ORIGINAL ARTICLE

Serum levels of interleukin-6 and Vitamin D at the onset of multiple sclerosis and neuromyelitis optica: A pilot study

Fereshteh Ashtari[1], Reyhanehsadat Madanian[1], Sayyed Hamid Zarkesh[2], Arshia Ghalamkari[1]

[1]Isfahan Neuroscience Research Center, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran,
[2]Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Interleukin-6 (IL-6) is an important mediator in the acute phase of inflammatory diseases such as neuromyelitis optica (NMO) and multiple sclerosis (MS). The level of IL-6 is higher in cerebrospinal fluid and serum of NMO patients compare to MS. Vitamin D has a regulatory effect on IL-6, so it may have a negative correlation with IL-6 in the acute phase of these diseases. This study was performed to evaluate the serum levels of IL-6 and Vitamin D in NMO and MS patients at the onset of disease to find differences that may help in early diagnosis. **Materials and Methods:** This case–control study was done on patients with the first episode of optic neuritis, transverse myelitis, and area postrema syndrome who were referred to Kashani MS Center in Isfahan, Iran, between January 2018 and January 2020. The serum levels of Vitamin D and IL-6 were assessed using enzyme-linked immunosorbent assay in blood sample taken at the time of first presentation in patients who had a definitive diagnosis of NMO and MS during subsequent workup. **Results:** During a 2-year follow-up, definitive diagnosis of NMO was given in 25 cases, and they were compared with 25 cases that were randomly selected from patients with definite MS. Nineteen patients in the NMO group and 21 patients in the MS group were female. The mean age of patients in the NMO and MS groups was 29.64 ± 1.47 and 30.20 ± 1.42, respectively (P = 0.46). The mean of serum level of Vitamin D was 24.88 ± 15.2 in NMO patients and 21.56 ± 18.7 in MS patients without significant difference (P = 0.48). The mean of IL-6 was 30.1 ± 22.62 in the NMO group and 23.35 ± 18.8 in the MS group without significant difference (P = 0.28). The serum levels of Vitamin D were insufficient in both groups. No correlation between Vitamin D and IL-6 levels was found in our study (P > 0.05). **Conclusion:** Our results showed that serum IL-6 levels were higher at the onset of NMO disease compared with MS. The serum levels of Vitamin D were low in both groups and there was no association between serum levels of Vitamin D and IL-6 in either group. Future studies with large sample size are needed to confirm these findings.

Key words: Interleukin-6, multiple sclerosis, Vitamin D

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INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune disease of the central nervous system (CNS) among the young adults.[1,2] Various aspects of the immune system are impaired in this disease. The activity of Th1 and Th17, which produces pro-inflammatory cytokines such as interleukin (IL)-6, is increased,[3] and regulatory T-cells, which control inflammation, are suppressed.[4] In addition, there is evidence of abnormal activity in B-cells.[5] In other words, MS is an inflammatory disease of the CNS, and inflammatory factors such as IL-6 are increased in the lesions.[6]

Neuromyelitis optica (NMO) is another inflammatory disease of the CNS that was previously considered a severe variant of MS. Although it is now recognized as a distinct disease, its clinical manifestations including optic neuritis, transverse myelitis, and area postrema syndrome are also common symptoms of MS.[7-9] In a significant number of patients, the antibodies against

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Address for correspondence: Mr. Arshia Ghalamkari, Isfahan Neuroscience Research Center, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran. E-mail: arshiaghalamkari@gmail.com
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aquaporin-4 (AQP4) confirm the diagnosis.[9] However, the antibody is absent in 20%–40% of cases, and the similarity of the common clinical symptoms in both diseases may lead to misdiagnosis.[7,10]

AQP4-AB is a specific biomarker in NMO disease, and once enters the CNS, leads to the activation of a complement cascade that damages neural tissue.[11] IL-6 is an important mediator in the acute phase response and may induce AQP4-immunoglobulin G (IgG) secretion, reduces blood–brain barrier integrity, activates pro-inflammatory T-cells, and may induce NMO disease activity.[12] Studies have shown increased IL-6 levels in cerebrospinal fluid (CSF) and serum of NMO and MS patients[13,14] In the acute phase of disease, the CSF levels of IL-6 in NMO patients are higher than in MS and other inflammatory neurologic diseases.[14] Indeed, the association between CSF level of IL-6 with disability was reported in NMO patients.[15] Therefore, inhibition of ILs may be effective in controlling the disease. Recently, satralizumab, an anti-IL-6 monoclonal antibody, has been approved for the treatment of NMO.[16]

