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Effect of Vitamin D deficiency and some hematological parameters in Iraqi female patients with Systemic Lupus Erythematosus

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ABSTRACT

Background: Systemic Lupus Erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects multiple organs. Vitamin D deficiency is considered potential environmental factor triggering some autoimmune disorders, including SLE. This study was performed for determination of effect of vitamin D deficiency in SLE infection or disease worsening, and the relationship of ESR, HB, and WBCs with level of vitamin D in SLE disease, in addition to determine which of the previous parameter may describe the disease case of patients.

Methods: This study included fifty three (53) SLE female patients and twenty six (26) healthy females were included. White blood count (WBC) and Heamoglobin (Hb) were determined in complete blood count (CBC) device, traditional Westergren method was used for Erythrocyte sedimentation rate (ESR) estimation, vitamin D was measured by using enzyme link immune-sorbent assay (ELISA) Kit.

Results: The results showed a significant decrease in the hemoglobin levels in the SLE patients; significant increase was noticed in the ESR of SLE patients and significant increase was noticed in the WBC counts compared to the control group's samples. A significant decrease in serum levels of vitamin D in patients compared to apparently healthy controls.

Conclusions: Photosensitivity to sun in SLE patients contributed in decrease of vitamin D blood-levels. According to the ROC results, each of Hb, WBC, ESR and vitamin D may describe the disease case of patients.

دور نقص فيتامين – د و بعض مَعلَمات الدم في النساء العراقيات المرضى بداء الذئب الإحمراري

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الملخص

الخلفية العلمية: داء الذئب الإحمراري (SLE) هو مرض مناعي ذاتي التهابي مزمن يؤثر على أعضاء متعددة. يعتبر نقص فيتامين د عاملاً بيئيًا قوياً يسبب بعض اضطرابات المناعة الذاتية، بما في ذلك داء الذئب الإحمراري. أجريت الدراسة لبيان تأثير نقص فيتامين د في الإصابة بداء الذئب الإحمراري أو في تفاقم المرض، وعلاقة معدل ترسيب كريات الدم الحمر و الهيموغلوبين وخلايا الدم البيضاء بمستوى فيتامين د في داء الذئب الإحمراري، بالإضافة إلى تحديد أي من العوامل السابقة قد تصف الحالة المرضية للمرضى.

طرق العمل: تضمنت الدراسة ثلاثة وخمسون (53) مريضة بداء الذئب الإحمراري وستة وعشرون (26) أنثى سليمة. تم قياس عدد خلايا الدم البيض والهيموغلوبين في جهاز تحليل صورة الدم الكاملة (CBC)، أستُخدِمت طريقة ويسترجرين التقليدية لقياس قيمة ترسيب كريات الدم الحمر، وتم قياس فيتامين– د بإستخدام جهاز تشخيص الإليزا.

النتائج: أظهرت النتائج إنخفاضاً معنوياً في مستوى الهيموغلوبين لدى مرضى داء الذئب الإحمراري. وقد لوحظت زيادة معنوية في معدل ترسيب كريات الدم الحمر لدى مرضى داء الذئب الإحمراري و لوحظت زيادة معنوية في تعداد خلايا الدم البيضاء مقارنة ب الاشخاص الاصحاء. لوحظ انخفاض معنوي في مستويات فيتامين د في مصل المرضى مقارنة بالاشخاص الأصحاء.

