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C4d in lupus nephritis and correlation with clinicopathologic findings

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ABSTRACT

Background: Lupus nephritis (LN) is a serious complication of systemic lupus erythematosus (SLE). Activation of complement system which leads to the production of C4 and its ultimate product, C4d, plays an important role in the pathogenesis of LN.
Objectives: Although serum C4d levels correlate with disease activity, there is almost no study on the correlation between tissue deposition of C4d and classes of LN.
Patients and Methods: Seventy-two patients with a diagnosis of SLE who met ≥ 4 criteria of American Rheumatism Association (ARA) were enrolled in this study. Blood levels of anti-nuclear antigens (ANA), anti-double stranded DNA (Anti-dsDNA), C3, C4 and antiphospholipid antibodies were measured. Renal tissue obtained by biopsy was examined regarding diffuse granular deposition of C4d along the glomerular capillary loops and classes of LN according to the World Health Organization (WHO) classification.
Results: LN class IV was the most prevalent and LN class I had the least prevalence. There was no correlation between positive C4d staining and classes of LN ($P>0.05$), but a significant correlation between positive Anti-dsDNA and C4d positive LN was found ($P=0.05$). Likewise no correlation was detected between the low levels of complements and classes of LN or C4d positivity.
Conclusions: The presence of C4d indicates activation of classical complement pathway in LN. C4d deposition in glomerular capillaries of LN does not indicate the present disease activity but may be a useful marker to predict the prognosis of LN. Anti-dsDNA is a valuable test for disease activity and is correlated with C4d positive staining.

Original Article

Implication for health policy/practice/research/medical education:
In a study on 72 patients with a diagnosis of SLE, we found anti-dsDNA is a valuable test in disease activity and is correlated with C4d positive staining.

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1. Background

Systemic Lupus Erythematosus (SLE) is an autoimmune multisystem disease, mediated by autoantibodies directed against nuclear antigens (ANA) and particularly against DNA (Anti-dsDNA), and immune complexes (1,2). The overall incidence of SLE ranges from 1.8 to 7.6 cases per 100 000 (2).

Antiphospholipid syndrome (APS) is an acquired auto-antibody mediated condition characterized by frequent arterial or venous thrombosis and/or pregnancy morbidity. APS may occur primarily or in association with any other autoimmune diseases. The major auto antibodies detected in these patients are directed against phospholipid (PL) binding plasma proteins, mostly against β2 glycoprotein I (anti-β2GPI), cardiolipin, and prothrombin (1).

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Lupus nephritis (LN) is a serious complication of SLE. Approximately 25% to 50% of lupus patients have clinical renal disease at onset, and as many as 60% of adults with SLE develop renal disease during their course (3).

Complement system has an important role in the pathogenesis of LN (4). In recent years, the potential importance of C4d, an ultimate product of complement C4 activation has been revealed in the pathogenesis of SLE (5). C4d binds to endothelial cell surfaces and the extracellular matrix material of vascular basement membranes (6). C4d deposition on endothelial cells induces insertion of membrane attack complex (MAC), which is followed by Von Willebrand factor release from endothelial cells, and then platelet aggregation (6). The presence of C4d and C1q deposits with C3 deposits in the glomerulus is considered to be the evidence for the activation of the classical pathway (7). In LN, diffuse granular deposition of C4d along the glomerular capillary loops is seen (8).

During past years, the presence of hypocomplementemia and high titer of anti-double stranded DNA (anti-dsDNA) in serum have been used for diagnosis of disease activity in SLE (1). Recently, other markers such as serum and tissue C4d seem to have important roles in the determination of lupus activity (9) and it seems that complement derivative products are more sensitive indicator of disease activity compared to the usual measurement of serum C3 and C4 (10-17).

2. Objectives

Although serum C4d levels correlate with disease activity (9), there is almost no study on the correlation between tissue deposition of C4d and classes of LN.

Moreover C4d is one of the main pathologic parameters to identify antibody-mediated rejection (18) but the importance of deposition of C4d in LN has been remained unclear.

Due to lack of comprehensive study in this regard, this study is designed to determine the incidence of tissue C4d deposition in LN and the importance of its detection in different classes of LN and correlation with clinicopathologic findings. This is important as many of the previously designated laboratory measurements do not accurately disclose this relation.

