy controls too were studied for the same parameters. The serum levels of zinc (Zn), copper (Cu) and selenium (Se) were analyzed by using flame and flameless atomic absorption spectrophotometer.

Results: There was a significant decrease in the mean serum levels of both zinc and selenium in both the groups of patients which received the treatment as compared to the patients who received no treatment and the control groups. The serum level of copper was found to increase significantly in the patient groups as compared to that in the controls, and there was also a significant increase in the serum level of copper in the patients who received Methylprednisolone as compared to that in the patients who received no treatment. There was no significant increase in its level in the patients who received interferon beta-1b as compared to that in the patients who received no treatment.

Conclusion: Methylprednisolone and interferon beta-1b treatments affect the serum levels of zinc, copper and selenium in patients with multiple sclerosis.

Key Words: Trace elements (zinc, copper, selenium), Multiple sclerosis, Methylprednisolone, Interferon beta-1b

INTRODUCTION

Multiple sclerosis (MS) is a chronic neurological disease which is characterized by the idiopathic inflammation of the central nervous system (CNS). This inflammation has been theorized to result from lymphocyte and macrophage infiltration, which causes demyelination and axonal injury and presents as neurological signs which are generally disseminated in both location and time [1,2]. The development of MS is theorized to be genetically determined and to be triggered by an environmental factor. Clinically, MS can be classified, based on its presentation into several categories, which include relapsing-remitting MS (RRMS) which is characterized by acute attacks, followed by complete or partial recovery; primary progressive MS (PPMS) has disease progression from the onset; secondary progression MS (SPMS) occurs when an an initial RRMS course progresses with or without occasional relapses, remissions, and disease stabilization; and progressive relapsing MS (PRMS) has progression from the onset of the disease with acute relapses, followed by full or partial recovery to the level of the prior disability [2]. Relapsing-remitting MS – the type which is present in 80% of the patients – typically begins in the second or third decade of life and it has a female predominance of approximately 2:1 [3].

Perturbation of the cellular oxidant/antioxidant balance has been suggested to be involved in the neuropathogenesis of several disease states which include stroke, MS, Parkinson's disease and Alzheimer's disease, as well as "normal" physiological aging [3,4].

The presumed role of the immunoinflammatory processes in the pathogenesis of MS has led to many attempts of treating the disease by immunosuppressive and immunomodulating drugs, and the recent introduction of interferon beta and other disease-modifying agents have changed the approach to the treatment of the patients with relapsing-remitting MS immensely [5].

Type I interferon beta has been theorized to impact the disease course of MS through the induction of a signaling pathway cascade, leading to the production of interferon-stimulated gene products with immunomodulatory, antiviral and anti-proliferative properties [6].

Glucocorticoids have anti-inflammatory and immunosuppressive effects, and the treatment with glucocorticoids has a long history in MS [7]. In a review which was published in 1991, the benefit of the glucocorticoid treatment in MS was, however, questioned [8].

Trace elements, despite their low concentration in the body, play an important role in various metabolic events. They are also important for the development of the nervous system, myelination of the nerve fibres, and also for neurnal excitability [9].

Many studies have shown persistent low levels of zinc in the MS patients. Zinc has an important role in the inhibition of potentially destructive immune reactions against the T lymphocytes, and in the predisposing inflammatory responses of MS. It is also an antioxidant which protects the cell membranes and myelin. The copper level also changes in MS. Copper is needed for the basal metabolic activities of the bone, skin, and the nervous system, and more importantly, it is needed for the enzyme reactions which are involved in the production of ATP, and for the transmission of impulses in

the nerves and the muscles [10]. Selenium is a component of the enzyme, glutathione peroxidase, and it is important, together with vitamin E in the protection against the damage by peroxide and free radicals. Selenium acts as an anti-toxic element, it can bind cadmium, mercury, and other metals and it mitigates their toxic effects. Even their toxic levels in the tissues remain unchanged. On the other hand, selenium may be toxic when it is ingested through water which contains high amounts of it [11,12].

The aim of this study was to evaluate whether interferon beta-1b and methylprednisolone affected the serum trace element levels.

