ORIGINAL ARTICLE

Histological Features of Antiphospholipid Nephropathy in Patients with Systemic Lupus Erythematosus

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Abstract

Objective: To determine the histological features of renal biopsies of Systemic Lupus Erythematosus (SLE) patients with and without antiphospholipid antibodies in Saudi population.

Study Design: Cross-sectional, comparative study.

Place and Duration of Study: King Khalid University Hospital, Riyadh, Saudi Arabia, from January to December 2013.

Methodology: Consecutive SLE patients admitted to King Khalid University Hospital, Riyadh for renal biopsy for evaluation of proteinuria or deterioration of renal function were recruited. SLE patients with renal involvement were divided in two groups. Group one included patients with positive APS antibodies and group two included patients with negative APS antibodies. The histological features of renal biopsies of the two patients groups were compared. Data was analyzed using simple statistical analysis.

Results: The mean age of APS antibodies-positive patients was 30.37 ± 10.714 years while mean age of APS negative patients was 33.62 ± 11.717 years (p=0.224). Twenty five (83.33%) patients were females and 5 (16.67%) patients were males in APS positive patients while 42 (89.36%) were females and 5 (10.63%) were males in group two. Acute lesions like thrombotic microangiopathy were in 2 (6.7%) of APS positive patients while chronic lesions like focal cortical atrophy was found in 6 (20%) and fibrous intimal hyperplasia was found in 9 (30%). Other significant histological findings in APS antibodies positive group were glomerular basement membrane wrinkling in 12 (40%), glomerular double wall contour in 17 (56.7%), fibrous adhesions in 11 (36.7%) patients with APS antibodies.

Conclusion: Systemic Lupus Erythematosus (SLE) patients with positive APS antibodies has specific histological findings suggesting an important role of APS antibodies in the pathogenesis of APS nephropathy.

Key Words: *Antiphospholipid syndrome nephropathy. Lupus nephritis. Thrombotic microangiopathy. Systemic lupus erythematosus.*

INTRODUCTION

Antiphospholipid Syndrome (APS) is a systemic autoimmune disease characterized by recurrent arterial or venous thrombosis and/or pregnancy losses, in the presence of persistently elevated levels of Antiphospholipid Antibodies (APLA).[1]

The updated laboratory criteria for definite Anti-Phospholipid Syndrome (APS) includes the Lupus Anticoagulant (LAC), the Anticardiolipin Antibodies (ACLAs) and the beta-2-glycoprotein-I antibodies.[2] These antibodies use beta 2-glycoprotein like co-factors which have an important role in thrombotic events in patients with APS and SLE.[3] Antiphospholipid antibodies promote activation of endothelial cells, monocytes, and platelets; and overproduction of tissue factor and thromboxane A2. Activiation of complement generates split products that leads to thrombosis.[4]

APS may develop independently of any underlying disease (primary APS) or in association with autoimmune diseases, mainly SLE. The kidney is a major target organ in both primary and secondary APS more recently called antiphospholipid nephropathy.[5] APSN is clinically manifested by hypertension, acute renal injury, chronic renal injury and low grade proteinuria. Antiphospholipids antibodies were detected upto 49.7% of SLE patients in Saudi Arabia in one study, however, the renal histological features of APS antibodies positive patients were not studied before in the Middle East region.[6] Previous studies of histological features in other parts of world found different features in different ethnic groups. It was not clear if these parameters were similar in our patients population. This propelled the authors to conduct this study.

In addition to macrovascular thrombosis, in number of cases only small vessels are affected like Thrombotic Microangiopathy (TMA) which may not be clinically apparent, reflecting so called microvascular APS.[7] However, less frequently an overt renal involvement is present in primary APS and fear of complications of renal biopsy in primary APS related to concomitant thrombocytopenia and anticoagulant therapy are the other reasons why renal biopsy is rarely done in primary APS and the histological features of APS nephropathy is mainly relied on analysis of kidney biopsies of patients affected by Lupus Nephritis(LN).[8]

In LN, renal lesions related to APSN may be overshadowed by those related to the LN itself. Two types of

