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Evaluation of the Immunological and Hematological parameters of Systemic Lupus Erythematosus in Iraqi Women

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***Abstract*—Systemic lupus erythematosus is an autoimmune disease involving several systems, mainly affects young adult women, and causes a significant deterioration in quality of life. Different environmental aspects are known to facilitate the development of lupus in predisposed individuals. Hemoglobin concentration (HB), granulocyte level, hemoglobin level, saturation (ESR), and platelets (PLT) were among the clinical blood indicators that were found to be associated with the beginning of SLE in adults and teenagers. Blood PLT levels in both SLE patients were significantly lower than in the G1 control group (P< 0.001). When compared to the control group, G1, the two SLE patient groups (G2 & G3) had noticeably higher ESRs (P < 0.01). The study employed many immunological markers, such as C-reactive protein (CRP), antinuclear antibody (ANA), Anti-double stranded DNA (Anti-dsDNA), complement components C3 and C4 levels. Serum CRP levels were significantly higher in G2 and G3 SLE patients (P<0.001) than in the G1 control group. Research revealed that two SLE patient groups (G2 & G3) had blood levels of ANA and Anti-double stranded DNA (Anti-dsDNA) that were considerably higher than those of the control group (G1).**

***Keyword*— lupus, Anti-dsDNA, ANA, CRP, HB, ESR.**

I. INTRODUCTION

Systemic lupus erythematosus be classified as a multi-organ systemic immune disease [1,2] The complex interaction between genetic and environmental factors is due to immune dysregulation and loss of immune tolerance [3].Several studies on people and animals have shown the outcomes, which include multiple organ involvement and autoantibodies [4]. SLE is a deadly autoimmune illness with clinical signs ranging from moderate to temporary to fatal. Interest,ingly, SLE typically affects women in their reproductive years, between the ages of 15 and 44 [5, 6]. SLE typically accompanies prolonged disease activity and organ damage from medication-related side effects [7]. There are several indicators that SLE is less common. Even though there is a lack of reliable, up-to-date data on SLE incidence and prevalence, Europeans and their descendants have a higher incidence of SLE than any other race [8]. When compared to patients of African, Asian, and other indigenous peoples, patients of European heritage typically experience less severe clinical signs of SLE. Differences in SLE incidence and clinical manifestation are likely influenced by genetic, environmental, socioeconomic, demographic, and sociocultural factors [9]. Familial SLE recurrence rates appear to be 8–10%, comparable among Europeans, Latin Americans, African Americans, and Afro-Caribbeans [10]. In contrast, the percentage of infections reported globally has increased in women at a rate of 9:1 compared to males. Additionally, [10] On the other hand, the global incidence of infections among women increased by a ratio of 9:1 compared to males [11, 12]. The incidence of SLE is 2.1 times higher among African Americans and non-Arab whites than in Arab Americans [12]. In contrast, the percentage of infections reported globally has increased in women at a rate of 9:1 compared to males [11,12].

II. MATERIALS AND METHODS

A. *Subject Collection*

A total of 180 females were recruited from July to October 2021 at the Medical City. The study included a control group of 60 healthy girls (G1) and 60 females with SLE (comprising 60 early-diagnosed patients (G2) without treatment and 120 treated patients (G3)). Relevant medical history was also considered.

B. *Blood Collection*

10ml of blood was extracted from the veins of the control and patient groups after the withdrawal region had been cleaned with 70% ethanol. The following sample distribution was made into test tubes: 3 milliliters The assessment of total hemoglobin, erythrocyte sedimentation rate (ESR) using the Westergren's technique, and blood cell count (CBC) using an automated CBC analyzer were conducted

