Hypovitaminosis D and Systemic Lupus Erythematosus Activity and Related Neuropathy: Clinical Correlation

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease. Vitamin D has a modulating effect on immune responses. Hypovitaminosis D is highly prevalent in SLE patients, and it may lead to SLE activity and SLE-related neuropathy.

Aim of the study: To recognize the role of serum vitamin D levels in SLE activity and also to investigate its relation to SLE-related neuropathy.

Patients and Methods: The current study was a cross-sectional study performed on 100 SLE patients, who were divided into two groups, Group I: included 50 patients with disease activity. Group II: included 50 patients without disease activity. They were tested for serum vitamin D levels, serum electrolytes, complement levels and nerve conduction. Results: Vitamin D was significantly low in group 1 (median = 9.0 ng/ml) compared to the group 2 (median = 19.3 ng/ml and P-value of<0.001). Hypovitaminosis D was statistically significantly correlated with lower levels of complement (both C3 and C4) in the activity group but not in the non-activity group. Vitamin D levels were significantly associated with delayed nerve conduction in both groups, suggesting that neuropathy was linked to vitamin D level rather than SLE activity.

Conclusion: Hypovitaminosis D is statistically significantly correlated with SLE activity and SLE-related neuropathy.

Keywords: SLEDAI-2k, Systemic Lupus Erythematosus, Vitamin D.

INTRODUCTION

Vitamin D is considered a multifunctional steroid hormone with many actions. Little about its function is understood. It is becoming more clear that vitamin D is not only engaged in calcium homeostasis and bone metabolism, but also has multiple biological actions mediated by vitamin D receptors (VDR) (¹), present in even more than thirty tissues including the kidneys, brain, intestine, pituitary, parathyroid gland, prostate, breast, heart muscle, skeletal muscles, hepatic cells, immune system and the endothelial cells (³).

Vitamin D is obtained from certain food stuffs and from exposure to ultraviolet light. Activation of vitamin D is done through two steps; the first is activating vitamin D through hydroxylation of cholecalciferol into the 25-hydroxyvitamin D (25-OHD) metabolite that occurs in the liver (⁴).

This is the major circulating metabolic form of vitamin D. It circulates bound to the vitamin D-binding protein carrier protein (DBP) with a 15-21 day half-life. Second activation process for 1, 25 dihydroxy vitamin D occurs mainly in the kidney and to a lesser extent in a range of all other tissues such as bone, mammary gland, brain tissue, monocytes, parathyroid gland and placenta. This active metabolite has a shorter 10–20 hour half-life. Accordingly, the level of vitamin D is commonly assessed by measuring circulating 25-OHD (⁴,⁵).

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterized by autoantibody attacks and the deposition of complement-fixing immune complexes (ICs), which causes inflammation and can lead to systemic injuries (⁶). Hypovitaminosis D is common in SLE patients, likely due to photoprotection, renal insufficiency, glucocorticoid usage (⁷) and the presence of anti-vitamin D antibody (⁸). SLE activity has been linked to hypovitaminosis D (⁹) and the development of peripheral neuropathies (¹⁰).

Vitamin D has a modulating effect on immune responses (¹¹). Both the innate and adaptive immune systems rely on vitamin D for immune stability, as it stimulates the innate immune response while inhibiting the adaptive immune response (¹²). This role is mediated by VDR, which is expressed on immune cells (¹³). Vitamin D enhances regulatory T cells and macrophage antimicrobial effects, while decreases Th1 CD4 T cells and cytokines including IL-2, interferon, and tumor necrosis factor, it also inhibits dendritic cell differentiation and prevents activated B cells from proliferating (¹¹).

As a consequence, vitamin D would be more anti-inflammatory and immune-regulatory. Additionally, it is essential for calcium homeostasis and its deficiency may lead to calcium metabolism disorders and autoimmune diseases (¹⁴).
SLE activity has been linked in several studies to hypovitaminosis D (9), whereas others have linked it to the development of peripheral neuropathy (10).

The pathogenesis of SLE-related neuropathy is unknown; axonal degeneration, inflammatory changes, and vasculitis have all been suggested, but vitamin D plays many roles in the nervous system, including biosynthesis of neurotrophic factors, production of enzymes for neurotransmitter synthesis, inhibition of inducible nitric oxide synthase (iNOS) synthesis and it increases the levels of glutathione and gamma glutamyl-transpeptidase (15).

