Complement Component C3 and C4 Levels in Juvenile and Adult SLE Iraqi Patients

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المتممات سي3 و سي4 في المرضى الأطفال والبالغين العراقيين المصابين بداء الذئبة الاحمراري

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Abstract

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease, with about 15-20% of patients being children. The causes of the disease are a mix of genetic and environmental factors. Complement C3 and C4 play a significant role in the diagnosis and monitoring of disease severity. Additionally, renal function tests are important for monitoring the disease, as the disease affects the kidneys in a large percentage of patients. **Aim**: The study was primarily conducted to compare complement C3 and C4 levels in Iraqi adult and juvenile patients with systemic lupus erythematosus. **Patient and methods**: The study consisted of 70 patients, 35 children and 35 adults, in addition to 30 individuals as a control group. The patients visited the Rheumatology Clinic at Baghdad Hospital and the Nephrology Clinic at the Children's Welfare Hospital in Medical City, Baghdad, during the period between January and May 2024. Human C3 and C4 tests was measured by Immunoturbidimetric assay by using Roche/Hitachi cobas C311 analyzer. **Results**: Complement levels were low in both groups, but more notice in juveniles, particularly complement C3 $p<0.01$, with higher levels of urea and creatinine in the juvenile group $p<0.01$, $p<0.05$, respectively. There were no significant differences in blood counts between the two groups; however, all patients suffered from anemia, as it is one of the common hematological manifestations of the disease. **Conclusion**: Measuring complement levels, kidney function, and blood counts is crucial in the regular monitoring of patients, as these tests are related to disease assessment and the development of an appropriate treatment plan.

Keywords: Juvenile-onset SLE, Adult onset SLE, Complements system.

المستخلص

المقدمة: داء الذئبة الاحمراري هو مرض مناعي ذاتي، وتشخيصه في الاطفال يُشكّل حوالي 15-20٪ من المرضى. تعود أسباب المرض إلى عوامل مختلطة بين الجينية والبيئية، تلعب المتممات 3 و4 دوراً مهماً في تشخيص المرض ومراقبة شدة الحالة. بالإضافة إلى ذلك، تعد اختبارات وظائف الكلى مهمة لمراقبة المرض، حيث يصيب المرض الكليتين في نسبة كبيرة من المرضى.

الهدف: أجريت هذه الدراسة لمقارنة مستويات المتممات 3 و4 , وظائف الكلى، صورة الدم الكاملة لدى المرضى العراقيين البالغين والأطفال المصابين بداء الذئبة الاحمراري. **المرضى وطريقة العمل:** تكونت الدراسة من 70 مريضاً، 35 طفلاً و35 بالغاً، بالإضافة إلى 30 فرداً كمجموعة ضابطة، أخذت العينات من المرضى خلال زيارتهم العيادة الاستشارية لأمراض المفاصل في مستشفى بغداد والعيادة الاستشارية لأمراض الكلى في مستشفى حماية الاطفال بمدينة الطب في بغداد، خلال الفترة من يناير إلى مايو2024، قيست المتممات بواسطة تقنية التعكر المناعي بواسطة جهاز./Roche Hitachi cobas c 311

النتائج: كانت مستويات المتممات 3 و4 منخفضة في كلا المجموعتين، لكنها كانت أكثر انخفاضاً لدى الأطفال وخاصة المتمم 3,

مع مستويات أعلى من اليوريا والكرياتينين في مجموعة الأطفال، $p<0.01$، $p<0.05$ على التوالي، لم يكن هناك فرق معنوي في تحليل صورة الدم لكلا المجموعتين. لكن جميع المرضى كانوا يعانون من فقر الدم كونه أحد اعراض الدموية الشائعة للمرض.

الاستنتاجات: يعد قياس مستويات المتممات ووظائف الكلى وتعداد الدم أمراً بالغ الأهمية في الفحوصات الدورية للمرضى، حيث ترتبط هذه الاختبارات بتقييم المرض وتطوير خطة علاج مناسب.

الكلمات المفتاحية: داء الذئبة الاحمراري لدى الكبار، داء الذئبة الاحمراري لدى الأطفال ونظام المتممات.

