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Free vitamin D and vitamin D binding protein in multiple sclerosis patients

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Abstract

Objective: In multiple sclerosis (MS), where low vitamin D level is a risk factor, there is no previous study evaluating the level of free vitamin D by direct measurement, and there is little information about vitamin D binding protein (DBP). This study assessed free vitamin D and DBP in patients with MS. *Methods:* The study subjects consisted of 43 MS patients and 25 controls. Free vitamin D and DBP levels were measured through an enzyme-linked immunosorbent (ELISA) kit. *Results:* The patient group had a mean age of 36.58 ± 9.4 (19-60) and the control group had a mean age of 33.56 ± 9.65 (24-52) years (p=0.210). The patient had free vitamin D level of 21.51 ±9.72 pg/ml and the free vitamin D level of the control group was 26.02 ±9.29 pg/ml. Free vitamin D levels did not significantly differ across the groups (p=0.065). The patient group (34.78 ±18.24 ng/ml) had a significantly higher DBP level than the control group (15.36 ±10.64 ng/ml) (p<0.001).
Conclusion: MS patients have higher DBP than healthy control, while the free vitamin D levels of the patients tend to be lower.

Keywords: Multiple sclerosis, vitamin D, free vitamin D, vitamin D binding protein

INTRODUCTION

It is thought that vitamin D level and vitamin D receptor (VDR) function may play a role in the etiopathogenesis of various neurodegenerative illnesses like multiple sclerosis (MS), due to environmental and genetic factors.[1] In previous vitamin D studies in MS, a clear correlation was found between a rise in serum 25-hydroxyvitamin D levels and a reduction in clinical and subclinical activity in MS.[2]

It is a moot point if the vitamin D binding protein (DBP)-bound fractions of vitamin D metabolites are active. The "free hormone hypothesis" stipulates that free metabolites cross the cell membrane.[3] According to this hypothesis, free and albumin-bound pools transfer 25 (OH) D to the cell, and 25 (OH) D bound to DBP is a systemic reservoir.[4] Free vitamin D may be a more biologically pertinent factor than the total 25 (OH) D.[5] The balance between free and bound vitamin D is contingent on DBP, which is a fundamental regulator of vitamin D in target cells. Genome-wide linkage research has also shown that the genetic determinant of 25 (OH) D vitamin is DBP.[6]

Although we know what role vitamin D plays in the etiology of MS, there is limited information on DBP in the literature, and there is no study on free vitamin D by direct measurement. This study aimed to evaluat DBP and free vitamin D levels in patients with MS.

METHODS

The study subjects consisted of 43 MS patients who presented to Cumhuriyet University Neurology Outpatient Clinic between July 2017 and January 2018 and 25 healthy volunteers. Exclusion criteria were having a disease that would affect vitamin D metabolism, having vitamin D replacement in the last 6 months, having an acute attack in the last 3 months, and receiving steroid therapy. This study received ethical approval from the Cumhuriyet University Clinical Research Ethics Committee no. 2017-07/33. Written consent was received from all participants.

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Free vitamin D and vitamin D binding protein

Approximately 5 cc of blood samples were collected and placed in BD vacutainer tubes with yellow caps. The samples were centrifuged in the biochemistry laboratory and stored at -80 ºC. The levels of free vitamin D (DIAsource, Louvain-la-Neuve-Belgium) and vitamin D binding protein (Elabscience, Wuhan, China.) were measured in patient and control sera using an enzyme-linked immunosorbent (ELISA) kit. The kit measures total Vit D (25(OH)D2 and D3.

Statistical analysis

In this study, where R (Sample Allocutian Ratio) = 1.7 α=0.05 ß=0.10 (1-ß)=0.90, the sample consisted of 68 participants. The power of the test was found as p=0.90343. Our data was uploaded to SPSS (IBM SPSS Statistics Software, version 22) at a significance level of 0.05. Normality was tested using the Kolmogorov-Smirnov test. Discrete data were analyzed through a Chi-square test. The continuous data were compared using the independent sample t-test. The correlation was assessed using Pearson r.

RESULTS

More than half of the participants were male (64.88%). The patient and control groups had a mean age of 36.58 ± 9.4 (19-60) and 33.56 ± 9.65 (24-52) years, respectively. There was no significant difference in gender (p=0.412) and age (p=0.210).

When the patient group was classified according to MS subtypes, 32 (74.42%) of 43 patients had RRMS, 9 (20.93%) had SPMS, and 2 (4.65%) had PPMS. The patient group had the illness for 1.5 years to 28 years (mean 7.1 ± 5.8). As for disease-modifying therapies (DMTs) use, 7 in the patient group were using interferon β-1a (sc), 2 interferon β-1a (im), 6 interfero n β-1b, 4 glatiramer acetate, 8 fingolimod, 3 dimethyl fumarate, 2 teriflunomide, 2 natalizumab and 4 of them were using ocrelizumab; while 5 patients were not using any disease-modifying treatment.

Although the patient group (21.51±9.72 pg/ml) had a lower mean free vitamin D level than the control group (26.02±9.29 pg/ml), the difference was not statistically significant (p=0.065). The patient group (34.78±18.24 ng/ml) had a significantly higher mean DBP level than the control group (15.36±10.64 ng/ml) (p<0.001) (Table 1).

