RESEARCH ARTICLE

The relationship between vitamin D status and disease activity in Iraqi systemic lupus erythematosus patients

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Abstract

Objective: To find the relationship between vitamin D deficiency and the activity of systemic lupus erythematosus in Iraqi patients.

Method: The case-control study was conducted at Baghdad Teaching Hospital, Baghdad, Iraq, from July to October 2018, and comprised systemic lupus erythematosus patients regardless of age and gender visiting the Rheumatology outpatient clinic. Serum levels of complement protein 3, complement protein 4, anti-double-stranded deoxyribonucleic acid and 25-hydroxy vitamin D were estimated. Based on disease activity scores, patients were divided into moderate activity group SLE-M and severe activity group SLE-S. Healthy subjects matched for age and gender were also enrolled as the control group. Data was analysed using Graph Pad Prism 5.0.

Results: Of the 150 subjects, 62(41.3%) were in SLE-S group, 38(25.3%) in SLE-M and 59(33.3%) in the control group. Among the patients, 97(97%) were females and 3(3%) were males, with a female-to-male ratio of 32:1. The patients' age range was <10-≥50 years, while the control group consisted of 2 (4%) males and 48 (96%) females with an age range of <10-≥50. The mean levels of serum complement protein 3, complement protein 4 and vitamin D levels were significantly lower in the patient groups compared to the controls ($p<0.05$).

Conclusion: Systemic lupus erythematosus patients suffered from either vitamin D deficiency or insufficiency, and low vitamin D levels were found related to disease activity.

Key Words: Rheumatology, Vitamin D, Lupus Erythematosus, Systemic, DNA, Proteins, Ambulatory Care
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Introduction

Systemic lupus erythematosus (SLE) is a systemic disease of autoimmune origin in which healthy organs and tissue are attacked by the body's immune system, leading to organ damage, such as kidneys, skin, joints and brain as well as cardiovascular, pulmonary and haematological systems.[1] A typical feature of SLE is the formation of immune complexes. The dysfunction within both the adaptive and innate immune systems lead to increased T-cell activity, cytokine production, and B-cell overproduction of autoantibodies. Autoantibodies have been linked to the human leukocyte antigen (HLA) Class II gene, including anti-deoxyribonucleic acid (DNA), anti-ribonucleoprotein (anti-nRNP), anti–Sjögren's-syndrome-related antigen autoantibodies anti-Ro and anti-La, and anti-Smith (anti-SM) antibodies. Pathogenesis mechanisms result from an interaction of environmental factors, like ultraviolet (UV) light, Epstein-Barr virus infection, etc., as well as genetic, hormonal and epigenetic factors. Among the standard scales used to assess SLE disease activity is the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), which evaluates SLE activity over the preceding 10 days and had 24 items collecting particular manifestations in various organ systems, including musculoskeletal, neurological, mucocutaneous, haematological, respiratory, vascular and renal systems. This index generates a global score ranging 0-105, as well as a weighted individual item score ranging 1-8.[2] Immune complex production and the subsequent activation of the complement pathway leading to complement consumption lies behind the pathogenesis of SLE. Thus, measurement of serum complements proteins 3 and 4 (C3, C4) is among the criteria used for diagnosing and monitoring SLE.[3] Furthermore, the origin and site of double stranded (ds) DNA antibodies beside the presence of autoantibodies are critical for the initiation and pathogenesis of SLE. Therefore, anti-dsDNA antibodies are highly specific for the diagnosis and monitoring of SLE.[4]

Vitamin D is a steroid hormone that has long been known for its role in bone and calcium metabolism. Lately, it has been recognised for its role in immune response and its immunomodulatory effects in autoimmune diseases, such as SLE.[5] Vitamin D has been linked to a number of

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actions involved in the regulation of the immune response, such as enhancement of phagocytosis, promotion of regulatory T-cell (T-reg) development, and inhibition of B-cell differentiation, which are usually found malfunctioning in SLE patients. Previous studies have observed vitamin D deficiency in SLE patients.[6, 7]

The current study was planned to find the relationship between vitamin D deficiency and SLE disease activity in Iraqi patients.

Patients and Methods

The case-control study was conducted at Baghdad Teaching hospital, Baghdad, Iraq, from July to October 2018. After approval from the ethics review committee of the Iraqi Ministry of Health and Environment, Baghdad, the sample was raised using convenience sampling technique from those visiting the Rheumatology outpatient clinic. Those included were SLE patients regardless of age and gender who were willing to participate in the study. Those not willing to volunteer were excluded. SLE was diagnosed according to the guidelines of the American College of Rheumatology (ACR) for SLE classification,[8] and SLEDAI2 was used to categorise the patients into SLE-Severe (SLE-S) and SLE-Moderate (SLE-M) groups. Apparently healthy individuals matched for age and gender were included as the controls group from among those coming for routine health check-ups and had normal results.

