Effect of vitamin D3 supplementation towards vitamin D serum levels and Myasthenia Gravis Composite Score (MGCS)

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Abstract. Myasthenia gravis (MG) is due to the functional impairment Treg cell that is as important as controlling the activation of T cells and inhibit the autoimmune response. Vitamin D3 is known to have an effect on increasing the quantity of Treg cells in the peripheral blood circulation and also functional so that it can suppress T cell activation. This study used a double-blind randomized controlled trial of 20 patients MG during the period from April to July 2017. The bivariate analysis using independent t-test and paired T-test. Multivariate analysis using linear regression. The mean change MGCS after vitamin D3 treatment group and placebo were not statistically significant (p = 0.531). On the addition of vitamin D3 MGCS 1:19 ± 0.1 (p = 0.798). There is a significant change (p = 0.041) in the mean levels of vitamin D after treatment between vitamin D3 and placebo groups. A significant increase (p = 0.005) vitamin D levels after administration of vitamin D3 in the amount of 17.88 ± 29.5 Ng/ml. Multivariate analysis showed that the initial MGCS and IMT values that most influence on the final MGCS value, and levels of vitamin D beginning the most influence on the final vitamin D levels. Vitamin D3 is 800 IU / day had no effect on the value of MGCS but the effect on changes in levels of vitamin D in the blood.

1. Introduction
Myasthenia gravis (MG) is an autoimmune disease that causes the neuromuscular junction transmission failure due to the nicotinic acetylcholine receptor antibody (AChR) which has a characteristic form of voluntary fluctuating muscle weakness. The prevalence of MG varies between 43-84 per 1,000,000 population and the incidence value reaches 1 per 300,000. The disease can affect all ages but reached the peak at age 20-30 years for women and 50-60 years for men [1,2].

MG is a disease mediated T-cell dependent antibody, which occupies antibody acetylcholine receptors at the synapse post produced by the B cells were first activated by T cells. Some research suggests the process of T cell activation is regulated by the Treg cells are a subpopulation of T cells that function modulate the immune system to maintain tolerance to self-antigens and prevent the onset of autoimmune disorders. The main role of Treg cells, in general, inhibits the process of T cell activation [3]. Further research revealed a decline in functional Treg cells in patients with MG,
accompanied by a decrease in Treg cells in peripheral lymphocyte Mg patients compared with normal subjects [4].

Vitamin D insufficiency has been reported to occur in patients with systemic lupus erythematosus. Vitamin D deficiency is also associated with autoimmune activity in some chronic autoimmune diseases such as rheumatoid arthritis and of multiple sclerosis. In a study of vitamin D3 in the case of multiple sclerosis found a significant reduction in symptoms [5]. In the study, Prietl B et al declared vitamin D3 has the effect of Treg cells increased, both in quantity in the peripheral blood circulation and functional so that it can suppress T cell activation [6].

Management of patients with MG is major thymectomy in patients with thymoma aimed at reducing the production of antibodies by B cells undergo activation in the thymus. Other Procedures such as medication use acetylcholine esterase inhibitor and immunosuppressant only reduce the symptoms, not cure definitively. This latter idea arose in the treatment of MG is to improve the function of Treg cells suppresses T cell activation using vitamin D3 supplementation [7]. This study aimed to analyze the effect of vitamin D3 on serum levels of vitamin D and MGCS in patients with MG.

2. Methods
This research uses experimental test design double-blind, placebo-controlled trial in patients with MG in the RS. Muhammad Hoesin Palembang period from April to June 2017. Subjects were divided into two groups: the treatment group and the control group. Making the subject of research conducted by consecutive sampling the inclusion criteria had been diagnosed with MG by a neurologist and agreed to participate in the study as well as not having acute exacerbations or crisis MG. The research subjects were excluded if dies or stop participating before the study ended and also experienced severe drug side effects that bet administration had to be stopped. Subjects selected randomly to be divided into two groups: the treatment group and the control group. The division of subjects is done by lottery in a sealed envelope which is not known either by the researcher and subject. List division opened when the group of subjects throughout the sample results and clinical data of patients post-intervention is complete and the data is ready for analysis. This study was approved by the ethics committee of health research Hoesin Mohammad Central Public Hospital and Faculty of Medicine, University of Sriwijaya.

All subjects will be counted MGCS research and examination of serum vitamin D levels before treatment. Subjects in the treatment group will receive standard therapy MG + Vitamin D3 2x400 IU / day for 30 days and the control group will receive standard therapy MG + placebo for 30 days. Vitamin D3 and placebo were given in the form of a soft gel capsule with the size, color, and packaging the same. Furthermore, vitamin D3 and placebo incorporated into the drug container be numbered and given in accordance with the lottery that is not known researcher and subject. After 30 days throughout the study, subjects underwent MGCS calculation and re-examination of vitamin D levels and all the tests assessed by investigators and analyzed.