الاستنتاجات: ساهمت الحساسية لضوء أشعة الشمس لدى مريضات داء الذئب الإحمراري في إنخفاض مستويات فيتامين د في مصل المريضات. ووفقاً لنتائج منحنى الـ ROC ، فإن كل من الهيموغلوبين و عدد خلايا الدم البيض و ترسيب كريات الدم الحمر و فيتامين–د قد يصف حالة المرض لدى المريضات.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease has an effect for multiple organs and systems of the body, including the cardiovascular, gastrointestinal, hematologic, integumentary, musculoskeletal, neuropsychiatric, pulmonary, renal, and reproductive systems, this chronic disease may result in recurrent cases of symptoms (getting worse if not treated properly). The main objective of controlling SLE is to reach complete recovery or maintain low disease activity, while reducing the need on glucocorticoids, avoiding exacerbations, and enhancing overall quality of life [1]. The start of this condition is caused by several factors and is characterized by the complicated development of autoantibodies. These autoantibodies impair the immune system of the body, resulting in an uncontrolled inflammatory response. On the other hand, an essential component play important role in SLE-pathogenesis which is the interaction between environmental factors and genetic variables, these genes participate in immunological autoregulation. As concluded in genome-wide association research technologies, over 80 loci have been detected in SLE-pathogenesis. These loci generate vital proteins that result in minor pathophysiological alterations and are linked to systemic lupus erythematosus (SLE) [2–3]. Since vitamin D possesses traits of a hormone, it is more of a pro-hormone than a real vitamin, starting vitamin D major activity within the body begins after attaching active form of vitamin D is to vitamin D receptors (VDR). Deficiency in vitamin D is one of the factors that affects SLE; vitamin D insufficiency is becoming more common worldwide [4, 5]. A sufficient vitamin D supplement is essential for SLE patients since they avoid the sun due to photosensitive rashes and the risk of an eruption of their condition [4–7]. Hypovitaminosis D condition also due to the use of pharmaceuticals such as glucocorticoids, anticonvulsants, antimalarials, and calcineurin inhibitors. These medications affect the way in which vitamin D is used in the body or reduce the activity of the vitamin D receptor. Additionally,

low vitamin D levels are connected with osteoporosis, tiredness, and certain cardiovascular risk factors in these individuals [8], in the treatment of SLE, corticosteroids are often used, and long-term usage has been linked to weight gain (ranging from less than 10 to about 30 pounds). Obesity is defined as the abnormal or excessive buildup of fat due to a prolonged imbalance between the amount of energy consumed and the amount of energy expended. In fact, the increased body weight seen in SLE patients using chronic corticosteroids raises their risk of developing cardiovascular disease, creating a recurrent cycle where weight gain might keep the illness active and require continued use of the drugs [9–11]. The non-specific measure of systemic inflammation known as the erythrocyte sedimentation rate (ESR) can be raised in cases of inflammation caused by an autoimmune disorder, an infection, or a malignancy. ESR increases have been linked to both lupus activity and infection, making it difficult to distinguish between a lupus flare and an infection in SLE patients when compared to those with rheumatoid arthritis [12, 13]. While ESR increases are closely linked to SLE illness flare-ups, they also increase with age and are greater in women than males [14, 15].

Methods

Subject and Blood sample collection

This study included fifty three SLE female patients "The diagnosis was made by the doctor and the immunology team in accordance with the established protocols" from Medical City /Baghdad Teaching Hospital and 26 healthy females, ages ranging from 15 to 52 years for both groups. During the period from 26th of August to 20th of October, 2022, the blood samples were collected.

Five millimeters of the collected blood samples were divided into two tubes (EDTA and gel tube); blood samples in a gel tube were centrifuged; and then the serum was collected into 0.5 ml Eppendorf tubes which were put at -20 °C until the evaluation of vitamin D in serum by ELISA. Blood samples in EDTA tubes were used for the estimation of hemoglobin, white blood cell count, and erythrocyte sedimentation rate.

Determination of hematological parameters

Whole blood samples were examined for complete blood count (CBC) by using Beckman-Coulter analyzer with five parts. Hematological parameters included in the CBC were white blood cell count (WBC) and hemoglobin (Hb) parameters were used in this study. Erythrocyte sedimentation rate was estimated according to traditional method of measuring ESR "Westergren method" [16].

Determination of vitamin D in serum

Vitamin D was estimated by using enzyme-linked immune-sorbent assay kit. (Sandwich Human- vitamin D. ELISA-Kit), then according to the manufacturer-instructions the serum levels of vitamin D were estimated (SunLong Biotech).

Statistical analysis

SPSS program version 26.0 (Armonk, NY: IBM Corp.) was used for determination differences between parameters. Continuous data represents the mean and standard error, and categorical data represents the number and percent. Independent samples, the T test was used to compare the means of parameters; the chi-square and receiver operating characteristic (ROC) were used to determine the area under curve (AUC), sensitivity, specificity, and cut-off value; and the Pearson correlation test was used to determine the correlations between parameters.

- NS: Non-Significant
- S.E: Standard Error
- P: Probability.
- $\chi 2$: Chi-Square.
- (**) Significant $P < 0.01$
- (*) Significant $P < 0.05$.

