Assessments of Biochemical, Immunological and Hematological Parameters in Iraqi Pediatrics SLE patient's Compared to Healthy Controls

Mohammed Abed Jawad kadhum[*1], Usama S. Al-timari [2] and Izzat AbdulSatar Al-Rayahi[3]

[1,2] Al-Nisour University College, Baghdad -Iraq.

[3] Medical laboratory Department of Middle Technical University /College of Health and Medical technology, Baghdad- Iraq.

Email(*) : mohammed.a.medical.lab@nuc.edu.iq

Abstract

Background: Systemic lupus erythematosus was defined as an autoimmune chronic systemic rheumatic disease. Pediatric systemic lupus erythematosus (pSLE) is a rare condition, representing approximately 10% of SLE cases.

Aims: Interleukins play an important role in the susceptibility to the disease. So, this study focused on some Interleukins and Complements to identify pSLE.

Material and Methods: this study was carried out in Baghdad teaching Hospital, Welfare Teaching Hospital Children and Teaching Laboratories in Medical City and included thirty Iraqi patients diagnosed as pSLE and thirty healthy control. The patients group divided into two subgroups according to the SLEDAI (description of the diseases activity) the first group included 19 patients which had SLEDAI value lower than 11 patients and considered as mild group while the second group included 11 patients which had SLEDAI value higher than 19 patients and this group considered as severe group. All the hematological and immunological (dsDNA antibodies, IL-9, IL-10, IL-12, and INF-Ɣ were measured in sera samples

by ELISA) parameters were conducted for both patients and control samples. Biochemical parameters (urea, uric acid, and creatinine) were also measured.

Results: The results showed alopecia is the most apparent clinical feature among the SLE patients, and for hematological parameters showed the hemoglobin is lower in patients while the erythrocyte sedimentation rate are higher in patients. For the immunological tests the level of dsDNA is higher in patients than the control, the complements (C3 and C4) levels are lower in patients. IL-9, IL-10, IL-12, and INF- ɣ) level showed higher level in patients.

Conclusion: The assessment of disease activity on Iraqi pSLE patients showed increment of all studied parameters accompanied with increased severity of the disease except (IL-12, C3, uric acid, urea)

Keywords: *pSLE, Systemic lupus erythematosus.*

دراسة تقيمية للأطفال المصابين بداء الذئب الاحمراري مناعياً و كيميائياً ولكافة مكونات الدم

م. محمد عبد جواد كاظم [1] ، أ.د. اسامة سلمان التماري [2] و أ.د.عزة عبد الستار الرياحي [3]

الخلاصة

تم دراسة التحضيرات البايوكيميائية والمناعية في المرضى الاطفال المصابين بداء الذئب الاحمراري وتم مقارنة التحضيرات في مستويات مكونات الدم المختلفة مع فئة الاطفال الغير مصابين بداء الذئب الاحمراري عند فئة من الاطفال بشكل 10% فقط من مجموع الاصابات الكلي بالمرض اعلاه .

اجريت هذه الدراسة لتوضيح اهمية الانترلوكين في تشخيص المرض حيث تم دراسة 30 طفل مصاب بالمرض وتم تقسيمهم الى مجموعتين حسب شدة المرض : المجموعة الاولى تتكون من 11 طفل مصاب اصابة غير شديدة ، المجموعة الثانية 19 طفل مصاب اصابة شديدة اجريت لهم الفحوص المناعية الخاصة بالمرض بواسطة جهاز الاليزا واجريت لهم ايضاً فحص اليوريا والكرياتين واليورك اسد (وظائف الكلى) .

اظهرت نتائج الدراسة ان الصلع من اهم الاعراض التي ظهرت على الاطفال وانخفاض نسبة الهيموغلبين في الدم .

في حين ارتفعت نسبة ترسب الكريات الحمراء في المرضى وزيادة في فحص DNA في جميع المرضى وزيادة الانترلوكينات IL-9, IL-10, IL-12 INF- ϒ وانخفاض في Complement C4- C3 .

خلاصتاً اظهرت الدراسة ارتفاع في كل المؤشرات المناعية مع ازياد شدة المرض عدا , C3, 1L-12 , يورك اسد ونسبة اليوريا في الدم .

