The Effect of Vitamin D Supplementation on Disease Activity and Neutrophyl-Lymphocyte Count Ratio in Systemic Lupus Erythematosus Patients with Hypovitaminosis D : A Preliminary Study

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ABSTRACT

Background : Previous studies showed a significant role of Vitamin D in modulating inflammation and immune abnormality in SLE. The correlation between vitamin D supplementation and SLE disease activity remains controversy. Neutrophyl-Lymphocyte count Ratio (NLCR) as an inflammation marker was significantly increased in SLE patients.

Objective : To evaluate the effect of vitamin D supplementation on disease activity and neutrophyl-lypmhocyte count ratio (NLCR) in SLE patients with hypovitaminosis D.

Methods : This is a pre-post test study without control group using a consecutive sampling method. SLE patients were enrolled from Rheumatology Clinic of Hasan Sadikin General Hospital from November 2013-March 2014. Subjects received vitamin D3 2000 IU/day for 3 months. Data was analyzed using Wilcoxon test.

Results : We analyzed 28 subjects with 89,3% of vitamin D deficiency and 10,7% of vitamin D insufficiency, which converted to 25% of vitamin D deficiency, 32,1% vitamin D insufficiency and 42,9% normal vitamin D plasma level at the end of the study. After supplementation, Mexican Systemic Lupus Erythematosus Disease Activity Index (MEX-SLEDAI) and NLCR was significantly decreased (median 4(3-8) to 2(0-6) and median 2,95(1,17-7,27) to 2,28 (1,07-4,87), $p<0,001$, respectively). SLE organ involvement such as mucocutan, hematology and renal also high BMI (>23 kg/m^2) were risks of hypovitaminosis D. Vitamin D supplementation increased mean 25(OH)D serum level by 164,7%, 46,7% decreased of MEX-SLEDAI, and 24,2% decreased of NLCR ($p<0,001$). Nine subjects (32,1%) achieved remission, 19 subjects (67,9%) at disease persistence and no subjects experienced flare up after supplementation.

Conclusion : The effects of vitamin D3 2000 IU/day supplementation for 3 months are reduced disease activity and NLCR in SLE patients with hypovitaminosis D. The role of NLCR as a simple inflammation marker in this pilot study needs further investigation.

Systemic Lupus Erythematosus (SLE) is a chronic systemic autoimmune disease marked by autoantibody tissue deposition, tissue damage and heterogen clinical manifestation. Prevalence of SLE in the United States is 10-400 in 100.000 people, with women to men ratio 9−14 : 1.[1] There was lack of data for prevalence of SLE in Indonesia. Rheumatology Clinic Hasan Sadikin General Hospital, Bandung in West Java recorded new cases of SLE between 2003-2005 were 6,4% from 3025 patients, which increased to 9,07% from 4037 patients in 2011 and 11,54% from 27.496 patients in 2012.[2,3]

Pathogenesis of SLE is multifactorial (genetic, environment and hormonal). Clinical manifestation of SLE developed through autoimmune predisposition, autoantibody formation, then disease exacerbation, remission, disease damage and comorbidity caused by chronic inflammation. Two main disease domain in SLE are disease activity and disease damage. Disease activity represent a reversible manifestation of disease which is the important parameter of SLE treatment.[4,5,6]

Vitamin D induces chemotaxis, macrophage phagocytosis, T*reg* activity, inhibits B cell differentiation and proliferation, and inhibits autoantibody production. The role of vitamin D in the immune system is the reversal of immune abnormalities in SLE. In vivo and in vitro studies showed beneficial effect of vitamin D supplementation on SLE immune abnormalities.[7-14]

SLE patients are at risk for hypovitamonis D due to chronic steroid and hydroxychloroquine usage, mucocutaneous and renal involvement, antibody to vitamin D, sun avoidance and VDR gene polymorphysm.[9,14-17] However, the correlation and overall effect of vitamin D on disease activity remain unclear from the available clinical data.[10-13,18-22]

Hematologic involvement has been found in 50-70% SLE patients, which is mainly leukopenia (46%), consist of 75-93% lymphopenia and 47% netropenia.[4,23] In severe disease activity, leukopenia and lymphopenia can be found.[24]

Neutrophyl-lymphocyte count ratio (NLCR) has been evaluated and used as inflammatory marker in various diseases.[25,26,27] NLCR was significantly higher in SLE patients than normal subjects (2,52 vs 1,64, $p=0,007$).[24] The role of NLCR in SLE disease activity monitoring is unknown.

