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Research Article

Serum Vitamin D3 and Osteocalcin Levels in Iraqi Females with Lupus Erythematosus and their Relationship to Disease Severity

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Abstract

Background: Lupus erythematosus (SLE) is a disease that affects two or more joints and is characterized by inflammation, effusion, or pain that lasts at least 30 minutes in the morning. Musculoskeletal involvement is one of the most prevalent signs of SLE and can be present in up to 90% of patients. ***Objective***: To evaluate the serum levels of vitamin D3 (Vit-D3) and osteocalcin (OC) in patients with SLE and their association with disease severity and other biomarkers. ***Methods***: This case-controlled observational study was conducted on 131 women with SLE and 50 healthy individuals over a period of 4 months, from November 2022 until March 2023. All participants were subjected to laboratory investigations, including measurements of CBC, blood urea, serum creatinine, and ANA levels. A consultant doctor carried out clinical examinations to classify disease severity into three categories—inactive, mild, moderate, and severe—according to the Roma Helper program. ***Results***: The levels of Vit-D and OC were significantly decreased in SLE patients, especially in the severe group (p<0.001). OC and Vit-D3 levels were negatively correlated with disease activity, while OC was positively correlated with HB. The results of Vit-D3 were positively correlated with RBC and negatively correlated with HB. ***Conclusion***: Vit-D and OC levels were significantly decreased in females with SLE and negatively correlated with disease activity.

Keywords: Osteocalcin, Vitamin-D3, Systemic lupus erythematosus, Blood urea, Serum creatinine, Anti-nuclear antibody.

مستويات فيتامين د3 وأوستيوكالسين في الإناث العراقيات المصابات بالذئبة الحمامية وعلاقتهما بخطورة المرض

الخلاصة

الخلفية: الذئبة الحمامية (SLE) هو مرض يصيب مفصلين أو أكثر ويتميز بالتهاب أو انصباب أو ألم يستمر لمدة 30 دقيقة على الأقل في الصباح. تعد إصابة الجهاز العضلي الهيكلي واحدة من أكثر علامات مرض الذئبة الحمراء انتشارا ويمكن أن تكون موجودة في ما يصل إلى 90٪ من المرضى. **الهدف**: تقييم مستويات فيتامين D3 وأوستيوكالسين (OC)في مصل المرضى الذين يعانون من مرض الذئبة الحمراء وارتباطهم بشدة المرض والمؤشرات الحيوية الأخرى. **الطريقة**: أجريت هذه الدراسة الرصدية التي تسيطر على الحالات على 131 امرأة مصابة بمرض الذئبة الحمراء و 50 فردا سليما على مدى 4 أشهر، من نوفمبر 2022 حتى مارس 2023. خضع جميع المشاركين لفحوصات مخبرية، بما في ذلك قياساتCBC ، واليوريا في الدم ، والكرياتينين في الدم ، ومستويات ANA. أجرى طبيب استشاري فحوصات سريرية لتصنيف شدة المرض إلى ثلاث فئات - غير نشطة وخفيفة ومتوسطة وشديدة - وفقا لبرنامجRoma Helper . **النتائج**: انخفضت مستويات Vit-D و OC بشكل ملحوظ في مرضى الذئبة الحمراء، وخاصة في المجموعة الشديدة. ارتبطت مستويات OC و Vit-D3 سلبا بنشاط المرض، بينما ارتبطت OC بشكل إيجابي مع Hb. كانت نتائج Vit-D3 مرتبطة بشكل إيجابي مع RBC ومرتبطة سلبا مع Hb. **الأستنتاج**: انخفضت مستويات Vit-D و OC بشكل ملحوظ لدى الإناث المصابات بمرض الذئبة الحمراء وارتبطت سلبا بنشاط المرض.