الكلمات المفتاحية *: داء الذئب الاحمراري للأطفال .*

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease which predominantly affects females. Approximately, children signify 15% to 20% of all SLE patients. They tend to have a more severe disease than adults at the onset [1]. The important medical and psychosocial cases that have a relationship with a pediatrician who specializes in children with SLE is due to the production of antibodies that attack cells of host organs, including the skin, muscles, joints, blood, kidney and brain [2].

Pediatric SLE (pSLE) is an uncommon disease presenting with an incidence of 0.3-0.9\ 100.000 children in year and a prevalence of 3.3-8.8\ 100.000 children. A higher frequency of pSLE has been reported in the populations of Asia, Africa, America, Hispanics and Native Americans Asians, Africans, Americans, Hispanics and Native Americans [1]. Also, several recent studies indicated that the average age at which pSLE expresses itself is between 11 and 12 years; and its appearance was very rare under the age of 5 years. While studies have proven that the presence of SLE in adults has registered nearly 80% of the patients, and most of them were female [3]. The common clinical features of pediatric SLE and the major signs are those related to mucocutaneous, musculoskeletal, vasculitis, serositis, photosensitivity hematological abnormalities and renal disorders [4]. Kidney disease occurs in pSLE patients in a range of 50% to 75%, and more than

90% of these patients develop kidney disease within the first two years after diagnosis [5].

Pediatric systemic lupus erythematosus (pSLE) exhibits an aggressive clinical phenotype and severe complications. This could be due to a pro-inflammatory cytokine milieu [6]. A cohort of pSLE patients and healthy controls, determined plasma levels of Th1 (IL-2, IFN-γ, TNF), Th2 (IL-4), Th17 (IL-17A, IL-6), and Treg (IL-10) cytokines and the results showed an association between these cytokines and disease activity. Patients with active disease had higher increased IL-6, IL-10, and IL-17A as candidate biomarkers for disease activity in pSLE patients [7]. The aim of the study is to assess disease activity in pediatric Iraqi patients as well as to elucidate the clinical–laboratory characteristics associated with those patients.

Materials and Methods

- **Subjects: t**his study was carried out in Baghdad Teaching Hospital, welfare Teaching Hospital Children and Teaching Laboratories in Medical City. It was conducted during the period from December / 2019 to December /2020.
- **Patients Group:** This study involved thirty Iraqi patients who had been diagnosed with pSLE. The age ranged between (6-16) years. They were sequentially visited Baghdad Teaching Hospital. The diagnosis based upon the patients' medical history, physical examination of the consultant and laboratory findings, which included immunological tests (Anti-nuclear antibody (ANA), ds-DNA, Anti-cardiolipin antibody (aCL) by ELISA technique in addition to hematological parameters such as ESR, Hb, WBCs count.
- **Control Group:** Thirty blood samples have collected from apparently healthy volunteers with age range from (6-16) years; as a healthy control

group. This group constituted of 23 males and 7 females. Those samples had been undergone the same immunological, hematological and biochemical as the patients group.

- **Study design:** This study has included 60 subjects and those subjects divided into two groups control and patients. Blood samples collected from both control and patients. The Kits which used in the present study are (Human anti-double stranded DNA (dsDNA) antibody (IgG) ELISA Kit; Human complement 3(C3) ELISA Kit; Human complement 4 (C4) ELISA Kit; Human Interleukin 9 (IL-9) ELISA Kit; Human Interleukin 10 (IL-10) ELISA Kit; Human Interleukin 12 (IL-12) ELISA Kit and Human Interferon alpha (INF-γ) ELISA Kit - CUSABIO/ China). Serum uric acid and urea were measured by spectrophotometric method.

- **Statistical Analysis:** In order to interpret and evaluate the results, appropriate statistical approaches were use the statistical package SPSS program version (26) and excel application (2016), which included the following: Descriptive statistics (For presentation of data using frequency table; Mathematical presentation (mean and standard deviation), Inferential statistics were used for statistical analyze Chi square test (Chi- test), Student test (t. test), Comparison of significant (p- value) were.

