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Assessment of Serum ACTH, Melatonin and Cortisol Levels in Patients with Hormone Imbalance and Multiple Sclerosis

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Abstract: Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease that affects the central nervous system due to an abnormal immune response. Hormonal and enzymatic changes in MS patients can influence disease progression and prognosis. This study aimed to evaluate the levels of cortisol, adrenocorticotropic hormone (ACTH), melatonin, and lactate dehydrogenase (LDH) in the blood of MS patients and their potential role as biomarkers. Methods: The study included 50 MS patients and 50 healthy controls. Blood levels of ACTH, melatonin, and cortisol were measured using specific enzyme-linked immunosorbent assay (ELISA) kits, while LDH levels were also assessed. Data were statistically analyzed using SPSS software to determine significant differences between the two groups. Results: The results showed a significant increase in cortisol and LDH levels in MS patients compared to healthy controls ($P<0.05$). In contrast, melatonin and ACTH levels were significantly decreased in MS patients ($P<0.05$). The elevated cortisol levels may be associated with chronic stress and inflammation related to MS, while increased LDH levels could indicate tissue damage due to myelin destruction. Reduced melatonin levels may contribute to sleep disturbances and increased stress, and lower ACTH levels may reflect disruptions in the hypothalamic–pituitary–adrenal (HPA) axis. Conclusions: The observed hormonal and enzymatic changes in MS patients highlight the extensive impact of MS on various physiological systems. The increase in cortisol and LDH levels, along with the decrease in melatonin and ACTH, could serve as potential biomarkers for monitoring disease progression and guiding therapeutic interventions. These findings align with previous studies and offer a deeper understanding of the pathological mechanisms of MS.

Keywords: multiple sclerosis; cortisol; ACTH; melatonin; inflammation; hypothalamic–pituitary–adrenal axis

1. Introduction

Currently, it is generally accepted that autoimmune diseases involve various groups of immune disturbances that cause aberrant B-cell and T-cell reactivity to normal constituents of the host [1]. Multiple sclerosis (MS) is a chronic, common autoimmune disease that affects young adults. The body's immune system attacks the protective covering of nerve cells in the brain, optic nerve, and spinal cord, called the myelin sheath [2,3]. Thus, MS can cause numerous symptoms, such as fatigue, weakness, cognitive impairment, memory loss, and paralysis, depending on the specific area of the body affected and the extent of nerve damage.

MS is a complex heterogeneous inflammatory disease influenced by various gene effects and environmental factors. Factors such as vitamin D or ultraviolet B light (UVB) exposure, Epstein–Barr virus (EBV) infection, stress, obesity, and smoking modestly increase disease susceptibility [4]. This leads to increased activity of T and B lymphocytes and macrophage infiltration, resulting in progressive demyelination, axonal damage with neuronal loss, and gliosis in both the white and gray matter of the central nervous system (CNS) [5,6]. Molecular research has indicated that MS is influenced by a series of biochemical changes in neuronal functions, which are common and

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significant factors in other neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [7,8].

Currently, magnetic resonance imaging (MRI) is used for evaluating disease progression, determining prognosis, monitoring disease activity, and responding to treatment; however, this tool is expensive, lacks sensitivity, is time-consuming, and is a semiquantitative imaging marker [9,10]. Therefore, a better understanding of biomarkers can be effective in determining the prognosis, definitive diagnosis, and development of new therapeutic methods for the treatment of MS, but to the best of our knowledge, there are no effective serum biomarkers for the diagnosis of MS [11].

Various studies have indicated the possible role of the neuroendocrine system in the diagnosis and prognosis of MS. Recent studies have shown that interactions and communication between immune system factors and neuroendocrine activity, as well as endocrine disorders related to the dysregulation of hormone secretion, can contribute to the expansion of diseases and exacerbate their course. Moreover, the neuroendocrine system has immune-modulatory potential, and the advantageous effects of important hormones, including thyroid and sex hormones, are well defined in MS experimental models [12,13]. In this respect, the present study was undertaken to assess specific neuroendocrine hormones and peptides, particularly those that play crucial roles in immune system-related diseases. We examined the circular concentrations of the adrenocorticotropic hormone (ACTH), melatonin, and cortisol hormones in MS.

