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The Differences of Serum Complements and Anti-dsDNA Levels Between Renal and Non-renal Manifestations in Systemic Lupus Erythematosus

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ABSTRACT

Background. Systemic lupus erythematosus (SLE) has diverse clinical manifestations, including renal and non-renal. Renal manifestation is related to significant morbidity and mortality. SLE is also characterized by serological aberrations, including decrease levels of complement 3, complement 4 and increase levels of anti-dsDNA, but the association of them with clinical manifestations including renal and non-renal is unclear. This study investigated the associations of complement 3, complement 4 and anti-dsDNA levels with renal and non-renal manifestations in SLE patients. **Method.** A cross-sectional study was conducted in the Polyclinic of Rheumatology, Dr. Saiful Anwar Hospital Malang. A number of 43 subjects fulfilled the 1997 American College of Rheumatology criteria participated in this study that consisted of 11 patients with renal manifestation and 32 patients with non-renal manifestations. Serum complement 3 and complement 4 levels were measured using immunoturbidimetry, and serum anti-dsDNA levels were measured using enzyme-linked immunosorbent assays (ELISA). The independent T-test was used to compare complement 3 levels and the Mann-Whitney U test was used to compare complement 4 and anti-dsDNA levels between groups. **Result**. SLE with renal manifestation had significant lower levels of serum complement 3 compare to non-renal manifestations (mean ± SD: 71.27 ± 32.65 mg/dL and 94.47 ± 26.29 mg/dL respectively, p=0.022). SLE with renal manifestation also had significantly lower levels of serum complement 4 compare to non-renal manifestations (mean ± SD: 14.55 ± 8.20 mg/dL and 25.50 ± 11.05 mg/dL respectively, p=0.002). Conversely, SLE with renal manifestation had significantly higher levels of serum anti-dsDNA compare to non-renal manifestations (mean ± SD: 249.27 ± 240.34 IU/mL and 109.91 ± 166.11 IU/mL respectively, p=0.014). **Conclusion.** SLE patients with renal manifestation have significantly lower levels of serum complement 3 and complement 4 and a higher level of serum anti-dsDNA than SLE patients with non-renal manifestations.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disorder with broad spectrum clinical manifestations in almost all organs.[1] Renal manifestation is an important predictor of poor outcome and is regarded as one of the most severe SLE manifestations and may have devastating consequences at any age.[2-5] Higher rates of renal manifestation, one of main systems involved in death, were occurred more in Asians than in whites, with 21–65% occurred at diagnosis and 40–82% all over time.[6,7] The mortality risk increased with longer disease duration.[8]

Renal manifestation of SLE, also named as lupus nephritis, is an immune complex (IC) glomerulonephritis. ICs and complement activation mediates the function of immune effector and leads to tissue injury. Failure in immune complex cleaning results in tissue deposition and tissue injury.[1] Large aggregates and insoluble ICs are cleared by phagocyte system in the liver and spleen. Tissue deposition of

soluble ICs is influenced by systemic factors, their physiochemical properties and hemodynamics of tissue.[4]

Increased titers of anti-dsDNA, along with hypocomplementemia, was associated with the disease activity, but there were only a few data about its association with renal manifestation.[5] The involvement of the complement system in autoimmune diseases is well known but its mechanism is not clear whether it is a cause or a consequence of autoimmune diseases.[6] Deficiencies in classical pathway complement components predisposed patients to SLE and activation of complement by ICs is proven in SLE.[6,9] Complement 3 and complement 4 levels reflect the circulating complements.[8] Studies to determine whether complement 3, complement 4 and anti-dsDNA serum levels reflect renal manifestation had conflicting results. In this study, we investigated the association of serum complement 3, complement 4 and anti-dsDNA levels with renal and non-renal manifestations of SLE.

2. Research Methods

Patients

Forty-three patients were included in this cross-sectional study during the period May 2013 to May 2015 at the Polyclinic of Rheumatology at Dr. Saiful Anwar Hospital Malang, Indonesia. All the patients fulfilled at least four of 11 American College of Rheumatology (ACR) 1997 SLE criteria (malar rash, discoid rashes, photosensitivity, oral ulcers, non-erosive arthritis, pleuritis or pericarditis, renal disorders, neurologic disorders, hematologic disorders, immunologic disorders and positive antinuclear antibody).[10] This study excluded subjects suffering from other autoimmune diseases, severe infection/sepsis, chronic infection, hypertension and diabetes mellitus. This research was approved by the Ethical Committee of Dr. Saiful Anwar Hospital.

Clinical Measurement

Renal manifestation is defined as evidence of lupus nephritis (renal disorder in the ACR 1997 SLE criteria list). Lupus nephritis is clinical and laboratory manifestations that meet ACR 1997 criteria specifically for renal, which includes: persistent proteinuria >0.5 g per day or greater than 3+ by dipstick and/or cellular casts including red cell, hemoglobin, granular, tubular or mixed (10); and includes also the criteria from review of the ACR 1997, which a spot urine creatinine/protein ratio >0.5 can substitute the 24 hour protein measurement, and active urinary sediment (>5 RBC/hpf, >5 WBC/hpf in the absence of infection, or cellular casts limited to RBC or WBC casts) can substitute cellular casts.[7] Non-renal manifestations are all other criteria in ACR 1997 except for renal disorders.