In this study, we evaluated the effects of vitamin D supplementation toward disease activity and NLCR as simple inflammation marker in SLE patients with hypovitaminosis D.

METHODS

This was a pre-post design study without control group (quasi-experimental study) to assess the effect of 3-month vitamin D supplementation on disease activity and NLCR. Samples were collected using consecutive sampling from admissions method from Rheumatology Clinic, Outpatient Department Hasan Sadikin General Hospital, Bandung. This study was held from November 2013 unit March 2014. The inclusion criterias were fulfilling the 2012 SLICC SLE diagnostic criteria[28], age 14-55 years old, disease activity score using Mexican-SLEDAI (MEX-SLEDAI) more than 2 until 9, and vitamin D serum level using 25(OH)D (the best indicator for vitamin D status) of less than 30 ng/mL. The exclusion criterias were chronic kidney disease stage 4-5 patients, patients with chronic liver disease, subjects with disease flare, acute infection and chronic inflammation other than SLE during the study, patients received high dose corticosteroid (equivalent to prednisone > 30 mg/day) in the last 30 day, pregnant and breast-feeding, hypolipidemic and anticonvulsant consumption, subjects with active tuberculosis and/or on TB treatment, and history of vitamin D supplementation during the last 3 month was excluded from the study. The study was approved by the Ethical Committee of the Hasan Sadikin General Hospital, Bandung. All patients agreed to participate and signed informed consent at the beginning of the study.

Vitamin D serum level was assessed by *Enzyme-linked Immuno Sorbent Assay* (ELISA) 25(OH)D serum level examination. Hypovitaminosis D defined as vitamin D insufficiency (21−29 ng/mL) or deficiency (< 20 ng/mL).[31] SLE disease activity was measured using *Mexican SLEDAI* (MEX-SLEDAI), with scores classified as follows : 0-1 for remission, 2-5 represented mild disease activity, 6-9 was moderate and more than 10 as severe disease activity. Disease flare defined as ≥ 3 score increment. Remission defined as more than 3 scores reductions and less than 3 scores changes as disease persistence.[6,29,30] NLCR was obtained from absolute neutrophyl count divided by absolute lymphocyte count attained from leukocyte differential count[25-27] Chronic kidney disease (CKD) stage 4-5 defined as glomerular filtration rate ≤ 29 ml/minute/1,73m^2 for more than 3 months by Cockroft-Gault equation.[32] Previous liver disease, physical examination and elevated transaminase needed to assess chronic liver disease.[33] Fitzpatrick skin type score was evaluated to classify skin coloration and response to sun exposure.[34] The duration of ultraviolet exposure (minute/day) held from interview with study subjects.

Subjects received 2000 IU/day vitamin D3 (cholecalcipherol) for 3 months based on clinical data from previous study that showed 100% SLE patients in our Rheumatology clinic was vitamin D deficiency (median 25(OH)D serum level was 3,84 ng/mL).[22] American College of Rheumatology (ACR) recommended 50.000 IU/week of vitamin D for 8 weeks, continued with 2000-4000 IU/day for maintenance. ACR also stated 2000 IU/day of vitamin D was sufficient to achieve optimal vitamin D status (30-60 ng/mL) in normal subjects. Vitamin D available in Indonesia was supplied as vitamin D3 (cholecalcipherol) 400 IU/soft capsule. During the study, we supplement study subjects with 5 soft capsule/day. We monitored every patients monthly to evaluate symptoms, SLE disease activity, compliance and any side effects to vitamin D supplementation. After 3-month supplementation (vitamin D serum level is not recommended to be remeasured for less than 3 months)[31,35], we repeated the measurement of 25(OH)D serum level and NLCR. Data analysis started with *Shapiro-Wilk* normality test (n<50) for quantitative data. The comparison of MEX-SLEDAI and NLCR at baseline and after supplementation were analyzed using paired T-test or Wilcoxon's test as appropriate using SPSS *for window ver* 17.