Many retrospective studies have shown that physical and psychological stress leads to immune dysregulation and altered or amplified cytokine production, resulting in the development of autoimmune diseases or decreased host defense [14,15]. Increased cytokine levels during inflammation, an innate immune system response, can affect glucocorticoid receptors and indirectly upregulate the synthesis of corticotrophin-releasing hormone (CRH), ACTH, and cortisol [2,16]. In response to stress, CRH binds to a surface protein of corticotrophic cells (pituitary cells) and stimulates their release of ACTH [17]. ACTH is derived from proopiomelanocortin (POMC), which is processed by the prohormone convertase PC1 in the anterior pituitary [18]. In the intermediate pituitary lobe and hypothalamus, POMC is further processed by PC2 and other enzymes into more active peptides, such as α-melanocyte-stimulating hormone and β-endorphin [19]. ACTH is one of the first neuropeptides shown to act on receptors on leukocytes and inhibit immune responses. However, certain functions, such as stimulating glucocorticoid synthesis and secretion in adults, can be enhanced. In addition, ACTH plays an important role in the immune system by acting on the adrenal cortex to regulate the production of cortisol [12].

Cortisol, also known as the primary stress neurohormone, is a steroid hormone that is classified within the glucocorticoid class of hormones [20]. It is typically anti-inflammatory, has a critical impact on regulating the immune system, and helps to control inflammation within the body [14,21]. Different studies have demonstrated that light conditions can influence cortisol levels, with peaks occurring in the morning. Interestingly, cortisol and melatonin respond oppositely to light: while cortisol levels rise in response to light, melatonin is synthesized strictly during the night [22]. Melatonin, a neurohormone, also plays a role in regulating sleep–wake cycles, antioxidant properties, and anti-inflammatory effects [23]. Emerging research indicates that melatonin can inhibit proinflammatory Th17 cells (immunosuppression) and stimulate inflammation in people with certain autoimmune disorders [24,25].

On the other hand, measurements of the circular concentration of creatine phosphokinase (CPK) and liver enzymes such as serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and lactate dehydrogenase (LDH) in serum blood can be indicative of liver diseases and may provide early signals for the risk of other chronic diseases. According to the alteration pattern, liver enzymes in various patients indicate increased SGPT and SGOT in dominant liver injury, increased alkaline phosphatase in cholestatic syndromes, and increased CPK in systemic lupus erythematosus-related myositis [26,27]. The main objective of the present study was to measure the serum levels of specific neuroendocrine hormones and peptides, particularly ACTH, melatonin, and cortisol, in patients with MS. Additionally, we aimed to explore the relevance of CPK and liver enzymes such as SGPT, SGOT, and LDH with MS.

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2. Materials and Methods

2.1. Sample Collection and Participants

This research aimed to evaluate the ACTH, cortisol, and melatonin concentrations in the serum of patients with MS and healthy controls without MS who were referred to the Specialized Center of Genetic Blood Diseases in Thi-Qar city (Iraq) between 16 March and 30 June 2023. For this purpose, 50 patients with MS and 50 healthy people were included in the study. The inclusion criteria for the patient group included a confirmed diagnosis of MS as defined by the McDonald criteria, with the condition having been present for over a year, and participants had to be at least 18 years old. Informed and written consent was obtained from all the participants. MS cases from other diseases were approved by the patient via a combination of clinical evaluation, imaging, and molecular testing. Patients with liver deficiency, kidney disorders, thyroid disorders, acute coronary syndrome, Alzheimer's disease, various cancers, or a family history of dementia were excluded from the study. The control group comprised healthy individuals, matched by age and sex, who had not been diagnosed with or suffered from heart disease, thyroid disorders, Alzheimer's disease, or other neurological disorders. Relevant sociodemographic, clinical, and laboratory data, such as age, sex, SGPT, SGOT, LDH, CPK, and body mass index (BMI), were obtained from the patients' medical records and recorded on data sheets. Anthropometric measurements, including weight and height, were also taken. Blood samples (5 ml) were collected from peripheral veins into K-EDTA tubes, plain tubes, and Na-containing tubes. The serum was separated immediately after clotting by centrifugation at 8000 rpm for 10 minutes. Serum samples were stored at -20°C for biochemical analysis, and the levels of ACTH, cortisol, and melatonin were determined via an ELISA kit.

2.2. Neurohormone and Neuropeptide Measurements via ELISA Kits

Human ACTH (LS-F39298, LSBio), human cortisol (LS-F10024), and melatonin (LS-F39279) ELISAs were used in this research. Additionally, a centrifuge instrument (KK, China) and an ELISA reader (Biotek 1800, USA) were used to obtain the serum samples.

2.3. Statistical Analysis

All experiments were independently replicated at least three times, and the results are presented as the mean ± standard deviation (SD) and were analysed via Graph Pad in Stat version 10.0.2 (Graph Pad Software, San Diego, CA). Statistical significance was determined via Student's t test with a significance threshold of $p < 0.05$.