3. Patients and Methods

3.1. Study population

This study was carried out in the nephrology department of Hasheminejad hospital in Tehran, Iran, from 2012 to 2013All cases were provided written informed consent before study registration. The diagnosis of SLE was established by the presence of certain clinical and laboratory features defined by the 1997 modified American Rheumatism Association (ARA) criteria (2). An increase in serum levels of anti-ds DNA and decrease in serum complement levels was used as an indicator of SLE activity. APS was defined based on laboratory findings including either abnormal anticardiolipin (aCL) Abs (IgM and IgG) and β2GP1 (IgG, IgM) or abnormal antiphospholipid antibodies (aPL antibodies) (IgM and IgG), β2GP1 (IgG, IgM), and low C3 and C4 levels. LN was classified according to the World Health Organization (WHO) into six classes (9) and lesions limited to the renal mesangium (LN II) were considered as less severe, while diffuse disease (LN IV) was a criterion of most severe disease (3).

Estimated glomerular filtration rate (eGFR) was calculated based on creatinine clearance (CrCl) using the Cockcroft and Gault formula (19). The GFR of ≤ 60 mL/min was considered abnormal.

Demographic data, history of arterial or venous thrombosis in both sex, miscarriage and other pregnancy complications in females were recorded.

3.3. Study design

Blood levels of ANA, anti-double stranded DNA (Anti dsDNA), C3, C4, creatinine, aCL, aPL antibodies and anti β2 GP1 were measured and 24 hours urine was collected. For measurement of aCL, aPL antibodies and anti β2 GP1, an AESKULISA ELISA kit was used. According to the kit package insert, a normal range for aCL, aPL antibodies and-anti β2 GP1 were showed in Table 1.

All patients underwent a renal biopsy and two samples were taken. One of the tissue samples was fixed in 10% formalin and then blocks were provided in 2-3 millimeters sections for hematoxylin & eosin (H&E), periodic acid–Schiff (PAS), silver methenamine and Masson's trichrome staining. The prepared sections were examined under light microscopy. The second sample was kept in normal saline and sections were prepared for immunofluorescence microscopic examination in which they were assessed for IgG, IgM, IgA, C1q, C3 and C4. All tissue samples were stained for C4d deposition and reported as C4d positive, if there was diffuse granular deposition of C4d along the glomerular capillary loops and peritubular capillaries C4d deposition, or C4d negative.

3.3. Ethical issues

1) The research followed the tenets of the Declaration of

Table 1. The values for disease defining tests and patient values

	Normal value	Patient % with abnormal levels
aCL IgG	8-12	4 (10%)
aCL IgM	8-12	0 (0%)
Anti-β2GP1 IgG	8-12	1 (2.8%)
Anti-β2GP1 IgM	8-12	1 (2.8%)
C3	0.89-1.87	35 (71%)
C4	0.165-0.38	31 (62%)
ANA	1-12	9 (18.75%)
Anti-dsDNA	8-12	51 (71.70%)

aCL: anticardiolipin; β2GP1: Beta 2 glycoprotein complex 1; ANA: anti-nuclear antibody; Anti-dsDNA: Anti-double stranded DNA.

Helsinki; 2) informed consent was obtained; and 3) This study was approved by the Ethics Committee of Iran University of Medical Sciences (ethical code# 93-02-30-24471). This study was conducted as the nephrology fellowship thesis of Atefeh Amouzegar in Iran University of Medical Sciences, Tehran, Iran.

3.4. Statistical analysis

Statistical analysis was performed using SPSS® 18 Software. Continuous variables in each group of subjects were stated as mean values ± standard deviation (SD). Differences between mean of two groups were done using the unpaired Student's *t* test and Mann–Whitney U-test for variables with normal and non-normal distributions, respectively. Chi-square and Fischer's exact tests to compare dichotomous variable were employed. In all cases, a *P* value of less than 0.05 was considered to be statistically significant.

4. Results

4.1. Patients' characteristics

Seventy-two patients with the diagnosis of SLE who met ≥ 4 criteria of ARA were enrolled in this study (2). All patients were new cases of SLE. Fifty-eight (80.6%) patients were female and 14 (19.4%) patients were male. The mean age of patients was 29.83 ± 10.47 years (mean ± SD), with a mean age of 29.86 ± 11.36 years in females and 30.68 ± 9.48 years in males.

The mean systolic blood pressure was 120.53± 15.88 mm Hg and the mean diastolic pressure was 75± 6.54 mmHg. Twenty-four hours urine collection revealed that all patients had proteinuria. There were 7 miscarriages and 4 preeclampsia and one case of thrombocytopenia but no case of stillbirth in our female patients.