Results and Discussion

Distribution of samples according to age

The current study showed non-significant difference in age of patients compare to control as shown in table 1. According to age groups, second age group (31-45 year) was the higher incidence than first and third group as mentioned in table 2.

Table 1: Distribution of samples according to age.

Studied Groups	Patients(no. = 53)	Control(no. = 26)
Age (Year) Mean ± SE	34.07 ± 1.3 (Range 15-52)	31.42 ± 2.0 (Range 17-52)
P-value	0.27 NS	

Table 2: Distribution of samples according to age groups.

Studied Groups	Patients	
Age Groups (Year)	Frequency	Percent
15-30	20	37.7
31-45	26	49.1
Over 45	7	13.2
Total	53	100.0
χ^2	10.679	
P-value	0.005**	

Many studies found that SLE affects people of all ages, especially women led to the belief that the

female hormone estrogen may play a role in SLE, however, more research is still needed to substantiate this theory [17]. The current study showed that mean of SLE ages was 34 year which was closed to what Motawei *et al*., [18] and Lu *et al*., [19] concluded (mean of aged were 32.99 and 33 year, respectively), on the other hand Khairallah *et al*., [20] found that mean of SLE patients was 28.30, the current study suggests that it may be that in mid-ages (especially twenties and thirties), the immune system will be more active than in childhood and elderly ages due to quick immune responses to any foreign body as well as autoimmune effects.

Brailovski *et al*., [21] who concluded that SLE-infected-female aged from 18-44 year was high prevalent than < 18 year group and > 45 year group, also Chung *et al*., [22] demonstrated that the highest occurrence of SLE (Systemic Lupus Erythematosus) was observed between the ages of 25 and 39. They also found that the incidence of SLE was lower during the early (20-24 years) and late (40-44 years) childbearing age periods. The researchers explained that the prevalence of SLE in women increased with age during their childbearing years, reaching its highest point between the ages of 40 and 44. Previously, it has been showed that the peak of disease incidence for women is during reproductive years (ages 20–30 years) [23, 24], which is closed to the current results and the previous studies.

According to the prevalence of SLE disease in female than male, this may because the presence of chromosome X, In their research, Chagnon *et al*., [25] proposed that there is a dosage impact of the X chromosome on the susceptibility to systemic lupus erythematosus (SLE): having two X chromosomes increases the risk of SLE, whereas having just one X chromosome decreases the risk of SLE.

Weight results

Weight in SLE patients and controls were measured and was significantly higher in the SLE patients' (67.94 ± 1.5 kg) than in the control group's weight (60.00 ± 2.2 kg), (*P*=0.005) as shown in table 3.

Table 3: Weight results of SLE patients and control.

Studied Groups	Patients	Control
Weight (kg) Mean ± SE	67.94 ± 1.5	60.00 ± 2.2
P-value	0.005**	

Many previous studies showed that that obesity is considered one the strong risk factors associated with worse SLE disease or it's activity, one of the causes that connects obesity with SLE is the prolonged use of corticosteroid therapy which due to cause obesity [26, 27], in SLE patients-obese subjects frequently observed a vitamin D-deficiency, also hypoactivity, and dysbiosis of the gut microbiota [28, 29].

The regular exercise and sport are one of the strengthening factors of the immune system which occurs by promoting the release of antinflammatory cytokines [30–32], also due to reducing neutrophil chemotaxis, increase circulating-leukocytes-concentration, decreasing levels of lymphocyte, and inhibiting monocyte as well as macrophage infiltration which occurs into adipose tissue [33–36]. Thus, lower-physical activity levels as well as non sedentary behavior may related to an unfavorable level of adiposity-associated inflammation [37].

Hematological parameters results

Blood parameters in both of cases and controls were measured, table 4 shows that HB significantly decreased in the SLE than in the control samples, while significant increase was noticed in WBC counts and ESR of SLE patients than in the control samples.

Table 4: Differences between SLE patients and control according to blood parameters.

