WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 45-55. Research Article ISSN 2277– 7105

IMMUNOMODULATION IN IRAQI PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS THROUGH VITAMIN D_3 SUPPLEMENTATION

Eman T.A. Al-Ahmed[1], Awatif H.issa[2]*, Saad Shaheen Hammadi[3]

[1]University of basrah, Collage of Pharmacy, Dept. of Pharmacology
[2]University of basrah, Collage of science, Dept. of Biology.
[3]University of Basra, Collage of Medicine, Dept. of Medicine.

Article Received on
20 September 2014,

Revised on 12 Oct 2014,
Accepted on 06 Nov 2014

***Correspondence for Author**
Awatif H. issa
University of basrah, Collage of science, Dept. of Biology.

ABSTRACT

Vitamin D deficiency is worldwide problem with serious health effects on SLE disease and one of the most important risk factors for immune system. 1, 25-Dihydroxyvitamin D3 (1, 25(OH) 2D3) can modulate immune responses. Systemic Lupus erythematosus (SLE) is an autoimmune disease more prominent in women characterized by wide variety of auto antibodies production, some of which are pathogenic, immune complex deposition and various clinical systemic manifestation that effect various organ. Patients with systemic lupus erythematosus, especially those with anti-dsDNAbs and increased disease activity, had decreased 1, 25(OH) 2D3 levels, suggesting that Vitamin D might play a role in regulating autoantibody production. To address this, we examined the effects of 1, 25(OH) 2D3 on anti-dsDNA concentration and its relation to organ involvement. Serum1, 25(OH) $_2D_3$ was significantly lower in total SLE patient (4.202 ± 5.3 ng/ml) ranged from (0.021-28.71) than healthy control was (34.78 ± 6.49 ng/ml), There was highly significant difference (p=0.000) between two groups of SLE patients, (SLE patients before receiving Ca/Vitamin D and SLE patients after receiving Ca/Vitamin D) depended on the serum 25(OH) D_3 level and Anti-dsDNA concentrations. Dramatically, levels of Serum 25(OH) D_3 increased under vitamin D supplementation from (3.204±3.6ng/ml to 28.90±4.84ng/ml) while anti-dsDNA levels decreased from (243.3±82.4to47.40±26.77I U/ml). Moreover, observed high significant negative correlation between vitamin D and anti-dsDNA. Also, strong significant association between vitamin D deficiency and organ involvement.

KEYWORDS: SLE, Vitamin D_3, anti-dsDNA.

INTRODUCTION

SLE is a systemic autoimmune disease which is characterized by abnormalities in both the B and T-cell compartments associated with loss of tolerance followed by activation and expansion of autoreactive lymphocytes. As occurs in other autoimmune diseases (Maria, 2011). Vitamin D_3 whose main function is the bone formation, regulation of calcium homeostasis and reabsorption through the interaction with the kidneys, parathyroid glands and bowel (Arnson, 2007). The endogenous production in the skin is the main source of vitamin D3 comes after exposure to ultraviolet B light (Leventis, 2008). Vitamin D_3 deficiency has been implicated as one of the environmental factors contributing to the prevalence of several autoimmune diseases, including SLE (Kamen, 2010) Vitamin D_3 seems to interact with the immune system through its actions on the regulation and differentiation of cells like lymphocytes, macrophages, and natural killer cells (NK), besides interfering in the in vivo and in vitro production of cytokines (Lemire, 1992; Ben-Zvi *et al.*, 2010).

Among the main functions of vitamin D3 in the immune system, we could mention: regulation of the differentiation and activation of CD4 lymphocytes (Szodoray, 2008; Cutolo, 2009) increase in the number and function of regulatory T cells (T_{reg}) in vitro inhibition of the differentiation of monocytes in dendritic cells; reduction in the production of cytokines, IFN-γ, IL-2, and TNF-α by Th1 cells, and stimulation of the function of Th2 helper cells inhibition of the production of IL-17 by Th1 cells (Steinman, 2007) and in vivo and in vitro stimulation of NK T cells (Yu, 2008). The vitamin D_3 has the ability to effect in the immune system through an enhancement of innate immunity associated with a multifaceted regulation of acquired immunity (Adorinin, 2008). Vitamin D_3 exerts multiple actions at the cellular level to balance the population of effectors cells (Bonelli, 2010).