RESULTS

We enrolled 33 eligible SLE patients. During this study, 1 subject reported skin rash, 2 subjects with nausea-vomiting and 2 subjects were excluded due to uncompliance to supplementation. At the end of the study, 28 subjects available to be analyzed. Table 1 showed that the prevalence of SLE among the study subjects was higher in women (92,9%). Mean age was 30,7±10,5 years old. Median disease duration was 21,25 months (4 -156 months). Median Body Mass Index (BMI) was 21,25 kg/m^2 (16,1 - 41,1 kg/m^2). Frequency of SLE manifestation consisted of 92,9% mucocutaneous, 25% muskuloskeletal, 67,9% hematology, 57,1% renal, 17,9% vaskulitis, 21,4% neuropsychiatric (NPSLE) and 14,3% serositis. Baseline Median (25(OH)D) serum level was 9,24 ng/mL (3-24,64 ng/mL). All subjects (100%) received methylprednisolone. Other SLE medications were chloroquine (57,1%), cyclophosphamide (14,3%), azathioprine (39,3%) and cyclosporine (3,5%). Only age (p=0,261) and vitamin D level after supplementation (p=0,170) were distributed normal (p>0,05). Baseline median MEX-SLEDAI score and NLCR were 4 (3 – 8) and 2,95 (1,17 - 7,27) (Table 2).

Table 1. Baseline characteristics of patients (n=28)

Characteristics	n = 28
Sex (n, %)	
Male	2 (7,1%)
Female	26 (92,9%)
Age (years)	
Mean ± SD	30,7 ± 10,5
SLE disease duration(months)	
Median (Range)	30,5 (4-156)
Body Mass Index / BMI (kg/m^2)	
Median (Range)	21,3 (16,1-41,1)
SLE manifestation (n,%)	
Mucocutan	26 (92,9%)
Musculoskeletal	7 (25%)
Hematology	19 (67,9%)
Renal	16 (57,1%)
Vaskulitis	5 (17,9%)
NPSLE	6 (21,4%)
Serositis	4 (14,3%)

SLE medications (n, %)	
Corticosteroid	28 (100%)
Chloroquine	16 (57,1%)
Azathioprine	11 (39,3%)
Cyclophosphamide	4 (14,3%)
Cyclosporine	1 (3,6%)
UV exposure (minute/day)	
Median (Range)	15 (5-60)
Skin colour (n %) *(Fitzpatrick skin type score)*	
2	5 (17,9%)
3	11 (39,3%)
4	7 (25%)
5	4 (17,9%)
Sunblock application SPF 30 (n, %)	
Applying	19 (67,9%)
Not applying	9 (32,1%)
Vit D Compliance (%)	
Median (Range)	96,7 (90-100)
Baseline 25(OH)D serum level (ng/mL)(n,%)	
Insufficiency (20-29)	3 (10,7 %)
Deficiency (<20)	25 (89,3 %)
Severe Deficiency (<12)	19 (67,9%)

The effects of vitamin D supplementation on MEX-SLEDAI and NLCR

Table 2 showed 3-month vitamin D supplementation resulted in statistically significance decreased disease activity (p<0,05). There was 46,7% decrease in MEX-SLEDAI, 24,2% decrease in NLCR, simultaneously with 164,7% increase in mean 25(OH)D serum level after supplementation.

Table 2. Vitamin D (25(OH)D) serum level, MEX-SLEDAI dan NLCR Pre and post

Variables	Before cholecalciferol supplementation (pre)	After cholecalciferol supplementation (post)	pre&post changes	*p*-value*
MEX-SLEDAI				
Median (Range)	4 (3-8)	2 (0-6)	46,7% Decrease	0,000
NLCR				
Median (Range)	2,95 (1,17-7,27)	2,28 (1,07-4,87)	24,2% Decrease	0,000
25(OH)D (ng/mL)				
Mean ± SD	10,2 ± 5,8	27 ± 12,1	164,7% Increase	0,000

Note * : *p*-value submitted from Wilcoxon test, p < 0,05 as level of significance.