3. Results

The clinical and demographic characteristics and biochemical parameters of the whole MS group and the control group are summarized in Table 1. The median age of the subjects was 39.83±7.79 years, ranging from 25--55 years. As shown in this table, 22.7% and 77.3% of the patients were female and male, respectively. The median BMI of the MS patients was 26.38±3.53 kg/m^2 (ranging from 16.85--33.95 kg/m^2). The levels of serum CPK (66.28±15.60 U/L vs. 65.98±14.87 U/L), SGOT (26.00±6.26 U/L vs. 23.72±6.56 U/L), and SGPT (27.49±7.14 U/L vs. 27.30±7.03 U/L) were measured in MS patients and the control group. The results revealed no significant differences between the two study groups. Furthermore, the serum levels of LDH (275.11±98.02 U/L vs. 217.74±74.15 U/L; p=0.001) and cortisol (218.52±34.57 ng/mL vs 137.90±43.46 ng/mL; p=0.0001) were significantly greater in MS patients than in the control group. However, the levels of ACTH (41.43±24.23 pg/mL vs 54.26±27.52 pg/mL; p=0.007) and melatonin (23.11±4.83 pg/mL vs 41.65±6.98 pg/mL; p=0.0001) were significantly lower in MS patients.

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Table 1. The clinical and demographic characteristics of the subjects.

Parameters	Patients Mean±SD (n=50)	Control Mean±SD (n=50)	P value
Age (years)	39.83±7.79	43.02±5.60	0.009**
BMI (kg/m²)	26.38±3.53	26.32±3.78	0.921
CPK (U/L)	66.28±15.60	65.98±14.87	0.910
LDH (U/L)	275.11±98.02	217.74±74.15	0.001**
SGOT (U/L)	26.00±6.26	23.72±6.56	0.043
SGPT (U/L)	27.49±7.14	27.30±7.03	0.872
ACTH (pg/mL)	41.43±24.23	54.26±27.52	0.006**
Melatonin (pg/mL)	23.11±4.83	41.65±6.98	0.0001**
Cortisol (ng/mL)	218.52±34.57	137.90±43.46	0.0001**

Correlations between different clinical factors were evaluated, and the findings are shown in Table 2. As depicted in this table, there was a negative and significant association between the serum levels of CPK and LDH (p=0.036). In addition, the serum level of LDH was negatively and significantly associated with SGOT and ACTH, with p values of 0.038 and 0.001, respectively. A positive and significant correlation was observed between LDH and cortisol levels. Furthermore, a positive and significant association (p=0.050) was detected between the serum SGPT and SGOT levels. On the other hand, the correlations between melatonin and both cortisol and ACTH were negative and positive, with p values of 0.0001 and 0.009, respectively.

Table 2. Correlations between different clinical factors.

Characteristics		EDSS	CPK	LDH	SGOT	SGPT	ACTH	Melatonin	Cortisol
EDSS	R	1	0.221	-0.041	0.034	0.200	0.076	0.096	0.132
	P value	-	0.056	0.724	0.772	0.085	0.516	0.412	0.241
CPK	R	0.221	1	-0.188*	-0.075	0.137	0.125	-0.038	0.132
	P value	0.056	-	0.036	0.404	0.129	0.166	0.671	0.142
LDH	R	-0.041	-0.188*	1	0.186*	-0.040	-0.291**	-0.131	0.231**
	P value	0.724	0.036	-	0.038	0.658	0.001	0.145	0.009
SGOT	R	0.034	-0.075	0.186*	1	0.176*	0.094	-0.156	0.109
	P value	0.772	0.404	0.038	-	0.050	0.295	0.082	0.228
SGPT	R	0.200	0.137	-0.040	0.176*	1	0.161	0.046	0.058
	P value	0.085	0.129	0.658	0.050	-	0.072	0.610	0.519
ACTH	R	0.076	0.125	-0.291**	0.094	0.161	1	0.233**	-0.221*
	P value	0.516	0.166	0.001	0.295	0.072	-	0.009	0.013
Melatonin	R	0.096	-0.038	-0.131	-0.156	0.046	0.233**	1	-0.577**
	P value	0.412	0.671	0.145	0.082	0.610	0.009	-	0.0001
Cortisol	R	0.134	0.132	0.231**	0.109	0.058	-0.221*	-0.577**	1
	P value	0.251	0.142	0.009	0.228	0.519	0.013	0.0001	-

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

MS is a chronic and inflammatory autoimmune disease that affects the CNS and causes destruction of the myelin sheath. This disease leads to various physical and cognitive disabilities in patients and has extensive effects on various biological systems of the body [28,29]. In this study, the serum levels of cortisol, ACTH, melatonin, and LDH were investigated in patients with MS. Various studies have investigated the changes in the serum levels of cortisol, ACTH, melatonin and LDH in

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patients with MS. Our study revealed increases in cortisol and LDH and decreases in melatonin and ACTH in patients with MS (Table 1), which are in accordance with the results of previous studies.