MATERIAL AND METHODS

Patients

Fifty patients with SLE (female 48, male2, Mean age (32.360± 9.405) years, ranged (15 -55) diagnostic by Al-ahmed, (2014) and treated under supervisions phystion in three main hospitals in the Basra. The patients are classified into two groups according to the specification of medical and therapeutic prescription depending on use vitamin D3, patients who used vitamin D3 classified as group (II) were taken for study and patients who devoted from vitamin D3 classified as group (I) and for the classification of SLE at least four or More

of the 11 ACR criteria were studied. No patients fulfilling these criteria were excluded. Thirty healthy control volunteers unrelated to the patients, without inflammatory or autoimmune disease as normal control subjects (female28, male2, mean age was (38.7± 7.240) were studied. Information for each case were obtained and recorded on predefined aspects of the following symptoms and clinical features that are common in patients with SLE were used for review of the medical record**.**

Specimens

Specimens of venous blood from all patients were taken during morning, Sera were separated by centrifugation at 3000 rpm for 3 minutes and separated as soon as possible from the clot of red cells and were kept in aliquots at-80 centigrade until the time of assay.

Laboratory Measurement

Vitamin D_3,Calicium and anti-ds DNA were evaluated in serum of SLE patients and healthy control before and after supplementation with Ca^+/ vitamin D_3.So, Study divided SLE patients into two groups (Group I : total 50 SLE patient not receiving Ca^+/ vitamin D_3) and(GroupII:25female SLE patients were taken from total 50 depending on the prescription of doctors, those receiving Ca^+/Vitamin D_3 supplementation with vitamin D_3 dose ranged from(800-1200)IU/day for six months during this study. Disease activity was assessed according to (Bombardier *et al*., 1992; Sdaile *et al*., 1996), two stages of SLE were considered as function of the SLEDAI score; active SLE when SLEDAI >12 points and inactive SLEDAI <12 points.

Statistical Analysis

The results were evaluated by the analysis of the variance (ANOVA), p-values at levels ($p<0.05$) was considered to be statistically significant. This calculation was carried out according to Statistical Package for Social Science (SPSS version 16), Group differences on normally distributed numerical variables were assessed by the independent samples-test (Groups 1 and 2) and ANOVA (Groups 1, 2) and the least significant difference (LSD) at level less than 0.05 by using Gene State 2009 and correlation(r) were used when appropriate at 0.01.

RESULTS AND DISCUSSION

SLE patients divided into four groups according to their concentrations. Very sever Vitamin D insufficiency in SLE patients was found in 41patients (82%) and sever deficiency in 5

patients (10%). While, 3(6%) SLE patients had vitamin D deficiency level. Only suboptimal vitamin D deficiency was found in 1 (2%). There was significant difference between these groups p=0.01as shown in figure (1). Furthermore, concentration of vitamin D_3 in patients with active and in active SLE disease was (4.202 ± 5.3 ng/ml) ranged from (0.021-28.71) and healthy control was (34.78 ± 6.49 ng/ml). There was a significant difference between the level of vitamin D_3 in SLE patients and healthy control (p=0.000) as shown in table (1) and figure (2). Moreover, the mean concentration of calcium in serum of SLE patients was (7.51±1.68mg/dl) while in healthy control was (9.806±0.415mg/dl) at (p= 0.04) and the difference was significant table (2).

