The Relationship Between Serum Orexin A, TGF-β, and Leptin Levels with Body Mass Index in Multiple Sclerosis Patients

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Research Article

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Abstract

Backgrounds: Multiple Sclerosis (MS) is a chronic inflammatory and autoimmune disease linked to several inflammatory and dietary parameters. This study was carried out to determine the relationship between serum leptin, orexin-A, and TGF-β levels with BMI in MS patients.

Methods and results: In this cross-sectional study, 25 relapsing-remitting multiple sclerosis (RRMS) patients and 40 healthy controls were enrolled. The serum level of Leptin, Orexin-A, and TGF- were measured by the Enzyme-linked immunosorbent assay (ELISA). The data was analyzed using descriptive statistics, t-test, Chi-square test, and Linear regression test. A total of 65 volunteers, including 25 MS patients and 40 healthy, were enrolled in the study. The mean age of individuals in the case and control groups was 38.04 ± 7.53 and 40.23 ± 5.88. There were no statistically significant differences between the case and control groups regarding gender, age, alcohol, and cigarette use (P>0.05). The mean serum levels of Orexin-A and TGF-ß were lower among multiple sclerosis patients than in healthy controls, but leptin was higher (42.8 vs. 18.9 ng/ml, P<0.001). The relationship between BMI and serum levels of Orexin-A, TGF-ß, and Leptin among Multiple Sclerosis patients was not statistically significant (P > 0.05).

Conclusion: Our results showed that the serum levels of Orexin-A and TGF-β were significantly lower. The serum level of leptin was higher among multiple sclerosis patients than among healthy controls. Also, there was no statistically significant relationship between BMI and serum levels of Orexin-A, TGF-ß, and Leptin among multiple sclerosis patients.

1. Introduction

Multiple Sclerosis (MS) is a potentially disabling disease of the brain and spinal cord with a high prevalence [1]. Despite many studies about MS, the leading cause of the illness remains unknown. There are more than 2.8 million MS patients worldwide [2]. In MS, the immune system attacks the protective sheath (myelin) that covers nerve cells and causes sensory-motor problems. Eventually, the disease can cause permanent damage or deterioration of the nerves [3, 4].

Moreover, MS is correlated with extended T-cell reactivity towards various antigens and abnormal B-cell responses [5, 6]. Because neurologic involvement sites differ among patients, different clinical observations are often recorded. The risk of developing MS is high in individuals with a genetic background of autoimmunity, exposure to various hazardous elements, including obesity, EBV (Epstein-Barr virus), vitamin D deficiency, smoking, and the absence of sunlight exposure [4–6]. Many studies have reported obesity and BMI on various health issues and neurological disorders, such as MS, Alzheimer's, and Parkinson's disease [7].

Orexin-A is a neuropeptide that controls some physiological activities such as sleep and diet behaviours [8]. The roles of Orexin-A in glucose and energy metabolism are well known. Multiple studies have reported that Orexin-A exhibits a neuroprotective role, which indicates its therapeutic implementation in neurological disorders associated with inflammation like MS [9, 10].

Leptin controls our dietary behaviour like seeking food, starvation, or suppressing it [11]. Also, leptin has pro-inflammatory properties and acts as an acute-phase protein, up-regulated by inflammatory mediators, can modulate the immune system by induction of cytokines. An imbalance in leptin levels may suppress immunity and facilitate development of an autoimmune disease such as MS and MS-related disabilities [12, 13].

TGF-β is produced by many immune and non-immune cells. It has been demonstrated that TGF-β influences the activity and development of both T helper cells and regulatory T cells that are dysfunctional in MS sufferers [14]. Regarding that T helper cells mediate auto-immunity, the correlation of TGF-β with neurodegeneration-related disorders like MS, Alzheimer's, and Parkinson's disease has been widely explored [15–17].

BMI is a statistical measure of a person's weight and height and is an excellent tool to estimate weight health; it shows how well your size and weight fit together [18]. Moreover, Leptin, TGF-β, and Orexins can be involved in MS pathology. The current study is aimed at evaluating the serum concentration of Orexin-A, TGF-β, Leptin and distinguishing the relationship between these factors and BMI in MS patients.