Data analysis was performed with SPSS version 23. The bivariate analysis using independent St test and paired T-test against MGCS and vitamin D3 levels before and after treatment. Multivariate analysis using linear regression on the variables of age, sex, body mass index, and long-suffering pyridostigmine dose MG.

3. Results
The study was conducted with a number of subjects who met the inclusion criteria of 20 people, of which 10 subjects into the treatment group and 10 subjects entered the control group. There are no subject dropouts in this study.

3.1. General characteristics
In this study, the mean age of the study subjects is 41.75 ± 11.92 years with the majority of subjects aged 31-40 years were 9 people (45%). More research subject is female is 15 people (75%) than men. Most patients had a normal BMI and excess (each 45%). The duration of study subjects most since 1-2 years (30%) followed by 2-4 years (25%) and pyridostigmine highest dose of 60 mg four times a day (75%).
Table 1. General characteristics of research subjects.

| Variables                  | Number (N) | Percentage (%) |
|----------------------------|------------|----------------|
| Age                       |            |                |
| <20 years                  | 0          | 0              |
| 21-30 years                | 3          | 15             |
| 31-40 years                | 9          | 45             |
| 41-50 years                | 3          | 15             |
| > 50 years                 | 5          | 25             |
| Gender                     |            |                |
| Man                        | 5          | 25             |
| Woman                      | 15         | 75             |
| Body mass index            |            |                |
| Less                       | 2          | 10             |
| Normal                     | 9          | 45             |
| Excess                     | 9          | 45             |
| Long-suffering MG          |            |                |
| <6 months                  | 3          | 15             |
| 6 months-1 year            | 2          | 10             |
| 1-2 years                  | 6          | 30             |
| 2-4 years                  | 5          | 25             |
| > 4 years                  | 4          | 20             |
| Doses pyridostigmin        |            |                |
| 2 x 60 mg / day            | 0          | 0              |
| 3 x 60 mg / day            | 4          | 20             |
| 4 x 60 mg / day            | 15         | 75             |
| 5 x 60 mg / day            | 1          | 5              |

3.2. MG long-suffering relationship with vitamin D levels prior to the administration of vitamin D3

Levels of vitamin D beginning not associated statistically with MG suffer duration (p = 0.727). Shapiro Wilk test showed abnormalities in vitamin D levels were not normally distributed (p = 0.000). Based test Spearman correlation test showed a very weak correlation (r = 0.083) with the direction of a positive correlation, meaning that the shorter the smaller MG suffer vitamin D levels early. More results showed in table 2.

Table 2. Relationship duration MG suffer from high levels of vitamin D.

| Levels of vitamin D          | R  | p *      | N  |
|------------------------------|----|----------|----|
| Long-suffering MG            |    |          |    |
| 0083                         |    | 0.0727   | 20 |

* Spearman Test (P <0.05)

3.3. Comparison Results MGCS and vitamin D before and after administration of vitamin D3

MGCS value before treatment in the group of vitamin D3 is 5.20 ± 3.79 whereas in the placebo group at 7:00 ± 3.65 (p = 0294). After treatment, the value of vitamin D3 MGCS group at 5:30 ± 3:53 and placebo groups at 6:30 ± 3:47 (p = 0531). MGCS changes in the group of vitamin D3 0.1 ± 1:19 showed clinical impairment (p = 0.789), and a change of -0.7 ± MGCS 1:34 in the placebo group showed that there was a clinical improvement (p = 0.135), although statistically nonsignificant respectively.

Table 3. Comparison of MGCS before and after treatment.

| Group | Myasthenia Gravis Composite Score (MGCS) | P a | P b |
|-------|------------------------------------------|-----|-----|
|       | Before                                   | After| Change| Before| After|       |
| Vit. D3| 5.20 ± 3.79                              | 5.30 ± 3.53 | 0.1 ± 1:19| 0.294 | 0.531| 0.798 |
| placebos| 7.00 ± 3.65                             | 6.30 ± 3:47 | -0.7 ± 1:34| 0.0132|       |

aT-independent test (P <0.05); bpaired T-test (P <0.05)
3.4. Changes in levels of vitamin D

In the group of vitamin D3 have obtained 4 subjects with vitamin D levels after administration of vitamin D3 2 x 400 IU per day for 30 days. In the placebo group, there were two subjects who had increased levels of vitamin D status and no subject who achieved the status of normal vitamin D levels (Table 4). Levels of vitamin D early in the vitamin D group at 12:52 ± 24.7 Ng / mL, while the placebo group amounted to 3.79 ± 2.97 Ng / mL (p = 0.012). After treatment, levels of vitamin D in vitamin D3 group amounted to 30.39 ± 8.5 Ng / mL, while the placebo group at 8:59 ± 6:35 Ng / mL (p = 0.041). Increased levels of vitamin D in vitamin D3 group was statistically significant (p = 0.005), as well as with the placebo group (p = 0.007). Comparative levels of vitamin D before and after treatment can be seen in Table 5.