The study exhibited that low vitamin D_3 level was frequent in SLE patients, and indicated that SLE patients had higher risk of insufficient vitamin D_3. The high prevalence of SLE patients who have vitamin D_3 level below normal was similar with most studies in the world (Mouyis *et al*., 2008; Toloza e*t al*., 2010; Kim *et al*., 2010; Broder *et al*., 2010; Mok *et al*., 2012and Robinson *et al*.,2012**).** This is mainly due to the long-term use of sunscreen by patients with SLE that advised to avoid sun light, common trigger of disease flares but also the primary source of vitamin D_3 .So there are multiple risk factors for 25(OH) D_3 deficiency, photosensitivity, chronic treatment with corticosteroid and hydroxychloroquine therapy seems to affect vitamin D_3 metabolism and decreased calcium concentration, and lack of dietary intake (Danby *et al*., 2007). The low level of vitamin D_3 causes impaired immunological response that is thought to increase disease activity in SLE (Cutolo, 2008). Study did not find any significant correlation between low vitamin D_3 levels and SLEDAI score as shown in table (3), this finding was matched to other studies (Ruiz-Irastorza *et al*., 2008, Toloza *et al*., 20101; and Souto *et al*., 2011) and no significant correlation between vitamin D and Age, disease duration table (3), and this was similar to one study (Afify *et al*., 2013). On the other hand, the serum 25(OH) D3 levels were correlated inversely with anti-dsDNA titer (r=--0.860*, p=0.000), table (3) and these results are in accordance with that of (Mok *et al*., 2012; Afify *et a*l., 2013***)***. Study demonstrated the strong significant association between vitamin D deficiency and organ involvement. That The presence of renal disorder in SLE is one of the most consistent clinical manifestations that had significantly lower vitamin D_3 level than those without renal manifestation. So, The mean level of vitamin D_3 in SLE patients with renal failure was (0.58ng/ml) while the mean levels of vitamin D_3in patients with others organ (skin, Kidney disorder, Liver, Lung, CNS, Spleen ,Heart, Serositis, Pancreatic, Thermocytopenia and joints) were (3.47, 2.93, 10.66, 3.38, 5.73, 1.8,14,75,5.58,

1.86,1.85 and 2.10ng/ml) respectively as shown in figure(3). This finding is similar to the some studies (Ruiz-Irastorza *et al*., 2008, Toloza *et al*., 20101; Souto *et al*., 2011 and Afify, 2013). Because SLE-related renal involvement may inhibit the conversion of 25(OH)D3 in the kidney to its biologically active form of $1,25(OH)_2D_3$ via inhibition of CYP27 B1enzyme which may increase 25-(OH)D on the expense of decrease $1,25(OH)_2D_3$, but the urinary losses of $25(OH)D_3$ and its binding protein which decrease half-life of serum $25(OH)D_3$ in proteinuric nephropathies contribute to the net result of vitamin D_3 deficiency in lupus nephritis and so it is considered as an important risk factor of vitamin D_3 deficiency in SLE patients**(**Ruiz-Irastorza, *et al*.,2008, ;Souto *etal*., 2011andAfify *etal*.,2013**).** Furthermore, Low vitamin D level was found as strong predictor of Cutaneous lupus and this similar to (Cutillas, 2010).

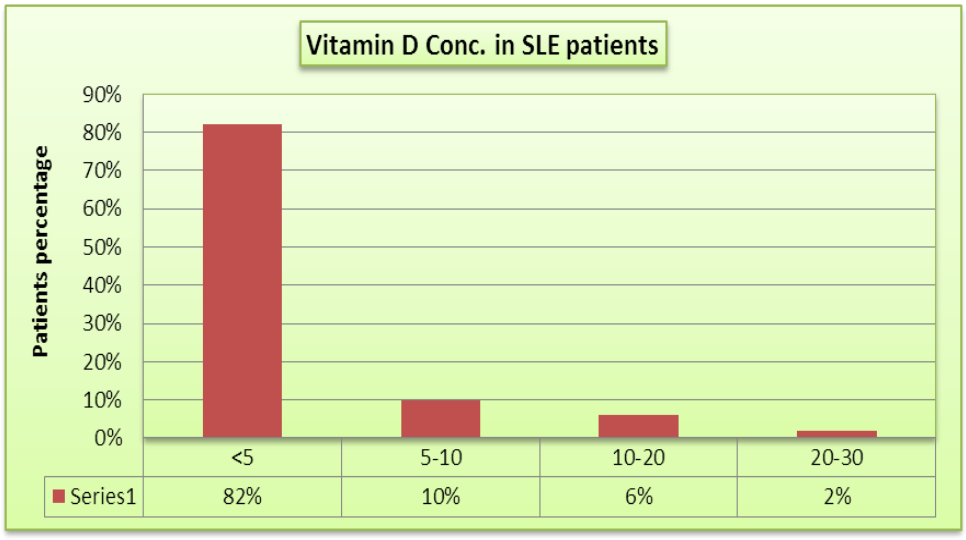

Figure (1): SLE Patients Divided into Four Groups' Depended on Concentration of Vitamin D_3 Deficiency.

