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Short communication

Low serum vitamin D levels in patients with myasthenia gravis

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ABSTRACT

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disease. Vitamin D has important roles both in the autoimmune response and in skeletal muscles. We investigated the levels of 1,25-dihydroxy vitamin D [1,25$(OH)_2$D] and 25-hydroxy vitamin D [25(OH)D] in patients with MG and healthy subjects. MG patients were classified by disease stage, age of onset and treatment status whether or not to taking immunosuppressive agents. MG patients had lower plasma 25(OH)D levels (mean, 18.8 ± 8.4 ng/mL) than healthy controls (26.3 ± 6.1 ng/mL) ($p < .05$). 1,25$(OH)_2$D levels showed slightly high in MG patients than healthy controls, but had no significant difference between two groups. In addition, no significant differences were observed between two groups divided by clinical characteristics. Serum 25(OH)D levels significantly lower in patients with MG compared with healthy controls. We recommend monitoring of vitamin D status in patients with MG to avoid direct negative effects on the muscles or autoimmune response.

1. Introduction

Myasthenia gravis (MG) is an autoimmune disease generally mediated by anti-acetylcholine receptor (AChR) autoantibodies. The pathogenic role of anti-AChR antibodies in MG has been clearly shown, and autoantibody response is T cell dependent. Regulation of potentially pathogenic T cells is important and one of the most important regulators of effector T cells is the regulatory T cell (Treg), which has been shown functionally defective in MG patients [1,2].

The two major metabolites of vitamin D, 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] are potent immunomodulators. The immune-regulatory effect of vitamin D is known to inhibit effector T cells directly and also induce Treg to decrease the production of inflammatory cytokines. Both vitamin D and Treg might be critical for T cell regulation [3,4]. Previous studies have shown that vitamin D levels are low in patients with autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis [5–7]. However, there are few studies on vitamin D status in autoimmune neuromuscular diseases.

The aims of this study were to evaluate serum levels of 1,25$(OH)_2$D and 25(OH)D in patients with MG and healthy controls and the association of vitamin D levels with clinical severity and treatment status.

2. Methods

2.1. Subjects

We reviewed data from the MG registry of Jeju National University Hospital and selected MG patients without taking vitamin D supplementation. We identified 34 patients and 9 patients refused to participate in the study. A total of 25 patients were ultimately enrolled. Diagnostic criteria of MG included clinical muscle fatigue and decremental response on repetitive nerve stimulation test in conjunction with the presence of AChR antibodies. We only enrolled MG patients with anti-AChR antibodies (AChR-MG). Forty healthy control subjects of volunteers who had no prior history of any medical disease and without prescribed vitamin D medication. MG patients were classified by disease stage (ocular or generalized according to the Myasthenia Gravis Foundation of America clinical classification), age of onset (early or late onset: >50 years) and treatment status whether or not to taking immunosuppressive agents. In addition, the MG composite (MGC) scale was assessed to evaluate the disease severity. All subjects gave their written informed consent prior to participation in the study, which was approved by the Ethics Committee of Jeju National University Hospital.

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2.2. Measurement of 1,25(OH)$_2$D and 25(OH)D

Serum samples for vitamin D measurements were obtained from one blood sample per individual and all samples were collected between January 1 and February 28, 2015. 1,25(OH)$_2$D and 25(OH)D levels were measured with chemiluminescence microparticle immunoassay in our hospital. The normal range of 1,25(OH)$_2$D value was between 19.60 and 54.30 ng/mL. 25(OH)D levels <10 ng/mL, 10 to 40 ng/mL, and >100 ng/mL were defined as insufficiency, sufficiency, and toxicity, respectively.

2.3. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Means of the patient group and healthy control group were compared by the non-parametric Mann-Whitney test *U* test. The relationship between serum vitamin D status and disease severity was analyzed by Spearman's correlation coefficient using the rank test. All statistical analyses were conducted using SPSS version 18 (SPSS Inc., Chicago, USA), and the level of significance was defined as a p value of <.05.

3. Results

3.1. Demographic and clinical characteristics

Twenty-five patients (mean age, 56.0 ± 14.5 years; female:male = 13:12) with AChR antibodies and forty healthy controls (mean age, 56.2 ± 12.2 years) were included in this study. No significant differences were observed in age and gender between two groups. According to the MGFA clinical classification, 25 patients with MG were divided ocular (9 patients) and generalized (16 patients) MG. The number of patients in early and late onset MG was 15 and 10, respectively. MGC score ranged from 0 to 11 with a mean value of 2.8. Ten patients had only acetylcholine esterase inhibitors with a daily dose ranging from 180 mg to 480 mg. The remaining patients had immunosuppressive agents, of which 9 patients had corticosteroid treatment with a dose ranging from 5 mg to 25 mg. Among the patients with corticosteroid therapy, 2 patients additionally had azathioprine and 1 patient had tacrolimus. Four patients had only tacrolimus, and 2 patients had only azathioprine (Table 1).

