



## ASSOCIATION BETWEEN SERUM VITAMIN D LEVEL AND CLINICAL MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

<b>Zuhrial Zubir</b>	Division of Pulmonology and Allergy Immunology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara / H. Adam Malik General Hospital, Medan, Indonesia
<b>Meivina Ramadhani Pane</b>	Division of Pulmonology and Allergy Immunology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara / H. Adam Malik General Hospital, Medan, Indonesia
<b>Darmadi*</b>	Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara / H. Adam Malik General Hospital, Medan, Indonesia *Corresponding Author

### ABSTRACT

**Introduction:** The role of vitamin D in immunomodulatory is established suggesting its association in affecting autoimmune diseases such as systemic lupus erythematosus (SLE). In spite of its importance, particularly in South Asia, vitamin D inadequacy is common. International studies have shown vitamin D deficiency has been frequently reported in SLE populations and is associated to multiple manifestations of SLE, though mixed results have been reported in terms of its relation to certain clinical manifestations of SLE.

**Aim:** In the current study, we aimed to evaluate the association between serum vitamin D level with specific clinical manifestations classically found in SLE patients.

**Methods:** This was retrospective study from medical records of patients with SLE who came to Adam Malik General Hospital, Medan, Indonesia during the year of 2015-2017. Data on clinical manifestations, serum vitamin D level, ANA and anti-dsDNA level were assessed. Vitamin D status was assessed by measuring serum 25(OH)D concentration using standard cut-off value.

**Results:** A total number of 50 SLE cases were included in this study. Among SLE patients, 48% was vitamin D insufficient whilst 32% was vitamin D deficient. Only 20% of subjects showed normal level of vitamin D. This current study found significant association between serum vitamin D concentration with photosensitivity (median 14.9,  $p = 0.004$ ), renal impairment (median 15.25,  $p = 0.021$ ), and hematology disorder (median 16.2,  $p = 0.009$ ). This study did not found significant correlation between vitamin D level with both ANA and anti-dsDNA.

**Conclusion:** This study showed a high prevalence of vitamin D inadequacy in SLE patients residing in our region. We reported significant associations between vitamin D level with photosensitivity, renal impairments and hematology disorders in SLE patients. In terms of SLE-specific antibody titer, we did not find significant correlation between vitamin D with anti-dsDNA.

**KEYWORDS :** Vitamin D, SLE, ANA, Anti-dsDNA

### Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder involving a wide range of clinical manifestations leading to possible organ damage.<sup>1</sup> One factor that is associated with the course of the disease is vitamin D.<sup>2</sup> In South Asia, vitamin D inadequacy is common.<sup>3</sup> Vitamin D deficiency has been frequently reported in SLE suggesting its role on the disease manifestations. Vitamin D deficiency has previously shown to be a risk factor in developing autoimmune diseases such as multiple sclerosis and SLE.<sup>2</sup> Not only as a risk factor, multiple data also suggests the role of vitamin D in affecting disease activity and damage in SLE patients.<sup>4</sup>

The role of vitamin D on immune function is established. It is involved in interleukin (IL-2) inhibition, lymphocyte proliferation and antibody production.<sup>5-7</sup> Tabasi et al showed vitamin D regulatory function on promoting cell cycle and apoptosis in SLE patients.<sup>8</sup> Vitamin D also previously showed protective effect on endothelial cells through aiding endothelial repair mechanisms thus reducing risk for developing cardiovascular-related events in SLE patients.<sup>9</sup> Various immune cells such as monocyte, macrophages, activated T and B cells among others possess vitamin D receptor allowing vitamin D to exert its effect, as well as contain 1-alpha-hydroxylase essential in converting vitamin D to its active form.<sup>10</sup>

Low level of vitamin D has been linked to multiple manifestations of SLE. Kamen et al reported photosensitivity and renal disease as a strong predictor for vitamin D deficiency.<sup>11</sup> Available evidences support in SLE populations, photosensitivity and the use of sunscreen is associated with vitamin D inadequacy, suggesting SLE leads to vitamin D deficiency due to lifestyle modification that limits sun exposure.<sup>12</sup> This is concordant to existing knowledge that majority of vitamin D is synthesized cutaneously through ultraviolet B radiation which later converted to 25(OH)D in the liver, and then in

the kidney. However, Rada et al in 2012 found no association between vitamin D level and photosensitivity, additionally, no association to skin lesions and arthritis.<sup>13</sup>

Mixed results have been reported in terms of vitamin D deficiency in relation to certain clinical manifestations of SLE. In the current study, we aimed to evaluate the association between serum vitamin D level with specific clinical manifestations classically found in SLE patients. This study also aimed to investigate the correlation between vitamin D concentration with antibodies commonly used in assessing SLE conditions.