Results

Age distribution: the distribution of results according to study group by age year in (table 1) show the difference between control and patients' group with age was non-significant (p=0.201). The age was divided in two groups first group including the sample who were younger are aged less than 10 years and the second

including samples who are 10 years or older. Higher percentage of both control and SLE patients (96.6% and 83.3%, respectively) were shown among age group ≥10 -15 years, while lower percentage of both groups of study (control and SLE-patients) 16.6% and 3.3%, respectively, were shown among age groups 6-10 years. This study focused on a pediatric aged of patients suffered from SLE for this reason the maximum age was shown in the subjects was 16 years old while the minimum age was 6 years. This study succeeded to achieve a good distribution between the patients and control groups.

Table (1): Distribution of the Study Groups according to age

AGE GROUPS (YEARS)	STUDY GROUPS		P- VALUE
	PATIENT	CONTROL	
(6-10)	1 (3.3%)	(16.6%) 5	0.201
(≥10 - 15)	29 (96.6%)	(83.3%) 25	(N. S.)

The systemic autoimmune/inflammatory condition systemic lupus erythematosus (SLE) manifests before the age of 16 years in 10-20% of all cases. Clinical courses are more severe, and organ complications are more common in patients with pediatric SLE [8]. A recent study showed how the pediatric patients suffer from severe features of SLE than in adults [9].

Gender distribution: The results showed a higher percentage in female (63.3%) than in male (36.6%), while the higher percentage of male (76.6%) than female (23.3%) was shown in control group, the difference between control group and patient group according to gender are highly significant Table (2).

Table (2): Distribution of samples according to gender

GENDER	STUDY GROUPS		P-VALUE
	CONTROL	PATIENTS	
Male	(76.6%) 23	11 (36.6%)	0.006 (H. S.)
Female	(23.3%) 7	19 (63.3%)	
Total	30	30	

The results of this study agreed with[10] who showed that the strongest risk factor for SLE is gender, SLE is much more frequent among women than men. In most studies, 90% or more of patients are women, and thus as expected, in studies in which gender-specific rates are given, the incidence and prevalence rates for men are approximately 1/10th those in women. gender distribution in different age groups and increasing severity with younger age and the presence of monogenic disease in early childhood indicate distinct differences in the pathophysiology of pediatric versus adult-onset SLE [8].

Hematological parameters of the studied groups: Levels of hematological parameters; WBC count, Hemoglobin, platelet count, Lymphocyte count, Monocyte count, Neutrophils, Eosinophil, Basophil and ESR was measured by in patients and controls serum. Data expressed as mean ± SD showed that the mean level of hematological parameters were altered in SLE patients compared to control group as shown in table (3).

Table (3): The mean ± S.D. values of hematological parameters

TEST	GROUP	MEAN± S.D.	P- VALUE
WBC	PATIENTS	7.95+5.01	0.563
	CONTROL	8.66+4.52	(N. S.)
HB	PATIENTS	5.6+1.64	0.010
	CONTROL	11.97+1.58	(S.)
PLT	PATIENTS	251.9+112.18	0.100
	CONTROL	318.76+78.06	(N. S.)
LYMPHOCYTE	PATIENTS	32.63+14.26	0.319
	CONTROL	35.9+10.6	(N. S.)
MONOCYTE	PATIENTS	9.56+6.95	0.490
	CONTROL	8.53+4.27	(N. S.)
NEUTROPHILS	PATIENTS	55.13+15.26	0.931
	CONTROL	54.83+11.19	(N. S.)
EASO	PATIENTS	1.93+1.48	0.050
	CONTROL	2.86+2.14	(S.)
BASO	PATIENTS	0.7+0.65	0.090
	CONTROL	1+0.69	(N. S.)
ESR	PATIENTS	28.63+14.98	< 0.001
	CONTROL	14.66+9.27	(H.S.)

Pediatric-onset SLE (pSLE) represents 10-20% of all SLE cases, they tend to have more fulminant onset, severe disease course and higher mortality rate [11]. In study conducted by Ma *et al*., (2019) [12], it is noticed that pSLE have High ESR and Pyuria which could be related to either to SLE flare versus active infection.

