

## Research Paper:

# Effect of Melatonin on Serum Levels of INF-1 $\beta$ and Vitamin B<sub>12</sub> in Patients With Multiple Sclerosis: A Randomized Controlled Trial



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## ABSTRACT

**Background:** Multiple Sclerosis (MS) is a chronic autoimmune disease due to the demyelination of axons in the central nervous system. Melatonin, as a hormone with potential anti-inflammatory effects, can reduce the MS symptoms by altering the levels of inflammatory mediators.

**Methods:** In this study, 50 MS patients referred to the MS Society in Markazi Province were randomly selected. Each patient completed and signed a consent form and was referred to the MS Center at Vali-Asr Hospital in Arak, Iran. All patients who were routinely receiving interferon, were divided into two groups of 25 each. Group 1 (control) received placebo, and Group 2 (treatment) received one dose of melatonin (3mg) per day for 24 weeks. Patients' recorded anthropometric data included height, weight, and age. Fasting blood samples were collected and the serum levels of INF-1 $\beta$  and VitB<sub>12</sub> determined. The blood samples were immediately centrifuged to separate sera, which were kept in a freezer at -80° C. The serum levels of INF-1 $\beta$  and Vitamin B<sub>12</sub> were determined, using ELISA kits.

**Results:** The data showed that there were significant differences in the serum levels of INF-1 $\beta$  and Vitamin B<sub>12</sub> between the control and treatment groups. The levels were significantly increased in the treatment group compared to those in the controls, suggesting that melatonin might have induced the changes.

**Conclusion:** Based on the results, the application of melatonin might be a valuable strategy in the treatment of patients with MS.

**Keywords:** Multiple Sclerosis; Melatonin; INF-1 $\beta$  and Vitamin B<sub>12</sub>; Anti-inflammatory effect; Autoimmune disease

## Introduction

**M**ultiple Sclerosis (MS) is a chronic inflammatory disease associated with the demyelination of the nerve fibers in the Central Nervous System (CNS) [1]. It is an autoimmune disease due to the pathological infiltration of mononuclear cells that

lead to the demyelination of neuronal axons, gliosis in the CNS white matter, and the formation of numerous plaques in the brain and spinal cord tissue [2]. Interferons are important inflammatory mediators, believed to be involved in the mechanism and progression of the disease [3, 4]. They belong to a group of intracellular messengers called cytokines with anti-tumor, anti-viral and anti-proliferative properties [5].

Interferons also have regulatory roles in the immune system [6]. The release of interferon beta (IFN- $\beta$ ) is induced by cytokines, such as IL-1, IL-2, TNF- $\alpha$ , T-cells, macrophages and some viruses [7]. One of the pharmaceutical treatment options for MS is IFN- $\beta$  [8, 9]. This agent reduces the relapse rate of MS by about 30% also lowers the incidence of neuronal lesions in the CNS [10]. The Administration of IFN- $\beta$  is beneficial against the relapse of MS, but it has no effect on the initial progression [11, 12].

The role of vitamin B<sub>12</sub> in the pathogenesis of MS has been reported over the past three decades [13]. Also, the regulatory effects of vitamin B<sub>12</sub>, such as modification of TNF- $\alpha$  activity in the immune system have been reported [14]. The concurrent deficiency of vitamin B<sub>12</sub> and the regulatory mechanisms can exacerbate MS by intensifying the inflammatory processes, demyelination or retarding the remyelination process, and the management [15]. Therefore, the application of vitamin B<sub>12</sub> may be considered as a complementary therapeutic strategy along with other treatment protocols in MS patients.

Melatonin (5-methoxy-n-acetyl-tryptamine) is a hormone secreted from the pineal gland and is synthesized from the neurotransmitter serotonin [16]. Melatonin receptors in the brain are found in the prefrontal cortex, cerebellum, hippocampus, basal ganglia, substantia nigra, nucleus accumbens, retina, and in various parts of the hypothalamus [17]. In addition, these receptors are found in such tissues as the digestive tract, adipose tissue, pancreas, ovaries, skin, lungs and heart, and lymphocytes [18, 19]. Melatonin is believed to be effective in regulating such cytokines as TNF- $\alpha$ , IL-8, IL-6, INF and other inflammatory mediators [20, 21].

Studies published in recent years on melatonin have suggested that melatonin may be used as a therapeutic supplement in many neurodegenerative conditions [20, 22, 23]. In patients with MS, serum melatonin levels are reduced due either to calcium deposition in the pineal gland or hypothalamic dysfunction [24, 25]. Also, melatonin plays a mediating role in the mechanisms of signal transduction by changing the ion flow through various ion channels [26]. In this study, the serum levels of INF-1 $\beta$  and Vitamin B<sub>12</sub> were investigated in two groups of patients with MS.

