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ORIGINAL RESEARCH ARTICLE

The impact of antiphospholipid antibodies in children with lupus nephritis

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Abstract *Background and objectives:* To evaluate the frequency of antiphospholipid antibodies (APLa) among patients with childhood lupus nephritis (cLN) and to assess their impact on long-term renal outcomes.
Design and setting: This is an observational hospital based study.
Patients and methods: Patients with cLN diagnosed by renal biopsy seen between January 2002 and June 2014 were included. APLa positivity was defined if detection was positive on 2 occasions 6—12 weeks apart during their follow up. Demographic features, age at disease onset, disease duration, follow-up duration and clinical and laboratory variables at the time of renal biopsy were collected. The renal biopsy was reviewed for the nephritis class, microthrombi, activity and chronicity indices. Renal outcome measures included the serum creatinine levels, protein/creatinine ratio and end stage renal disease (ESRD).
Results: Fifty-nine, (49 female) patients with a mean age of 19.8 years and mean disease duration of 6.8 years were involved. APLa were detected in 46 (78%) patients. Twenty-two patients had class IV nephritis, which was more prevalent in APLa positive patients. The frequencies of class III and V nephritis was similar in 10 patients in each class (7 patients in each class with APLa). The presence of APLa did not correlate with nephritis activity or the chronicity indices. Microthrombosis was found in 10 patients, and 8 of them had APLa. Patients with APLa had a higher frequency of elevated serum creatinine and hypertension, 9 developed ESRD, and 7 had APLa. There was no statistically significant association between the presence of APLa and the accrual damage index and clinical manifestations. Furthermore, there was no association between APLa and other autoantibodies.

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Conclusion: The frequency of APLa in cLN was high. While the association is not statistically significant, APLa positive patients tend to develop renal microthrombi and are probably at higher risk of ESRD.

1. Introduction

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease, and while it is predominantly a disease of young women, childhood SLE represents approximately 20% of all SLE cases [1,2]. The onset and clinical manifestations of childhood SLE are often aggressive, with widespread organ involvement [3,4]. Childhood lupus nephritis (cLN) occurs in almost 30–60% of cases, and it may present with proteinuria, haematuria, hypertension, and occasionally, renal impairment. Furthermore, the renal outcomes reported by several studies suggest that it is more frequent and aggressive compared to the adult disease [5,6].

In general, cLN remains indicative of poor outcomes, which is especially true of diffuse proliferative glomerulonephritis, the most severe type, which is most frequently associated with the development of end stage renal disease (ESRD) or death [7,8].

Like other autoimmune diseases, the aetiology of SLE remains unknown. However, the primary pathology results from massive autoantibody production followed by immune complex deposition. Among these autoantibodies, antiphospholipid antibodies (APLa), which are a heterogeneous group of pathogenic autoantibodies, are directed against negatively phospholipid-binding proteins. APLa, including lupus anticoagulant (LAC), anticardiolipin antibody (aCL), and anti-β2 glycoprotein I (β2GPI) antibody, are frequently observed in SLE patients and are associated with the increased risk and frequency of thrombosis at different sites [9,10]. A variety of renal manifestations, including intra-glomerular capillary thrombosis and nephropathy, have been reported in APLa positive SLE patients. However, the true significance of APLa on the progression of lupus nephritis is still controversial [11–14].

In this study, we evaluated the frequency of APLa among patients with cLN and assessed the impact of APLa on long-term renal outcomes. To the best of our knowledge, there is no available published data from the Middle East about the impact of APLa in cLN.

2. Patients and methods

This observational study was composed of all of the patients with childhood onset SLE with biopsy-proven nephritis who were followed at the lupus clinic at the King Faisal Specialist Hospital and Research Centre (KFSHRC)-Riyadh, Saudi Arabia, between January 2002 and June 2014. All included patients fulfilled the definition of SLE using the Systemic Lupus International Collaborating Clinic classification criteria for systemic lupus erythematosus [15].

The patients were classified APLa positive if they had at least one of APLa (aCL IgG >15 GPL/mL, IgM >7 MPL/mL, β2GPI IgA >3 SA U/mL, β2GPI IgG >10 SG U/mL, or β2GPI IgM >12 SM U/mL) detected on 2 occasions 6–12 weeks apart during their follow up. The measurement of APLa was performed by Automated ELISA (ETI -Max 3000) using commercial kits (Diasorin kit, Saluggia, Italy).

A renal histopathologist reviewed all of the renal biopsies independently without knowledge of the APLa status. The histopathology assessment included a classification of the nephritis class according to the lupus nephritis classification system of the International Society of Nephrology/Renal Pathology Society (ISN/RPS) and the activity and chronicity of nephritis and the presence of microthrombosis [16].