Introduction

Systemic lupus erythematosus (SLE) is a complicated autoimmune disorder that affects multiple body systems, it is complex and includes epigenetic, genetic, and environmental factors *(*Ameer *et al*., 2022a). Pathogenicity of SLE is heterogenetic, involving impaired removal of cells, increase in both innate and adaptive immune responses, activation of complement system, formation of immune complexes, and inflammation in tissues (Fava and Petri, 2019). Patients under 18 years old, they are often called to have juvenile-onset systemic lupus erythematosus (jSLE), which is uncommon, around 10% to 20% of youngsters with SLE diagnosed during childhood, and usually, (jSLE) demonstrates a more intense clinical progression compared to adult cases, often with a greater frequency of lupus nephritis and blood-related abnormalities (Harry *et al*., 2018). A combination of clinical criteria, laboratory testing, and sometimes imaging techniques are used to diagnose SLE, standardized guidelines regarding the diagnosis and classification of SLE are provided by the classification criteria created by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR), which incorporate both clinical and immunologic criteria (Aringer *et al*., 2019). Women *in 10 times risk of developing SLE compared to males,* men experience a more severe and aggressive course of the disease, which leads to a poor prognosis (Ameer et al., 2022b, Garcia et al., 2024). According to research, the prevalence of SLE worldwide is 43.7 (15.87 to 108.92) per 100,000 individuals, with 3.41 million persons affected (Tian *et al.*, 2023). SLE has a wide range of clinical features that can initially appear in almost any organ including the kidney (lupus nephritis) and a spectrum of mucocutaneous involvement including mouth ulcers,

photosensitivity, alopecia, and malar rash (Connelly and Morand, 2021). Renal inflammation is considered to be the most common clinical presentation in SLE, compared to adult-onset disease, (jSLE) has a higher frequency of lupus nephritis which can result in severe morbidity (Smith *et al.,* 2019, Li *et al*., 2021). Complement activation is a key event in the pathogenesis of tissue inflammation and injury in systemic lupus erythematosus (Weinstein *et al*., 2021). In general, most patients typically experience decreased complement C3 and C4 levels during the stages of the disease, which is due to the consumption of these complements as part of the immunopathogenesis process (Weinstein *et a*l., 2021). C3 is included in the common cascade after the convergence of the three pathways, by contrast, C4 is included in the classical and lectin pathways. Therefore, low levels of both proteins indicate the activation of these two pathways, whereas low C3 and normal C4 indicate the activation of the alternative pathway (Sandhu and Quan, 2017). The study aimed to compare complement levels (C3) and (C4), blood urea, serum creatinine, and complete blood count among adult and juvenile SLE patients.

Patient and Methods

The present research was to recruit 70 participants from different ages, all of whom have been diagnosed with SLE according to the 1997 American College of Rheumatology (ACR) criteria (Hochberg, 1997). They went to the Rheumatology Unit at Baghdad Teaching Hospital (Adults) and the Welfare Teaching Hospital (children) between January and May 2024. Patients were categorized into two groups according to their age: Group I consisted of 35 individuals with adult-onset SLE, aged over 20 years, while Group II comprised 35 patients with juvenile-onset SLE, aged under 16 years. The control group

comprises 30 healthy volunteers who were matched with patients in age and sex, and had no previous history of lupus or other autoimmune diseases. Participants could be enrolled at any point after their diagnosis. Human C3 and C4 tests was measured by Immunoturbidimetric assay, in which form a precipitate with a specific antiserum determined turbidimetrically by using Roche/Hitachi cobas c 311 analyzer. Blood urea, serum creatinine tests done by Mindray BS-240 chemistry full-automated analyzer, China. While for CBC, Mindray BC-5000 Hematology analyzer, China, was used. The SAS program / 2018 was utilized to identify how various groups impacted study parameters. A t-test was employed to compare means for significance. Chi-square test was utilized to compare significance between percentages with probabilities (p-value). The study was done with the permission of the Middle Technical University, College of Health and Medical Technology, Baghdad. Furthermore, approval has been obtained by The General Directorate of The Medical City, Baghdad Hospital, Baghdad. And all participants submitted written, informed consent. Approval numberd:193/3; date:09/01/2024.

Results

The study included seventy patients, categorized by age. Individuals over the age of 20Y were classified as having adult-onset systemic lupus erythematosus (aSLE), (mean ± SD, 34.73 ±1.76 years); Male: Female 3:32 respectively, and those aged under 16Y were categorized as juvenile-onset systemic lupus erythematosus (jSLE) (mean ± SD, 10.44 ±0.59 years); Male: Female ratio is 8:27 respectively, and 30 healthy control (15 children, mean ± SD, 8.44 ±1.50 years, Male: Female 3:12 respectively), and (15 Adult, mean ± SD, 33.2 ±1.62 years, Male: Female 2:13 respectively. (jSLE) group experienced the lowest levels of C3 than (aSLE) and there are statistically

high-significant between them p<0.01. But there was none statistical significance of C4 between the studied groups as presented in Table 1, (jSLE) had a greater level of both urea p<0.01 and creatinine p<0.05 compared to (aSLE) as presented in Table 2. Mean WBC count were non-significant changes among all patients, both study groups showed low levels of hemoglobin (anemia), with lower levels observed in the (jSLE) group, although there was no statistically significant difference between them, p>0.5, platelets were within normal values in the studied group and there were no statically differences between them, p>0.05 as presented in Table 3.