## Materials and Methods

**Sample Population:** The sample population consisted of 50 patients with MS referred to the Arak MS Society and subsequently to the Vali-Asr Hospital, Arak, Iran,

between April 2015 and August 2017. The sample size was calculated based on the Cochrane formula.

**Inclusion & Exclusion Criteria:** Patients with relapsing-remitting MS based on McDonald 2 criteria, age 18-50 years old, Expanded Disability Status Scale (EDSS) between 0-5.5, completion of the consent form, and the ability to participate in the study were included. A completed consent form was received from each patient prior to participation in the study. The exclusion criteria were the use of non-interferon medications, vitamin B<sub>12</sub>, alcohol, being pregnant and/or a having a diagnosis of autoimmune disease.

**Treatments:** All patients were treated routinely with interferon (30 $\mu$ g / week / 24 weeks). They were randomly divided into a control group (n=25) that received a placebo, and a treatment group (n=25) who received one dose of melatonin (3 mg) at night an hour before bedtime for 24 weeks. Venous blood samples from both groups were collected by a nurse under sterile condition at Vali-Asr Hospital in Arak, Iran.

**Biochemical Analyses:** The blood samples were immediately centrifuged and the sera were kept in a freezer at -80° C. The frozen serum samples were transferred to the Immunology Laboratory at Tabriz Medical University, in which the levels of INF-1 $\beta$  and Vitamin B<sub>12</sub> were assayed, using the ELISA methods. All biochemical kits were purchased from Bioassay Technology Laboratory (China) and the samples were evaluated based on the specific methodology for each assay kit. Finally, samples were read by an Elisa reading unit at an optic density of 450 nm.

**Data Analyses:** The recorded patients' anthropometric data included height, weight, and age. The normality of data distribution was analyzed by Kolmogorov-Smirnov test followed by Mann-Whitney non-parametric test. The results for both groups were analyzed by SPSS V. 21 and a randomized division method.

## Results

Demographic data of the patients participating in the two groups were analyzed on SPSS V. 21, using independent t-test. The two groups were compared in terms of age, gender, weight, and height and there was no significant differences between them (Table 1).

**Analyses of INF1 $\beta$  Data:** The serum levels of INF1 $\beta$  were lower in patients with MS compared to the normal standard range. The results indicated that the levels of the

**Table 1.** Patient's demographic information that there was no significant difference between two groups (n=25)

Demographic Factors	Control		Treatment		P
	No.	Means±SE	No.	Means±SE	
Age (Years)	-	37.96±6.88	-	37.76±6.45	0.91
Male (n=5)	2	-	3	-	0.64
Female (n=45)	23	-	22	-	0.64
Weight	-	73.63±10.71	-	74.33±9.20	0.80
Height	-	158.60±4.74	-	161.88±8.90	0.11

INF1 $\beta$  increased significantly ( $P<0.004$ ) only in patients taking a daily dose of melatonin for 24 weeks (Figure 1).

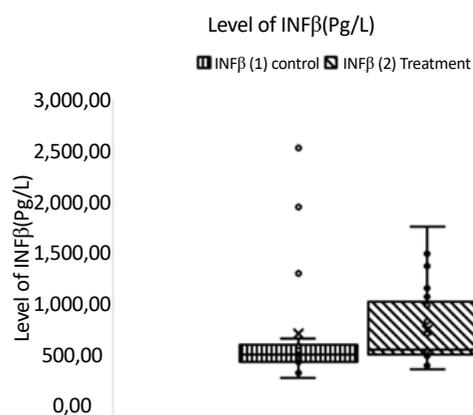
**Analysis of Vitamin B<sub>12</sub> Data:** The serum level of Vitamin B<sub>12</sub> was lower in patients with MS compared to the normal standard range. As shown in Figure 2, the serum vitamin B<sub>12</sub> increased significantly only in patients taking melatonin over 24 weeks compared to that in the control group ( $P<0.004$ ).

## Discussion

The results showed that there was a significant difference between the placebo and treatment groups in the serum levels of the Vitamin B<sub>12</sub>. It is likely that melatonin influenced the higher presence of vitamin B<sub>12</sub> in the serum. Studies on MS patients suggest that Vitamin B<sub>12</sub> deficiency plays a role in the pathogenesis of MS [27, 28]. Miller et al. [16] and Singh et al. [17] reported that neurological disorders caused by vitamin B<sub>12</sub> deficiency include such pathologies as demyelination and axonal degeneration [29, 30].