DBP levels were negatively correlated with free vitamin D levels (p=0.039 r=-0.251). However, when the patient and control groups were analyzed separately, DBP was not correlated with free vitamin D levels (p=0.153, r=0.222 and p=0.964 r=0.010, respectively).

DISCUSSION

Vitamin D deficiency is regarded as a risk factor for MS[8] because people with high vitamin D levels are associated with lower prevalence of MS[7] and the different distribution of MS according to latitudes. Vitamin D is active in cell proliferation, differentiation, neurotransmission, and neuroplasticity in the nervous system and plays a neurotrophic and neuroprotective role. Vitamin D is also considered a neurosteroid in the central nervous system.[4] While the role of vitamin D in MS has been clearly demonstrated, there is little previous study on the role of DBP and free vitamin D in MS.

A few studies have also attempted to understand how blood DBP levels change in MS patients. Pediatric MS patients had higher blood DBP than healthy individuals.[10] Rinaldi *et al*. (2015) also reported higher plasma DBP levels in MS patients than in healthy individuals.[11] Contrary to the results of this study, there are also studies reporting that blood DBP levels are not different in CIS, RRMS, and SPMS patients as compared to healthy control and those with other neurological

Table 1: Comparison of MS patients and control group

	Patient Group (n:43) Mean ± Std (Min-Max)	Control Group (n:25) Mean ± Std (Min-Max)	P value
Age	36.58 ± 9.4 (19-60)	33.56 ± 9.65 (24-52)	0.21
Free vitamin D level, pg/ml	21.51±9.72	26.02±9.29	0.065
DBP level, ng/ml	34.78±18.24	15.36±10.64	<0.001

illnesses.[12-14] In addition, in a study conducted in Japan, no relationship was found between DBP gene polymorphism in MS.[15] It has also been shown that DBP levels are not correlated with relapse and vitamin D supplementation in MS patients.[13]

As mentioned, data on blood DBP levels in MS patients are contradictory. There may be many reasons for the differences. One is heterogeneity; investigators often recruit healthy individuals or patients with varied neurological illnesses. There is also considerable variability in patient populations. In addition, the methods used for DBP detection and measurement also differ from study to study. DBP polyclonal ELISA kits should be used to recognize only actin-free DBP. Axonal degeneration in MS patients may lead to actin release in the CNS. Circulating release of DBP-bound G-actin is possible after loss of integrity of the BBB associated with axonal damage and disease relapses. Thus, there may be variation in the ratio of actin-free and actin-bound DBP in different disease phenotypes and/or phases.

There are also previous studies on CSF and serum DBP levels in MS patients. Although these studies do not support the use of DBP level in CSF as a biomarker for MS, it suggests that MS patients and those with other neurological diseases or healthy individuals may differ from each other in terms of DBP levels in CSF, and MS subtypes may be characterized by different CSF DBP levels.[16-21] The reason why DBP levels in CSF differ in MS patients is unclear. Is there intrathecal synthesis of DBP? Or is DBP passing into CSF as a result of blood-brain barrier (BBB) disorder? Gressner *et al*. also reported that DBP levels were increased in CSF in all neurological diseases except trauma. The authors thought that although there was increase in intrathecal synthesis of DBP in patients with CNS damage, DBP in the CSF was primarily due to BBB leakage.[22]

This study compared free vitamin D levels, which is a more specific molecule in determining the active level of vitamin D. Our results show that free vitamin D levels are lower in MS patients than in healthy controls, although this is not statistically significant. To date, no study has reported on serum free vitamin D levels by direct measurement in MS patients. Behrens *et al*. measured 25 (OH) D3 and DBP in CIS patients, estimated the free vitamin D levels and determined that 25 (OH) CIS patients had lower D3 levels than healthy controls, and concluded that but DBP and free vitamin D levels were no different from healthy controls.[23] However, Behrens *et al*. did not directly measure free vitamin D levels. Therefore, this is the first study to directly measure free vitamin D levels in MS patients.

In conclusion, DBP levels were significantly higher and free vitamin D levels were low, although not significantly, in MS patients. We do not know whether vitamin D bioavailability is altered as a result of high blood DBP levels, but high DBP levels may promote immune cell infiltration into the CNS.

This study had three limitations. First, the sample size was small. Second, we did not address total vitamin D and albumin levels. Third, the EDSS and cranial MRI findings in the patient group could not be compared with the free vitamin D and DBP values. There is a need for further studies aiming to compare MS subtypes with a larger number of patients concerning free vitamin D, total vitamin D, albumin and DBP levels, and EDSS and cranial MRI findings. Finally, the ELISA kit used in our study measures total Vit D (25 (OH) D2 and D3). The D3 component level is seasonal. However, D3 variation according to seasons was not evaluated in our study.

DISCLOSURE

Financial support: None

Conflicts of interest: None

Ethics: Permission was obtained from the hospital ethics committee (Date: 20/10/2021 Number: E1-21-2077)

Availability of Data: Data available on request from the authors.

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