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using an ethylenediaminetetraacetic acid (EDTA) tube. Next, 7 milliliters were put in a gelatinous tube, and the serum was extracted using a centrifuge set to 3000 rpm for ten minutes. The C-reactive protein (CRP) content of the serum was measured. Next, 0.5 ml Eppendorf tubes were filled with the serum. They were frozen at -20°C right away, pending additional assessment. The study criteria encompassed complement (C3, C4), antinuclear antibody (ANA), and anti-double-stranded DNA antibody testing (DSDNA). Evaluating the total erythrocyte sedimentation rate (ESR) was one of the diagnostic criteria. The study criteria encompassed complement (C3, C4), antinuclear antibody (ANA), and anti-double-stranded DNA antibody testing (DSDNA). The present result is also consistent with (24) studies. The appearance of a low level of HB may be one of the causes of hypersensitivity (type 2) to IgG and IgM antibodies that attack self-antigens on cells, causing hemolytic anemia, white blood cell count, and measurement of total erythrocyte sedimentation rate (ESR). A professional also makes the first diagnosis. The hospital physician reviewed the patient's initial complaints and admission indicators. Apart from the aforementioned laboratory tests.

C. Statistical Analyses

Using IBM SPSS version 28, the data was analysed. One-way ANOVA and the independent t-test are two suitable statistical techniques for identifying the least significant differences (LSD). By means of the Pearson correlation coefficient, the components that were being studied were correlated. Standard error (S.E.) was included in the mean data report, and significant differences were classified as $p<0.001$.

III. Result and Discussion

This study included collecting 180 Iraqi patients (SLE) which were distributed according to several parameters.
The study found that among the categories under investigation, early-diagnosed patients had significantly higher blood levels of ANA ($P < 0.001$) than the control group. Table 1 displays the mean values for G1, G2, and G3 as follows: 0.66 ± 0.03) U/ml, 4.77 ± 0.21) U/ml, and 1.80 ± 0.17) U/ml. The ANA percentage of the sick group was 1.95 5.51) U/ml, compared to 0.53 0.23) U/ml for the control group, indicating a highly significant difference between the two groups. Conversely, it was found by ELAMIR and Wichainun [13, 14] that 90–100% of individuals with SLE showed positive ANA findings. In relation to (G3), a significant decrease was revealed in the ANA level compared to the (G2). The ratio reached an average of (1.80 ± 0.17) U/ml, and the percentage remained higher than (G1). The low ANA rate in the (G3) may be related to their treatment. An overactive immune response to foreign antigens that damages healthy body tissues (allergy reaction) and an immunological response against the body itself (autoimmune illness) are brought on by the immune system's production of antibodies to body proteins that attach to the nucleus of cells. This is what's causing the elevated ANA level.

Table 1. The serum level of ANA in the patients and control.
* Serum level Significant difference between groups

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
ANA (U/ml)	Control	0.66±0.03	-	0.001*	0.001*
	Early diagnostic patients	4.77±0.21	0.001*	-	0.001*
	Treated Patients	1.80±0.17	0.001*	0.001*	-

A. ds-DNA

As shown in Table 2., which describes the serum level of Ant double-stranded deoxyribonucleic acid (anti-dsDNA), a significant increase was noted ($P < 0.001$) among the under-studied groups. The means were (22.25±0.9), (84.13±1.6), and (65.22 ± 2.40)] IU/ml in G1, G2 and G3 groups, respectively. The higher level was in G2, while the lowest level was in G1, and significant variations were also found between the G2 and G3 groups. The ds-DNA may become low after the disease improves with treatment, so the anti-DNA antibody can provide the basis for treatment monitoring [15,16].The percentage of ds-DNA is high in most cases of autoimmune diseases, including [17]. The explanation for the positivity of anti-ds-DNA in the patient group is an antinuclear antibody, but what distinguishes it is that it is mainly associated with SLE. B cells release antibody, which is D-type switched into IgG antibodies and bind to the nucleus. Also, when apoptosis occurs, the cells fragment and remove the remaining molecules from the cellular organelles, as well as the macrophages that phagocytosis some microorganisms, and as a result, the antibodies are released and combined with antigens (forming immune complexes). The complement component works to degrade these complexes; perhaps most SLE patients have a deficiency in the complement component, and these antibodies will accumulate and appear higher in patients [17]. Because the immune system is triggered by nucleosomes on peptide cell debris, which is not removed well in SLE patients, the impact develops by binding nucleosomes rather than free ds-DNA.