MATERIAL AND METHODS

Patients and Controls

A total of 96 patients (72 females and 24 males) with relapsing-remitting multiple sclerosis from the Multiple Sclerosis Center, Department of Neurology, Baghdad Teaching Hospital were included in this study: group I- 32 patients (24 females and 8 males) received intravenous methylprednisolone (IV-MP) therapy; group II- 32 patients (24 females and 8 males) received interferon beta-1b (IFNβ-1b); and group III-32 patients (24 females and 8 males) received no treatment.

The mean±SD age of the groups was 31.6±6.6 years for the patients who received no treatment and it was 32.3±6.4 years and 30.2±6.1 years for the patients who received IV-MP and IFNβ-1b respectivity. 32 age and sex matched individuals [the control group (mean±SD age, 31.3±5.4)] were recruited as healthy blood donors. The exclusion criteria for all the groups were: a history or present status of cardiological, respiratory, kidney or liver diseases, intestinal absorption abnormalities and infections, assumption of the thyroid hormones, lithium therapy, intake of vitamin or mineral supplements, vegetarian dietary, artificial metallic bodies.

Treatment

The patients in group I received intravenous methylprednisolone at a dose of 1 gram per day for 3 to 5 days, and group II received 250 µg of IFNβ-1b every other day (subcutaneous).

Metal Analysis

After overnight fasting of the study subjects, 1 ml of blood was drawn from them with polyethylene syringes and it was transferred into plastic tubes in the absence of any anti-coagulant to prevent the possibility of an exogenous source of the metals. The blood was allowed to stand for 30 minutes at room temperature and it was further centrifuged at 3000 rpm for 15 minutes to obtain the serum. Zinc, copper and selenium were further quantified by a flame and flameless atomic absorption spectrophotometer.

Statistical Analysis

All the statistical work and the reporting of the obtained data were carried out by using the The SPSS program (version 10). The differences of the means were considered to be of significance according to the t-test at the levels of p≤0.05 and ≤0.01 .

RESULTS

The zinc and the selenium levels were significantly decreased, while the copper level was increased significantly in all the patients groups as compared to their levels in the control group and baseline [Table/Fig-1,2,3,4,5] and [Table/Fig-6,7,8]. [Tables/Fig-9,10] and [Table/Fig-6,7,8] have shown a significant decrease in the serum levels of zinc and selenium in the patients who received methylprednisolone (group I) and interferon beta-1b (group II) as compared to their levels in the patients who received no treatment (group III); there was a significant increase in the copper level in the patients who received methyl prednisolone (group I) and there was no significant increase in the level of this element in the patients who received interferon beta-1b (group II) as compared to that in the patients who received no treatment (group III).

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group I	32	773.59	81.30	2208.34	0.0001
	Control	32	1502.18	32.89		
Cu	Group I	32	1265	66.42	746.28	0.0001
	Control	32	917.96	27.41		
Se	Group I	32	54.15	20.68	165.55	0.0001
	Control	32	102.15	4.19		

[Table/Fig-1]: Mean of serum trace elements level in patients receiving methylprednisolone (group I) and control.

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group II	32	1347.5	45.43	243.31	0.0001
	Control	32	1502.18	32.89		
Cu	Group II	32	1094.21	129.69	183.29	0.0001
	Control	32	917.96	27.41		
Se	Group II	32	78.93	8.74	1666.29	0.0001
	Control	32	102.15	4.19		

[Table/Fig-2]: Mean of serum trace elements level in patients receiving interferon beta-1b (group II) and control.