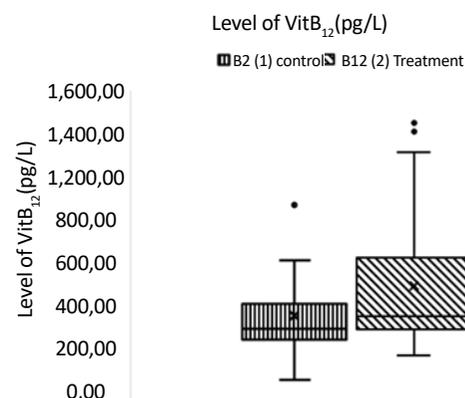
Also, previous studies demonstrated that the vitamin B<sub>12</sub> levels in serum and/or CSF in MS patients decreased and there was a significant relationship between MS and vitamin B<sub>12</sub> deficiency [31, 32]. We expected that receiving melatonin in our patients would increase the serum level of vitamin B<sub>12</sub>, because its deficiency can exacerbate the symptoms of MS by intensifying the resultant inflammatory processes, demyelination, and retarding the remyelination process and prolonging the recovery. Therefore, taking vitamin B<sub>12</sub> in MS patients can be considered as a positive therapeutic complement along with other clinical strategies [33].

Studies have also shown that there is a direct link between vitamin B<sub>12</sub> intake and the amount of melatonin produced in the CNS, i.e. the vitamin B<sub>12</sub> intake increases the CNS melatonin level [34, 35]. It has been shown that in patients with MS, the serum levels of both vitamin B<sub>12</sub> and melatonin decrease dramatically [36]. In the current study, it was assumed that the serum level of vitamin B<sub>12</sub> in the patients receiving melatonin would change. Our results were consistent with this concept. Our results also indicated that there was a significant dif-



**Figure 1.** The comparison of the means between the control and treatment groups (INF1 $\beta$ )

There was a significant difference in the INF1 $\beta$  levels between the two groups.



**Figure 2.** Significant differences were found between the means of serum VitB<sub>12</sub> levels in the control and treatment groups.

ference in the serum levels of INF1 $\beta$  between the placebo and melatonin treatment groups.

In the patients receiving melatonin, a significant increase was noted in their serum level of INF1 $\beta$ . Based on our findings, we may argue that there is an association between the serum melatonin and INF1 $\beta$ . As an effective ingredient of Cinnovex, the drug used to treat patients with recurrent MS symptoms, INF1 $\beta$ , reduces the recurrences and prevents the clinical exacerbations.

Also, IFN $\beta$  is widely used as the first-line treatment in MS [37]. It also minimizes or delays the development of motor disabilities. Hence the reason for this drug to be currently one of the nine known medications that alters the clinical course of the disease [38]. IFN $\beta$  reduces the crossing of inflammatory mediators through the Blood-Brain Barrier (BBB) and subsequently increases the neuronal repairs in MS patients [39]. Therefore, melatonin may be used as an auxiliary, safe and affordable medication with minimal side effects and low toxicity risk to effectively manage the MS symptoms. Several studies have also shown that melatonin has anti-inflammatory properties and leads to the alleviation of fatigue states in MS patients [40-43]. Also, Jamshidi-Fard et al. [42] and Wurtman [43] have shown that melatonin is effective in treating the visual impairment in MS patients.

## Conclusions

The level of serum melatonin was lower in MS patients than that in standard normal range. This hormone is likely to play a protective role in MS and other biological processes, such as immune responses and circadian rhythm. Also, this hormone is known to have anti-inflammatory, immunomodulatory and antioxidative effects. Further, melatonin serves to reduce oxidative and nitrous oxide (nitrosative) stresses, and can lower the serum inflammatory mediators in MS patients. The protective effects of this hormone could play an important role in the pathogenesis of the disease. In this study, the serum melatonin levels were associated with increases in the levels of INF1 $\beta$  and VitB<sub>12</sub>. Therefore, it can potentially be a valuable therapeutic agent in the management of this human neurological disorder.

## Ethical Considerations

### Compliance with ethical guidelines

The present study was registered in the Iranian Registry of Clinical Trials Code: IRCT2017072520258N55 and approved by Arak Medical University Ethics Committee

with the Code of ethics confirmation: IR.ARAKMU.REC.1394.9.

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### Author's contributions

Performed experiments, analysed data, writing original draft preparation and co-writing the paper: Masoomeh Yosefi-Fard; Review and editing original draft and Supervised the research: Gholamhassan Vaezi; Designed methodology and supervised the research also reviewed and editing original draft: Ali Akbar Maleki-Rad; Provided Multiplied Sclerosis (MS): Fardin Faraji; Reviewed and editing original draft: Vida Hojati.

### Conflict of interest

The authors declared no conflict of interests.

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