Cortisol, a steroid hormone secreted by the adrenal glands in response to stress, plays an important role in regulating the immune system and inflammatory responses [30]. Elevated cortisol levels in MS patients may be due to chronic stress and disease-induced inflammation. Studies have shown that cortisol levels in MS patients are usually higher than normal. This increase may be due to the body trying to control the inflammation and stress associated with the disease. For example, a study by *Fassbender* et al. [31] revealed that cortisol levels are increased in patients with MS, and this increase can be considered a compensatory mechanism to reduce inflammation and tissue damage. In patients with MS, serum cortisol levels are increased. This increase may be due to the following reasons: chronic stress can cause chronic stress in patients, which leads to increased secretion of cortisol. Inflammation: Cortisol has an anti-inflammatory role, and its increase can be the body's response to inflammation caused by disease.

ACTH is a peptide hormone secreted by the pituitary gland that stimulates the secretion of cortisol from the adrenal glands [32]. A decrease in ACTH levels in patients with MS can indicate a disturbance in the hypothalamic–pituitary–adrenal (HPA) axis. Previous studies have shown that the HPA axis may be disrupted in patients with MS. For example, research by *Wei and Stafford* [33] has shown that the decrease in ACTH levels in patients with MS could be due to an insufficient response of the pituitary gland to chronic stress and inflammation. This reduction may lead to defects in cortisol regulation and further elevation of cortisol levels. A decrease in ACTH levels and disruption of the HPA axis have also been reported in previous studies as signs of hormonal regulation problems in these patients [34]. The HPA axis may be disrupted in patients with MS, which can lead to decreased ACTH production. The pituitary may not be able to produce enough ACTH in response to chronic stress and inflammation.

Melatonin is a hormone secreted by the pineal gland and plays a critical role in regulating the sleep–wake cycle [35,36]. Decreased melatonin levels in patients with MS can lead to sleep problems and severe fatigue, which are common symptoms in these patients. Research has shown that melatonin levels are decreased in patients with MS. For example, *Sandyk* and *Awerbuch* [37] reported that a decrease in melatonin can lead to an exacerbation of MS symptoms, including fatigue and sleep problems. Additionally, melatonin has antioxidant properties, and its reduction can lead to increased oxidative stress and aggravation of nerve damage in patients with MS [38]. A decrease in melatonin levels has also been reported in previous studies as an effective factor for aggravating MS symptoms, including fatigue and sleep problems. MS patients often suffer from sleep problems, which can lead to reduced melatonin production. On the other hand, chronic stress and inflammation can affect melatonin production [39]. LDH is an enzyme that is involved in glycolysis, and an increase in its level usually indicates tissue damage [40]. The increase in LDH levels in patients with MS may be caused by the destruction of nerve tissues and inflammation. Previous studies have also shown that LDH levels in patients with MS are usually greater than normal, and this increase can be used as a biomarker for disease severity. For example, research by Philip G. et al. [41] has shown that increased LDH levels can be related to the severity of nerve tissue destruction and disease progression. An increase in LDH levels, a biomarker for MS disease severity, has also been reported in previous studies. The increased LDH levels in MS patients may be due to tissue damage. The destruction of nerve tissues and myelin in MS can lead to an increase in LDH levels. In addition, chronic inflammation in MS can increase LDH production.

The HPA axis plays an important role in regulating stress responses. Disturbances in this axis can lead to changes in cortisol and ACTH levels. Increased cortisol may act as a compensatory mechanism to reduce inflammation, whereas decreased ACTH may result from an inadequate pituitary response to chronic stress. Melatonin and cortisol act as two important hormones that regulate the sleep–wake cycle and stress responses. Decreased melatonin can lead to sleep problems and increased stress, which in turn can lead to increased cortisol levels. An increase in LDH can indicate tissue damage caused by chronic inflammation. Chronic inflammation in MS can increase cortisol production, which acts as an anti-inflammatory mechanism.

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5. Conclusions

In our study, the serum levels of ACTH, cortisol, melatonin and LDH were evaluated in MS patients. The results of our study revealed that cortisol and lactate dehydrogenase levels were increased and that melatonin and ACTH levels were decreased in MS patients. These changes may be due to chronic inflammation, stress and HPA axis disorders in these patients. The results of our research are consistent with the findings of previous studies and can contribute to a better understanding of the pathophysiological mechanisms of MS and the development of new therapeutic methods.

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