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INTRODUCTION

Systemic lupus erythematosus is a systemic autoimmune illness characterized by immune system activation [1]. SLE is a clinically heterogeneous disease characterized by the production of autoantibodies against nuclear antigens [2]. SLE can affect the skin, joints, kidneys, lungs, neurological system, serous membranes, and/or other organs [3]. The causes of SLE are unknown, but they could be caused by infection. UV radiation, demethylating therapies, infectious or endogenous viruses [4], antibiotics, infections, and hormones [5,6] are all environmental causes. Non-collagenic bone protein osteocalcin (OC) binds calcium and phosphate, two indications of dynamic bone-forming activity [7]. It is produced by osteoblasts and binds to the mineralized bone matrix during bone formation [8]. Osteocalcin exists in two forms: carboxylated, which is involved in calcium binding, and hydroxyapatite-bound, which allows for osteocalcin deposition in mineralized bone matrix. Non-carboxylated osteocalcin, on the other hand, is more easily released into the bloodstream and has a low affinity for hydroxyapatite. However, both the carboxylated and non-carboxylated forms of OC, as well as the total OC level, which is frequently evaluated as a marker of bone formation, are detectable in peripheral blood [9]. Several studies [10,11] found a decrease in OC levels to be negatively linked with disease activity. Vitamin D, or calciferol, is made up of a series of fat-soluble secosterols [12]. Vitamin D, as a steroid hormone, regulates immune system cell growth, proliferation, apoptosis, and function, which are associated with the pathophysiology of SLE [13]. Because vitamin D3 deficiency has been related to a variety of diseases and appears to be ubiquitous around the world, interest in the vitamin has expanded considerably in recent decades. Multiple physiological and pathological processes involving various organs and systems of the human body have currently been connected to Vit-D3's action as a hormone [14]. Evidence suggests that vitamin D3 deficiency, which is common, may worsen morbidity and death in several chronic disorders, including SLE [13]. The current study sought to determine the relationship between OC and Vit-D3 levels, disease severity, and other biomarkers in Iraqi women with SLE.

METHODS

Study design

This case-control study was conducted on 131 SLE women over a period of 4 months, from November 2022 until March 2023. SLE women included in our study were recruited from the Baghdad Teaching Hospital in Medical City, Baghdad. Biochemical analysis was performed at the Research Laboratories in the International Center for Research and Development, Kadhimiya City, and the Teaching Laboratories of the Medical City. All SLE women who are included in the study were subjected to laboratory investigations, including urine examination and evaluation of certain markers, including ANA, CBC, B. urea, and S. Cr.

Inclusion criteria

All women with SLE, whose age ranged from 15 to 65 years, and have been examined clinically by a consultant physician.

Exclusion criteria

All patients with tumors under 10 years old who are taking nutritional supplements, have other inflammatory diseases such as RA, OA, myositis vasculitis, a history of hypersensitivity, are treated with cyclophosphamide, and are pregnant were excluded, as were those with chronic conditions such as asthma or Crohn's disease, thyroid disease, or are pregnant.

Outcome measurements

Serum calcium, B. urea and S. Cr levels were analyzed using fully automated Selectra device. The blood markers were measured by the CBC device using non-coagulated blood. The ANA level was measured using IDS-ISYS machine that is powered by chemiluminescence immune assay.

Ethical considerations

This research was approved by the Institutional Higher Scientific and Ethical Committee, and before participation all women were informed about the study and their written informed consent was taken.

Statistical analysis

Using Statistical Package for Social Science (SPSS 16 IBM, Armonk, USA), a one-sample Kolmogorov-Smirnov test was used to know how the values were distributed. If the values were distributed normally, then the *t*-test is used, and if the values were not distributed normally, the Mann-Whitney test should be applied. The results were expressed as mean±standard deviation (SD), and the differences in means of the variables between control and patient groups (inactive, mild, and severe SLE groups) were analyzed by an analysis of variance (one-way ANOVA) test. Correlations between all of the studied variables were evaluated using Pearson's correlation coefficient (r), and linear regression analyses were used for the evaluation of the data. A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 shows that the average ANA levels of inactive, mild, moderate, and severe patients and healthy people were 1.51±0.74, 1.73±0.81, 2.21±1.18, and 0.154±0.07

IU/ml, respectively. The positive ANA in all SLE patients was observed in 97 (74.04%) when the index value was greater than 1, while the negative ANA was present in 34 (25.95%) when the index value was below 0.97.

