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Research Article

Comparative Analysis of C4d Deposition with the Severity, Laboratory Tests and Histopathologic Scores in Lupus Nephritis

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Abstract

Background: Lupus nephritis (LN) is a serious renal condition and is evident in about 60% of systemic lupus erythematosus (SLE) patients. The complement system and its product, complement factor 4 degradation (C4d), have an important role in immune-mediated nephritis. The aim of this study is to assess the prognostic value of C4d deposition in comparison with the disease severity, laboratory results, and histopathologic activity scores in LN.

Methods: Thirty-five LN patients who underwent renal biopsy at AL-Kafeel Center of Nephrology and Kidney Transplantation were included in this retrospective study. The renal specimens were classified according to the International Society of Nephrology, Renal Pathology Society classification. Pathological activity and chronicity scores were calculated according to the modified National Institute of Health (NIH) scoring system. The clinical data including patient age, gender, laboratory tests including complements value (C3, and C4), 24 hr urine collection, and serum levels of anti-nuclear antibody and anti-dsDNA antibody were recorded. All cases were stained immunohistochemically with C4d and assessed regarding their deposition in renal capillaries.

Results: Glomerular C4d was expressed in 40% of LN patients without peritubular capillary deposition. There was a positive correlation between glomerular C4d and urinary protein excretion (p=0.001) and also with anti-double stranded DNA antibody (anti-dsDNA) (P =0.048), but not with LN classes or activity score or low complements.

Conclusions: Glomerular C4d deposition of LN does not match up with disease activity score but may aid in categorizing a subset of patients with a worse prognosis of their disease which may help to guide treatment decisions.

Keywords: Lupus nephritis, glomerular C4d, proteinuria, *anti-dsDNA antibody*.

Introduction

Lupus nephritis is an immune-mediated glomerulonephritis developed in about 60% of systemic lupus erythematosus (SLE) patients during their life [1]. Renal biopsy is essential in the renal assessment of patients with SLE and is indicated in all patients with abnormalities of urine sediment or renal function. In patients with these abnormalities, the clinical presentation does not accurately predict the disease severity, and the renal biopsy findings provide prognostic information and aid in the formulation of the treatment plan [2]. In addition, repeated biopsies have been suggested for proper post-treatment evaluation [3-4].

Histologic markers of the disease severity in patients with LN are LN class IV, class V, significant interstitial fibrosis, tubular atrophy, vascular involvement, and thrombotic microangiopathy [5]. In addition to the pathologic classes of LN, activity, and chronicity indices are scored pathologically and predict the progression of renal disease. Renal lesions with a high activity index are more likely to respond to aggressive therapy, while renal lesions with high chronicity are not [6]. Generally, elevated levels of anti-dsDNA and anti-C1q, decreased levels of serum complement C3 and C4 and proteinuria are associated with disease activity in LN [5]. C4d is a complement degradation product of the complement C4, commonly used in the diagnostic criteria for antibody-mediated tissue rejection. C4d

is formed when the classical pathway is activated by the deposition of circulating immune complexes or from an in situ combination of antigen and antibody on the renal endothelium, leading to glomerular injury in LN [7-8]. Several studies recorded C4d as a potential disease activity and severity biomarker in patients with LN [9-13].

The study aims to assess the incidence and distribution of C4d in different renal compartments of LN patients, assess the prognostic value of its deposition in pathologic classes of LN, and correlate with serologic markers and histopathologic activity scores.

