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Effects of vitamin D3 (cholecalciferol) supplementation on systemic lupus erythematosus patients with hypovitaminosis D on serum pro-inflammatory cytokines (IL-6, IL-7, IFN-γ), anti-inflammatory cytokine (TGF-β) and anti-ds DNA levels

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ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease and can attack almost all organs and tissues of the human body. Beside genetic factors, environmental factors are thought to play a role, such as ultraviolet light, viral infections and smoking, causing a breakdown of self-tolerance which can trigger an autoimmune response. The study was conducted in the outpatient and inpatient units of the Rheumatology Division of the Department of Internal Medicine Saiful Anwar General Hospital/Faculty of medicine Universitas Brawijaya, Malang. Subjects were female patients, aged > 18 years who had been diagnosed as SLE by internist-rheumatologist based on the 1997 ACR criteria, with SLEDAI score > 3. After 3 months of supplementation, there was a significant decrease in serum levels of the three pro-inflammatory cytokines (IL-6, IL-17, IFN-γ), as shown in table 3, compared to before treatment, as well as anti-dsDNA levels. Serum TGF-β1 levels increased significantly, while 25 (OH) D3 levels also increased significantly.

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease and can attack almost all organs and tissues of the human body. Beside genetic factors, environmental factors are thought to play a role, such as ultraviolet light, viral infections and smoking, causing a breakdown of self-tolerance which can trigger an autoimmune response [1]

This disease mainly attacks women of reproductive age with a ratio of women : men ranging from 9:1 to 15:1. The prevalence of SLE is reported to range from 20 to 150 cases per 100,000 population with high mortality and morbidity rates, especially in developing countries[2]. In developed countries, in general the 5-year life expectancy in SLE sufferers increases to 97% and around 95% for 10 years in 2010 and above. Whereas in Indonesia, life expectancy is still quite low, namely 80% for a period of 5 years and 75% for a period of 10 years[3].

The pathogenesis of SLE is very complex, it involves mainly an immune system autoregulation

abnormality[4]. Many studies suggest that the main cause of SLE is an imbalance in the number of CD4 + T cell subsets, i.e. an increase in Thelper 1 (Th1), Thelper 2 (Th2) and Thelper 17 (Th17) cells accompanied by a decrease in the number of regulator T cells (Treg). The imbalance of immune cell activation is a process that can induce the production of inflammatory mediators (especially pro-inflammatory cytokines) which are toxic to tissues while anti-inflammatory cytokines decrease, it will increase the progression of the disease[5].

Vitamin D apparently plays a role in the immune system in addition to its classic function on calcium and bone metabolism. The non-classical role of vitamin D is because it turns out that immune cells have a vitamin D receptor (VDR), and are able to metabolize vitamin D in cells, because these immune cells have enzymes for vitamin D metabolism (Holick, 2013). Moreover, vitamin D deficiency in SLE causes increased differentiation and maturation of dendritic cells, impaired Th1 / Th2 balance and Treg / Th17, which is reflected in increased pro-inflammatory cytokines, decreased anti-inflammatory cytokines and increased B cell activation and autoantibody production.[6-7]

The role of vitamin D in several autoimmune diseases including SLE has been widely studied, but in Indonesia there is still not enough research on the effect of vitamin D supplementation on SLE patients. This study intends to look at the effects of vitamin D3 supplementation (cholecalciferol) on pro-inflammatory cytokine serum levels (IFN-γ, IL-6, IL-17) and anti-inflammatory cytokines (TGF-β1), and serum anti-dsDNA levels in patients SLE with hypovitamin D[8].