| Levels of vitamin D status | Vitamin D before treatment | Vitamin D after treatment |
|---------------------------|-----------------------------|---------------------------|
|                           | vitamin D3 | placebos | vitamin D3 | placebos |
| deficiency                | 8 (80%)    | 9 (90%)   | 4 (40%)    | 7 (70%)   |
| insufficiency             | 1 (10%)    | 1 (10%)   | 4 (40%)    | 3 (30%)   |
| Normal                    | 1 (10%)    | 0         | 2 (20%)    | 0         |
| Total                     | 10 (100%)  | 10 (100%) | 10 (100%)  | 10 (100%) |

3.5. MGCS Multivariate analysis and blood levels of vitamin D

3.5.1. MGCS

Based on multivariate analysis obtained IMT correlated very weakly with MGCS end (r = 0.1660 and towards negative correlation and statistically significant (p = 0.039). In addition, MGCS early have a very strong correlation with MGCS end (r = 0.921) and direction and positive correlation was statistically significant (p = 0.000).

3.5.2. Levels of vitamin D

Based on the results of the multivariate analysis obtained initial vitamin D levels had a strong correlation with the levels of vitamin D final (r = 0.674) and the direction of the positive correlation and statistically significant (p = 0.001).

4. Discussions

Vitamin D has been studied has an important role in the pathophysiology of autoimmune diseases such as MG. In MG, vitamin D plays a role both in the regulation of autoimmune response and also maintain muscle function through the mechanisms involved in vitamin D receptors in the muscles [8]. The active form of vitamin D is 25 (OH) D is believed to have an effect as a regulator of the immune system by increasing the number of regulatory T cells [6]. MG patients proved to have a deficiency of vitamin D [9] and the administration of high-dose vitamin D (80000-120000 IU / day) have been reported to cause complete remission in patients with severe MG [10].

This research obtains 80% of the subjects in the group of vitamin D3 deficiency before treatment and 10% insufficiency, after treatment of the subject deficient dropped to 40%, 40% of the subjects became insufficiency and the rest to be normal. In the D3 group recorded only 1 subject who experienced an increase in serum levels of vitamin D3 to its normal value, so this should be considered to be one cause of the change is meaningless MGCS positively to the group of vitamin D3. Geographical conditions also need to be considered in the study. Indonesia tropical conditions with
high sun exposure and diet will affect the metabolism of vitamin D in the body. It became one of the weaknesses of this study.

In this study, the initial value MGCS difference in both groups but was not statistically significant. MGCS also mean changes in both groups were also not significant. Although a double-blind randomized placebo-controlled trial [11] the effectiveness of vitamin D3 in normal subjects with levels of 25 (OH) D serum low shows the results of the change score Fatigue Assessment Scale (FAS), no change in the value of MGCS in both arms of this study support the hypothesis that although the muscle receptors of vitamin D, levels of 25 (OH) D levels did not correlate with muscle weakness as measured by MGCS. Muscle weakness thought to be affected by the regulation of the central nervous system. Vitamin D receptors have been found in many areas of the brain [12]. The mechanism of muscle weakness thought to be caused by the imbalance of dopamine in the central nervous system and vitamin D receptors are found in the midbrain dopaminergic neuron mice and humans, these neurons is believed to be fully regulated by the active form of vitamin D [13].

In this study, both groups had increased levels of vitamin D were statistically significant. Vitamin D3 group experienced higher levels compared to the placebo group. It supports Askmark, et.al. research that showed that vitamin D supplementation has the effect of improving peripheral blood vitamin D levels.

Multivariate analysis showed that body mass index had a very weak correlation to the value MGCS end with the direction of negative correlation, meaning that the lower the person’s body mass index, the higher the value MGCS person. These findings support the hypothesis that higher body mass index, serum levels of vitamin D it would be lower. Higher body mass index correlated with low levels of vitamin D, because the inactive form of vitamin D binds to adipose tissue [14]. In addition, the value of the initial MGCS has a very strong correlation with the direction of the end MGCS positive correlation, meaning the value of the initial MGCS greatly affect the value of the final MGCS. Multivariate analysis of vitamin D levels showed that only the initial vitamin D levels that affect levels of vitamin after treatment.

Another limitation of this study is the sample size is still small and the duration of administration of vitamin D3 is short, so it has not obtained MGCS changes are statistically significant. However, the findings of the high percentage of vitamin d3 deficiency in patients with MG and a significant association between BMI and MGCS value can be further research material.

5. Conclusions
Giving vitamin D3 800UI / day increases blood levels of vitamin D but did not cause significant changes in the value MGCS.

6. References
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