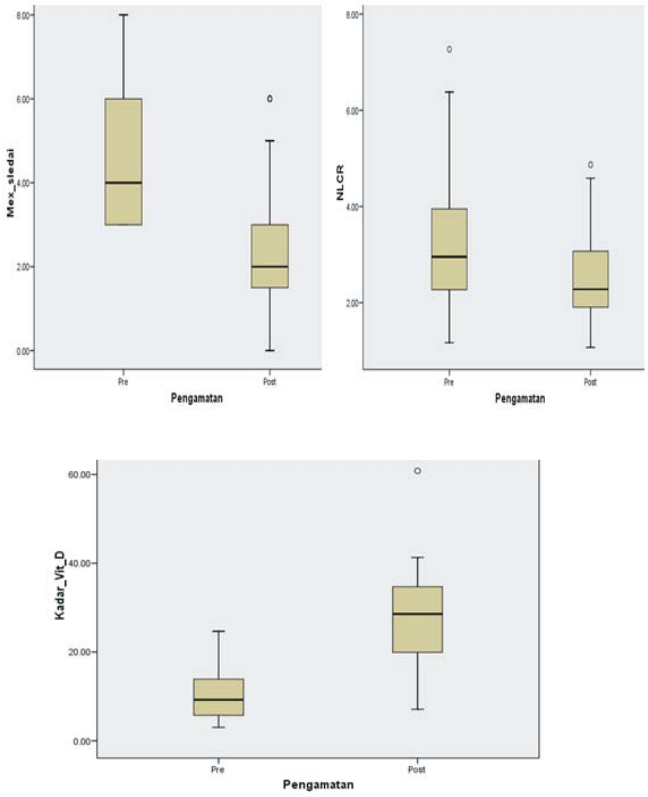

Figure 1. MEX-SLEDAI, NLCR and 25(OH)D serum level pre and post.

The effect of vitamin D supplementation (along with SLE immunosupresive therapy) on disease activity comprised of 32,1% subjects with disease remission, 67,9% experienced persistence and no subject with disease flare during observation. (Table 3)

Table 3. MEX-SLEDAI after cholecalcipherol supplementation

MEX-SLEDAI decreasing ≥3 (remission) (n,%)	MEX-SLEDAI decreasing <3 (persistent) (n,%)	MEX-SLEDAI increasing ≥ 3 (flare up) (n,%)
9 (32,1%)	19 (67,9%)	-
TOTAL		28 (100%)

There were 89,3% subjects with vitamin D deficiency at baseline. After 3-month cholecalcipherol supplementation, 25% subjects remains deficiency but 42,9% subjects achieved optimal vitamin D serum level. (Table 4)

Table 4. Characteristics of 25(OH)D serum level pre and post

	Before cholecalciferol supplementation (*pre*) n=28	After cholecalciferol supplementation (*post*) n=28
25(OH)D serum level (ng/mL)		
Normal (30-60)	-	12(42,9%)
Insufficiency (20-29)	3(10,7%)	9 (32,1%)
Deficiency (<20)	25 (89,3%)	7 (25%)
Severe deficiency (<12)	19 (67,8%)	5 (17,8%)

DISCUSSION

This is a pre-post design study without control group (quasi-experimental study) to assess the effect of 3-month vitamin D supplementation on disease activity and NLCR in SLE Patients. This study design was selected because we found limitation in obtaining placebo or control group as comparison. On the first month follw-up, one subject reported skin rash, which is concluded as hypersensitivity reaction (co-evaluation with Dermatovenereologist). Moreover, two subjects reported gastrointestinal intolerance (nausea and vomitting). On the second month, two other subjects reported compliance less than 90%. Those five subjects' participation were discontinued from our study.