Table (1): Concentrations of Vitamin D_3 in Serum of SLE Patients and Controls.

Groups	No	Mean	SD	Rang	P
SLE(Active ,Inactive)	50	7.5	1.688	2-8.4	0.04
Control	30	9.806	0.415	8.6-11	

Table (2): Concentrations of Calcium in Serum of SLE Patients and Controls P-Value is significant at level 0.05.

Groups	No	Mean	SD	Rang	P
SLE(Active ,Inactive)	50	4.202	5.3	0.021-28.71	0.000
Control	30	34.78	6.49	25.76-50.881	

P-Value is significant at level 0.05 determined by T-Test.

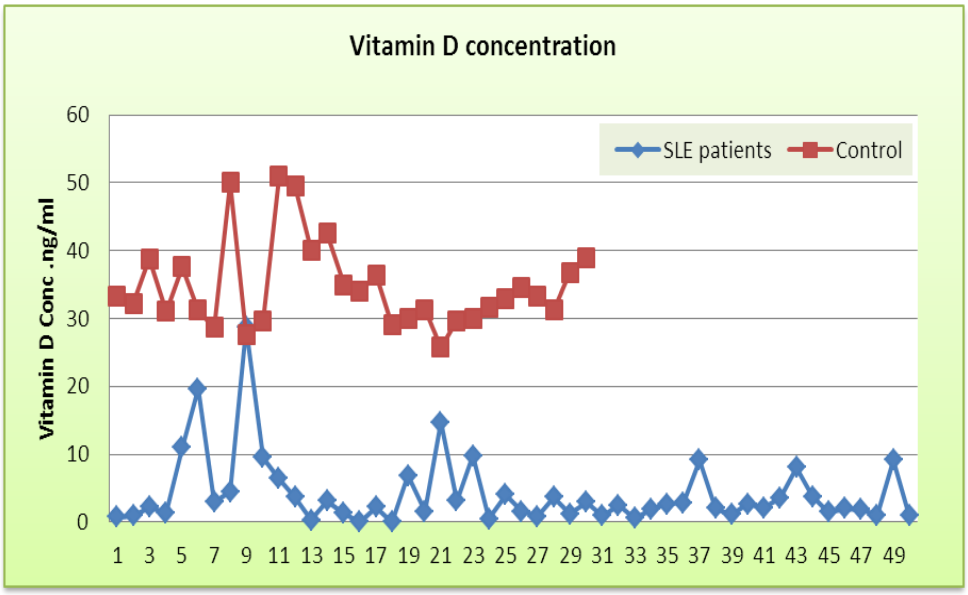

Figure (2): Vitamin D_3 Concentrations ng/ml in SLE Patients and Control.

Table (3): Pearson Correlation between Vitamin D_3 and Anti-dsDNA for Total SLE.

		Anti-dsDNA	SLEDAI	ESR	Age	Duration
Vitamin D	Pearson Correlation	-0.860*	0.047	0.032	0.134	0.085
	Sig-2tailed	0.000	0.7	0.8	0.3	0.5
	N	50				