### Methods

This retrospective study utilized data from medical records of patients with SLE who came to Adam Malik General Hospital, Medan during the year of 2015-2017. Diagnosis of SLE was made based on the American College of Rheumatology (ACR) 1997 revised criteria for the classification of SLE. Inclusion criteria were medical records of SLE patients with complete data on clinical manifestations, serum vitamin D level, ANA and anti-dsDNA level. Medical records of SLE patients with incomplete data were excluded. Baseline data was gathered including patients characteristics, clinical signs and symptoms, associated impairment, vitamin D status, and ANA and anti-dsDNA level.

Vitamin D status was assessed by measuring serum 25(OH)D concentration as the form of circulating vitamin D. Cut-off value used was based on local laboratory classification which were: normal ( $> 30$  ng/ml), insufficiency (15-30 ng/ml), and deficiency ( $\leq 15$  ng/ml). Serum 25(OH)D level was measured using ECLIA (electrochemiluminescence immunoassays) on the Roche Cobas E601 apparatus. Data was analyzed using Chi-Square test to find the association as well as Pearson test for further correlation on numerical data. Data analysed using SPSS version 22 (SPSS Inc.,

Chicago),  $p < 0.05\%$  was considered as statistically significant.

**Results**

**Patients' demography and vitamin D status**

A total number of 50 medical records of patients with SLE were included in this study. Majority of patients was female (84%), with median age of 28 years old (ranging from 17-58 years old). In terms of clinical manifestations, arthritis was the most commonly reported (96%), followed by hematology disorder (74%), malar rash (68%), photosensitivity (40%), and oral ulcer and renal impairment (36%). Laboratory study showed median ANA value of 87.4, median anti-dsDNA value of 258, and median 25(OH)D value of 18.2. Nearly half of the SLE patients was vitamin D insufficient (48%). Frank vitamin D deficiency was found in 32% of patients, whilst only 20% of subjects showed normal level of vitamin D (table 1).

**Table 1. Demographic data and clinical characteristics of subjects**

Characteristics	n = 50
Gender, n(%) a Male Female	8 (16%) 42 (84%)
Age, years b	28 (17 – 58)
Occupation, n(%) a	11 (22%)
Student	21 (42%)
Housewife	7 (14%)
Farmer	8 (16%)
Entrepreneur	3 (6%)
Others	
<b>Clinical Manifestation, n(%)<sup>a</sup></b>	
Malar rash	34 (68%)
Discoid rash	13 (26%)
Photosensitivity	20 (40%)
Oral ulcer	18 (36%)
Arthritis	48 (96%)
Serositis	14 (28%)
Renal impairment	18 (36%)
Neurologic disorder	5 (10%)
Hematology disorder	37 (74%)
<b>Laboratory<sup>b</sup></b>	
ANA level, IU/mL	87.4 (2.4 – 303)
Anti-dsDNA level, IU/mL	258 (31.4 – 145)
25(OH)D level, ng/mL	18.2 (8 – 40.4)
Vitamin D Status a	
Normal	10 (20%)
Insufficient	24 (48%)
Deficient	16 (32%)

<sup>a</sup>categorical data : n (%)

<sup>b</sup>numeric data, median (minimum – maksimum)

**Association between vitamin D level with SLE clinical manifestations**

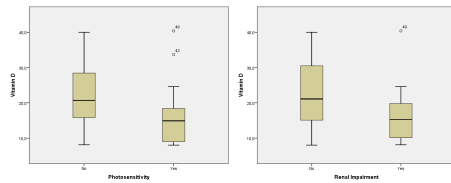
The present study also evaluate the association between vitamin D level with the reported clinical manifestations of SLE patients. Of all the clinical manifestations (table 2), this study found significant association between serum vitamin D level with photosensitivity (median 14.9,  $p = 0.004$ ), renal impairment (median 15.25,  $p = 0.021$ ), and hematology disorder (median 16.2,  $p = 0.009$ ). No significant association was found between vitamin D level and malar rash, discoid rash, oral ulcer, arthritis, serositis and neurologic disorder.