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APS nephropathy lesions have been described previously. Acute lesions are described as thrombotic microangiopathy and chronic lesions include Fibrous Intimal Hyperplasia (FIH), tubular atrophy and focal Cortical Atrophy (FCA).[9]

There is no consensus on the management of APS nephropathy. It may occur despite full-dose anti-coagulation and it may not improve if anticoagulation is initiated after its diagnosis.[10]

Purpose of the current study was to evaluate presence of APS nephropathy in kidney biopsy specimens obtained from SLE patients with or without APS antibodies to assess any association between APS nephropathy and APS antibodies; and also to determine the laboratory associations with APS nephropathy.

METHODOLOGY

The authors prospectively recruited 77 consecutive SLE patients admitted to King Khalid University Hospital, Riyadh, for renal biopsy for evaluation of proteinuria or deterioration of renal function, from January to December 2013. SLE patients with renal involvement were divided in two groups. Group one included patients with positive APS antibodies and group two included APS antibodies negative patients.

Sample size was calculated using formula for simple random sampling. Prevalence of APS nephropathy in SLE was known to be 32%. Confidence interval was set at 95% with Z-set at 1.96. Margin of error was set at 10%. The total sample size was computed to be 84. Due to time limitations, the authors were only able to include 77 patients in this study. All patients satisfied at least four of American College of Rheumatology (ACR) 1982 revised classification criteria for SLE.

Biopsy specimens from patients in which renal concomitant thrombotic microangiopathy could be secondary to systemic sclerosis, Thrombotic thrombo-cytopenic purpura, and patients on cyclosporine therapy were excluded from the study.

For each patient following information was collected; Demographic data, laboratory data at the time of diagnosis and at the time of renal biopsy and duration of disease till time of renal biopsy. The patients were tested for each of the antiphospholipid antibodies (anti-cardiolipin antibodies, beta-2-glycoprotein, and lupus anticoagulant) on two occasions at least 12 weeks apart.

The presence of IgG and IgM anticardiolipin antibodies (ACL) were detected by using quantitative ELISA kits, the levels of ACL were expressed as standard units for either G-phospholipid (GPL) units/ml or M-phospholipid (MPL) and values more than 18 GPL units /ml or more than 18 MPL units/ml were considered positive. IgG and IgM anti-beta-2-glycoprotein were measured with Elisa kits and values more than 20 RU/ml were considered positive for either IgG and IgM. Lupus anticoagulant was detected by mixing study and confirmed by Staclot LA and dilute Russell Viper Venom Time (dRVVT).

Renal involvement is defined as 24-hour urinary proteinuria more than 0.5 gram per day or Creatinine clearance less than 60 ml per minute. Informed consent was obtained for renal biopsy from each patient. The study was approved by the Institutional Review Board (IRB) College of Medicine, King Saud University.

Renal biopsies were performed transcutaneously under ultrasound guidance in the absence of contra-indications. Renal biopsy specimens were examined under light and electron microscopy and also for immunofluorescence. Histopathologist was blinded for APS status of patients. Lupus Nephritis (LN) was classified according to the International Society of Nephrology and Renal Pathology Society (ISN/RPS) 2004 lupus nephritis classification system.

The following data from each biopsy sample was collected: (I) type of lupus glomerulonephritis according to ISN/RPS 2004 classification system with activity and chronicity indices; (II) lesions suggestive of acute APS nephropathy such as TMA, consisting of the presence of fibrin thrombi in arteries, arterioles and/or glomeruli; (III) lesions suggestive of chronic APS nephropathy such as Focal Intimal Hyperplasia (FIH), consisting of myofibro-blastic cellular proliferation in the intimal with luminal narrowing of small arteries, and Focal Cortical Atrophy (FCA) with or without tubular thyroidization.

The diagnosis of APSN was made when at least one of the lesions suggestive of APSN was found. Simple arteriosclerosis was not included in the lesions suggestive of APSN. Renal tissue injury was evaluated using activity and chronicity indices as previously reported by Austin and colleagues. Statistical software SPSS version 20.0 was used for statistical analysis. Quantitative data were expressed as mean ± SD and median range (minimum, maximum). Frequencies and percentages were presented for categorical variables. For comparison of histological features of patients student t-tests, Chi-square tests and Fishers exact test were used. Statistical significance was considered as p-value less than 0.05.