Cytopenias are common in SLE, with more than 50% of patients presenting a decrease in at least one cell line.Mild leukopenia (white blood cell count 3,000 – 4 000/mm3) is the most common hematologic manifestation, and is usually due to lymphopenia (<1500 cells/mm3), and less frequently neutropenia. The

thrombocytopenia observed in SLE patients spans the spectrum from mild (<150,000) to profound (<10,000) [13] .

Anti-dsDNA in studied groups: Anti- dsDNA antibodies serum level were measured by ELISA and the results are expressed as mean ± SD as summarized in table (4) which showed that the mean level of anti-dsDNA was higher in SLE patients (4.3± 1.0 IU/ml) than the control group (2.9 ± 0.99 IU/ml).

Table (4): Compare between patients and control by anti-dsDNA serum level

TEST	GROUP	N	MEAN	P- VALUE
dsDNA ng/ml	PATIENT	30	4.367 ± 1.09	0.001 (H.S.)
	CONTROL	30	2.033 ± 0.99	

The higher level of anti-dsDNA in patients of this study can be resulted from the defective clearance of apoptotic material, together with neutrophil extracellular traps (NETs), provides abundant chromatin or self-dsDNA to trigger the production of anti-dsDNA antibodies. In SLE patients, the immune complex (IC) of dsDNA and its autoantibodies trigger interferon (IFN-I) production through intracellular DNA sensors, which drives the adaptive immune system to break down self-tolerance [14].

Complements (C3 and C4) serum levels in studied groups: The results of complements level are summarized in table (5), both C3 and C4 showed highly significant results in control than patients (P= 0.001).

Table (5): Compare between patients and control group by complements serum level between patients and control

TEST	GROUP	N	MEAN	P- VALUE
C3 (NG/M)	PATIENT	30	18.67 ± 4.76	0.001 (H. S.)
	CONTROL	30	25.1 ± 3.24	
C4 (NG/M)	PATIENT	30	22.07 ± 7.24	0.001 (H. S.)
	CONTROL	30	30.17 ± 11.75	

The results of this study showed a lower level of C3 and C4 were accompanied with the SLE disease, The decrease level of complement C3 and C4 in the serum of SLE patients means that the patient has higher autoantibodies concentration, which is the manifestation of immune activation [15].

Serum levels differences of the studied biochemical parameters: The three factors taken into consideration are; uric acid, urea, and creatinine. The results showed non-significant different between the patients with SLE and control group, table (6).

Table (6): Compare between patients and control group by biochemical parameters serum levels

TEST	GROUP	N	MEAN	P – VALUE
URIC ACID MMOL/L	PATIENT	30	3.82 ± 0.80	0.121 (N.S)
	CONTROL	30	3.967 ± 0.87	
UREA MMOL/L	PATIENT	30	28.217 ± 10.57	0.431 (N.S.)
	CONTROL	30	28.3 ±7.21	
CREATININE MMOL/L	PATIENT	30	0.514 ± 0.13	0.323 (N.S.)
	CONTROL	30	0.49 ± 0.15	

In patients with SLE, urea and uric acid has been recognized as a potential marker of endothelial dysfunction and renal disease, as an association has been found between active lupus nephritis and hyperuricemia, as well as with cerebral infarction and peripheral neuropathy. But the results of this study showed no difference between the level of uric acid in patients and control this might be due to the patients of this study are pediatric and they are not suffering from nephritis SLE [16].

Serum level of interleukin- 9: Analysis showed highly significant difference ($p < 0.01$) with respect to serum IL-9 levels among patients and control groups. The levels were distinguished to be increased in SLE patients compared to controls (284.0 ± 72.5 vs. 151.1± 50.6) Table (7).

Table (7): comparison of IL-9 serum level between patients and control

TEST	GROUP	N	MEAN ± SD	P-VALUE
IL9 (PG/ML)	PATIENT	30	284.07 ± 72.60	0.001 (H.S.)
	CONTROL	30	151.10 ± 50.62	

Serum level of interleukin- 10: The serum level of IL-10 is indicated in table (8) a higher level in patients (75.3± 21.2) group than in control (45.1± 13.6).