Vitamin D deficiency is one of the possible environmental risk factors in MS and NMO. Besides having a significant role in calcium and phosphorus homeostasis, Vitamin D plays a role in regulating the immune system and has anti-inflammatory effects.[17]

Recent studies have shown the positive effect of Vitamin D3 on various clinical parameters in MS patients. Vitamin D plays an essential role in repairing damaged myelin peptides and regulating T-cell proliferation. It also has a positive immune-modulating effect on pro-inflammatory cytokines such as IL-6 and low Vitamin D levels may associate with disease activity in NMO patients.[18] A study in patients with rheumatoid arthritis showed a better response to IL-6 antibody therapy in the presence of adequate serum Vitamin D.[19] Based on these effects, Vitamin D deficiency may be associated with high level of IL-6 and increase disease activity. Although Vitamin D deficiency is one of the risk factors of MS and IL-6 levels may be high in MS patients, the role of IL-6 is more prevalent in NMO and may play a role in disease progression and even in the onset of the disease. To the best of our knowledge, no studies have been performed at the onset of NMO and MS to assess serum levels of Vitamin D and IL-6. This study was performed to compare serum levels of Vitamin D and IL-6 at the first presentation of NMO and MS disease. We aimed to find a correlation between Vitamin D levels and IL-6 levels at the onset of these diseases.

MATERIALS AND METHODS

This is a case–control study to assess serum levels of Vitamin D and IL-6 in MS and NMO patients at the disease onset.

All patients with the first episode of optic neuritis, myelitis, and area postrema syndrome that were referred to Kashani MS Center, affiliated to Isfahan University of Medical Sciences (IUMS), between January 2018 and December 2019, were selected. Inclusion criteria were: The age over 18 years, presence of the first episode of optic neuritis, myelitis, or area postrema syndrome, not receiving corticosteroids during the past 4 weeks, and no underlying diseases including diabetes mellitus, collagen vascular, and cardiovascular diseases.

This study was approved by the IUMS Ethics Committee (no: IR.MUI.REC.1396.2.014), and patients provided informed written consent before enrollment.

Before receiving corticosteroids, a demographic questionnaire was completed and 7 ml of blood was taken from a peripheral vein of each participant. The serum of these samples was separated and kept at 20°C. The patients were then treated with 1000 mg intravenous methylprednisolone for 5 consecutive days. Additional workup including brain and cervical magnetic resonance imaging, AQP4-AB, and vasculitis tests, and if necessary, CSF analysis was used to aid in diagnosis. The patients were followed up for 2 years to confirm the diagnosis of MS according to the McDonald criteria[20] and NMO based on the international consensus diagnostic criteria for NMO spectrum disorders.[21]

Finally, among the patients who were followed up, 25 cases were definitively diagnosed with NMO disease and 95 definite MS.

Twenty-five cases were randomly selected from 95 MS patients and compared with 25 NMO patients. Demographic characteristics (e.g., age and gender) and serum levels of Vitamin D and IL-6 were measured in these patients by enzyme-linked immunosorbent assay (Boster Biological Technology Co., Ltd. Wuhan, China). Regard to our laboratory reference, Vitamin D3 level <10 ng/mL was defined as Vitamin D deficiency, 10–30 ng/mL was defined as Vitamin D insufficiency, and more than 30 ng/mL was defined as Vitamin D sufficiency.

All data were entered to the Statistical Package for the Social Sciences version 22 (SPSS crop. Chicago, IL, USA). Qualitative variables data have been shown as frequency (percentage), and for quantitative variables, data were shown as mean ± standard deviation. To assess normal distribution, Kolmogorov–Smirnov test was performed on different variables; the Mann–Whitney U-test is used to compare differences between nonparametric groups when the dependent variable is either ordinal or continuous, and for categorical groups, Pearson's Chi-square test was used.

RESULTS

In this study, 135 patients with the first episode of optic neuritis, myelitis, or area postrema syndrome were evaluated. After a complete evaluation, NMO was definitively diagnosed in 25 patients and MS definitively in 95 patients. Fifteen patients have no specific diagnosis and were classified as clinically isolated syndrome. Twenty-five patients from the MS group were randomly selected and compared with the NMO group.