Table 1: Anti-nuclear antibody levels in healthy individuals and SLE women with SLE of different severity

Parameter Mean±SD	Inactive SLE *n*=30	Mild and Moderate SLE *n*=49	Severe SLE *n*=52	Healthy individuals *n*=50
ANA	1.51±0.74	1.73±0.81	2.21±1.18	0.154±0.07

The comparison in levels of ANA between SLE groups (inactive, mild, moderate, and severe) and healthy individuals revealed a significant increase (p<0.001) in the means of ANA in inactive, mild, moderate, and severe groups compared with healthy individuals, while a highly significant increase (p<0.01) in the means of ANA was reported in the severe disease group compared with the mild and moderate disease groups. However, there was a non-significant difference (p< 0.05) between mild and moderate disease and inactive disease groups. The mean of ANA for all SLE patients (1.87±1.00) was statistically significantly higher at p<0.001 when compared to the mean of healthy individuals (0.154±0.07) (Table 1). The results of the CBC of the study groups are illustrated in Table 2.

Table 2: CBC level in SLE groups (inactive, mild and moderate and severe) and healthy individuals group

Parameters Mean±SD	Inactive SLE *n*= 30	Mild & Moderate SLE *n*= 49	Severe SLE *n*= 52	Healthy individuals *n*= 50
WBC	5.8±1.7	5.4±1.5	4.69±1.3	6.04±1.2
Hb	11.1±1.5	10.9 ±1.45	10.5±1.4	12.4±0.7
RBC	4.4±0.62	4.1±0.45	3.96±0.48	4.9±0.52
PLT	248.3±72.5	236.7±71	226.2±48.6	288.9±30.1

When the WBC values of SLE patients were compared with those of healthy individuals, there was a significant decrease (p<0.001) in the means of WBC in the severe group versus the healthy individuals group, while the mild and moderate groups compared with the healthy individuals group were significantly decreased (p<0.05), but the inactive group was insignificantly different (p>0.05). Significantly decreased (p<0.05) in means of WBC in severe groups versus mild and moderate disease groups, and significantly decreased (p<0.001) versus inactive disease groups; yet there was an insignificant difference (p<0.05) between mild, moderate, and inactive disease groups. The levels of Hb in the SLE groups (inactive, mild, moderate, and severe) were compared with those of healthy individuals, which revealed a significantly decreased (p<0.001) level of Hb in all groups versus healthy individuals. However, we found no significant differences (p<0.05) in the comparison between the severe and inactive groups. The comparison in levels of RBC between SLE groups (inactive, mild, moderate, and severe) implies a very significant decrease (p<0.001) versus the healthy individuals group, and when comparing the severe group with the inactive group, a significant decrease was noted (p<0.05), but when comparing the severe disease group to the mild and moderate disease groups, there were no significant differences (p<0.05). The levels of PLT in the SLE groups (mild, moderate, and severe) were compared with those of healthy individuals and showed a very significant decrease (p<0.001), while the inactive group noted a significant decrease (p<0.05) when compared to the healthy individuals group. There are no significant differences (p<0.05) among groups (Table 2). The results of B. urea levels in SLE patients revealed a high and significant increase (p<0.001) compared to healthy individuals. The mean concentration of blood urea (BU) for SLE patients was 31.56±9.96 in comparison to the mean concentration in healthy individuals (20.38±4.05). The results of S. Cr concentration in SLE patients revealed a significant increase (p<0.05) compared to healthy individuals. The mean concentration of S. Cr for SLE patients was 0.74±0.30 and for healthy individuals was 0.60±0.20 as shown in Table 3, and significantly increased when comparing the inactive group (p<0.001) to the healthy individuals group.