**Correlation is significant at level 0.05.

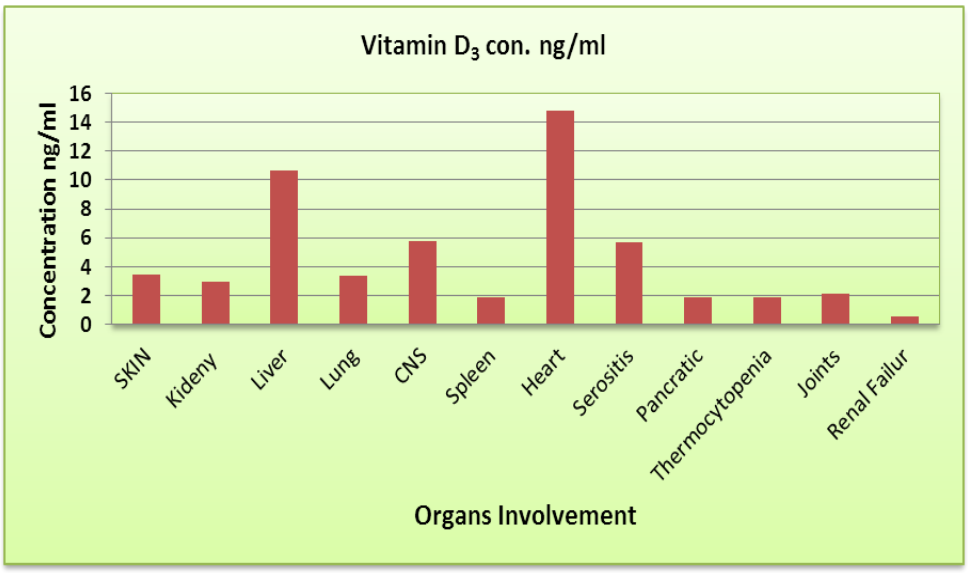

Figure (3): The Assoiasstion between Vitamin D_3 and Organ Involvement.

No study to date in Iraq has assessed in vivo the benefit of vitamin D_3 supplementation in patients with SLE. Current study for the first time has assessed the immunological effects and safety the of vitamin D_3 supplementation in patients with SLE. Study demonstrated those

treated patients with vitamin D_3 was safe and induced a decrease of-dsDNA autoantibodies levels. Most SLE patients were included in the Study with different disease activity to determine the specific effects of vitamin D_3 in the presence of modification of associated therapy, such as use of immunosuppressive agents or an Increase of the prednisone dosage. Study found high significant difference (p= 0.000) between two groups of SLE patients (SLE patients before receiving Ca^+/Vitamin D and SLE patients after receiving Ca^+/Vitamin D) depended on the serum 25(OH) D_3 level and Anti-dsDNA concentrations. dramatically levels of Serum 25(OH) D_3 increased under vitamin D supplementation from (3.204±3.6ng/ml to 28.90 ± 4.84ng/ml) while anti-dsDNA levels decreased from (243.3 ±82.4 to 47.40 ±26.77 IU/ml) as shown in table (4).On the other hand, the serum 25(OH) D3 levels were correlated inversely with anti-dsDNA titer(r=-0.889**, P=0.000) as shown in table (5).Morover, the study found the mean concentration of Calcium(Ca^+) before supplementation was (7.05±1.15 mg/dl) range (2-7.9) while after supplementation the mean concentration of Ca become (8.21±0.56) rang(6-9) table(6). No significant increase of serum calcium that demonstrated the safety of Treatment. No patients require Modification of the prednisone dosage or initiation of new immunosuppressant agents, these results are in accordance with that of (Mok, 2012; Afify *et al*., 2013**)**. During the 6 months follow-up period, study did not observe SLE flare. This may explained, that high dose of vitamin D_3 have ability to inhibit or decreased B cell proliferation, or decreasing autoantibodies production that lead to decreased the concentration of anti-dsDNA in serum of patient and this may effect on the SLEDAI score ,clinical manifestation and other factors that consider to be risk factors for SLE development, this finding matched to (Terrier *et al.*,2013*)*,he found that Vitamin D_3 supplementation provides beneficial immunological effects in patients with SLE, with a decrease of memory B cells that decreased of anti-dsDNA autoantibodies levels and effector T cells and an increase of regulatory T cells, and to induced the differentiation or expansion of FoxP3 T-reg and an increased expression of CTLA4 . Also, it have the ability to inhibit Th17 responses, probably owing to its ability to inhibit IL-23 production .Vitamin D_3 supplementation in patients with SLE caused an increase in beneficial of each of Treg cells ,CD4+ cells, and a decrease of effector Th1 and Th17 cells (Daniel *et al.*,2008; Jeffery *et al*.,2009).