Method

The study was conducted in the outpatient and inpatient units of the Rheumatology Division of the Department of Internal Medicine Saiful Anwar General Hospital/Faculty of medicine Universitas Brawijaya, Malang. Subjects were female patients, aged > 18 years who had been diagnosed as SLE by internist-rheumatologist based on the 1997 ACR criteria, with SLEDAI score > 3. Subjects were examined vitamin D3 (25 (OH) D3) levels, if the results were <30ng / L, laboratory tests were performed: complete blood count, ESR, ureum/creatinine, SGOT/SGPT, anti-dsDNA, serum calcium, at the start of the study. The levels of cytokines (IL-6, IL-17, TGF-β1) were also examined serum. Patients were given supplementation of vitamin D3 (cholecalciferol) soft capsul (cholecalciferol) 3 x 400 IU per day for 3 months, after that, then we repeated clinical, and the same laboratory examination again. Standard treatments were still given for every subjects. This research was carried out from October 2016 to May 2017. The study was conducted after obtaining approval from the ethics committee of Saiful Anwar hospital (no: 400/125/K.3/302/2016). All subjects who were included in this study were asked to sign informed concent before[9-10].

Result

There were 20 SLE patients included in this study with mean of age was 30.3 ± 10.1 years, the mean of disease duration was 2.2 (between 1.0 - 4.0) years, while mean of SLEDAI score was 15.1 ± 7.6. The initial manifestations of SLE in this study were varied, but at most was a manifestation of mucocutaneous (43.6%) and arthritis (23.1%). All study subjects received steroids (methylprednisolone) in varying doses unSLEs one patient did not receive glucocorticoid therapy. All subjects of this study also received immunosuppressant treatment for SLE and the most used immunosuppressant was azathioprine (25.6%), as shown in table 1.

Table 1. Baseline characteristic

Characteristic	n= 20
Age (year)	30.3 ± 10.1
Duration of disease (month)	32.2 ± 23.5
Early clinical manifestation (%)	
Mukcocutaneous	17 (43.6%)
Arthritis	9 (23.1%)
Nephritis (proteinuria, hematuria, pyuria, cylindruria)	4 (10.3%)
Hematology (AIHA, leukopenia, thrombocytopenia)	2 (5.1%)
Vasculitis	1 (2.6%)
Serositis	1 (2.6%)
Cerebral	3 (7.7%)
Pharmacotherapy	
• Methylprednisolone	19 (48.7%)
• Calcium	19 (48.7%)
• Chloroquine	10 (25.6%)
• Cyclosporine	2 (5.1%)
• Cyclophosphamide	1 (2.6%)
• Azathioprine	10 (25.6%)

All routine laboratory variable examined (complete blood count, AST/ALP, ureum, creatinine, serum calcium were normal, while vitamin D (25[OH]D) was low (14.9 ± 7.5 ng/ml) as shown in table 2.

Table 2. Laboratory characteristic

Characteristic	(n= 20)
Hemoglobin (g/dL)	11.2 ± 1.6
Total Lymphocyte Count	1321.5 ± 608.2
Vitamin D3 (25(OH)D3	14.9 ± 7.5
Calcium (mg/dl)	8.9 ± 0.6
SGOT/AST(U/L)	25.4 ± 3.6
SGPT/ALT (U/L)	25.4 ± 4.6
Ureum (mg/dl)	28.4 ± 22.5
Creatinine (mg/dl)	0.6 ± 0.2

After 3 months of supplementation, there was a significant decrease in serum levels of the three pro-inflammatory cytokines (IL-6, IL-17, IFN-γ), as shown in table 3, compared to before treatment, as well as

anti-dsDNA levels. Serum TGF-β1 levels increased significantly, while 25 (OH) D3 levels also increased significantly.