RESULTS

A total of 77 renal biopsies of SLE patients were included. Among APS positive patients 25 (83.33%) were females and 5 (16.67%) were males. Forty two (89.36%) were females and 5 (10.63%) were males in APS negative patients. The mean age of thirty SLE patients with APL antibody-positive was 30.37 ± 10.714 years while mean age of 47 antibody-negative SLE patients was 33.62 ± 11.717 years (p=0.224).

The time from SLE diagnosis to renal biopsy was 71.30 ± 56.071 months in antibody-positive patients and 95.70 ±

64.44 months in antibody-negative patients (p=0.093). Creatinine clearance calculated from 24 hours urine collection at time of renal biopsy revealed no significant difference in two groups (p=0.108). Serology of APS at time of renal biopsy revealed Anticardiolipin antibody IgG (ACA IgG) low positive (19 - 39) in 6/30 (20%) patients and high positive (40 and more) in 8/30 (26.7%) patients, while anticardiolipin antibody IgM (ACA IgM) was low positive (19-39) in 10/30 (33.33%) and high positive in 3/30 (10%) of patients.

Lupus anticoagulant was positive in 9/30(31%). Beta-2-glycoprotein was done in 19 patients due to limited resourses which revealed weak positive IgA (4 - 20) in 14 (73.7%) patients and high positive (more than 20) in 4 (21.1%). IgM was low positive (4 - 20) in 1 (5.3%), and high positive (more than 20) in 3 (15.8%). IgG was low positive (13 - 19) in 2 (10.5%) and high positive (more than 20) in 2 (10.5%).

Histological features of two groups are shown in Table I. Renal histology showed statistically significant features

Table I: Histological features of lupus nephritis related to APS nephropathy.

Histological characteristics	APS antibodies positive patients (Group 1) (n=30)	APS antibodies negative patients (Group 2) (n=47)	p-value
Thrombotic microangiopathy	2 (6.7%)	0	0.149
Focal cortical atrophy	6 (20%)	3 (6.4%)	0.142
Fibrous intimal hyperplasia	9 (30%)	3 (6.4%)	0.009
Glomerular cap wall wrinkling	12 (40%)	5 (10.6%)	0.004
Glomerular double wall contour	17 (56.7%)	13 (27.7%)	0.016
Fibrous crescents	10 (33.3%)	4 (8.5%)	0.013
Fibrous adhesion	11 (36.7%)	6 (12.8%)	0.023
Glomerular sclerosis	18 (60%)	20 (42.6%)	0.165
Cellular or fibro-cellular crescent	9 (30%)	10 (21.3%)	0.38
Glomerular fibrinoid necrosis	7 (23.3%)	6 (12.8%)	0.227
Vasculitis	0	0	NA
Lupus vasculopathy	1 (3.3%)	0	0.390
Lupus nephritis class			
Class I	0	4 (8.5%)	0.436
Class II	7 (23.3%)	8 (17%)	
Class III	9 (30%)	9 (19.1%)	
Class IV	11 (36.7%)	18 (38.3%)	
Class V	3 (10%)	7 (14.9%)	
Class VI	0	1 (2.1%)	
Activity index			
0 - 5	14 (46.7%)	29 (61.7%)	0.386
6 - 9	11 (36.7%)	11 (23.4%)	
10 and more	5 (16.7%)	7 (14.9%)	
Chronicity index			
0 - 3	16 (53.3%)	27 (57.4%)	0.540
4 - 5	13 (43.3%)	16 (34%)	
6 and more	1 (3.33%)	4 (8.5%)	