The duration of disease was between 2 months and 20 years with a mean of 29.63 months (2.5 years). The mean plasma creatinine level was 2.51± 2.71 mg/dL, mean body weight (BW) was 64.69± 11.94 kg and mean GFR with the use of C-G formula was 61± 34.54 mm/min. Around 49.2 % had low and 50.8% had normal GFR.

Table 2. Frequency of different classes of lupus nephritis

LN class	Number of patients	Percent
LN 1	0	0
LN 2	4	15.6%
LN 3	13	18%
LN 4	42	58.3%
LN 5	12	16.7%
LN 6	1	3.4%
Total	72	100

LN: lupus nephritis.

The incidence of APS was 20% (CI; 95%). In this study all 72 patients underwent renal biopsy and LN was categorized for them according to the WHO classification. C4d staining was successfully done in 47 patients. Twenty-seven cases (57.4%) had positive C4d staining and 20 cases (42.6%) had negative results. No glomerular microthrombosis (GMT) was reported in biopsy samples.

LN class 4 was the most prevalent which was seen in 42 patients (58.3%) and LN class 1 had the least prevalence. (Table 2). There was no correlation between C4d positive LN and different classes of LN (CI; 95%). We did not find any positive correlation between positive ANA or anti-dsDNA tests and classes of LN ($P > 0.05$). However, we found a significant correlation between positive anti dsDNA and C4d positive LN ($P = 0.05$). There was also no positive relation between APS and positive C4d staining ($P = 0.47$).

Likewise we did not find any correlation between the low levels of complements and classes of LN or C4d positivity ($P > 0.05$).

5. Discussion

We observed a positive correlation between anti-dsDNA positivity, which is one of the parameters of LN activity and C4d positive staining. This finding shows that activation of the classical pathway of complement has a crucial pathogenic role in activation or flare of LN.

In the group of patients enrolled in this study we did not find any correlation between the intensity of C4d staining and different classes of LN.

In the normal kidney, the glomerular mesangium and GBM are stained for C4d in a granular and segmental pattern and the wall of some renal arterioles stain in a linear pattern (20). However, in LN, diffuse granular deposition of C4d along the glomerular capillary loops

and peritubular capillaries are seen (8). The presence of C4d and C1q deposits in combination with C3 deposits in the glomerulus is considered to be the evidence for the activation of the classical pathway which has an important role in the pathogenesis of LN (4,7). In the current study, the validity of C4d staining by immunofluorescence method was evaluated as a useful marker of different classes of LN. Several studies have assessed the correlation between C4d staining and LN activity (18-21).

In a study by Shen et al the intensity of glomerular C4d staining was strongly associated with the presence of IgG anti-b2GPI antibodies (22), however we did not find any correlation between anti β2GP1 and C4d staining that could be due to small sample size.

In a study by Senaldi et al no correlation was found between reduced C4 levels and degrees of disease activity (9). We also did not find any correlation between low complements level and classes of LN and C4d staining. Kim and Jeong et al studied glomerular C4d deposition in 21 cases diagnosed as LN. They found a correlation between the intensity of C4d with those of capillary IgG, IgA, C4, C1q, and fibrinogen but C4d staining intensity did not correlate with the LN activity index (8). We also did not find any correlation between the intensity of C4d staining and classes of LN. Furthermore, Sahin et al observed a relationship between glomerular C4d staining and activity of LN (22). We also found a positive correlation between anti-dsDNA positivity, which is one of the parameters of LN activity and C4d positive staining but we did not find any correlation between C4d Positivity with classes of LN.

In two studies conducted by Bheemavathi et al – which evaluated the patterns of glomerular C4d deposition in patients with LN – and Kim and Jeong for the validity of C4d staining as a marker of LN activity, they did not find peritubular capillaries deposition of C4d (8,18). We also did not find peritubular capillaries C4d deposition in our biopsy samples.

In the same study by Kim and Jeong, they found that C4d deposition could be a more sensitive marker of classical complement pathway activation but not a useful marker of disease activity (8).

6. Conclusions

C4d positive indicates activation of classical complement pathway in LN. C4d deposition in glomerular capillaries of LN does not indicate the present disease activity but may be a useful marker to predict the prognosis of LN. Anti-dsDNA is a valuable test for disease activity and is correlated with C4d positive staining.

Limitations of the study

It would be more valuable to have a proper follow up to find valid associations between disease activity and C4d deposition. Moreover adequate sample size is required.

Authors' contribution

TM: Conception, study design, literature review, final approval of manuscript; AA: Acquisition of data, literature review, data analysis, interpretation of data, drafting the article; MA: Acquisition of data; TS: Acquisition of data.

Conflicts of interest

All authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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