2. Materials And Methods

2.1. Study population

All participants were assigned the consent forms and were justified about the aim of the study. This study included 25 MS patients (21RRMS/4CIS) and 40 healthy as control groups. MS patients who visited Razi hospital (Tabriz, Iran) were diagnosed according to the standard serum and CSF biomarkers by an experienced neuroscientist. McDonald's criteria were used for MRI finding interpretation. We consider the absence of gadolinium contrast-enhancing as no evidence of disease activity (NEDA). Alcohol consumption, corticosteroids, blood lipid-lowering, and immunosuppressive drugs are considered exclusion criteria.

2.2. Sample preparation

For measuring biochemical factors, 10ml of blood was collected after overnight fasting from each individual. The samples were poured into tubes and centrifuged at 3000 rpm for four minutes at 4°C and stored at -80°c.

2.3. Measuring methods

2.3.1. Measurement of Orexin-A

Orexin-A was measured by the Human Orexin-A ELISA Kit (cat number: CSB-E08859h) of the CUSBIO company. The principle of the assay is the quantitative sandwich enzyme immunoassay technique. A pre-coated antibody captured Orexin-A. After washing, a biotin-conjugated antibody specific for Orexin-A was used, an unbound antibody removed, and avidin conjugated Horseradish Peroxidase (HRP) was added to

the wells. Unbound avidin conjugated removed, and substrate used to produce color. The intensity of color was measured at 450nm.

2.3.2. Measurement of TGF-β

The Biosensis ELISA Kit (cat number: BEK_2093_1P) was used to determine the concentration of TGF-β. The capture antibody is immobilized in the wells. Samples were added to these wells, and after washing, a biotinylated TGF-β antibody as a detector was used. The Avidin-Biotin-Peroxidase complex, which binds to the second antibody, was added. The peroxidase substrate was used to induce a colored reaction product and measured at 450nm.

2.3.3. Measurement of Leptin

We used the ALPCO ELISA kit (cat number: 11-LEPHU-E01, USA) following company instructions to determine leptin concentration. Like Orexin-A and TGF-β, the sandwich enzyme immunoassay technique was the basis of the assay. Leptin is present in the samples bound to the immobilized antibody and the biotinylated antibody, thus forming a sandwich complex. After adding streptavidin-HRP and substrate, the OD of the colored product was measured at 450nm.

2.3.4. Measurement of cholesterol and triglyceride

The pars azmoon assay kit (cat number: 110 500 BT, Iran) for automatic biochemistry analyzer (BT3000) was used to measure cholesterol concentration. The formation of quinone imin, produced by hydrolysis and oxidation of cholesterol, directly shows the amount of cholesterol and is easily measurable photometrically at 550nm.

Also, for measuring triglyceride concentration, the pars azmoon assay kit (cat number: 132 504 H917) was used for the Hitachi 917 automatic biochemistry analyzer. The measuring principle produces quinone imin by the reaction of glycerol with 4-amino antipyrine and phenol, which can be measured photometrically (550nm).

2.3.5. Calculating BMI

The patient's weight and height were measured, and the BMIs were calculated according to the available standard tables. The following formula calculated BMI; BMI (kg/m^2) = weight (kg)/height (m^2).

2.4 Statistical Analysis

To display categorical variables, frequency, percentage, and continuous variables, mean and standard deviation (SD) were used. To assess the normal distribution of continuous data, the Kolmogorov-Smirnov test was applied. The t-test and Chi-square tests were used to compare continuous and categorical variables between case and control groups. A logistic regression test was used to assess the relationship between some serum biomarkers between the case and control groups. A linear regression test was also used to determine the relationship between some serum biomarkers and BMI among MS patients. A P-

value of < 0.05 was considered statistically significant. All data was analyzed using SPSS version 21 and Graph Pad Prism 8.

3. Results

3.1. Demographic data

A total of 65 volunteers, including 25 MS patients and 40 healthy controls, were enrolled in this study. The demographic characteristics of the study population are shown in Table 1. There were no statistically significant differences between the case and control groups regarding gender, age, alcohol and cigarette use (P > 0.05).