Materials and Methods

This retrospective study included 35 patients with SLE who underwent renal biopsy at AL-Kafeel Center of Nephrology and kidney transplantation from 2019 to 2023. The diagnosis of LN was based on histological assessment of formalin-fixed, paraffin-embedded tissue blocks with hematoxylin and eosin (H&E), Masson trichrome, periodic acid-Schiff, methenamine silver and Congo red for light microscopy, and fresh frozen tissue for IgM, IgG, IgA, C1q, C3, C4, Kappa and Lambda immunofluorescence staining. The renal specimens were classified according to the International Society of Nephrology, Renal Pathology Society classification [14]. Activity and Chronicity scores were calculated according to the modified National Institute of Health (NIH) lupus nephritis activity and chronicity scoring system [15]. The activity index is calculated by the assessment of the following six histologic parameters: glomerular endocapillary proliferation, glomerular leukocyte infiltration, wire loop deposits, hyaline thrombi, glomerular fibrinoid necrosis and karyorrhexis, cellular crescents, and interstitial inflammation. The severity of each of these features is scored on a scale of 0 to 3+, and the fibrinoid necrosis, karyorrhexis, and cellular crescents scores are doubled. Thus, the maximum activity score is 24. The chronicity index is computed by the sum of individual scores (0–3+) of the following four parameters: global glomerulosclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis. Thus, the maximum chronicity score is 12. The clinical data including patient age, gender, laboratory tests including complements value (C3, and C4), 24 hr urine collection, and serum levels of anti-nuclear antibody and anti-dsDNA antibody were recorded. Two sections were taken from each paraffin block for histopathologic revision and C4d immunohistochemical staining. Cases with incomplete investigations and insufficient renal tissue (less than 10 glomeruli) remaining in the paraffin block were excluded from the study.

Immunohistochemical procedure of tissue C4d

Complement factor 4 degradation immunohistochemical staining was performed using mouse monoclonal antibody (clone: C4D204, PathnSitu, USA). A section from kidney transplanted tissue with rejection was considered as positive control and the negative control was obtained by the elimination of the primary antibody. Formalin-fixed, paraffin-embedded sections were cut into 4-µm by semi-automated rotary microtome, then the tissue section was dewaxed in xylene which was followed by rehydration in which the slide submerged through descending concentrations of alcohol. After that, the tissue was subjected to heat epitope retrieval using Tris/EDTA buffer at pH 9, then incubated at 95°C for 30 minutes in a water bath followed by putting the slide in a peroxidase blocker for 5 minutes. The pre-diluted primary antibody C4d was applied and incubated for 60 minutes, then the tissue was covered with PolyExel polyHRP and incubated for 10 minutes. Finally, the slides were immersed in Mayer`s hematoxylin then one to two drops of mounting medium were applied. A light microscope was used in the examination of the slides, then the image was captured in high definition using the camera of a digital light microscope.

Scoring of C4d staining

The slides were examined for diffuse C4d positivity along capillaries of glomeruli and peritubular capillaries and scored semiquatitatively according to their intensity from 0 to 2: 0 (absent), 1+ (weak to intermediate), and 2+ (strong) [10].

Ethical approval

The authors followed the ethical standards and the research was approved by the Ethics committee of the College of Medicine at the University of Kerbala (ethical No: 24-14 on 14 March 2024) and it was managed as the Histopathology fellowship thesis in the College of Medicine at the University of Kerbala.

Statistical Analysis

Data was analyzed using a statistical package for social sciences (SPSS version 26) by choosing the chi-square test, and Pearson correlation. P value <0.05 was considered statistically significant.

Results

The study involved thirty-five cases with LN. Their ages range from 11 to 65 years (mean: 25.89 years). 31(88.6%) of cases were females and 4(11.4%) of them were males. According to the collected data,

ANA was identified in the serum of 27 patients (77.1%), and 30 (85.7%) of these cases. Anti-dsDNA antibody was also detected. For serum complements, 21(60%) and 17(48.6%) had low C3 and C4 respectively. Although most of the patients (94.2%) were affected by proteinuria, only 9 patients (25.7%) were in the nephrotic range. Lupus nephritis class 4 was the most frequent class encountered in 13 patients (37.1%). Activity score ≥ 12 was seen in 10 patients (28.6%), while activity score < 12 was seen in 25 (71.4%). Positive glomerular C4d immunohistochemical deposition was observed in 14 cases (40%), 1+ in 9 cases (25.7%), and 2+ in 5 cases (14.3%), while C4d immune deposition was not observed in 21 cases (60%). The pattern of deposition was coarsely granular, along the entire capillary circumference. No C4d deposition was observed in the peritubular capillary of renal biopsies (Figure 1).

Clinically, patients with glomerular C4d deposition showed more severe urinary protein excretion than those with glomerular C4d negativity (P=0.001). On the other hand, neither low complement levels nor activity index correlated with glomerular C4d deposition (Table 1). Also, no correlation was found between classes of LN and glomerular C4d intensity (Table 2). However, a positive correlation was found between anti-dsDNA antibody and glomerular C4d (P= 0.048) (Table 1).