**Table 2. Association between vitamin D level with SLE clinical manifestations**

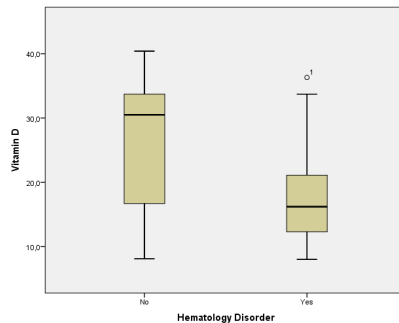
Clinical Manifestation	25(OH) D level	P
Malar rash	16.25 (8 – 40.4)	0.693
Yes	20.65 (8.1 – 33.7)	
No		

Discoid rash	17.9 (8.1 – 36.3)	0.319
Yes	24.6 (8 – 40.4)	
No		
Photosensitivity	14.9 (8 – 40.4)	0.004*
Yes	20.65 (8.1 – 40)	
No		
Oral ulcer	15.6 (8.1 – 36.3)	0.322
Yes	20 (8 – 40.4)	
No		
Arthritis	11.65 (8.1 – 15.2)	0.163
Yes	18.2 (8 – 40.4)	
No		
Serositis	19.1 (8.1 – 34.8)	0.689
Yes	17.3 (8 – 40.4)	
No		
Renal impairment	15.25 (8.1 – 40.4)	0.021*
Yes	21.1 (8 – 40)	
No		
Neurologic disorder	18 (10.1 – 24.9)	0.846
Yes	17.9 (8 – 40.4)	
No		
Hematology disorder	16.2 (8 – 36.3)	0.009*
Yes	30.5 (8.1 – 40.4)	
No		

\* $p < 0.05$



**Figure 1 & 2. Boxplot of 25(OH)D levels in SLE patients with and without photosensitivity (left); Boxplot of 25(OH)D levels in SLE patients with and without renal impairment (right)**



**Figure 3. Boxplot of 25(OH)D levels in SLE patients with and without hematology disorder**

**Correlation between vitamin D level with ANA and anti-dsDNA titer**

Pearson test was used to determine the correlation between serum vitamin D level with the disease activity, represented by titer of ANA and anti-dsDNA. Both ANA and anti-dsDNA titer showed weak negative linear correlation with vitamin D level showing both  $p > 0.05$ , hence no significant correlation was found.

**Table 3. Correlation between vitamin D level with ANA and anti-dsDNA**

	r	P
Anti-dsDNA	-0.228	0.112
ANA	-0.065	0.654

**Discussion**

Low level of vitamin D has been a topic of interest in many SLE researches. The role of vitamin D in regulating immune cells had been established in multiple studies, implying deficiency of such regulator carries a risk in the development and progression of autoimmune disease such as SLE. In spite of its importance, multiple evidence showed high prevalence of vitamin D deficiency in SLE populations. Kamen et al in 2006 conducted a population-based cohort involving 123 recently diagnosed SLE patients and 240 matched controls and found a significantly lower level of 25(OH)D in SLE groups compared to control. The study reported vitamin D deficiency was found in one-third among SLE patients, whilst severe deficiency (<10 ng/ml) was found in one-fifth of patients.<sup>11</sup>

In line with previous evidences, out of 50 subjects of SLE included in the current study, we found nearly half of the patients was vitamin D insufficient, whilst one-third of the population was vitamin D deficient. Several factors had been suggested to contribute to the commonly found vitamin D inadequacy in SLE patients. Avoidance of sunlight exposure due to photosensitivity, the use of sunscreen, lacking dietary sources of vitamin D, renal insufficiency or by the disease itself were among those factors.<sup>14,15</sup> Medication used in SLE patients especially those altering vitamin D metabolism or impairing the function of vitamin D receptor had also been linked to lower vitamin D.<sup>16</sup>