Table 1
Demographic and disease-related characteristics of the study population

Variable	Total	Case	Control	P-value*
Number	65	25	40	
Gender, N (%)	33 (50.8%)	12(48%)	21(52.5%)	0.46
Male	32 (49.2%)	13(52%)	19(47.5%)	
Female				
Mean age (SD)	39.38 (6.59)	38.04 (7.53)	40.23 (5.88)	0.224
OCP history, N (%)	-	-	-	-
Cigarette history, N (%)	3 (4.6%)	3(12%)	0	0.053
Alcohol use, N (%)	-	-	-	-
Diabetic disease, N (%)	2 (3.1%)	2 (8%)	0	0.14
Cancer history, N (%)	-	-	-	-
Duration of Multiple Sclerosis disease (year)		2.84 (1.8)	-	-

*chi-squared test was used.

3.2. Biochemical and Anthropometric characteristics

As shown in Table 2, there were significant differences in serum levels of triglyceride and cholesterol between the case and control groups (P < 0.0001). The anthropometric characteristics of the study population were not statistically significant (P > 0.05). The serum levels of Orexin A and TGF-ß were lower in MS patients than in healthy controls. The Leptin level was higher in MS patients, and the differences were also statistically significant (P < 0.001) (Fig. 1).

Table 2
Biochemical and Anthropometric characteristics between cases and controls

Variable	Total	Patients with Multiple sclerosis (n = 25)	Control groups (n = 40)	P-value*
	Mean (SD)	Mean (SD)	Mean (SD)	
TG(mg/dl)	159.49 (77.5)	242 (64)	107.8 (15)	**< 0.0001**
Chol (mg/dl)	155.92 (62.5)	228.4 (33)	110.6 (16.4)	**< 0.0001**
Orexin A(ng/ml)	0.58 (0.22)	0.41 (0.21)	0.69 (0.14)	**< 0.0001**
TGF-B (ng/ml)	69.09 (18.1)	49.4 (10)	81.35 (8.7)	**< 0.0001**
Leptin (ng/ml)	28.09 (12.4)	42.8 (5.9)	18.9 (2.8)	**< 0.0001**
weight	71.23 (13.9)	72.04(15)	70.73 (13.4)	0.502
height	167.1 (10.5%)	166.4 (11)	167.5 (10.3)	0.640
BMI	25.4 (3.7)	25.8 (3.9)	25 (3.6)	0.490

* T-test was used; TG: triglyceride; Chol: cholesterol; BMI: Body mass index

3.3. The Relationship between serum level of orexin-A, TGF-β, Leptin and BMI in Multiple Sclerosis patients

The results of the linear regression test showed that for every unit increase in the BMI of MS patients, the serum levels of Orexin-A and TGF-ß increased by 0.2% and 24%, respectively, but the level of Leptin decreased by 34%. However, the relationships were not statistically significant (P > 0.05) (Table 3).

Table 3
The relationship between blood level of Orexin A, TGF-β, Leptin, and BMI in Multiple Sclerosis patients

	Unstandardized Coefficients		Standardized Coefficients	t	P-value*
	B	Std. Error	r		
Orexin A(ng/ml)	0.002	0.012	0.028	0.136	0.893
TGF-B(ng/ml)	0.245	0.537	0.095	0.456	0.653
Leptin(ng/ml)	-0.340	0.310	-0.223	1.096	0.284
*Linear regression test was used.					

4. Discussion

Multiple sclerosis (MS) is a neurodegenerative disorder that causes inflammation in the central nerve system (CNS), which leads to neuropathy [19, 20]. According to epidemiological studies, its prevalence is closely associated with nutrition, and it commonly occurs in people with high levels of fatty acid and Vitamin D deficiency [21]. This study evaluated serum levels of Leptin, Orexin-A, and TGF-β in 25 MS patients and investigated the relationship between these factors and BMI.

We found low levels of TGF_B and Orexin-A and high levels of Leptin in MS patients compared to healthy controls, and the stories had changed significantly. Also, significant differences were observed in the levels of triglycerides and cholesterol between test groups. But the relationship between BMI and serum levels of Orexin A, TGF_B, and Leptin was not statistically significant.