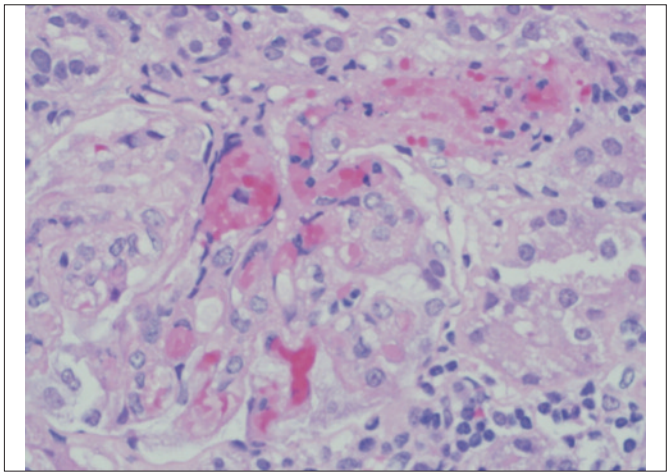

Figure 1: Photomicrograph showing an afferent arteriole and glomerular capillary loops with intraluminal thrombi (H&E x 400).

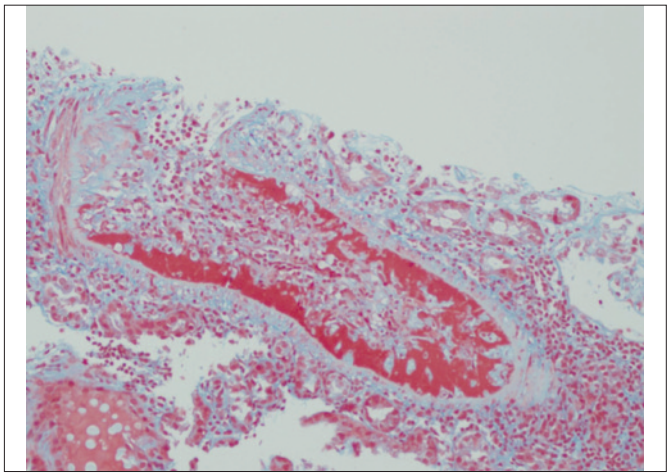

Figure 2: Photomicrograph of an artery with thrombus highlighted by the trichrome stains (Trichrome x 200).

suggestive of APSN in 12/30 (40%) in APS antibodies positive patients while in 4/47 (8.5%) in group-2 ($p < 0.001$). In terms of classical lesions of APS nephropathy, although the incidence of TMA and FCA were more common in those with positive antibodies, the numbers did not reach statistical significance. There were however, significant differences in FIH (30% vs. 6.4%, p=0.009), glomerular basement wrinkling (40% vs. 10.6%, p=0.004), glomerular double wall contour (56.7% vs. 27.7%, p=0.016), fibrous crescent (33.3% vs. 8.5%, p=0.013), and fibrous adhesions (36.7% vs. 12.8%, p=0.023) which were more in group with positive antibodies. Simultaneous acute and chronic APSN lesions in APS positive patients were found in one patient. Two patients who had thrombotic micro-angiopathy had severe forms of lupus nephritis class (Class III and IV). Acute lesions of APSN like TMA are shown in Figure 1 and 2.

DISCUSSION

Role of APS antibodies in lupus nephritis was first described in 1980 by Kant and then specific histological

features of APSN related to APS positivity were described many years later in other studies done by Griffith.[11,12] The histological features of SLE patients with positive antibodies were not studied previously in the Middle East region. The current study has tried to address this aspect.

APS nephropathy was found in 40% of SLE with APS antibodies while only in 8.5% of patients in group two suggesting significant association between the presence of APS nephropathy and APS antibodies ($p < 0.001$) which matches the findings of Tektonidou *et al*.[13] The result of this study revealed that APSN not only occurs in primary APS syndrome but also in secondary APS syndrome especially SLE. No difference in renal activity or chronicity scores was seen between two groups of patients similar to a previous study.[14]

Moreover, we have shown that ISN/RPS Class occurred independent of presence of APS antibodies as no difference in frequency of severe nephritis (lupus nephritis 4) in two groups of patients. The finding are similar to what has been reported previously, however, a larger sample size would be required to ascertain the relationship between APSN and LN Class.[15,16]