All patients or their parents/guardians furnished written informed consent.

Blood samples were collected from all the subjects, and serum was separated and stored at -20°C until needed. Serum levels of anti-dsDNA was measured using enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Germany, EU) following the manufacturer's instructions. Serum levels of C3, C4 and 25-hydroxy vitamin D (25[OH]D) were measured using a relevant analysed (c4000, Abbott, United States).

Detailed clinical history was taken for each SLE patient. The concentration of 25(OH)D <20ng/mL was considered vitamin D deficiency, and 20-30ng/mL was regarded to be insufficient.[9]

Data was analysed using Graph Pad Prism 5.0. Data was expressed as means ± standard deviation and frequencies and percentages, as appropriate. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used for intergroup comparison. Linear regression was used to investigate the relationship among the variables. P<0.05 was considered significant.

Results

Of the 150 subjects, 62(41.3%) were in SLE-S group, 38(25.3%) in SLE-M and 59(33.3%) in the control group. Among the patients, 97(97%) were females and 3(3%) were males, with a female-to-male ratio of 32:1. The patients' age range was <10-≥50 years. The control group comprised of 2 (4%) males and 48(96%) females with an age range of <10-≥50. The most common clinical manifestations were arthritis, rash, alopecia, lupus headache, fever, visual disturbance, cranial nerve disorder, proteinuria, pyuria, vasculitis, mucosal ulcers, organic brain syndrome, pericarditis, cerebrovascular accident (CVA), seizure, myositis, pleurisy, psychosis and haematuria (Table 1).

Table-1: Demographic data and clinical characteristics of SLE patient.

Descriptor	SLE-S	SLE-M
Age groups		
(<10-19)	3.2%	18.4%
(20-29)	27.4%	31.5%
(30-39)	38.7%	23.6%
(40- ≥50)	30.6%	26.3%
Gender		
Female	95.2%	100 %
Male	4.8 %	0.0%
Clinical features		
Arthritis	87.92%	12.08%
New rash	89.7%	10.3%
Alopecia	93.5%	6.5%
Lupus headache	88.6%	11.4%
Fever	72.7%	27.3%
Visual disturbance	77.8%	22.2%
Cranial Nerve Disorder	78.9%	21.1
Organic Brain Syndrome	84.8%	15.2%
Psychosis	93%	7%
Seizure	86%	14%
CVA	86.7%	13.3%
Vasculitis	66.7%	33.3%
Myositis	78.6%	21.4%
Haematuria	85.7%	14.3%
Proteinuria	84.2%	15.8%
Pyuria	94.6%	5.4%
Mucosal Ulcers	80%	20%
Pleurisy	85.7%	14.3%
Pericarditis	83.9%	16.1%

SLE: Systemic lupus erythematosus, SLE-S: Systemic lupus erythematosus-Severe, SLE-M: Systemic lupus erythematosus-Moderate, CVA: Cerebrovascular accident

Among SLE-S patients, 2(3.22%) had normal vitamin D level, 14(22.58%) had insufficient level and 46(74.19%) had vitamin D deficiency. Among SLE-M patients, the corresponding values were 5(13.15%), 8(21.05%) and 25(65.78%). All 50(100%) controls had normal vitamin D levels.

Table-2: Immunological parameters and vitamin D concentration among the groups.

Parameters	SLE-S (n=62)	SLE-M(n=38)	Control (n=50)
Mean Vitamin D level (ng/ml)	18.67± 17.70***	17.44 ±12.76***	35.34 ±4.18
Normal vitamin D 30-40 (ng/ml)	3.22 %	13.15 %	100%
vitamin D Insufficiency 20-30(ng/ml)	22.58 %	21.05%	0.0 %
Deficiency vitamin D <20 (ng/ml)	74.19 %	65.78 %	0.0 %
Anti-dsDNA (IU/ml)	83.35±62.786***	48.58 ±26.676**	16.32±4.460
C3 (mg/dl)	70.03 ± 41.24***	79.39±29.54**	103.58±14.63
C4 (mg/dl)	17.31 ±11.96**	18.18±11.30*	24.36 ±8.21

SLE-S: Systemic lupus erythematosus-Severe, SLE-M: Systemic lupus erythematosus-Moderate, dsDNA: Double stranded deoxyribonucleic acid, C: Complement protein.

The mean levels of C3, C4 vitamin D levels were significantly lower in the patient groups compared to the controls, while the mean level of anti-dsDNA was higher in the patient groups compared to the controls (Table 2). The differences were significant when each patient group was individually compared with the control group ($p<0.05$).