Table 2. The serum level of ds-DNA in the patients and control.

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
Ds-DNA (IU/ml)	Control	22.25±0.9	-	0.001*	0.001*
	Early diagnostic patients	84.13±1.6	0.001*	-	0.001*
	Treated Patients	65.22±2.40	0.001*	0.001*	-

* Significant difference between groups

B. The Serum of Complement component C3 and C4:

The complementary elements important components that activate the immune system's complement pathways are C3 and C4. According to Table 3, the current study discovered that the understudied patients' serum levels of C3 and C4 were much lower than those of healthy people. In the G1, G2, and G3 groups, the mean C3 was (1.4±0.03), (0.77±0.022), and (1.08 ± 0.05) g/l, respectively. Conversely, the averages of C4 in the G1, G2, and G3 groups were, respectively, 0.82±0.04, 0.13±0.03, and 0.45 ± 0.05 g/L.. Moreover, there was a notable distinction between G2 and G3. The present discovery aligns with the earlier research [18]. This suggests that there was a rise in auto-antibodies in the serum, a sign of immunological activation (the rising levels of Anti-ds DNA and ANA in this investigation have demonstrated this). SLE patients with low levels of C3 and C4 also have higher levels of autoantibodies [19].

Table 3. The serum level of (C3 and C4) in patients and control.

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
C3 (g/L)	Control	1.4±0.03	-	0.001*	0.001*
	Early diagnostic patients	077±0.02	0.001*	-	0.001*
	Treated Patients	1.09±0.05	0.001*	0.001*	-
C4 (g/L)	Control	0.82±0.04	-	0.001*	0.001*
	Early diagnostic patients	0.13±0.03	0.001*	-	0.001*
	Treated Patients	0.45±0.05	0.001*	0.001*	-

C. C-reactive protein (CRP) Serum level

The CRP serum level in the investigated groups was substantially greater ($P < 0.001$) than in the G1 (Control) group, as Table 4 demonstrates. The values for G1 and G2 were 15.10 ± 0.7 and 8.64 ± 0.4 mg/dl, respectively. (3.2 ± 0.22) mg/dl was the average CRP level. Moreover, there was a significant difference ($P<0.001$) between the two examined groups (G2 & G1). Patients with SLE may have higher CRP values due to an increase in immunological complexes [20, 21]. Additionally, a significant decrease is seen in the G3 group when compared to the G2 group ($P < 0.001$)., indicating a sustained level of higher than that of the G1 group. Given that CRP drops after hydroxychloroquine treatment, the decrease in CRP level in G3 may be due to this drug's effects [20,21]. An inflammatory reaction is indicated by an increased C-reactive protein level. Acute inflammation causes a 100–1000 fold increase in the protein CRP; an indication of inflammation is the ESR. The cytokines and several inflammatory mediators are among the blood proteins that elevate during chronic inflammation, which is why this elevation is a prevalent sign in these patients.

Table 4. The serum level of (CRP) in patients & control.

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
CRP (mg/dl)	Control	3.2±0.22	-	0.001*	0.001*
	Early diagnostic patients	15.10±0.7	0.001*	-	0.001*
	Treated Patients	8.64±0.4	0.001*	0.001*	-

* Significant difference between groups

D. Erythrocyte Sedimentation Rate (ESR)

Table 5 shows the ESR results. Statistical analysis revealed significant results in the rate of blood cell sedimentation rate between the two tested groups and the control group. In G1, the men's ESR was 14.2 ± 0.6; in G2, it was 80.33 ± 1.9; and in G3, it was 43.77 ± 2.4 mm/h. Furthermore, there was a notable distinction between G2 and G3. The present finding is consistent with Aringer [21] who reported a modest rise in ESR. Furthermore, as the disease progresses and becomes more severe, the elevation of ESR rises [22]. This kind of ESR level appearance indicates the presence of inflammation as well as the build-up and clumping of blood cells.