Parameter	Patients	Control	*P*-value
Hb (g/dL) Mean ± SE	11.84 ± 0.27	13.67 ± 0.15	0.001**
WBC (10^{-3}/µL) Mean ± SE	7.77 ± 0.57	5.96 ± 0.28	0.037*
ESR (ml/hr.) Mean ± SE	29.98 ± 2.5	14.19 ± 1.14	0.001**

A complete blood count (CBC) and most of hematology parameters are estimated in many researches, such parameters have clinical values for diagnosis or assessing certain blood conditions leading to tracking treatment response. The current study showed decrease in HB levels compare to control this result was closed to what Motawei *et al*., [18], Lu *et al*., [19] and Abdalhadi *et al*., [38] and Jassim *et al*., [39] who showed that hemoglobin was decreased in SLE patients. According to ESR results, the current result showed an increase in ESR levels which was agreed with what Abdalhadi *et al*., [38] and Jassim *et al*., [39] who concluded that ESR was increased in SLE patients. The common hematologic-disorders at the time of diagnosis

and throughout the disease may be included in as a manifestation of SLE, and/or may be caused by the treatment [40], decrease in Hb may be related to the injuries in tissues of SLE patients due to bleeding which affects in Hb content, on the other hand, the increase in WBC may be related to the high activity of immune cells in disease defense. As well as, the current study suggests that ESR in general provides only a guide to the extent of inflammation, increased ESR may be occurred because SLE disease causes chronic inflammation in several tissues and organs; and as medically known; ESR increases in inflammation.

Vitamin-D results

Serological parameter in both of SLE patients and apparently-healthy-controls were measured like vitamin D (table 5). vitamin D was significantly higher in the apparently-healthy-controls group's serum than in the SLE-patients.

Table 5: Differences between SLE patients and control according to vitamin D levels.

Parameter	Patients	Control
Vitamin D (gm/dl) Mean ± SE	12.93 ± 0.26	19.21 ± 0.54
P-value	0.001**	

The low levels of Vitamin D in patients with Systemic Lupus Erythematosus (SLE) may be attributed to decreased exposure to sunlight, since SLE patients often have sensitivity to light [41,42]. Additionally, it might be associated with the use of corticosteroid medication [43], however there is disagreement among researchers on this matter. Patients with systemic lupus erythematosus (SLE) may have decreased levels of Vitamin D as a result of hydroxychloroquine medication, which might impact the metabolism of Vitamin D [44]. There may be a connection between low levels of Vitamin D and the existence of autoantibodies against Vitamin D [45]. Kamen *et al*., [5] discovered a significant decrease in serum Vitamin D levels among recently diagnosed SLE patients compared to a control group with similar characteristics. This finding aligns with the current results. The

researchers explained that individuals of African American descent and those with photosensitivity experienced the most severe deficiency in Vitamin D, which supports this interpretation [5]. The current results also, closed to what Motawei, *et al*., [18] found, Vit.D levels in SLE patients 18.5 ± 5.9 which due to that patients had deficient in Vit.D. Borba et al. [9] discovered that SLE patients with high disease activity had lower levels of 25OHD and 1,25 (OH) 2 D3 compared to controls and patients with limited disease activity, which might influence their vitamin D levels. The possible reason of decreased-vitamin D-production may be because of the lack of sunlight exposure or use of sunblock (which minimize the sunlight absorption), or by the disease itself which included the deficiency that observed in medical in patients, this vitamin deficiency almost observed in summer due to the using of these sun-protectors, or avoidance of sunlight-exposure [5, 10].

ROC analysis and Pearson correlation

According to the Receiver Operating Characteristic (ROC) results, positive true results were observed in each of HB, WBC, ESR and vitamin D, these parameters may describe the disease case of patients (Figure 1, Table 6). The correlations between the studied parameters illustrated in table 7.
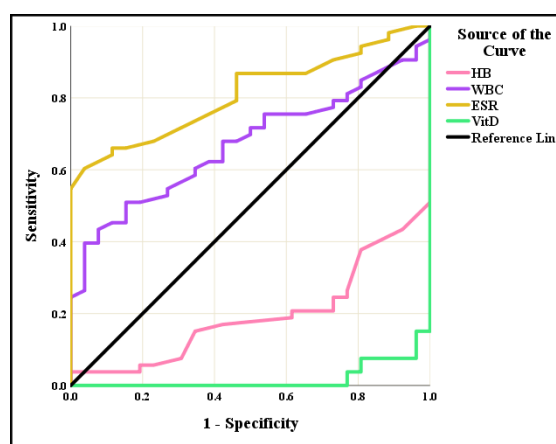

Fig. 1: ROC curve analysis of studied parameters.

Table 6: Area under the curve of studied parameters.