Among clinical manifestations of SLE, we found serum vitamin D level was significantly associated with photosensitivity ( $p=0.004$ ). This study took place in Southeast Asia where sun exposure was considerably constant throughout the year. Similar to the current study climate, recent study by Kamel et al in Middle east region where sun exposure was high, reported a 53.3% of SLE patients were vitamin D insufficient and 46.7% were vitamin D deficient and that vitamin D level was significantly lower in SLE patients compared to controls.<sup>17</sup> Samanhoudy et al reported similar finding in Saudi SLE patients with a higher prevalence of 98% vitamin D insufficiency and 89.7% vitamin D deficiency.<sup>18</sup> In spite of residing in areas where sun exposure was abundant, photosensitivity, the use of sunscreen and other measures that blocks the sun exposure were considered to contribute to the reduction of subsequent cutaneous vitamin D synthesis.<sup>12</sup>

Significant association between vitamin D level with renal impairment was also seen in our study ( $p=0.021$ ). Vitamin D deficiency had also been associated with renal impairments in SLE patients. Robinson et al reported serum vitamin D level were associated with serum albumin level and inversely correlated with urine protein to creatinin ratio (UP/C) and urinary vitamin D binding protein to creatinine ratio (DBP/C).<sup>19</sup> Previous data suggested an increase of 20 ng/mL vitamin D was associated with 15% decrease of developing clinically important proteinuria.<sup>20</sup> Renal involvement was frequent during course follow-up thus putting SLE patients at risk for developing advanced renal disease. Subsequently, 1-hydroxylation of vitamin D, essential in converting to its active form, greatly reduced. Sumethkul et al confirmed the finding stating that in lupus nephritis patients, vitamin D level was significantly lower.<sup>21</sup> This study also found significant association between vitamin D level with hematology disorders in SLE patients ( $p=0.009$ ). Hematology disorders was commonly found in 50-70% of SLE patients. It mainly involved leukopenia, specifically lymphopenia (75-93%) and neutropenia (47%).<sup>22,23</sup> Bogaczewicz J et al reported an increased risk of vitamin D deficiency in SLE patients with leukopenia and renal impairments.<sup>24</sup>

Low level of serum vitamin D had been related to other several manifestations of SLE. In this study, we did not find a significant correlation between vitamin D level with discoid and malar rash, neurologic disorders and arthritis. Study by Rada et al found no significant difference between incidence of photosensitivity, skin lesions and arthritis between vitamin D insufficiency and deficiency groups in SLE populations.<sup>13</sup> Recent study observed vitamin D deficiency was associated with worse cognitive function, though

needs further confirmations by other studies. Other study by Yasser et al also did not find any association between vitamin D level with cerebrovascular events.<sup>25</sup>

Other than its association to certain clinical findings, lower vitamin D level was also related to higher disease activity in SLE patients.<sup>26-29</sup> Borba et al reported patients with highly active SLE had significantly lower 25(OH)D compared to those with low activity and controls.<sup>15</sup> Previous data suggested vitamin D deficiency might aggravate disease activity and promoted autoantibody production in SLE favoring progression of the disease. Studies involving SLE patients in Europe and Middle East showed an inverse relationship between 25(OH)D level with disease activity score.<sup>26,27</sup> Other study stated an increase of 20 ng/mL of serum 25(OH)D was associated with 21% reduction risk of having a high activity score.<sup>20</sup> Lauren et al also reported high prevalence of vitamin D deficiency in ANA-positive healthy individuals. This finding suggested vitamin D deficiency might develop early in the course of autoimmune disease long before clinical manifestations occurred. This also implied vitamin D deficiency in SLE was not solely develop due to photosensitivity thus the avoidance of UV exposure, but rather it had a causative role in innitiation and progression of the disease.<sup>30</sup>

In this study we investigated serology biomarker to evaluate disease activity and the presence of antibodies commonly involved in SLE. Our study did not find a significant correlation between vitamin D level with both ANA and anti-dsDNA titer. Similarly to our study, other study by Rada et al did not find an association between vitamin D level with the presence of ANA and anti-dsDNA antibodies.<sup>13</sup> Our result however was different from other study by Kamel et al that reported significant negative correlation between vitamin D and anti-dsDNA titer.<sup>17</sup> Previous studies also reported similar negative correlation.<sup>27,31</sup> Other study also found anti-dsDNA was strongly associated with the presence of anti-vitamin D autoantibodies, suggesting the presence of these antibodies might associate with vitamin D deficiency.<sup>32</sup>