Table 1: Comparison Between the Mean Complement 3 (C3) & Complement 4 (C4)

Group	Mean ±SE	
	C3 (mg/dl)	C4 (mg/dl)
Juvenile-onset SLE n= (35)	67.66 ±6.91	16.74 ±1.93
Adult-onset SLE n= (35)	101.35 ±5.64	18.41 ±1.80
P-value	0.0004	0.531

*P-value ≥0.05 is non-significant; P-value ≤0.01 is highly-significant.

Table 2: Comparison Between the Mean of Urea and Creatinine Among Studied Groups

Group	Mean ±SE	
	Urea (mg/dl)	Creatinine (mg/dl)
Juvenile-onset SLE n= (35)	39.20 ±3.70	1.12 ± 0.12
Adult-onset SLE n= (35)	25.69 ±1.93	0.66 ± 0.04
P-value	<0.01	<0.05

*P-value ≤0.05 is significant; P-value ≤0.01 is highly-significant.

Table 3 Comparison Between the Mean of WBC, Hb, PLT and ESR Among Studied Groups

Group	Mean ±SE		
	WBC (x103)	Hb (g/dl)	PLT (x103)
Juvenile-onset SLE n= (35)	9.73 ±0.96	10.19 ±0.45	288.77 ±19.54
Adult-onset SLE n= (35)	7.65 ±0.54	11.60 ±0.84	262.23 ±17.57
P-value	0.0497	0.167	0.317

WBC, white blood cells; Hb, hemoglobin; PLT, platelet; ESR, erythrocyte sedimentation rate. *P-value ≤0.05 is significant; P-value ≥0.05 is non-significant.

Discussion

Female gender is domain in the study groups (P≤0.01). Duo to SLE is an autoimmune disease that typically shows sexual dimorphism, with about 90% of patients being female (Bose and Jefferies, 2022). The ratio of males to females in the two age groups notably showed disparity that strongly suggests the impact of puberty (hormonal changes) witch increase adult females susceptibility to the disease (Margery-Muir *et al.*, 2017), same results observed in previous studies (Aggarwal and Srivastava, 2015, Aeschlimann et al., 2019). Creatinine and urea levels correlate with renal injury (Gounden *et al*., 2018). And renal damage is more frequent in child (Tektonidou *et al*., 2017). Since lupus nephritis is observed in approximately 50-82% of cases, while in adults it affects about 20-40% of cases (Samanta *et al*., 2017). Disease activity tends to be higher in (jSLE) as previously mentioned, it likely leads to greater organ damage, which in turn results in elevated urea and creatinine levels compared to (aSLE). In addition, research indicates a stronger connection between genetic loci linked to SLE susceptibility and LN in SLE diagnosed during childhood (Webber *et al.*, 2020).

Hypocomplementemia is an important indicator in adults and children with SLE, showing the presence of active disease, and organ damage, especially in lupus nephritis and helping to determine treatment plans (Sharma *et al.*, 2020). In current study we demonstrated low levels of C3 in (jSLE) patients were found to be strongly linked to higher disease severity (based on physician assessment), while low C4 showed no connection with disease severity, this implies that C4 could be a less accurate indicator of disease activity in patients with (jSLE). disease activity tends to be higher in (jSLE). However low levels of complement C3 and C4 in (jSLE) compared to (aSLE) is mainly due to immune system differences as the immune system of children may respond differently to immune triggers and accordingly (jSLE) would have a more aggressive course leading to rapid complement depletion (Afzali *et al.*, 2018). The WBCs count means were within the normal range it concurred with recent research on Iraqi individuals with SLE (Ali *et al.,* 2024) , indeed, there were cases in both groups of mild leukopenia, but some patients had leukocytosis, leading to this normal average result overall. Patients with systemic lupus erythematosus often exhibit leukopenia, neutropenia, and lymphopenia as common clinical manifestations, with a significant decrease seen in all blood cell counts (Lu *et al.*, 2021). Both study groups showed low levels of hemoglobin (anemia), with lower levels observed in the (jSLE) group, although there was no statistically significant difference between them, $p>0.5$. In (jSLE) autoimmune hemolytic anemia is observed more than adults as discussed in clinical features, according to (Kisaoglu *et al*., 2022), AIHA and anemia of chronic disease are most common with (jSLE), although iron deficiency is not uncommon, the seriousness of inflammation is linked to the seriousness of anemia. AIHA develops as a

result of the presence of antiphospholipid antibodies, which are frequently found in patients with SLE (Zhang *et al*., 2023, Garcia *et al.*, 2024). Platelets were within normal values in the studied group and there were no statistically differences between them, $p>0.05$.

Conclusion

Systemic lupus erythematosus is an autoimmune disease that affects multiple parts of the body. It is relatively common in adults but rare in children, and tends to be more severe in children than in adults. The study showed a decrease in complement C3 and C4 levels in all patients, but the decrease was more pronounced in children. This reduction in complement levels is associated with increased disease severity and exacerbation of systemic symptoms. Additionally, serum urea and creatinine levels were higher in children, as they are more prone to kidney damage. All patients also had anemia due to the disease.

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