Regression analysis showed a direct relationship of vitamin D level with C3 and C4 levels ($p<0.01$), while there was an inverse relationship of vitamin D with anti-dsDNA and SLEDAI values ($p<0.05$) (Figure).

Discussion

SLE) is a heterogeneous, clinical, pathophysiological condition. People of all ethnic groups, races, genders and inherited backgrounds can develop SLE. This disease is more common and severe in African and Asian subjects than in Europeans. Mucocutaneous lesions and arthritis/musculoskeletal complaints are among the commonest manifestations.[10]

In the 1980s, SLE was reported as one of the most common inflammatory rheumatic diseases among Iraqi people with an incidence of 53/100,000 in the Iraqi population.[11] Although Iraq has witnessed an increase in the incidence of autoimmune disease, there is currently no large-scale study giving an estimation of the incidence of SLE in Iraq. In the current study, the age range of SLE patients was >10->50 years, which is comparable to a previous report[12]. The female: male ratio in the present study (32:1) was very high, indicating that females are more prone to this disease and any discrepancies could be due to the complex aetiopathogenesis of SLE. A study noted that SLE occurs among females in childbearing years.[13] The high SLE incidence in females is thought to be attributed to the sex steroid oestradiol via a receptor-based mechanism that remains poorly understood.[13]

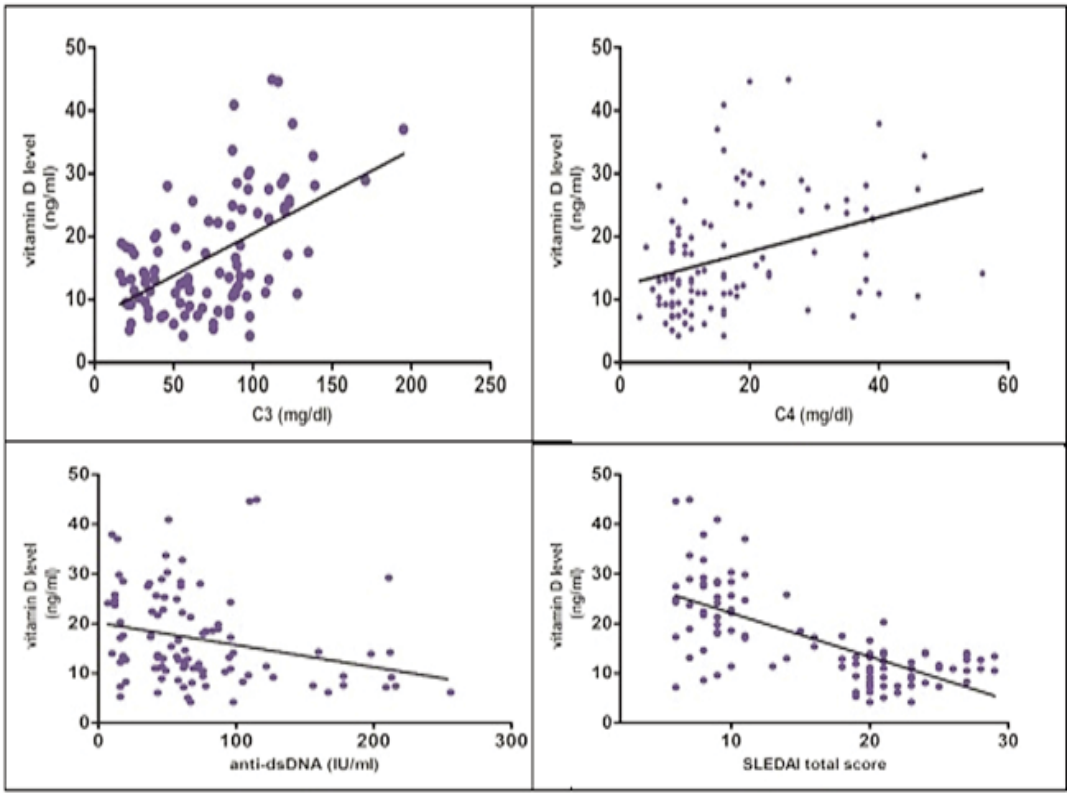

Figure: Simple liner regression showing the association of vitamin D with C3, C4, anti-dsDNA and SLEDAI score.
dsDNA: Double stranded deoxyribonucleic acid, C: Complement protein, SLEDAI: Systemic lupus erythematosus disease activity index.