Table (4): Comparison between Two Groups of SLE Patients before and after Receiving Ca+/Vitamin D_3 Depend on the Serum Level of 25(OH) $D_3$and Anti-dsDNA.

	Vitamin D(ng/ml)	Anti-dsDNA(IU/ml)
Group 1	**SLE patients before receiving Ca^+/Vitamin D_3**	
Means±SD	3.204±3.6	243.3±82.4
Rang	0.021-14.75	26.15-300
P-value	0.000	0.000
Group 2	**SLE patients after receiving Ca^+/Vitamin D_3**	
Means±SD	28.90±4.84	47.40±26.77
Rang	23.5-42.04	1.333-96.68
P-value	0.000	0.000

P-value is significant at 0.05 levels.

Table (5): Pearson Correlation between Two Groups (after and before) of SLE Patients.

		Anti- dsDNA
Vitamin D	Pearson Correlation	-0.889**
	Sig-2tailed	0.000
	N	25

**Correlation is significant at 0.05 levels.

Table (6): Concentration of Calcium in serum of SLE patient before and after supplementation.

	Ca concentration mg/dl
Group 1	**Conc.of Ca before receiving Ca^+/Vitamin D_3**
Means ± SD	7.056±1.15
Rang	2-7.9
P-value	0.02
Group 2	**Concentration of Ca after receiving Ca^+/Vitamin D_3**
Means ± SD	8.212±0.567
Rang	6-9

CONCLUSION

Vitamin D supplementation 1200IU per day provides beneficial immunological effects in patients with SLE that maintains normal levels with decrease of Anti-dsDNA autoantibodies levels in serum of SLE patients. Their possible therapeutic implications in the treatment of SLE in the future deserve wide scale trials.

REFERENCE

1. Maria – Konstantia C, Michael R, Ehrenstein. Regulatory T-cells in systemic lupus erythematosus and rheumatoid arthritis. 2011. Ehrenstein / FEBS Letters, 2011; 585: 3603–3610.

2. Arnson Y, Amital H, Shoenfeld Y, Vitamin D and autoimmunity: new etiological and therapeutic considerations. Ann Rheum Dis, 2007; 66:1137-42.
3. Leventis P, Patel S, Clinical aspects of vitamin D in the managementof rheumatoid arthritis. Rheumatology, 2008; 47:1617-21.
4. Kamen DL, Tangpricha V, Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J Mol Med (Berl), 2010; 88:441-50.
5. Lemire, J.M. Immunomodulatory role of 1, 25-dihydroxyvitaminD3. J of cellular biochemistry, 1992; 49(1): 26-31.
6. Ben-Zvi I, Aranow C, Mackay M, Stanevsky A, Kamen DL, and Marinescu LM. the impact of vitamin D on dendritic cell function in patients with systemiclupus erythematosus. PLoS One, 2010; 5: 91-93.
7. Szodoray P, Nakken, B, Gaal J, Jonsson R, Szegedi A, and Zold E, The complex role of vitamin D in autoimmune diseases. Scand JImmunol, 2008; 68(3):261-269.
8. Cutolo M, Vitamin D and autoimmune rheumatic diseases. Rheumatol, 2009; 48:210-220.
9. Steinman L, A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell-mediated tissue damage. Nat Med, 2007; 13(2):139-145.
10. Yu S, Cantorna MT. The vitamin D receptor is required for iNKTcell development. Proc Natl Acad Sci USA, 2008; 105:5207-5212.
11. Adorini A, Penna, G. Control of autoimmune diseases by the vitaminD endocrine system. Nat Clin Pract Rheumatol, 2008; 4:404-12.
12. Bonelli M, Treg and lupus. Ann Rheum Dis, 2010; 69(l):i65-66.
13. Tan E.M, Cohen A.S, Fries J.F, Masi A.T, McShane, D.J. and Roth field, N.F. The revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum, 1982; 25:1271–1277.
14. Hochberg, M.C. The application of genetic epidemiology to systemic lupus erythematosus. J Rheumatol, 1987; 14:867–869.
15. Bombardier C, Gladman D.D, Urowitz, M.B. Caron, D. and Chang, C.H. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthr Rheum, 1992; 35:630-640.
16. Sdaile E, Abrahamowic Z.M, and Joseph L. Laboratory tests aspredictors of disease exacerbations in systemic lupus erythematosus, Arth Rheum, 1996; 39:370-378.
17. Mouyis M, Ostor A.J.K, Crisp A.J, Ginawi A, Halsall D.J, Shenker, N. Hypovitaminosis D Among Rheumatology Outpatients in Clinical Practice. Rheum, 2008; 47:1348-1351.