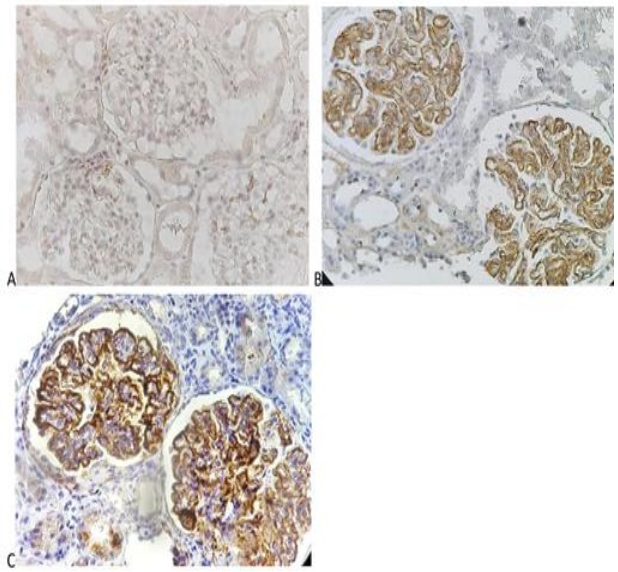

Figure 1. Representative images of C4d intensity scoring in kidney biopsies of lupus nephritis patients. Each score at 4ox magnification. A: score 0, B: score 1 and C: score 2.

Discussion

Biomarkers of renal outcome in LN are needed to predict general flares and to verify treatment response. This study was focused on the impact of glomerular C4d deposition in LN patients. C4d IHC in the current study was applied in a total of 35 kidney biopsy samples.

The glomerular staining of C4d in the total sample size was 40%. Regarding peritubular capillary staining of C4d, this study did not show any PTC deposition in LN renal biopsies which is also found by the study of Yadav *et al.* (2019) [16] and in contrast to the study by Li *et al.* (2007) who included 455 cases in his study only 6.8% patients had peritubular C4d deposition [17]. The reason for this variation is due to the limited number of cases in the current study.

The results of this study did not show a significant correlation between the LN activity index and low complement levels and these findings are in line with those previous studies [10,18-19].

Table 1. Glomerular C4d staining in correlation with the laboratory and pathologic parameters

	Positive	Negative	total	p-value
Activity index (>=12)	4(40%)	6(60%)	10	1.00*
Proteinuria (nephrotic)	8(88.9%)	1(11.1%)	9	0.001#
Low C3	7(38.1%)	14(61.9%)	21	0.324*
Low C4	6(35.3%)	11(64.7%)	17	0.581*
Anti-dsDNA antibody	14(46.7%)	16(53.3%)	30	0.048#

**non-significant, #significant*

Table 2. Glomerular C4d intensity scores for lupus nephritis classes

Pathological class	Glomerular C4d intensity score				
	Score 0	Score 1	Score 2	Total	P-value
Class 2	7	1	0	8	
Class 3	5	2	3	10	
Class 4	8	4	1	13	
Class 5	0	0	1	1	0.135*
Class 6	0	1	0	1	
Class 3+5	1	1	0	2	

*non-significant

It seems possible that these results are due to the immune deposits in the kidney which were responsible for complement cascade activation and further C4d deposition is derived from an in-situ combination of antigen and antibody rather than from circulating complexes. This explanation further supports the association between glomerular C4d deposition and nephrotic proteinuria which was documented by this study and the previous studies by Sahin *et al.* (2013) and Wang *et al.* (2023) [10,20]. Sahin *et al.* (2013) also found an association between activity index score >12 and glomerular C4d positive patients this finding doesn't match the results of the present study and it may be due to the majority of patients in this study having activity scores less than 12.

The current study also found a positive correlation between anti-dsDNA blood levels and glomerular C4d positive staining. These results are similar to the study of Malakoutian *et al.* (2018) [21]. This finding is significant in the respect that anti-dsDNA is one of the LN disease activity biomarkers and indicates classical complement pathway activation.

Conclusions

Glomerular C4d staining of LN correlates with anti-dsDNA antibody positivity and with severe proteinuria but it does not correlate with activity index. Glomerular C4d may aid in categorizing a set of patients with worse prognosis which may help guide treatment decisions for SLE with LN. A prospective study with a large sample size with proper follow-up for understanding the impact of C4d staining on disease severity in LN is recommended.

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