Table (8): comparison of IL-10 serum level between patients and control

TEST	GROUP	N	MEAN	P-VALUE
IL10 (PG/ML)	PATIENT	30	75.30 ± 21.21	< 0.001 (H.S.)
	CONTROL	30	45.13 ± 13.64	

Serum level of interleukin- 12: Serum level of IL-12 recorded with a higher level in patients than in control as shown in table (9) the IL-12 in patients equal to (91.7± 32.7) and the control level equal to (51.7± 19.9).

Table (9): comparison of IL-12 serum level between patients and control

TEST	GROUP	N	MEAN	P- VALUE
IL12 (PG/ML)	PATIENT	30	91.70 ± 32.8	< 0.001 (H.S.)
	CONTROL	30	51.73 ± 19.97	

Serum level of interferon- ɣ: The analysis showed a significant difference with respect to serum IFN-*γ* levels between the patients and control ($p < 0.01$). The levels were distinguished to be increased in SLE patients compared to healthy controls (132.9 ± 53.7 pg/mL *vs.* 42.8± 35.0 pg/mL) Table (10).

Table (10): Comparison of INF - ɣ serum level between patients and control

TEST	GROUP	N	MEAN	P- VALUE
INF-Γ (PG/ML)	PATIENT	30	132.93 ± 9.80	0.001
	CONTROL	30	42.86 ± 6.40	

Pediatric systemic lupus erythematosus exhibits an aggressive clinical phenotype and severe complications and this could be due to a pro-inflammatory cytokine environment [17]. A previous study also conducted for pediatric patients and agreed with the results of this study in both levels of IL-10 and INF-gamma [6]. IFN-*γ* might contribute to autoimmune disease by inducing production of

IgG2a and IgG3 isotype antibodies that activate complement and furthermore by activating macrophages and promoting tissue inflammation [18].

Relation between the complements levels: The relation between the both C3 and C4 serum levels have been studied and it has been represented in figure (1) and it showed a highly significant positive correlation (R=0.699, p= 0.001).

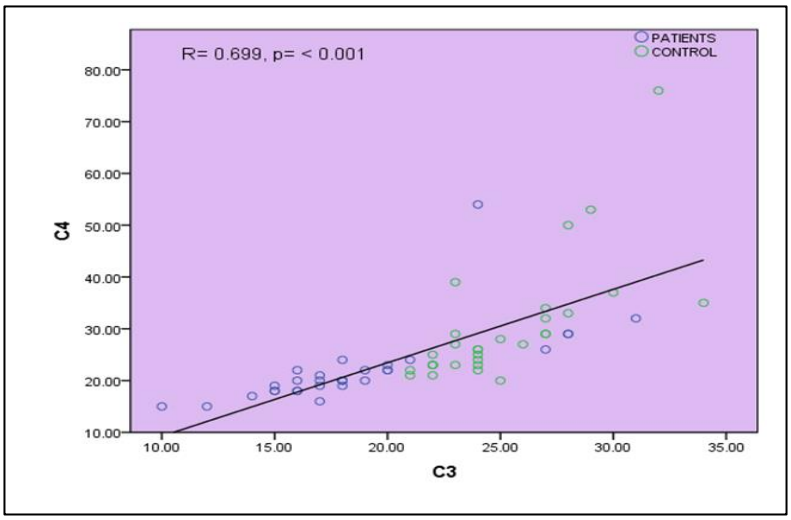

Figure (1): Simple linear regression and correlation between the C3 serum level and C4 serum level.

Autoantibodies, and immune-complex deposition which eventually leads to multi-organ damage. While the etiology of SLE is not yet fully elucidated, numerous dysregulations of the immune system have been observed among these patients. Despite numerous reports on the clinical and laboratory features of the adult population, less is known about the immunological dysregulation occurring in the pediatric population affected by SLE. Recent evidence has demonstrated that homozygous deficiency of any of the early components of the classical pathway of complement activation (C1q, C1r, C1s, C4, and C2) predisposes individuals to the development of SLE [19].

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