18. Toloza S, Cole D, Glandman D, Ibanez D, Urowitz M, Vitamin D Insufficiency in Large Female SLE Cohort. Lupus, 2010; 19:13-19.
19. Kim H.A, Sung J.M, Yoon J.M, Jeon J.Y, and Suh, C.H. Vitamin D may not be a Good Marker of Disease Activity in Korean Patients with Systemic Lupus Erythematosus. Rheumatol Int, 2010; 2(2):35-40.
20. Broder A.R, Tobin J.N, Putterman C, Disease-specific Definitions of Vitamin D Deficiency Need to be established in Autoimmune and Non-autoimmune Chronic Disease: A Retrospective Comparison of Three Chronic Disease. Arthr Resea and Thera, 2010; 12:191.
21. Mok C.C, Birmingham D.J, Ho L.Y, Vitamin D deficiency as marker for disease activity and damage in systemic lupus erythematosus: a comparison with anti-dsDNA and anti-C1q. Lupus, 2012; 21: 36–42
22. Robinson A.B, Palmer, M.T Gibson K.L, and Rabinovich C.E, Disease Activity, Proteinuria, and Vitamin D Status in Children with Systemic Lupus Erythematosus and Juvenile Dermatomyositis. J Pediatr, 2012; 160:297-302.
23. Danby C.S, Cusack C, Kelly P.O, Murray, B. and Murphy GM, Vitamin D Deficiency in Photosensitive Lupus Patient in Ireland, 2007; 1(2):171-176.
24. Cutolo M, Otsa K, Vitamin D, Immunity, and Lupus. Lupus, 2008; 17:6-19.
25. Ruiz-Irastorza G, Egurbide M.V, Olivares, N.; Martinez-Berriotxoa, A. and Aguirre, C. Vitamin D deficiency in systemic lupus erythematosus: prevalence, predictors and clinical consequences. Rheumatology, 2008; 47:920-923.
26. Souto M, Coelho A, Guo C, Vitamin D insufficiency in Brazilian patients with SLE. prevalence, associated factors, and relationship with activity. Lupus, 2011; 20:1019-1026.
27. Afify T.H, Mirvat A, El-Tokhi O.Z, Shehata A.G, Husina S, and Alyan M. Serum concentrations of 25-hydrixy vitamin D on patients with systemic lupus erythematosus; its relation with disease activity. Z.U.M.J. 2013; 19(4).
28. Mok C.C, Birmingham D.J, Ho L.Y, Vitamin D deficiency as marker for disease activity and damage in systemic lupus erythematosus: a comparison with anti-ds DNA and anti-C1q. Lupus, 2012; 21:36–42.
29. Cutillas-Marco E, Morales-Suarez-Varela M, Marquina-Vila A. and Grant W, Serum 25-hydroxyvitamin D Levels in Patients with Cutaneous Lupus Erythematosus in Mediterranean Region. Lupus. 2010; 0:1-5.

30. Terrier B, Nicolas D, Yol and S. and Wahiba C, Restoration of regulatory and effector T cell balance and B cell homeostasis in systemic lupus erythematosus patients through vitamin D supplementation. Arthr Res Ther. 2012; 14:R221.
31. Daniel C, Sartory N.A, Zahn N, Radeke H.H, and Stein, J.M. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 andregulatory T cell profile. J Pharmacol Exp Ther, 2008; 324:23-33.
32. Jeffery L.E, Burke F, Mura M, Zheng Y, Qureshi O.S, Hewison M, Walker L.S, Lammas D.A, Raza K, and Sansom D.M, 1, 25-Dihydroxyvitamin D3 and IL-2combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol, 2009; 183:5458-5467.