Vitamin D exerted its role on multiple immune cells and an association between its serum level with SLE has been frequently reported, whether as a consequence or a cause. Vitamin D deficiency is prevalent among SLE patients and had shown mixed findings in relation to specific clinical manifestations of SLE. Our study has several limitations in which no matched controls were included and that included SLE subjects were not classified based on disease activity or severity, which may contribute to different findings in our study. In conclusion, this current study showed a high prevalence of vitamin D inadequacy in SLE patients residing in our region. We reported a significant association between vitamin D level with photosensitivity, renal impairments and hematology disorders in SLE patients. In terms of SLE-specific antibody titer, we did not find significant correlation between vitamin D with anti-dsDNA.

## References

1. Agmon-Levin N, Mosca M, Petri M, Shoenfeld Y. Systemic lupus erythematosus one disease or many?. *Autoimmun Rev.* 2012;11:593–595.
2. Yang C-Y, Leung PS, Adamopoulos IE, Gershwin ME. The implication of vitamin D and autoimmunity: a comprehensive review. *Clin Rev Allergy Immunol.* 2013;45:217–226.
3. Yetley EA. Assessing the vitamin D status of the US population. *Am J Clin Nutr.* 2008; 88:558.
4. Sakthiwarthy R, Raymond AA. The clinical significance of vitamin D in systemic lupus erythematosus: a systematic review. *PLoS One* 2013;8:e55275.
5. Maruotti N, Cantatore FP. Vitamin D and the immune system. *J Rheumatol.* 2010;37:491–495.
6. Iruetagoiena M, Hirigoyen D, Naves R, Burgos PI. Immune response modulation by vitamin D: role in systemic lupus erythematosus. *Front Immunol.* 2015;6:513.
7. Cutolo M, Otsa K, Paolino S, Yprus M, Veldi T, Serio B. Vitamin D involvement in rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis.* 2009; 68:446.
8. Tabasi N, Rastin M, Mahmoudi M, Ghoryani M, Mirfeizi Z, Rabe SZT et al. Influence of vitamin D on cell cycle, apoptosis, and some apoptosis related molecules in systemic lupus erythematosus. *Iran J Basic Med Sci.* 2015;18:1107.
9. Reynolds JA, Haque S, Williamson K, Ray DW, Alexander MY, Bruce IN. Vitamin D improves endothelial dysfunction and restores myeloid angiogenic cell function via reduced CXCL-10 expression in systemic lupus erythematosus. *Sci Rep* 2016;6.
10. Handal AE, Handunnetthi L, Ebers GC, Ramagopalan SV. Type 1 diabetes mellitus and multiple sclerosis: common etiological features. *Nat Rev Endocrinol.* 2009;5:655–664.
11. Kamen DL, Cooper GS, Bouali H, Shafta SR, Hollis BW, Gilkeson GS. Vitamin D