An increase in BMI is an MS risk factor, and MS is more common in people who are genetically predisposed to high BMI [22]. The mechanism of BMI increase in MS has not yet been well studied. But some studies have reported a direct relationship between BMI and disability in RRMS patients. One study reported that women who experienced higher BMIs in adulthood were more likely to develop MS at an earlier age. This could be prevented by choosing a proper lifestyle and dietary habits [23]. Several studies over the past decade have shown that early obesity in childhood and adulthood are significant risk factors for MS [24]. With the onset of MS, obesity can worsen the disease and impair the response to the treatment (s). Therefore, obesity causes sensitivity to MS and acts as an essential risk factor for the disease [14].

High levels of pro-inflammatory cytokines and leptin and low anti-inflammatory cytokines were observed in obese MS patients [25]. Generally, MS patients under a therapy regimen show lower leptin levels in serum and CSF, whereas leptin levels during the acute phase of MS are usually high [21, 26]. Reducing leptin levels leads to amelioration of experimental autoimmune encephalomyelitis (EAE), an experimental

animal model for human MS. Also, the onset of disease was delayed, and the clinical symptoms improved [27].

Nowadays, we know more about leptin's role in the immune system [26–28]. Leptin directly acts on the leptin receptor (LepR) on $CD4^+$ T cells and induces metabolic and functional changes in T cells. Leptin-resistant $CD4^+$ T-cells can not regulate cytokine production properly [29]. This receptor-mediated problem is the basis of some autoimmune diseases such as MS.

Leptin induces anorexia during acute inflammatory responses [30]. Various studies have shown that nutritional intake is significantly lower in MS patients than in healthy controls [31]. A significant increase in leptin levels can explain the reduction in nutrient intake. On the other hand, Orexin-A is essential for eating behavior. Orexin-A increases appetite while decreasing metabolism [32, 33]. Our results showed significant changes in both Orexin-A and leptin concentration in MS patients compared to healthy controls following these reports. Orexin-A secretion is increased during physical activity [34]. Some diseases affecting the CNS, such as MS, can reduce the secretion of this neuropeptide.

Unfortunately, the importance of nutritional status is often underestimated by patients and therapists. Probably, an imbalance in Orexin-A and leptin levels affects the eating behaviors of patients. On the other hand, there is dietary advice available for MS patients, which is contradictory and not investigated [35].

Interestingly, we could not find any association between BMI values in the patient and control groups. This finding was also reported by some other studies [31, 36]. What is the explanation for this contradictory finding? Although there was enough appetite and suitable hormonal levels, high leptin level and low Orexin-A level, the BMI did not change in the patients group compared to the control group. Studies have shown leptin resistance when people have a diet rich in fat [37, 38]. As we have shown in Table 2, MS patients had significantly higher triglycerides and cholesterol levels than healthy participants, which may induce leptin resistance in patients. Also, MS is a nervous system disorder that may disrupt many nervous system-associated processes, such as vagal nerve functions, appetite, metabolism, and obesity. In addition, MS can affect many cytokines and inflammatory mediators [39, 40]. Orexins also have neuroprotective and immune-regulating (i.e. anti-inflammatory) properties. Studies show that orexins may have therapeutic potential in several pathologies with a single immune component, including multiple sclerosis, Alzheimer's disease, obesity, septic shock, and cancer [41]. We suggest more investigations into dietary-dependent neuropathy in MS patients and more in-depth examinations of the role of BMI in MS patients.

TGF-β, a cytokine produced by monocytes, smooth muscle cells, and endothelial cells, is involved in tissue development, extracellular matrix production, and immune system regulation via blocking inflammatory responses and promoting regulatory T cells. Furthermore, TGF-β plays a pivotal role in obesity and some significant disorders [42, 43]. Mice lacking TGF-β receptors developed the inflammatory disease. Also, the inability of T cells to produce TGF-β led to the development of autoimmune disorders

[44]. Also, according to a study, the imbalance in IL-10 and TGF levels leads to obesity via inefficient regulation of the inflammatory cytokines [45].