Table 3. Cytokines (IL- 6, IL-17, IFN-ɣ), anti-dsDNA and 25(OH)D3 serum levels before and after cholecalciferol supplementation

Variable	Before supplementation	After supplementation	p
IL-6 (pg/ml)	12 ± 6.7	3.6 ± 1.5	0.000*
IL-17 (pg/ml)	4.4 ± 3.5	1.9 ± 1	0.001*
IFN-γ (pg/ml)	139 ± 104.6	10.3 ± 3.1	0.007*
TGF- β1 (pg/ml)	289.1 ± 73.6	365.1 ± 79.2	0,000*
anti dsDNA (IU/ml)	115.2 ± 97.3	56.2 ± 66.6	0.001*
25(OH)D3 (ng/ml)	14.9 ± 7.4	26.8 ± 3.7	0.047*

*= p<0.05

After treatment, it appeared that some cytokines levels correlate with serum anti-dsDNA levels, i.e. TGF-β were negatively correlated with anti-dsDNA levels while proinflammatory cytokines, IL-6, IL-17, IFN- ɣ, were all positively correlated with anti-dsDNA. The correlation of the cytokines with anti-dsDNA can be seen in table 4.

Table 4. Correlation of cytokines serum levels and anti-dsDNA

Cytokines	Anti-dsDNA	p
IL-6	r = 0.340	p = 0.056*
IL-17	r = 0.515	p = 0.025*
IFN-γ	r = 0.408	p = 0.012*
TGF- β1	r = -0.500	p = 0.034*

*= p < 0,05

Discussion

In this study, cholecalciferol 1200 IU supplementation for 3 months can significantly increase serum 25 (OH) D3 levels, but has not reached normal levels (> 30 ng / ml). This might be due to the fact that 25 (OH) D3 serum levels of the study subjects were indeed low (deficiency); <20 ng / ml) at the begining of the study, or maybe we need larger dosages. To reach the normal 25 (OH) D3 level, supplementation must be given in a longer period of 4-6 months or in larger doses, but does not exceed tolerable upper intake of 50 µg/day which is equivalent to vitamin D3 2000 IU[11].

There was a significant decrease of serum IL-6 levels after vitamin D3 supplementation for 3 months. Decreased levels of IL-6 as a pro-inflammatory cytokine here were supposed due to the response of immunological modulation by VDR, where MKP5 (mitogen-activated protein kinase phosphatase 5) is a direct target of 1.25, D which is regulated positively by VDRE (Vitamin D Responsive Element) in the promoter region of genes. Increased

MKP5 synthesis will inactivate p38 needed for IL-6 production[12-13].

Interleukin-6 is a cytokine that has been shown to have an important role in SLE, both in humans and experimental animals. Interleukin-6 functions to stimulate the differentiation and maturation of B cells into plasma cells that produce antibodies[14-15]. Inhibition of IL-6 will reduce the severity of the disease in lupus mice and inhibit the production of anti-dsDNA. High levels of interleukin-6 are found in active serum SLE and are associated with an increased degree of disease activity[16]. There is a reciprocal relationship in the development of Th17 and Treg from naive TCD4 + (Th0) cells which is related to IL-6 levels. Th17 cells, which are proinflammatory T cells, in their development from Th0 cells, require stimulation from a combination of IL-6 and TGF-beta. When IL-6 levels fall, Th0 will be more motivated to become Treg cells which are anti-inflammatory immunoregulatory cells[17]. Thus, the decrease in IL-6 levels will cause the SLE immunoregulation to become anti-inflammatory so that the disease activity and anti-dsDNA levels decreases.

The other serum levels of proinflammatory cytokine, IL-17, was also found to decrease significantly after vitamin D3 treatment for 3 months. The immunomodulatory function of vitamin D was demonstrated from studies in mice by Chang (2010), where in the administration of 1,25-dihydroxyvitamin D3 (1,25D3), an improvement was seen in autoimmune encephalomyelitis, followed by decreased expression of IL-17 and IL-17F. In vitro, administration of 1,25D3 to CD4+T cells inhibits cytokine production by Th17. The administration of 1,25D3 induces the expression of C/EBP homologous protein (CHOP), a molecule that inhibits translation and is involved in the endoplasmic reticulum. Excessive CHOP expression can suppress cytokine production. Another study conducted by Da Costa et al. (2016) in patients with multiple sclerosis, showed that administration of 1,25-dihydroxyvitamin D3 in vitro can reduce Th17 and the resulting cytokines, one of which is IL-17[18].