Variable	AUC	Sensitivity	Specificity	Cut of Value	*P* value
Hb (g/dk)	0.194	0 %	100 %	15.15	**<0.001****
WBC (10^{-3}/uL)	0.671	39 %	96 %	7.87	**0.014***
ESR (mm/hr.)	0.810	60 %	96 %	24.0	**<0.001****
Vitamin D (pg /ml)	0.019	0 %	100 %	< 23.67	**<0.001****

Table 7: Pearson Correlation of the studied parameters.

Parameters	r- value	*P*-value	Correlation
Age *vs*. Weight	0.665	**< 0.001****	**Strong positive**
Age *vs*. Hb	- 0.036	0.75 NS	Weak negative
Age *vs*. WBC	0.022	0.85 NS	Weak positive
Age *vs*. ESR	0.237	**0.035***	**Strong positive**
Age *vs*. vitamin D	- 0.154	0.17 NS	Weak negative
Weight *vs*. Hb	- 0.160	0.1 NS	Weak negative
Weight *vs*. WBC	0.127	0.26 NS	Weak positive
Weight *vs*. ESR	0.375	**0.001****	**Strong positive**
Weight *vs*. vitamin D	- 0.295	**0.008****	**Strong negative**
Hb *vs*. WBC	0.04	0.7 NS	Weak positive
Hb *vs*. ESR	- 0.398	**< 0.001****	**Strong negative**
Hb *vs*. vitamin D	0.488	**< 0.001****	**Strong positive**
WBC *vs*. ESR	- 0.120	0.29 NS	Weak negative
WBC *vs*. vitamin D	- 0.188	0.09 NS	Weak negative
ESR *vs*. vitamin D	- 0.463	**< 0.001****	**Strong negative**

Motawei *et al*., [18] showed that vitamin D was positively correlated with age which disagreed with the current study which found that vitamin D negatively correlated with age, the current results also disagreed with the same study which showed that vitamin D negatively correlated with HB, while agreed with the same study which found that vitamin D was negatively-correlated with ESR like the current results which found that vitamin D had strong negative correlation with ESR. Additional research has demonstrated a notable association between a lack of Vitamin D and an increased body mass index (BMI). This correlation is particularly evident in studies conducted on Chinese individuals, the Mediterranean region, and a cohort study on pediatrics with systemic lupus erythematosus (SLE) conducted by Wright *et al*., (2009), where the statistical significance was indicated by a p-value of 0.004. The research conducted on the Chinese population revealed that there was no substantial correlation between disease activity and BMI. The negative correlation between age and Vitamin D may be due to the impact of aging on the production of 1,25-dihydroxyVitamin D (1,25[OH]2D; calcitriol), which is considered the active form of Vitamin D. The formation of these complexes is reduced by 50% as a result of age-related decline, particularly in renal function.

However, serum 1,25 (OH)2D levels are partially maintained through secondary hyperparathyroidism. Additionally, there is a decline in calcium absorption as individuals age, which may be attributed to a fall in 1,25(OH)2D levels after 10 to 15 years. Vitamin D shortage arises due to insufficient availability of the substrate Vitamin D, which is necessary for the production of 1,25(OH)2D. This shortfall further decreases the creation of 1,25(OH)2D. Assessing Vitamin D insufficiency may be done by measuring the metabolite 25OHD, which is the most often used measure for this purpose [48].

The current results agreed with Elshourbagy *et al*., [49] study who found a significant negative correlation between ESR and vitamin D, they explained that this fact due to the effect of cytokines in the induction of acute phase reactant. The current results also agreed with Kareem *et al*., [51] who showed that vitamin D levels inversely correlated with the ESR and according to their findings they suggested that findings support the theory concerning the potential relationship between auto-immune-diseases and hypovitaminosis D.

Current findings indicate that individuals with SLE are very susceptible to vitamin D insufficiency due to their sensitivity to sunlight and frequent use of sun protection treatments.

Given that vitamin D has an immune-modulating effects, it is acceptable to conclude that vitamin D insufficiency is both a risk factor and an indication of SLE.

Conclusion

In conclusion, photosensitivity to sun in SLE patients contributes in decrease of vitamin D blood-levels. According to the ROC results, each of Hb, WBC, ESR and vitamin D may describe the disease case of patients.

Acknowledgement

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