Table 3: Blood urea and serum creatinine levels in SLE groups (inactive, mild, moderate and severe) and healthy group

Groups	B. Urea mg/dl	S. creatinine mg/dl
Healthy *n*=50	20.38±4.05	0.60±0.2
Inactive *n*=30	26.83±6.37	0.61±0.13
Mild & Moderate *n*=49	30.17±7.51	0.72±0.26
Severe *n*=52	35.63±12	0.83±0.37

When comparing the severe group with the mild and moderate groups, there was a significantly higher increase (p<0.001) and a very high significant increase (p<0.001) with the inactive group; when comparing the mild and moderate groups, there were insignificant differences (p<0.05) with the inactive group. The severe group B. urea levels, when compared to the healthy individuals group, were significantly higher (p<0.001), and when comparing the mild and moderate groups with the healthy individuals group, they were significantly increased (p<0.05), while comparing the inactive group, there were insignificant differences (p>0.05). Comparing creatinine levels in the severe group showed significant increases (p<0.05) in the mild and moderate groups and a very significant increase (p<0.001) in the inactive group, but when comparing between the mild, moderate, and inactive groups, there were insignificant differences (p<0.05). It was shown that rising B. urea and S. Cr levels were positively related to disease activity. In Table 4, the results of Vit-D3 were different

for each group. The severe disease group had a severe lack of vitamin D3, with a mean of 10.11.66.

Table 4: Vit-D3 levels in healthy individuals and SLE patients with various disease severity (inactive, mild, moderate and severe)

Parameter Mean±SD	Inactive SLE *n*=30	Mild & Moderate SLE *n*=49	Severe SLE *n*=52	Healthy individuals *n*=50
Vit-D3	22.82±20	16.6±2.0	10.1±1.66	33.46±4.84

The results of the mild and moderate disease groups were higher than those of the severe disease group, which was 16.6±2.0. The results of the inactive disease group were higher than those of the mild, moderate, and severe disease groups. The highest results were reported in the healthy group (33.46±4.84). Comparing patient groups (inactive, mild, moderate, and severe) with healthy individuals revealed a significant decrease (p<0.001). Also, when comparing the severe group to the inactive and mild and moderate groups, the very high score decreased significantly (p=0.001), and when comparing the mild and moderate groups to the inactive group, the very high score decreased significantly (p=0.001). The correlation of the Vit-D values in the severe group showed a positive significant correlation with RBC (r= 0.33, p<0.05) and a negative significant correlation with Hb (r = -0.38, p<0.01) as shown in Table 5.

Table 5: The correlation analysis using Pearson correlation of Vit-D3 in severe group with Hb and RBC parameters

Severe SLE		Hb	RBC
VitD3	*r*	-0.381**	0.339*
	p-value	0.005	0.014

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

The results of OC concentration in SLE patients and healthy individuals are clarified in Table 6. The results of OC showed a clear decrease in the patient group compared to the healthy individuals. Comparing any patient group (inactive, mild, moderate, and severe) versus healthy individuals, we noticed a very significant decrease (p<0.001), while also noticing slight differences between the patient groups.

Table 6: Osteocalcin levels in healthy individuals and SLE patients with various disease severity (inactive, mild, moderate and severe)

Parameter Mean±SD	Inactive SLE *n*=30	Mild & Moderate SLE *n*=49	Severe SLE *n*=52	Healthy individuals *n*=50
Osteocalcin	35±6.2	30.3±5.8	24.4±5.4	101.2±31.2

When comparing the severe group to the inactive group, we noticed a significantly decreased (p<0.05), but when comparing the severe group to the mild and moderate group, we noticed insignificant differences (p<0.05), and when comparing the mild and moderate group to the inactive group, we noted insignificant differences (p<0.05). The correlation of the OC with the OPG for the severe group showed a significant positive correlation with Hb (r = 0.47, p<0.01) as shown in Table 7.