The formularisation of the criteria for SLE was first suggested by ACR in the 1970s. Classification of SLE disease severity was the main purpose of these criteria which afterwards became widely used as diagnostic criteria in clinical status. Subsequently, there have been at least 2 modifications in 1982 and 1997. Diagnostic criteria that can differentiate healthy people from patients with SLE include clinical manifestations, such as arthritis, skin lesions, haematological changes, neurological disorders, renal disorders and others. Serum anti-Smith (anti-Sm) antibody, anti-nuclear antibody, and anti-dsDNA antibodies are crucial biomarkers for SLE patients.[2] In agreement with previous studies, arthritis was the most common clinical manifestation in the current study (78%). Nearly all joints can be affected by SLE, but the most typical involvement is found in the knee and hands.[10,14] In SLE, vitamin D insufficiency seems to be involved with immune system abnormalities. Vitamin D appears to influence the activity and differentiation of T and B lymphocytes, as well as the formation of autoantibodies, according to some in vitro findings.[15] Deficiency in vitamin D is highly prevalent in SLE patients due to various factors, such as renal insufficiency, avoidance of sunshine, photo protection, and the use of drugs, like anticonvulsants, glucocorticoids, calcineurin inhibitors and anti-malarial, by downregulating the functions of the vitamin D receptor, or altering vitamin D metabolism.[16]

Furthermore, female gender is a classical risk factor for low levels of vitamin D, particularly in SLE patients and in the general population. Differences in clothing or outdoor activities, lower body surface area (BSA), in addition to androgen-associated differences in vitamin D binding protein levels are possible reasons for the impact of gender in vitamin D status. Compared to the controls, the current study found significantly lower vitamin D level in SLE patients. The role of Vitamin D insufficiency in the development of autoimmune diseases has revealed new information about the vitamin's activity. Vitamin D's immunomodulatory properties include lowering T helper cell (Th1) immunological responses and decreasing Th stimulation of immunoglobulin synthesis by B cells, resulting in a shift in cytokine profiles towards the Th2 phenotype and preservation of the innate immune response.[17]

The presence of anti-dsDNA in SLE patients is associated with disease activity, and usually coincides with renal involvement.[18] They are consistently associated with glomerulonephritis in SLE, and predict flares.[4] The initiation and pathogenesis of SLE is influenced by the origin and site of dsDNA besides the presence of autoantibodies. Compared to the control group, the anti-dsDNA level was significantly higher in SLE patients, which was in accordance with previous studies[18]. The failure of neutrophil extracellular traps (NETs) to fully remove apoptotic material results in self-dsDNA or excessive chromatin, which triggers the formation of anti-dsDNA antibodies, but the mechanisms are unknown.[19]

Low C4 and C3 levels are indicators of active SLE disease, and their presence in tissues serves as a diagnostic tool. Significantly lower mean C3 and C4 levels than the control group were found in the current study. A study reported that the low serum levels of C3 and C4 in SLE patients is attributed to increased autoantibodies, which is a manifestation of immune activation, and it may also be due to cytotoxic effect of complement components.[20] Since the classical complement pathway is the central complement pathway in SLE patients, it would be expected that C3 may either be normal or low, while C4 levels are always low in SLE patients.

The study by Fabrizio and colleagues has suggested that the formation of anti-dsDNA is influenced by complement receptors through regulation of B-cell activation and/or contributing in immune-complex clearance.[21] The current study found that low levels of C3 and C4 were associated with increased anti-dsDNA levels The finding was in accordance with previous studies.[22,23] The association between low levels of C3 and C4, with decreased vitamin D levels observed in the current study, agrees with earlier findings.[24]

The level of anti-dsDNA antibodies is specific and fluctuates with SLE activity. The decrease in vitamin D level is associated with B-cell hyperactivity and hyper-production of autoantibodies. The observation of a strong negative correlation between 25(OH)D and high anti-dsDNA antibodies has been reported earlier.[6,7] The current finding that the majority of SLE patients suffer from a decrease in vitamin D levels has been previously reported.[6] Nevertheless, whether this deficiency is considered a consequence or a risk factor is still under debate. Evidence suggests Vitamin D deficiency may serve as a causative factor in some autoimmune diseases, such as rheumatoid arthritis, but extensive, longitudinal studies with emphasis on the exact time point (whether in utero, during childhood or adolescence) are needed to prove such a hypothesis.[25]

The inverse association of vitamin D stasus with both anti-dsDNA levels and SLEDAI total score found in the current study underlined a potential effect of disease activity on vitamin D levels.

Limitation: The current study has its limitations. There

were 97% females among SLE patients, which could be an element of bias. Besides, the study was conducted at a single centre for a short duration and without a follow-up after vitamin D supplements, affecting the generalisability of the findings. Finally, the sample size was not calculated which could have affected the power of the study.

Conclusion

Majority of Iraqi SLE patients were found to be suffering from vitamin D insuffeciency or defeciency. Low levels of vitamin D were found to be associated with higher disease activity.

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