Is it possible that hypovitaminosis D is the primary cause of SLE-related neuropathy, and that it is thus linked to SLE activity and neuropathy development?

The aim of this study was to see if there's a connection between hypovitaminosis D and the occurrence of SLE activity and SLE-related neuropathy.

PATIENTS AND METHODS

A total of 100 consecutive SLE patients were recruited from Benha University Hospital internal medicine and rheumatology clinics, as well as hospital admissions, between August 2019 and December 2020 who met four or more of the American College of Rheumatology revised criteria of SLE (16). The level of 25-hydroxy vitamin D in the blood was determined.

Sample size estimation:

The minimal sample size for two-sample comparison means was carried out using STATA STATA/SE version 11.2 for Windows (STATA Corporation, College Station, Texas). The difference in serum Vit. D levels between the two groups was assumed as 2 ng/ml with a standard deviation of 3 ng/ml, 5% type I error (P<0.05) and at a power level of 90%.

The clinical activity of SLE was assessed using standard instruments, and regression tests were performed between 25-OHD levels and disease activity scores. A full detailed history taking and complete rheumatological and neurological examination was performed for each patient. Patients were examined and investigated for neuropathy, then the blood levels of 25-OHD were compared between patients with and without neuropathy.

The study's inclusion criteria were female patients between the ages of 17 and 60 who were willing to participate. Infection, other rheumatologic conditions, pregnant women, non-adherent patients, peripheral neuropathy caused by a condition other than SLE, hepatic cirrhosis, malignancies, chronic kidney disease, history of steroid/immunosuppressant use, smoking, and obesity were all ruled out.

Ethical clearance:

Informed consent was obtained from all subjects after taking the approval of the Institutional Review Board, Faculty of Medicine, Benha University. The work had been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Process:

Assessment of SLE activity:

SLE activity was defined as per SLEDAI-2k (Systemic Lupus Erythematosus Disease Activity Index 2000) score (15,16). This score allows the documentation of persistent disease activity in the descriptors: rash, alopecia, mucosal ulcers, and proteinuria. The cutoff point for an active disease is 4. In general, the score is a global index consisting of 24 weighted clinical and laboratory variables for nine organ systems that assesses lupus disease activity over the previous 10 days. The descriptors' scores will vary from 1 to 8, with a total possible score of 105 for all 24 variables (19).

Assay of vitamin D and definition of hypovitaminosis D:

Blood samples were collected and processed immediately to determine serum 25-OHD levels. The sample was centrifuged at 1500 for 15 minutes, after which the serum was frozen at 20°C and only thawed once. A competitive binding ELISA (Enzyme Linked Immune Sorbent Assay) was used for quantitative determination of total 25-OHD in human serum using a commercial 25-OHD ELISA Kit for diagnostic use only (Cat#: VD220B, Calbiotech USA). The detection limit was set at 1 ng/Ml. A level of less than 20 ng/ml was considered deficient, while a level of 20-29 ng/ml was considered insufficiency, and a level of ≥30 ng/ml was considered normal (20).

The electrophysiological assessment of the peripheral nerves:

A bilateral motor conduction examination of the median, ulnar and tibial, as well as a bilateral sensory conduction examination of the median, ulnar, and sural nerves, were performed with standard electrophysiological equipment provided by major manufacturers (Neuroworx Germany technology). To ensure that skin temperatures were at least 32°C, a digital skin thermometer with a 0.1°C accuracy was used, and studies were conducted out by trained electrophysiologists. Both nerve conduction and amplitude were measured.

Statistical analysis

The collected data were summarized in terms of mean ± Standard Deviation (SD) and range for numerical data, and frequency and percentage for categorical data. Skewness and kurtosis normality test was used to examine the distribution of numerical data.
Comparisons between the different study groups were carried out using the Student t-test and the Mann Whitney test to compare parametric and non-parametric data respectively. The Chi-square test and the Fisher Exact test were used to compare categorical data. The Pearson correlation coefficient (r) was used to test for the correlation between serum vitamin D levels and estimated parameters. Statistical significance was accepted at P<0.05.

All statistical analyses were carried out using STATA/SE version 11.2 for Windows (STATA Corporation, College Station, Texas).