3.2. Comparison of serum 1,25(OH)$_2$D and 25(OH)D levels between MG patients and healthy controls

Mean serum 25(OH)D levels were significantly lower in the AChR-MG patients (mean, 18.8 ± 8.4 ng/mL; range, 6.7–36.7) than in the healthy controls (mean, 26.3 ± 6.1 ng/mL; range, 19.4–37.4) (p < .001). 1,25(OH)$_2$D levels showed slightly high in AChR-MG patients (mean, 46.4 ± 21.9 ng/mL; range, 14.0–91.6) than healthy controls (mean, 42.1 ± 7.0 ng/mL; range, 28.4–49.6), but had no significant difference between two groups. Five patients (20%) showed insufficient levels of 25(OH)D, but all healthy controls had sufficient 25(OH)D levels (Fig. 1). Among 5 patients having insufficient 25(OH)D levels, four had immunosuppressive agents.

Table 1
Demographic and clinical characteristics of patients with MG and healthy controls.

	Patients with MG (n = 25)	Healthy controls (n = 40)	*P* value
Age (years)[a]	56.0 ± 14.5	56.2 ± 12.2	0.13
Sex (female/male)	13/12	20/20	0.65
Season at sampling	Winter	Winter	
1,25(OH)$_2$D (ng/mL)	46.4 ± 21.9	42.1 ± 7.0	0.28
25(OH)D (ng/mL)	18.8 ± 8.4	26.3 ± 6.1	<0.001
MGC score	2.8 ± 1.8		
Ocular/Generalized (n)	9/16		
Anti-cholinesterase agents only (n)	10		
Immunosuppressive agents (n)	15		

n = Number of patients; MGC = Myasthenia gravis composite scale; MG = Myasthenia gravis; 1,25(OH)$_2$D = 1,25-dihydroxy vitamin D; 25(OH)D = 25-hydroxyvitamin D.
Values are presented as the mean ± standard deviation.

3.3. Association between vitamin D and clinical severity and treatment status

In AChR-MG patients, levels of 1,25(OH)$_2$D did not significantly differ between ocular (mean, 50.9 ± 28.7 ng/mL) and generalized MG (mean, 48.6 ± 25.7 ng/mL). Similarly, levels of 25(OH)D did not differ between two groups (ocular: 17.2 ± 4.9 ng/mL; generalized: 19.8 ± 10.1 ng/mL). There is no significant difference in vitamin D levels between early and late onset MG patients. In addition, levels of 1,25(OH)$_2$D and 25(OH)D did not significantly differ between AChR-MG patients under immunosuppressive therapy and taking anti-cholinesterase only (Table 2). No correlation was observed between MGC scale score and 25(OH)D levels (Fig. 2 $\rho = -0.1439$, $p = .362$).

4. Discussion

Vitamin D has been shown to exert a multitude of effects on the autoimmune systems and its deficiency is increasingly associated with a wide range of immune-mediated disorders. However, there are few studies about association of vitamin D in autoimmune neuromuscular disorders. MG is a prototypic autoimmune disease and autoantibodies to the AChR are present in approximately 80% of patients. Various peripheral immunomodulatory mechanisms, including suppression of autoreactive clones by regulatory T cells (Treg), normally eliminate autoreactive cells. Treg is essential for self-tolerance and defects in Treg can cause experimental autoimmunity [8]. Functional defects of Treg have been reported in

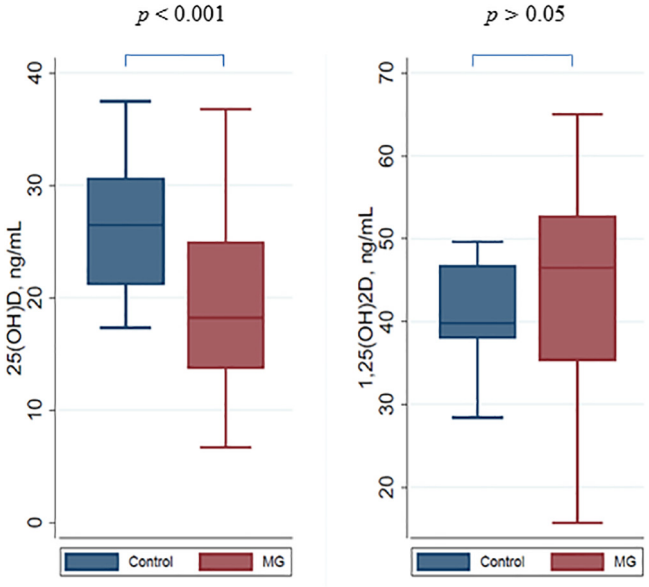

Fig. 1. Vitamin D levels in patients with myasthenia gravis (MG) (n = 25) and healthy controls (n = 40). Patients with MG showed significantly lower 25(OH)D levels compared with healthy controls, but 1,25(OH)$_2$D levels did not show significant differences between two groups.

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