Nineteen patients in the NMO group and 21 patients in the MS group were female. The mean age of patients in the NMO and MS groups was 29.64 ± 1.47 and 30.20 ± 1.42, respectively ($P = 0.46$).

There was no significant difference between serum levels of Vitamin D and IL-6 in the two groups ($P = 0.48$ and 0.28, respectively) [Table 1]. Moreover, based on the bivariate Pearson correlation test, the serum level of Vitamin D and IL-6 was not correlated ($P > 0.05$).

DISCUSSION

Our study showed that the serum level of Vitamin D was insufficient in the new-onset MS and NMO groups, without any significant difference.

This result was consistent with a study by Gao *et al*., which found a low level of 25-OH D in NMO patients.[22]

As our research, a study in Indonesia found that despite being near the equator, the prevalence of Vitamin D deficiency is high in MS and NMO patients.[23]

Min *et al*. showed a correlation between disability score and relapse rate with Vitamin D status in NMO patients.[24] On the other hand, another study in the Thai population showed low serum level of Vitamin D in NMO patients without any correlation with disease activity.[25]

Table 1: Demographic and laboratory findings in two groups

	Mean±SD/(%)		*P*
	MS	NMO	
Age (years)	30.20±1.42	29.64±1.47	0.46
Gender (female/male)	21/4 (84/16)	19/6 (76/24)	0.09
Clinical presentation			
ON	18 (72)	14 (56)	
Myelitis	7 (28)	8 (32)	
APR syndrome	0 (0)	3 (12)	
Serum level of Vitamin D	21.56±18.7	24.88±15.2	0.48
Serum level of IL6	23.35±18.8	30.1±22.6	0.28

ON=Optic neuritis; APR syndrome=Area postrema syndrome; MS=Multiple sclerosis; NMO=Neuromyelitis optica disorder; IL-6=interleukin-6; SD=Standard deviation

In addition, the immunoregulatory effect of Vitamin D in NMO has been reported by Wu Y.[18] The positive effect of Vitamin D supplementation on the improvement of symptoms during the treatment of acute relapse in NMO patients has been reported.[26]

Ascherio *et al*. showed a low level of Vitamin D as an important risk factor for long-term activity and progression in MS patients.[27]

Although the relationship between Vitamin D deficiency and the degree of disability in NMO patients has been contradictory, the result of our study showed that Vitamin D deficiency might be common in NMO, the same as MS. It makes sense to evaluate Vitamin D levels in NMO patients and to take supplements if low levels are detected.

In our study, serum IL-6 level was higher in the NMO group than in the MS group, with no significant difference. Perhaps, the reason for the lack of significant difference was the small sample size.

IL-6 is one of the stimulating factors, which differentiates B-cells from plasma cells and leads to the production of immunoglobulin.[12] It is recognized as an important cytokine in inflammatory diseases of CNS and is produced by different cells. IL-6 increases the survival of plasmablasts that produce AQP4-IgG antibodies, that can cause further tissue damage.[12] High concentrations of CSF IL6 have been reported in initial NMO attacks and suggested CSF IL-6 as an early diagnostic marker of NMO disease.[8] Although a prognostic marker for NMO patients has not yet been established, CSF levels of IL-6 may be considered a biomarker for NMO activity.

The modulatory effect of Vitamin D on IL-6 has been reported recently during COVID-19 infection.[17] In addition, Wesselink *et al*. found an inverse relationship between Vitamin D and IL-6 levels in the first 2 years after diagnosis of colorectal cancer.[28] We evaluated the association between serum levels of Vitamin D and IL-6 but found no such association.

Considering all these cases, maintaining a high serum level of Vitamin D using supplements to reduce IL-6 levels and disease activity in NMO patients should be recommended.

CONCLUSION

Our study showed higher serum level of IL-6 at the onset of NMO disease compared with MS but not significantly, and Vitamin D insufficiency in both diseases.

Based on our findings, there was no association between serum levels of Vitamin D and IL-6 in either group. As

our study is a pilot study with small sample size, future studies with larger sample sizes are needed to evaluate the correlation between serum IL-6 levels and Vitamin D at the onset of these diseases.

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Conflicts of interest
There are no conflicts of interest.

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