**RESULTS**

The patients had a mean age of 36.77±10.44 years (ranging from 17 to 60 years), a BMI of 24.70±3.59 and an average disease duration of 4.45±2.41 years. The most common symptom was renal dysfunction (72%). The activity group (group 1) had significantly higher levels of parathormone and alkaline phosphatase (group 2). Regarding serum vitamin D levels, the activity group had significantly lower levels than the non-activity group, with the majority of patients (67%) had vitamin D deficiency and only 33% having vitamin D insufficiency (Table 1).

<table>
<thead>
<tr>
<th>Table (1): Demographic data and comparison between both groups</th>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
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<tr>
<td><strong>Age(years)</strong></td>
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<td><strong>BMI</strong></td>
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<td><strong>Disease Duration (years)</strong></td>
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<td><strong>Clinical Manifestations</strong></td>
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<td><strong>Peripheral Neuropathy</strong></td>
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Group 1= patients with active SLE, Group 2= patients with non-active SLE, SLE= systemic lupus erythematosus, No.=number, *= expressed in Mean±SD, SD= standard deviation, BMI = body mass index, **= expressed in number and percentage, ***=expressed in Median and range, ****= Statistically significant difference, Low C3= Low levels of complement (C3), Low C4 == Low levels of complement (C4), Ca= serum calcium level, Phos = serum phosphorus level, PTH = parathyroid hormone level, ALP= alkaline phosphatase level

On correlation between vitamin D and other laboratory parameters in the two groups, we found that hypovitaminosis D was statistically significantly correlated with lower levels of complement (both C3 and C4) and anti-ds DNA in the activity group, suggesting that SLE activity is linked to vitamin D level. Hypovitaminosis D was also statistically significantly correlated with lower calcium and phosphorus levels and elevated levels of CRP in both groups (Table 2).
Table (2): Pearson correlation coefficient between vitamin D and other laboratory results.

<table>
<thead>
<tr>
<th></th>
<th>Serum Vitamin D Level</th>
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<tr>
<td></td>
<td>Activity group (group1)</td>
<td>Non-activity group (group2)</td>
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<tr>
<td></td>
<td>Pearson Correlation coefficient</td>
<td>P-Value</td>
<td>Pearson Correlation coefficient</td>
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<tr>
<td>C3</td>
<td>0.57</td>
<td>0.041*</td>
<td>0.031</td>
</tr>
<tr>
<td>C4</td>
<td>0.46</td>
<td>0.032*</td>
<td>0.081</td>
</tr>
<tr>
<td>ds-DNA</td>
<td>0.067</td>
<td>0.011*</td>
<td>0.067</td>
</tr>
<tr>
<td>Ca</td>
<td>0.53</td>
<td>0.003*</td>
<td>0.53</td>
</tr>
<tr>
<td>Phos</td>
<td>0.092</td>
<td>0.043*</td>
<td>0.111</td>
</tr>
<tr>
<td>ALP</td>
<td>-0.26</td>
<td>0.283</td>
<td>-0.15</td>
</tr>
<tr>
<td>PTH</td>
<td>-0.127</td>
<td>0.366</td>
<td>-0.127</td>
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<tr>
<td>CRP</td>
<td>-0.447</td>
<td>0.035*</td>
<td>-0.47</td>
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C3= levels of complement (C3), C4 = levels of complement (C4), ds-DNA= double strand DNA, Ca= serum calcium level, Phos = serum phosphorus level, PTH = parathyroid hormone level, ALP= alkaline phosphatase level, CRP= C reactive protein, *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed).

On correlating vitamin D and nerve conduction study in the two groups, we found that, hypovitaminosis D was significantly associated with delayed (decreased) never conduction in both groups as shown in table 3.

Table (3): Pearson correlation coefficient between vitamin D and nerves conduction study.

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<tr>
<th></th>
<th>Serum Vitamin D Level</th>
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<tbody>
<tr>
<td></td>
<td>Activity group (Mean±SD)</td>
<td>Non activity group (Mean±SD)</td>
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<tr>
<td></td>
<td>Median</td>
<td>Ulnar</td>
<td>Tibial</td>
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<tr>
<td>Latency (msec)</td>
<td>4.1±8.5</td>
<td>3.9±7.8</td>
<td>6±9.5</td>
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<tr>
<td>Amplitude (mv)</td>
<td>7±6.2</td>
<td>6±4.5</td>
<td>2±1.6</td>
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<tr>
<td>NCV (m/sec)</td>
<td>39±7</td>
<td>35±6.4</td>
<td>26±6</td>
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<tr>
<td>F-wave</td>
<td>33±4</td>
<td>35±4</td>
<td>44±3.2</td>
</tr>
</tbody>
</table>

SD=standard deviation, NCV = nerve conduction velocity, F-wave = F-wave latency of motor study, *= Statistically significant,

DISCUSSION

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterized by autoantibody attacks and the deposition of complement-fixing immune complexes (ICs), which causes inflammation and can lead to systemic injuries (6).