Interleukin-17 is produced mainly by Th17, and also NK cells and neutrophils. Interleukin-17 acts to cause inflammation by stimulating the production of inflammatory chemokines and cytokines, encouraging the migration of monocytes and neutrophils to tissues, causing inflammation. Interleukin-17 is also known to stimulate B cells to increase antibody production[19]. From the above data, it can be concluded that a decrease in IL-17 levels in SLE will decrease disease activity through decreased inflammation and decreased production of pathogenic antibodies by B cells. This is in line with the results of this study which shows that there is a positive correlation between IL-levels 17 with serum anti-dsDNA levels.

We found a significant decrease in serum IFN- γ levels, after vitamin D3 administration for 3 months. In innate immune responses, IFN-γ is produced by NK cells, whereas in adaptive immunity, it is mainly produced by Th1 cells so that they are called Th1 cytokines[20]. The effect of IFN-γ is very broad, including the activation of monocytes and macrophages, and if present in large quantities, will involve in tissue damage. Other activities are stimulating other proinflammatory cytokines, such as TNF-α, and stimulating renal cell apoptosis[21].

The effect of IFN-γ on the pathogenesis of SLE has been extensively studied in mice. Experiments using mice lacking IFN-γ (MRL / lpr mice and NZB × NZW F1) showed that for the occurrence of nephritis and the production of anti-dsDNA IgG2, IFN- γ was needed. There is a high IFN- γ expression in the kidneys of SLE patients which might increase the expression of chemokines which recruit inflammatory cells and cause tissue damage[22]. This is in line with the results of this study, where a decrease in IFN-γ levels is followed by a decrease in serum anti-dsDNA levels.

An increase in serum TGF-β1 levels was obtained in this study after 3 months of vitamin D3

supplementation. The increase in TGF-β1 here was due to the fact that vitamin D plays a role in the synthesis of TGF-β1 which binds to VDR to control transcription of target genes through VDR, namely SMAD3. This SMAD3 can mediate cross-talk between vitamin D and TGF-β1, so giving vitamin D will cause an increase in TGF-β1 expression[22].

Transforming growth factor β (TGF-β) consists of 3 subtypes, (TGF-β1, TGF-β2, and TGF-β3), and TGF-β1 are the most powerful as immunoregulators and immunosuppressants. These cytokines function as negative regulators of B cell differentiation and proliferation, as well as immunoglobulin production. It also inhibits T-cell activation so that elevated levels of TGF-β1 can reduce SLE disease activity. (Metawie, et al., 2015). This is consistent with the results of this study where TGF-β1 levels are negatively correlated with anti-dsDNA levels.[23]

There was a significant decrease in total anti dsDNA (IU/ml) after being given cholecalciferol for 3 months, in this study. Research conducted by Attar and Siddiqui (2013) in SLE patients with hypovitamin D shows that there is a negative correlation between vitamin D levels and anti-dsDNA levels, where the lower the vitamin D level, the higher the anti-dsDNA levels. These results are supported by previous studies by where similar results were obtained. In PBMC incubation, SLE patients with 1.25OH2D3 decreased proliferation and production of anti-dsDNA originating from B-lymphoblasts and inducing apoptosis in active B-lymphoblasts. This shows that vitamin D also works on B cells because B cells express VDR, and in turn will dcrease autoantibodies production.[24,25].

Study limitation

This study was only carried out for 3 months, so the effectiveness of supplementation in the longer term is not yet known, as well as its side effects. The limited number of research subjects is also a weakness of this study.

Conclusion

Vitamin D3 (cholecalciferol) supplementation of for 3 months significantly decreases pro-inflammatory cytokine levels (IFN-IL, IL-6, IL-17) and serum anti-dsDNA levels, and increases TGF-β1 levels in SLE patients with hypovitaminosis D.

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