Demographic data, age at disease onset, disease duration, and follow up duration data were extracted from patients' medical records. Clinical and laboratory data, including serum urea and creatinine, urinalysis, protein/creatinine ratio, antinuclear antibody (ANA), anti-double stranded DNA antibody (ds-DNA), and serum complement (C_3, C_4) levels, were collected at the time of the renal biopsy.

The renal outcomes were assessed according to the serum creatinine level, the protein/creatinine ratio, and the ESRD. The previous items are indicated in the pediatric adaptation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index score (pSDI) [7].

All of the collected data were analyzed confidentially, and no identifying data were needed. All of the clinical and laboratory assessments were a result of routine medical care, and informed consent was obtained at the time of the renal biopsy. The proposal was approved by the Research Affairs Council at KFSHRC.

3. Statistical methods

To determine the impact of APLa on nephritis, we compared APLa positive cLN patients with APLa negative cLN patients. SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software was used for the statistical analyses. The variables were compared using 2-sample *t*-tests, chi-square tests and Fisher's exact tests. The results are expressed as the mean ± standard deviation (SD) for continuous variables and as percentages for categorical variables. Regression analysis was carried out to examine the impact of APLa on long-term renal outcomes. *P* values $< .05$ were considered significant.

4. Results

A total of 59 (49 females) patients with biopsy-proven nephritis were included, with a mean age of 19.8 years

(±4.4), a mean disease duration of 6.8 years (±3.7) and a mean follow-up duration of 6.2 years (±3.6). The distribution of the ISN/RPS classification was follows: 22 patients had class IV nephritis, while class III and V nephritis had similar frequencies, with 10 patients in each class; 7 patients had class II nephritis; 5 patients had mixed class IV and V; 4 patients had mixed class III and V; and one patient had class VI nephritis. During the follow-up period, in addition to nephritis, haematological, mucocutaneous, neurological and cardiovascular involvements were the most frequent clinical manifestations. Twenty-one patients had haematological manifestations in the form of direct-coombs test related haemolytic anaemia, leukopenia, and thrombocytopenia, although most of these findings were transient. Twelve patients had mucocutaneous manifestations, including oral ulcerations and facial rashes, 2 had discoid rashes, and none had livedo reticularis. Nine patients had neurological manifestations, 4 of whom developed lupus cerebritis complicated by cerebral ischaemia, as shown by brain magnetic resonance imaging and angiography; 4 patients had seizure disorders; and one patient had psychosis. Six patients had cardiovascular involvement: 4 patients developed pericardial effusion; one had depressed myocardial contractility, as manifested by a decreased ejection faction; and 2 patients were complicated by mitral valve regurgitations, none of whom had valve vegetation.

All patients received corticosteroids, hydroxychloroquine and immunosuppressive treatment. Table 1 presents the frequency of the use of immunosuppressive drugs. The main indication of cyclophosphamide therapy was renal involvement, mainly class IV, followed by lupus cerebritis. However, rituximab was used mainly for nephritis class IV or class V.

Overall, 35 (59.3%) patients had disease damage; the mean accrual damage was 1.52 (±1.8). The damage was mostly in growth followed by renal then neuropsychiatric domains.

APLa were detected in 46 (78%) patients; anti β2GPI IgG, β2GPI IgA, and β2GPI IgM antibodies were detected in 60.9%, 50% and 32.6%, respectively; and aCL IgM and aCL IgG antibodies were detected in 39.1% and 32.6% of the 46 APLa positive patients, respectively.

Table 2 presents the differences between APLa positive patients and APLa negative patients. There were no significant differences in patient age, disease duration or follow-up duration. Among the 22 patients with class IV nephritis, 19 were APLa positive. The frequencies of class III and V nephritis were similar, with 10 patients in each class (7 APLa positive patients in each class). The activity and chronicity indices were comparable. However, microthrombosis was more frequent in APLa positive compared to APLa negative patients (8 patients versus 2 patients). Two out of the 8 APLa positive patients had microthrombi in the small arterioles, while the other patients had intra-glomerular microthrombosis. All patients with microthrombosis had proliferative glomerulonephritis. Additionally, APLa positive patients had higher serum creatinine levels and proteinuria. Furthermore, established renal damage was observed at the last follow-up visit in 7 APLa positive patients compared to 2 APLa negative patients. These differences did not reach statistical significance (P values $> .05$). There was no statistically significant association between the presence of APLa and accrual damage and clinical manifestations, including hypertension and arterial thrombosis. Additionally, there was no association between APLa and other autoantibodies and complement levels.

Table 1 A summary of the immunosuppressive drugs in 59 patients with cLN.