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group III	32	1066.25	214.03	129.68	0.0001
	Control	32	1502.18	32.89		
Cu	Group III	32	1072.18	232.45	13.89	0.0001
	Control	32	917.96	27.41		
Se	Group III	32	87.81	6.59	107.65	0.0001
	Control	32	102.15	4.19		

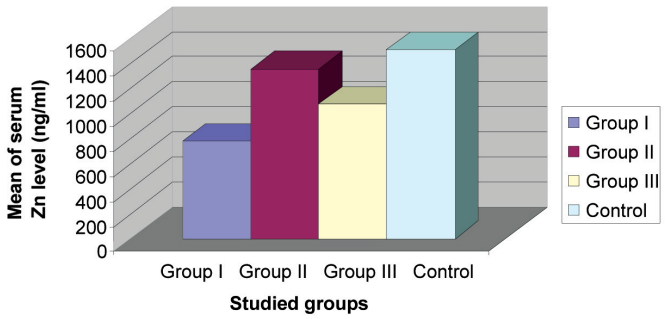
[Table/Fig-3]: Mean of serum trace elements level in patients receiving no treatment (group III) and control.

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group I	32	773.59	81.30	385.25	0.0001
	Baseline	32	1127.3	61.5		
Cu	Group I	32	1265	66.42	149.48	0.0001
	Baseline	32	1090	46.31		
Se	Group I	32	54.15	20.68	32.03	0.0001
	Baseline	32	12.72	78.44		

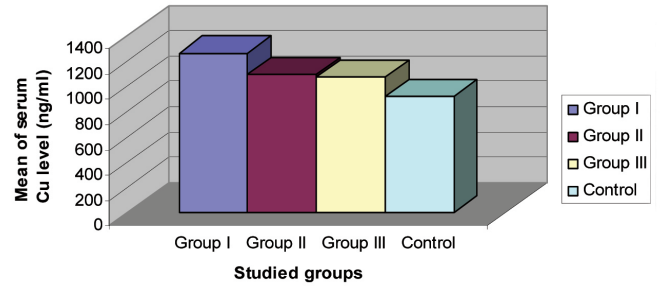
[Table/Fig-4]: Mean of serum trace elements level in patients receiving methylprednisolone (group I) and Baseline.

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group II	32	1347.5	45.43	53.06	0.0001
	Baseline	32	1424.2	38.52		
Cu	Group II	32	1094.21	129.69	10.99	0.0001
	Baseline	32	1005.44	78.25		
Se	Group II	32	78.93	8.74	39.83	0.0001
	Baseline	32	90.84	6.13		

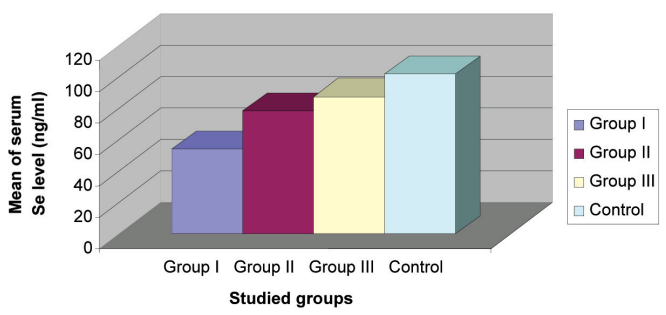
[Table/Fig-5]: Mean of serum trace elements level in patients receiving interferon beta-1b (group II) and Baseline.



[Table/Fig-6]: Mean of serum zinc (Zn) level among the studied groups.



[Table/Fig-7]: Mean of serum copper (Cu) level among the studied groups



[Table/Fig-8]: Mean of serum selenium (Se) level among the studied groups.

DISCUSSION

The results of the present study showed that the serum levels of zinc in all the groups of patients were lower than its level in the control group. These results were in agreement to those which had been published in the literature [13,14]. The effect of zinc on the immune system is very obvious since the deficiency of zinc causes lymphopaenia and as it reduces the immune capacity among the affected humans [15]. The roles of this redox-active metal in the human response and during inflammation have been extensively investigated. Zinc could modify the cytokine production by means of the matrix metallo-proteinases, it could stabilize the association of the myelin basic protein with the brain myelin membranes and also initiate the autoimmunity response [16].