The most frequently observed pathological lesions were TMA, FIH and FCA. Thrombotic Microangiopathy (TMA) is the best known and most characteristic lesion of APSN, and represents acute variant of APSN. It is characterized by presence of fibrin thrombi in the glomeruli and small arterioles in the absence of vascular immune deposits or inflammatory cells. TMA lesions is similar to changes observed in thrombotic thrombocytopenic purpura and haemolytic uremic syndrome. When making a diagnosis of APS nephropathy, clinical context must be considered rather than relying only on histological features. TMA was found in 2 (6.7%) of APS antibodies positive patients while Silvarino found in 33% of patients.[14] In several studies TMA was associated with worse prognosis.[17]

Delay in lupus nephritis diagnosis might explain the low prevalance of acute lesions and high incidence of chronic histological lesions. Both patients in this study with thrombotic microangiopathy had severe classes of lupus nephritis (Class 3 and 4) which similar to those found by Glueck and colleague.[18]

As acute lesions of APS nephropathy heal, leaving focal areas of fibrosis and secondary atrophy develop, it results in renal impairment depending upon degree of severity. A group of lesions reflecting chronicity were also noted in patients with positive APS antibodies in this study like glomerular double wall contour which was also noted by Griffiths.[19] Fibrous intimal hyperplasia is different from that observed in ageing and is more due to more intense cellular proliferation. It was found more frequently in APS positive than other group, confirming the importance of such finding of APS nephropathy.

Focal cortical atrophy is considered very important finding of APS nephropathy and can lead to interstitial fibrosis and tubular atrophy. FCA may be seen in other renal diseases such as malignant hypertension which was not present in this group of patients. Nonetheless, presence of FCA in young patient with lupus especially when associated with other lesions of APSN is highly suggestive of APSN. FCA is usually noted in sub-capsular cortex of kidney and may not be sampled on tru-cut biopsies which explains it relatively low percentage of occurrence in this study.

Glomerular hyaline thrombi were found more frequently in 6 (20%) in APS positive patients while 4 (8.5%) in APS antibody negative patients ($p=0.144$). Glomerular hyaline thrombi were described as poor prognostic factor in a previous study.[20]

This study emphasized the less frequent but possibly more specific renal lesions found in patients with APS antibodies. Electron microscopy revealed redundant and wrinkled segments of basal membrane accompanied by a duplicate thin membrane adjacent to the endothelium which is a pathognomonic finding of the anti-phospholipid syndrome reported by others.[21] Ischemic changes in glomerular capillary wall leads to retraction, wrinkling and folding of capillary wall. These changes were found more frequently in APS antibodies positive patients suggesting its significance in APS nephropathy.

It was of note in this study that non-specific arterio-sclerosis and arterial hyalinosis had same prevalence in two group of patients raising the possibility that these findings are not specific for APS nephropathy. Furthermore, no vascular lesions of polyarteritis type was found in any of 77 renal biopsies of lupus patients studied. Thus, this type of lesions turned out to be rare, as has been reported by others.[22]

This study has some limitations due to small number of patients with APSN which may hamper the ability to identify some significant associations such as the relationship of lupus anticoagulant and APSN. Moreover, patients in group two of LN nephritis patients were not tested for beta-2-glycoprotein, so the possibility that some of the group two patients were anti-beta-2 glycoprotein positive could not be ruled out.

The authors suggest that renal pathologists should carefully examine renal biopsies obtained from SLE patients with positive APS antibodies for the presence of APSN, as this may have significant implications on therapeutic decisions, especially in the acute phase TMA, may benefit from anticoagulation.

CONCLUSION

This study established the existence of APS nephropathy within SLE suggesting that APS nephropathy is a second renal problem superimposed on lupus

nephropathy. APS nephropathy occurred in 40% of SLE patients with positive APS antibodies but only in isolated SLE patients without APS antibodies suggesting an important role of APS antibodies in the pathogenesis of APS nephropathy. The review of renal biopsies also highlighted other important findings in APS nephropathy like glomerular capillary wall wrinkling and glomerular double wall contour formation.

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