At the end of the study, we analyzed 28 subjects with women subjects in majority (92,9%), and mean age of 30,7±10,5 years old, which is in line with the epidemiology of SLE.[2] Baseline data showed mucocutaneous (92,9%), hematology (67,9%) and renal (57,1%) manifestation of SLE because vitamin D metabolism involves skin and kidney.[31] Also Bogaczewicz J et al[12] found that SLE patients with hematologic (leukopeni) and renal involvement had higher risk of vitamin D deficiency. Fifty percent subjects had BMI>23 kg/m2 (overweight) which increased the risk of hypovitaminosis D as vitamin D was sequestrated in the adipose tissue.[31] Table 1 showed 100% subjects had corticosteroid, 39,3% subjects with Fitzpatrick skin type 3 and 67,9% applied sunscreen that also increased the risk of hypovitaminosis D. [9,14-17]

Initially, 89,3% subjects were vitamin D deficiency (25(OH)D <20ng/mL) and convert to 25% subjects after 3-month supplementation (table 4). We observed 42,9% subjects with normal vitamin D status, but 32,1% still in insufficiency after supplementation. Five subjects (17,8%) persist as severe vitamin D defiency (<12 ng/mL) due to less compliance, high BMI and gastrointestinal symptoms during the study. Robinson AB et al found that 400 IU per day vitamin D supplementation will increase mean 25(OH)D, but few subjects still hypovitaminosis D yet recommend higher dose of vitamin D, especially for patients with proteinuria and high disease activity.[8]

Table 2 demonstrate 46,7% decrease mean MEX-SLEDAI (p <0,001) and table 3 showed no subjects suffered flare up during study. According to a metaanalysis by Sakthiswary R et al[18], theres is a strong association between level of vitamin D and disease activity and no association with disease damage. Kurniati et al reported vitamin D 2000 IU/day followed by significant MEX-SLEDAI score decrease (6,85±2,71 vs 3,98±3,53, p<0,005) without marked difference of $1,25(OH)_2D_3$ serum level.[20] Handono et al[21] also reported negative correlation between 25(OH)D serum level and disease activity (r=-0,659, p=0,000). Altogether with Abou-Raya et al[13],Borba et al[19] and Petri et al[36] whose reported significantly better inflammation marker after vitamin D3 supplementation, vitamin D3 2000 IU/day could be considered as "additional" therapy for SLE.

High risk hypovitaminosis D patients (such as overweight, darker skin colour, sun avoidance, sun screen application, mucocutaneous, hematologic and renal involvement and drugs) should be considered for early vitamin D status examination, higher vitamin D supplementation and frequent monitoring of vitamin D serum level.[35]

We observed 24,2% decrease median NLCR, from 2,95 (1,17-7,27) to 2,28 (1,07-4,87) p<0,001). Dan JQ dkk reported decrease NLCR afte Radio Frequency Ablation in 178 hepatocelullar carcinoma patients, which indicate better survival.[37] Wolfsminke et al observed that NLCR was not a useful predictive value in 440 severe malaria patients.[38] Oehadian et al reported significantly higher NLCR in SLE patients compared to normal (2,52 vs 1,64, p=0,007). Cut-off NLCR of ≥ 1,93 resulted in 70% sensitivity and 67% specifity to differentiate SLE and normal subjects.[24] Though our study result is consistent with Oehadian et al, but the role NLCR as a simple inflammation marker useful for disease activity monitoring in SLE still need further investigation.

Few subjects reported fatigue as part of baseline MEX-SLEDAI assessment (data not shown). Sterling et al reported fatigue as frequently undiagnosed disease burden but influenced the quality of life of SLE patients.[39] We observed that after vitamin D3 2000 IU/day for 3 months, those subjects reported less fatigue and one subjects can back to work (through patient interview).

Our study limitation were no placebo or control group and relatively short duration of study. We expected more apparent vitamin D effect on disease activity and NLCR in longer supplementation duration. Polymorphism of vitamin D receptor needed to investigated for 17,8% subjects persist in severe vitamin D deficiency.

CONCLUSION

In the present study, there was a significant reduced disease activity and NLCR in SLE patients with hypovitaminosis D after the administration of vitamin D along with the main immunosuppresive SLE therapy. The role of NLCR as a simple inflammation marker in SLE disease activity monitoring needs to be further investigated. Furthemore, fatigue evaluation in SLE patients with objective measurement and the correlation with vitamin D supplementation also need further research.

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