Drug	Frequency
Cyclophosphamide	39 (66.1%)
Mycophenolate mofetil	27 (45.8%)
Rituximab	15 (25.4%)
Azathioprine	14 (23.7%)

Table 2 A comparison of nephritis classes, renal indices and renal outcomes between APLa positive and negative patients.[a]

	APLa positive	APLa negative
Number of patients	46	13
Age (years)	19.1 (3.6)	19.4 (4.1)
Gender (F:M)	38:8	11:2
Disease duration (years)	6.6 (3.2)	7.7 (4.9)
Follow-up duration (years)	6 (3.2)	7 (4.9)
pSDI	1.54 (1.82)	1.36 (1.96)
Creatinine/protein ratio	0.31 (0.48)	0.13 (0.15)
Serum Creatinine μmol/L	102 (93.9)	70.9 (50.4)
Lupus nephritis class		
II	6	1
III	7	3
IV	19	3
V	7	3
VI	1	0
IV + V	4	1
III + V	4	0
Activity	4.9 (3.7)	5.4 (4.3)
Chronicity	2.6 (2.1)	2.8 (2.0)
Microthrombi	8	2
ESRD	7	2

APLa = antiphospholipid antibodies, pSDI = pediatric adaptation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index score, ESRD = End stage renal disease.
[a] The differences are not statistically significant.

5. Discussion

SLE is an autoimmune disease that is characterized by multiple organ involvement and a frequently relapsing disease course with an increased risk of comorbidities, including renal impairment. The presence of APLa in childhood SLE and even transient or intermittent APLa, could be a risk factor for a poor prognosis [17]. The actual impact of APLa in the pathogenesis and progression of renal lesions in SLE patients requires further research, particularly in children, and data regarding the correlations

between the presence of APLa and long-term renal outcomes in cLN are scarce.

In the current study, we demonstrated that 78% of patients with cLN were APLa positive, which is comparable to the findings previously reported previously in childhood SLE [18]. All enrolled patients had nephritis with a long follow up period. Most patients had class IV nephritis, which was more prevalent in APLa positive patients. However, this difference was not statistically significant. In previous studies, glomerular changes and nephritis classes were not related to the presence of APLa [14,19,20]. Similarly, we did not find an association between the presence of APLa and different nephritis classes. Our results are comparable with the previous findings that APLa positivity does not correlate with renal histological activity and chronicity indices [14,19,21]. APLa are often observed in SLE patients and increase the risk of thrombosis at different sites and probably increase thrombotic complication in lupus nephritis. Previous reports demonstrated that the presence of APLa is a strong predicator for intra-glomerular capillary thrombosis. Furthermore, patients with intra-glomerular microthrombosis had more renal tissue injuries and poorer renal outcomes compared to patients without intra-glomerular microthrombosis [22,23]. On the other hand, another study found that the detection rate of APLa was higher in patients with intra-glomerular microthrombosis, which indicates the pathogenic significance of APLa in lupus nephritis [24]. In this study, we did not find a significant correlation between the presence of APLa and microthrombosis. Nonetheless, 10 patients had renal microthrombosis, and two of the eight APLa positive patients had microthrombi in the arterioles and severe disease manifested by refractory hypertension, lupus cerebritis and ESRD. However, it is still not known whether the presence of APLa accelerates renal impairment in SLE patients, although a few studies have suggested that APLa is a risk factor for increased serum creatinine levels and the elevated prevalence of hypertension and probably contributes to poor long-term renal outcomes [13]. Our findings show that patients with APLa had a higher frequency of elevated serum creatinine levels and hypertension; nine developed ESRD, and seven had APLa. The association of APLa with different clinical and laboratory findings, particularly in patients with life-threatening disease, is still debatable, although APLa were significantly associated with anti-ds-DNA antibody levels and haemolytic anaemia [18,25]. In our study, however, no statistically significant association was observed between the presence of APLa and the accrual damage index or clinical manifestations, such as hypertension and arterial thrombosis or other autoantibodies and complement levels.

We did not examine APLa level fluctuations during the study follow-up period or distinguish between different APLa subtypes. Additionally, we did not attempt to evaluate the efficiency of antiplatelet or anticoagulant treatment in patients with intra-glomerular microthrombosis because of the study limitations and design.

In summary, this is the first study from the Middle East regarding the relationship between the presence of APLa and renal outcomes in childhood SLE. Our findings suggest a probable prognostic consequence of APLa on the long-term outcomes of cLN. We realize that the studied sample is small; therefore, these results should be interpreted with caution. Further prospective investigation into the role of APLa in the long-term renal outcomes of childhood SLE is warranted.

Conflict of interest

The authors declare that they have no financial or other relationship that constitutes conflict of interest.

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