Vitamin D is considered a multifunctional steroid hormone with many actions. It has a modulating effect on immune responses (11). Hypovitaminosis D is common in SLE patients, there are many risk factors for this in our study's patients, since 58% of our patients had photosensitivity and 63% had a facial rash, about half of our patients were advised to use sun protection, which could reduce vitamin D synthesis from the skin as well as wearing clothing that cover the whole body which is a cultural and religious tradition. Furthermore, chronic glucocorticoid use (found in more than half of our patients) can cause hypovitaminosis D by increasing clearance, as well as renal impairment (found in 72% of our patients which may inhibit the conversion of 25(OH)D in the kidney to its biologically active form of 1,25(OH)2D3). Additionally, the low frequency of vitamin D supplement use (found in only 19% of our patients) may be another factor contributing to the low vitamin D levels observed.

Vitamin D deficiency affects up to two-thirds of our patients, most of whom have active SLE, with 33% having vitamin D insufficiency and 67% having vitamin D deficiency. This matches with the findings of Gao et al., who found that vitamin D deficiency is highly prevalent in patients with SLE and severe vitamin D deficiency increases the risk of disease activity (21). The same conclusion has been reached in a number of studies (22, 23).

This study discovered a strong inverse relationship between 25(OH)D3 levels and SLE activity scores, with 78% of active SLE patients having vitamin D deficiency compared to 56% of non-active
SLE patients. These results are in line with previous research that has linked hypovitaminosis D to SLE activity (24, 25).

Our study revealed that Hypovitaminosis D was associated with lower levels of complement (both C3 and C4) in the activity group, as well as lower calcium and phosphorus levels and elevated CRP levels in both groups. In accordance with the study that detected that vitamin D supplementation was associated with elevated C3 and C4 complement levels (26).

The connection between hypovitaminosis D and SLE activity can be explained by the fact that vitamin D has an immune-regulatory function and is linked to autoimmune disorders. Several epidemiologic findings support this theory, including the fact that people residing near the equator have a lower risk of developing autoimmunity; and lower vitamin D levels are mostly seen in patients with a variety of systemic autoimmune disorders (11, 27). In addition another connection was established between vitamin D supplementation and decreased disease activity in patients with juvenile-onset SLE (28).

Peripheral neuropathy, which is relatively common in SLE patients, has a significant impact on their quality of life. It is more common in patients with a high SLE disease activity index and affects the lower extremity, especially the peroneal and sural nerves (29). Twenty two percent of our patients had peripheral neuropathy, but when we compare our two groups, we find that 40% of active SLE patients had peripheral neuropathy compared to only 2% of non-active SLE patients. SLE has been shown to impair peripheral nerve function even before patients develop electrophysiological or clinical neuropathy, according to multiple studies (30-32). Further analysis of our data using Pearson correlation reveals a statistically significant correlation between peripheral nerve dysfunction and vitamin D levels in both SLE groups, indicating that vitamin D plays a role in the development of SLE-related neuropathy. This is in line with the findings of Yee et al. who found that patients with SLE related neuropathy had lower vitamin D levels than SLE patients without neuropathy (33). Thus, SLE-related neuropathy is highly linked to the levels of vitamin D rather than SLE activity, and vitamin D supplementation may minimize its incidence.

The limited number of patients is one of the study's limitations. It is recommended that the relationship between vitamin D and SLE activity, especially peripheral neuropathy, be studied in a larger number of patients to confirm the current study's findings. Another field of potential research is to look into the impact of vitamin D supplementation on the prognosis of SLE activity and SLE related neuropathy.

CONCLUSION

Hypovitaminosis D is highly prevalent in SLE patients, and it may lead to SLE activity and SLE-related neuropathy. Vitamin D estimation and supplementation should be regarded as part of the SLE management plan. However, clinical significances of vitamin D supplementation are still inconclusive. Therefore, there is still a need for well-conducted trials to check whether correcting vitamin D levels will reduce morbidity in SLE patients.

Data Availability:

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Funding: None.

Conflicts of interest: None.

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