TGF-β ameliorates EAE in mice. It seems that TGF-β is associated with an increase in specific cytokines such as interleukin 2 (IL2), IL6 and IL12 [46]. Regulating the TGF-β level can be utilized as a therapeutic approach. A study demonstrated that TGF-β is low in MS patients, associated with increased IL2 and IL6 [47]. In one study, a significant reduction in TGF-β expression was observed in patients with RRMS, consistent with our research [48]. In one study done in 2011, TGF-β signalling protected mice from obesity and diabetes. These results suggest that TGF-β signalling regulates glucose tolerance and energy homeostasis and suggest that modulating TGF-β activity may be an effective treatment strategy for obesity [49]. TGF-β is a central regulator in many pathophysiological processes in MS. We demonstrated there was no significant association between BMI and TGF-β in MS patients.

5. Conclusions

Our results demonstrated that the serum level of leptin among patients with relapsing-remitting multiple sclerosis was high, and the serum levels of TGF-β and Orexin-A were low. Also, there was no significant relationship between BMI and serum levels of Leptin, TGF-β, and Orexin-A among Multiple Sclerosis patients.

Declarations

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

All data obtained in the study can be accessed if desired.

Authorship contribution statement

Sepideh Moharami: Writing - original draft. **Alireza Nourazarian**: Conceptualization, Writing - original draft. **Masoud Nikanfar:** Data curation. **Delara Laghousi:** Methodology. **Behrouz Shademan:** Methodology, Data curation. **Omid Joodi khanghah:** Methodology. **Fatemeh khaki-khatibi:** Conceptualization, Methodology, Writing - review & editing.

Satisfaction with publication

All authors agree to publish the data of this study.

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Figures

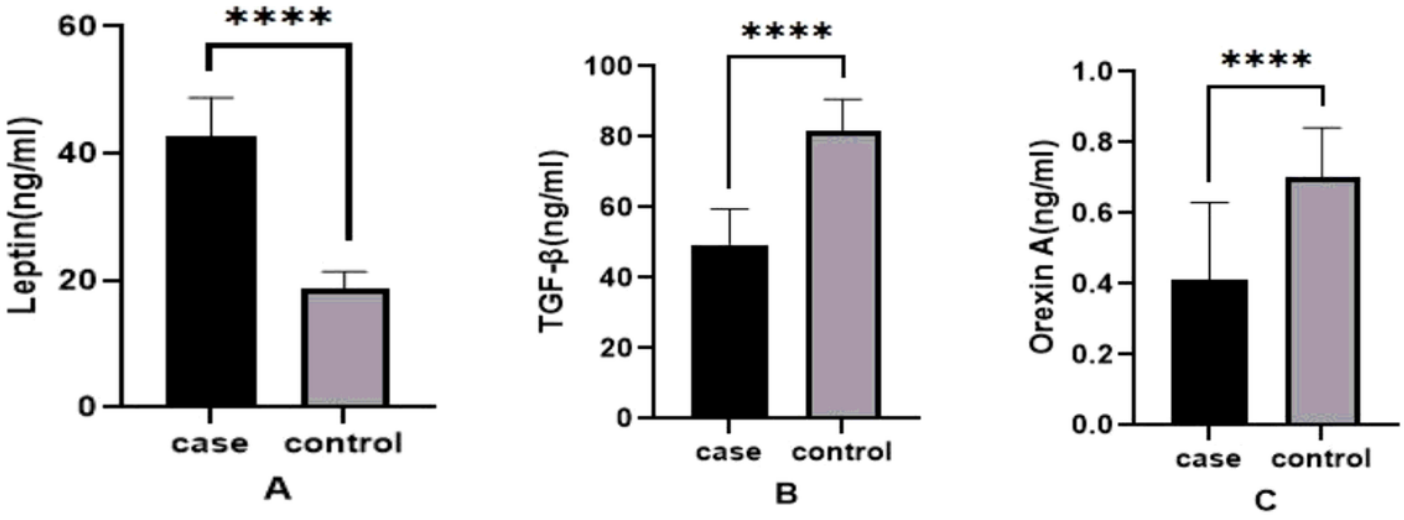

Figure 1

Comparison of the serum level of Leptin (A), TGF-β (B) and Orexin A (C) among MS patients and the control group.