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group I	32	773.59	81.30	52.28	0.0001
	Group III	32	1066.25	214.03		
Cu	Group I	32	1265	66.42	20.35	0.0001
	Group III	32	1072.18	232.45		
Se	Group I	32	54.15	20.68	76.94	0.0001
	Group III	32	87.81	6.59		

[Table/Fig-9]: Mean of serum trace elements level in patients receiving methylprednisolone (group I) and patients receiving no treatment (group III)

Trace elements	Studied groups	No.	Mean	SD	Comparison of significance	
					t-test	p-value
Zn	Group II	32	1347.5	45.43	52.87	0.0001
	Group III	32	1066.25	214.03		
Cu	Group II	32	1094.21	129.69	0.22	0.6412
	Group III	32	1072.18	232.45		
Se	Group II	32	78.93	8.74	21.02	0.0001
	Group III	32	87.81	6.59		

[Table/Fig-10]: Mean of serum trace elements level in patients receiving interferon beta-1b (group II) and patients receiving no treatment (group III).

This study revealed that the serum zinc level was related to the type of treatment which was given. Its level could be decreased in the patients who received methylprednisolone; a decrease in the levels of zinc had been also reported in other disease studies [17,18], while its levels had been reported to increase in the patients who received interferon beta. However, studies have shown that corticosteroid therapy affects zinc homeostasis in a dose-dependent, time-dependent fashion [19]. The plasma trace element levels initially rise and then fall in response to the glucocorticoid administration. Additionally, the nature of the circulating trace elements was altered, i.e., an increased level of diffusible zinc was observed in a system which utilized a membrane which was permeable to proteins with a molecular weight of <20000 [20]. In contrast to plasma, the tissue zinc levels were found to rise in response to steroid treatment, as a result of the de novo metallothionin synthesis [21]. Other studies have shown that the plasma zinc levels rapidly decreased and that the zinc concentrations increased in response to interferon beta treatment, which induced both the synthesis of metallotionin-mRNA and that of metallothionin proteins [22].

This study showed that the serum copper levels increased in all the patient groups as compared to those in the controls, which was agreeable with what had been reported in the literature [14,23]. The increase in the serum copper concentration was found to decrease the zinc absorption. Several reports have proved the existence of competition in the intestinal absorption and the inverse relationship between serum copper and the zinc concentration [24]. This relationship was found in patients who were treated with interferon beta and methylprednisolone as compared to that in the patients who were not treated and in the control groups.

In the present work, it was shown that there was a decrease in the serum selenium level in all the patients group as compared to that in the control group. The explanation for this reduction in the selenium level is that selenium itself has a fundamental role in the

regulation of the immune system, as selenium has been found at the active sites of the enzymes which are involved in the oxidation reduction reaction [25]. Thus, selenium can act as an antioxidant in the extracellular space and in the cell cytosol, in association with the cell membranes, all of which have the potential to influence the immune processes [26].

The present study showed that the serum levels of selenium decreased in the patients who received methylprednisolone, which had been also reported in other disease studies, and that it increased during the interferon beta-1b treatment, respectively. The explanation for this variability is that selenium is essential for an optimum immune response, although the mechanism of this requirement has not always been fully understood. Selenium influences both the innate "non-adaptive" and the aquired "adaptive" immune systems [27-32].

Methylprednisolone is a synthetic glucocorticoid drug; glucocorticoids are steroid hormones that are among the most potent immunosuppressive and the anti-inflammatory drugs, while endogenously produced glucocorticoids play essential and complex roles in the regulation of the immune response [33]. They have been shown to affect both the innate and the adaptive immune responses by influencing cell trafficking and proliferation, expression of the surface molecules and the co-stimulatory and the adhesion molecules, and the synthesis of many inflammatory mediators which include cytokines [33]. Glucocorticoids exert most, if not all, of their effects through their binding to the glucocorticoid receptor, a ligand-activated transcription factor [34,35]. Additional studies which were done on patients with rheumatoid arthritis who received corticosteroid therapy also demonstrated a decrease in the plasma selenium levels.

Zinc and selenium have a protective effect against free radical generation and oxidative stress [26,36,37,38]. So, the serum levels of zinc and selenium were elevated in the patients who received interferon beta as compared to those in the patients who received methylprednisolone, which may be attributed to the increased oxidative stress in the patients who received methylprednisolone.

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