- deficiency in systemic lupus erythematosus. *Autoimmun Rev.* 2006; 5:114-117.
12. Ruiz-Irastorza G, Egurbide M, Olivares N, Martinez-Berriotxo A, Aguirre. Vitamin D deficiency in systemic lupus erythematosus: prevalence, predictors and clinical consequences. *Rheumatology.* 2008;47:920–923.
  13. Miskovic R, Plavsic A, Raskovic S, Jovicic Z, Bolpacic J. Vitamin D Status in Patients with Systemic Lupus Erythematosus in Serbia: Correlation with Disease Activity and Clinical Manifestations. *OA Maced J Med Sci.* 2015;3(2):256-261.
  14. Kamen DL. Vitamin D in lupus: new kid on the block?. *Bull Hosp Jt Dis.* 2010;68:218.
  15. Borba V, Vieira J, Kasamatsu T, Radominski S, Sato E, Lazaretti-Castro M. Vitamin D deficiency in patients with active systemic lupus erythematosus. *Osteoporos Int.* 2009;20:427–433.
  16. Mok CC. Vitamin D and systemic lupus erythematosus: an update. *Expert Rev Clin Immunol.* 2013;9:453–463.
  17. Kamel H, Gado, Tarek H, Gado, Rasha M, Abdel Samie, Noha M, Khalil, Safa L, Emam, Hanan H, Fouad. Clinical significance of vitamin D deficiency and receptor gene polymorphism in systemic lupus erythematosus patients. *The Egyptian Rheumatologist.* 2017;39:159–164.
  18. Damanhoury LH. Vitamin D deficiency in Saudi patients with systemic lupus erythematosus. *Saudi Med J.* 2009;30(10):1291–5.
  19. Robinson AB, Thierry-Palmer M, Gibson KL, Rabinovich CE. Disease activity, proteinuria, and vitamin D status in children with systemic lupus erythematosus and juvenile dermatomyositis. *J Pediatr.* 2010;160:297–302.
  20. Petri M, Bello KJ, Fang H, Magder LS. Vitamin D in systemic lupus erythematosus: modest association with disease activity and the urine protein-to-creatinine ratio. *Arthr Rheum* 2013;65:1865–1871.
  21. Sumethkul K, Boonyaratavej S, Kitumnuaaypong T et al. The predictive factors of low serum 25-hydroxyvitamin D and vitamin D deficiency in patients with systemic lupus erythematosus. *Rheumatol Int.* 2012;33: 1461-7.
  22. Hahn BH. Overview of pathogenesis of systemic lupus erythematosus. *Dubois' Lupus Erythematosus.* 7th ed. Los Angeles: Lippincott Williams & Wilkins; 2007. p.47–52.
  23. Quismorio FP. Hematologic and lymphoid abnormalities in Systemic Lupus Erythematosus. In: Wallace DJ, Hahn BH, editors. *Dubois' Lupus Erythematosus.* 7th ed. Philadelphia: Lippincott William & Wilkins; 2007:801–20.
  24. Bogaczewicz J, Sysa-Jedrzejska A, Arkuszewska C, Zabek J, Kontny E, et al. Vitamin D status in systemic lupus erythematosus patients and its association with selected clinical and laboratory parameters. *Lupus* 2012;21:477-484.
  25. Yasser Ezzat, Safaa Sayed, Wafaa Gaber, Abeer M. Mohey, Tamer Wahid Kassem. 25-Hydroxy vitamin D levels and its relation to disease activity and cardiovascular risk factors in women with systemic lupus erythematosus. *The Egyptian Rheumatologist.* 2011;33, 195–201.
  26. Amital H, Szekanez Z, Szücs G, Danko K, Nagy E, Csepany T et al. Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D?. *Ann Rheum Dis.* 2010;69:1155–1157.
  27. Mok CC, Birmingham DJ, Leung HW, Hebert LA, Song H, Rovin BH. Vitamin D levels in Chinese patients with systemic lupus erythematosus: relationship with disease activity, vascular risk factors and atherosclerosis. *Rheumatology* 2012;51:644–652
  28. Yap K, Northcott M, Hoi AB, Morand E, Nikpour M. Association of low vitamin D with high disease activity in an Australian systemic lupus erythematosus cohort. *Lupus Sci Med.* 2015;2:e000064.
  29. Abou-Raya A, Abou-Raya S, Helmi M. The effect of vitamin D supplementation on inflammatory and hemostatic markers and disease activity in patients with systemic lupus erythematosus: a randomized placebo-controlled trial. *J Rheumatol.* 2013;40:265–272.
  30. Lauren LR, Sherry RC, Timothy BN, Diane LK, Susan RM, Virginia CR, et al. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. *Ann Rheum Dis.* 2011; 70(9): 1569–1574.
  31. Szodoray P, Tarr T, Bazso A, Poor G, Szegedi G, Kiss E. The immunopathological role of vitamin D in patients with SLE: data from a single centre registry in Hungary. *Scand J Rheumatol.* 2011;40(2):122-126.
  32. Carvalho JF, Blank M, Kiss E, Tarr T, Amital H, Shoenfeld Y. Anti-vitamin D, vitamin D in SLE: preliminary results. *Ann NY Acad Sci.* 2007;1109:550–7.