Table 5. ESR rate in patients and control.

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
ESR (mm/hr.)	Control	14.2±0.6	-	0.001*	0.001*
	Early diagnostic patients	80.33±1.9	0.001*	-	0.001*
	Treated Patients	43.77±2.4	0.001*	0.001*	-

* Significant difference between groups

E. Hemoglobin (Hb) concentration

As shown in Table 6., it describes the level of (Hb) level, and there was a significant decrease ($P<0.001$) in the SLE patients (G2&G3) as compared to the control group (G1). The means of Hb were (13.11 ± 0.11), (9.6 ± 0.2) and (9.8 ± 0.33) g/dl in G1, G2 and G3 groups, respectively. However, there was no significant difference between G2 and G3, which indicates that the hemoglobin value remains low in SLE patients even in the case of treatment. The present result was also consistent Gangadharaiah [23] study. The appearance of a low HB level may be one of the reasons for it being Hypersensitivity (Type 2) IgG & IgM antibodies attacking self-antigens on cells caused hemolytic anemia. Many causes lead to anemia during SLE, including renal failure, where the level of erythropoietin hormone is reduced [24].

Table 6. The concentration of hemoglobin (Hb) in patients and control.

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
HGB (g/dl)	Control	13.11±0.11	-	0.001*	0.001*
	Early diagnostic patients	9.6±0.2	0.001*	-	0.001*
	Treated Patients	9.8±0.33	0.001*	0.001*	-

*** Significant difference between groups**

F. Platelets (PLT) count

This study included the count of platelets in the studied groups. It revealed a significant decrease (P<0.001) in the platelet number in both types of SLE patients as compared to healthy individuals. The mean platelets count was in G1 (200.11 ± 4.8), in G2 (125.7 ± 5.5), and in G3 (163.07 ± 6.1)10^9 /L (Table 7). Additionally, there was a significant difference between G2 and G3, meaning there is a substantial decrease for early diagnosed SLE patients compared to treated patients. These results agreed with another study by Jiang [25], which indicated that PLT decreases by less than 150 (109/L) in patients with SLE. Furthermore, the agreement was also with Wang[26], who observed that the PLT rate was (200.11±4.8)for healthy people other than SLE patients; the decrease in our study may be because most patients had other diseases associated with SLE. Also, exposure to steroid medications leads to a reduction in PLT in patients. Autoantibodies play an essential role in thrombocytopenia by attaching it and destroying the platelets via immunological mechanisms such as opsonization.

Table 7. The count of (PLT) in patients and control.

Parameter	Group	Mean±S.E	P value		
			Control	Early diagnostic	Treated
Platelets (109/L)	Control	200.11±4.8	-	0.001*	0.001*
	Early diagnostic patients	1251.7±5.5	0.001*	-	0.001*
	Treated Patients	163.07±6.1	0.001*	0.001*	-

* Significant difference between groups Conclusion

Serum ANA, dsDNA and CRP levels were significantly higher in SLE compared to the control group. Moreover, the levels of C3 and C4 were relatively decreased in SLE patients and increased in patients under treatment, which was evidence of the improvement of the patient's immune system.

IV. Conclusion

Serum ANA, dsDNA and CRP levels were significantly higher in SLE compared to the control group. Moreover, the levels of C3 and C4 were relatively decreased in SLE patients and increased in patients under treatment, which was evidence of the improvement of the patient's immune system.

Ethics Approval and Consent to Participate

The Institutional Review Board approved experimental research involving humans or animals prior to study execution and sample collection (Ministry of Higher Education and Scientific Studies, University of Anbar, Scientific Research Ethics Committee, number 190, date 3-4-2021).

Conflict of Interest

The authors have declared that no conflict of interest exists.

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