Table 7: The correlation analysis using Pearson's correlation of OC in Mild & Moderate group with Hb levels

Mild and Moderate SLE		Hb
OC	*r*	0.477**
	p-value	0.001

** Correlation significant at the 0.01 level (2-tailed).

DISCUSSION

Anti-nuclear antibodies (ANA) are found in more than 90% of SLE patients [2]. Carey et al. discovered that 88.2% of the patients had positive ANA values. The ANA test should be used to diagnose the majority of patients because it is positive in more than 85% of instances, typically when symptoms first emerge. Positive ANA findings, on the other hand, do not invariably indicate SLE. Almost all SLE patients have ANA-positive but negligible titers [15]. SLE is distinguished by the presence of ANA, which is found in more than 95% of affected patients. The likelihood of SLE is low in people with negative ANA who do not have the entire constellation of symptoms. the rash and joint pain [16]. These findings show clear disparities in CBC between patients and healthy groups, which is consistent with the findings of much other research, including [17-21]. SLE typically develops as anemia for a variety of reasons. There is evidence to suggest that some immunopathologic events may have an impact on erythropoiesis at various stages prior to red blood cell maturation. Numerous autoantibodies that block erythropoiesis at various stages of red blood cell production can be the cause of primary anemia in SLE [22]. Antibodies directed against white cells are highly prevalent in SLE. SLE patients have a lower-than-normal lymphocyte count on a full blood count. This is related to the existence of antibodies against lymphocytes, which cause the antibody-coated cells to be destroyed [23]. In SLE, high-grade inflammation is associated with a reduction in PLT, presumably because of increased consumption of big platelets at rheumatoid inflammatory sites [24]. Many studies have shown that SLE patients have elevated blood urea and creatinine levels. Elevation levels might be moderate, mild, or severe. In our investigation, we discovered a difference in the levels, and while all patients did not have any kidney symptoms, these findings were considered predictive of kidney disease progression [25–28]. Urea is the major nitrogenous byproduct of protein metabolism in humans. The kidney transports and

eliminates it after it dissolves in the blood as a component of urine. The body continuously produces creatine, a byproduct of creatine phosphate, which is present in muscle. The kidney is the only organ responsible for removing creatinine from the blood. The glomerulus filters it, and the proximal tubules secrete a tiny amount into the glomerular filtrate. Because of decreased renal clearance, blood creatinine levels rise [29]. The main renal disease mediators in SLE are assumed to be glomerular immune complexes. A recent study has revealed that autoantibodies of diverse specificities are involved in the development of immune complexes that are deposited in the kidneys. Renal failure caused by lupus glomerulonephritis progresses primarily as a result of T cell, macrophage, and dendritic cell infiltration [30]. Several studies [31, 32] found a high frequency of vitamin D deficiency in people with autoimmune disorders, including SLE. Numerous studies have found that low vitamin D levels are associated with increased SLE disease activity and that SLE patients have a fracture risk that is double that of people without SLE [32]. Many investigations have found that Vit-D3 levels are low in SLE patients [32,33,35,11,36]. Because of their photosensitivity and frequent use of photoprotection, SLE patients are at high risk of 25(OH)D deficiency [37]. Even though research has established a link between SLE and vitamin D, which suggests that SLE may induce lower vitamin D levels [13], it is critical to consider the possibility that a vitamin D shortage may play a causal role in the genesis of SLE. Other research [33,7] demonstrated a drop in OC level in SLE patients, and many investigations [10,36,11] validated a decrease in OC in SLE patients. Our findings agreed with those of Baker-LePain et al. and Guo et al., who found that SLE disease activity is also adversely linked with osteocalcin [10,11]. As previously stated, OC is activated by 1,25-dihydroxycholecalciferol (125D); hence, a lack of 1,25-dihydroxyvitamin D causes a drop in OC levels. A drop in OC was seen in SLE patients, indicating an apparent decoupling of bone remodeling, which resulted in bone loss and a decrease in bone mass density [36]. Osteocalcin is released from the bone matrix and triggered by osteoclast resorption activity, which is blocked by OPG. Through its influence on the sympathetic signaling system, leptin is a strong inhibitor of osteocalcin [34].

Conclusion

Serum levels of vitamin D3 and osteocalcin were found to be considerably lower in Iraqi females with SLE, and this finding was found to have a negative correlation with disease activity.

Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author based on a reasonable request.

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