Laboratory Measurement

Complements

Measurement of serum complement 3 and complement 4 levels was performed using immunoturbidimetry (reagents from Abbott®, catalog number: 9D96-21 and 9D97-21, respectively). Complement 3 or complement 4 in the sample were combined with antibodies to complement 3 or complement 4 to form immune complexes. These complexes increased the intensity of light scatter in the reaction cuvette. The turbidimeter monitored the change in absorbance at 340 nanometers which was proportional to the concentrations of complement 3 or complement 4. Complement 3 or complement 4 concentrations were automatically calculated from the calibration curves. The normal range for serum complement 4 is 10-40 mg/dl. The normal range for serum complement 3 is 85-160 mg/dl for person aged 12-18 years old, 82-160 mg/dl for person aged 20s years old, 84-160 mg/dl for person aged 30s years old and 90-170 mg/dl for person aged 40-70 years old.

Anti-dsDNA

Measurement of quantitative serum IgG anti-dsDNA levels was performed using ELISA test system (kit from EUROIMMUN®, catalog number: EA 1572-9601 G). The test was based on an indirect enzyme linked immune reaction. Antibodies in the serum bound to the antigen coated on the reaction wells. After incubation, a washing removed unbound and unspecifically bound

components. Then, enzyme was conjugated to the antibody-antigen-complexes. After incubation, a second washing removed unbound enzyme. Substrate solution hydrolysed the substrate generating a blue coloured product. An acid stopped the reaction generating a yellow end-product. The intensity of the yellow color measured photometrically at 450 nm correlated with the concentration of the antibody-antigen-complex. Serum anti-dsDNA titer <100 IU/mL is considered as negative and ≥100 IU/mL is positive.

Other Laboratory Measurements

Routine laboratory tests from the SLE patients included complete blood count (flowcytometry), urinalysis (dipstick and microscopic), and plasma ureum and creatinine (spectrophotometry).

Statistical Methods

To compare between groups, we used T-test for normal distributed data and Mann-Whitney U test for abnormal distributed data. Independent T-test was used to compare complement 3 levels between renal and non-renal manifestation of SLE. Mann-Whitney U test was used to compare C4 and anti-dsDNA levels between renal and non-renal manifestations of SLE. Continuous variables were expressed as mean ± standard deviation. All *p* values were two-tailed and differences at ≤0.05 were considered significant. Statistical analysis was performed using SPSS 16.0.

3. Results

A total of 43 female patients with SLE were enrolled in this study with mean age of 34.35 ± 10.35 years old, 11 patients (25.58%) with renal manifestation and 32 patients (74.42%) with non-renal manifestations. Non-renal SLE subjects consisted of 21 patients (48.84%) with musculoskeletal disorders, 28 patients (65.12%) with mucocutaneous disorders, three patients (6.98%) with neurological disorders and 28 patients (65.12%) with hematologic disorders. Baseline patient characteristics of the two groups, renal and non-renal manifestations of SLE are summarized in **Table 1.** Hemoglobin levels in patients with renal manifestation were significantly lower than those in patients with non-renal manifestations. Other parameters were similar in both groups.

The mean of serum complement 3 levels was normal in patients with non-renal manifestations, but low in renal manifestation **(Figure 1A).** The mean of serum complement 4 levels was normal in patients with non-renal and renal manifestations, but profoundly lower in patients with renal than non-renal manifestations **(Figure 1B).** There was no patient had decreased serum complement 4 without decreased serum complement 3. The mean of serum anti-dsDNA levels in patients with renal and non-renal manifestations was positive (>100 IU/mL), but it was significantly higher in patients with renal than non-renal manifestations **(Figure 1C).** The findings are summarized in **Table 2.**

Table 1. Baseline patient characteristics.

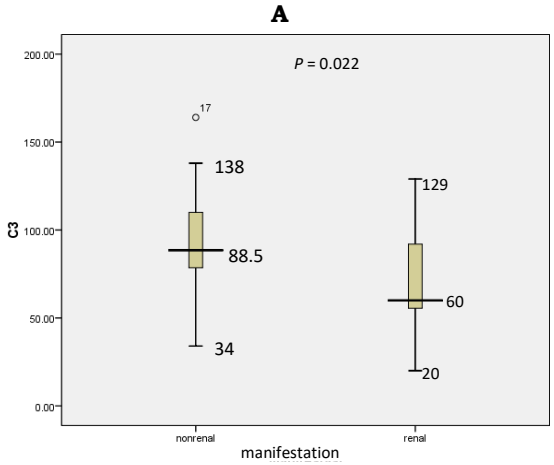
Patient characteristics	**Renal (n=11)**	**Non-renal (n=32)**	***p*-value**
Age in years; mean (± SD)	31.00 ± 5.53	35.53 ± 11.38	0.091
Race; n (%)			
Asian	11 (100)	32 (100)	
Ethnicity; n (%)			
Javanese	11 (100)	32 (100)	
Disease duration in months	24 (12 - 38)	26.35 ± 13.37	0.561
Blood pressure:			
Systole in mmHg	117.10 (100 - 160)	117.10 ± 12.49	0.670
Diastole in mmHg	77.00 ± 13.89	74.19 ± 8.72	0.436
Hemoglobin (g/dL)	10.75 ± 2.50	13.18 (11.3 - 16.5)	0.005
Lymphocyte (/μL)	1.324 ± 720	1.575 ± 1.008	0.750

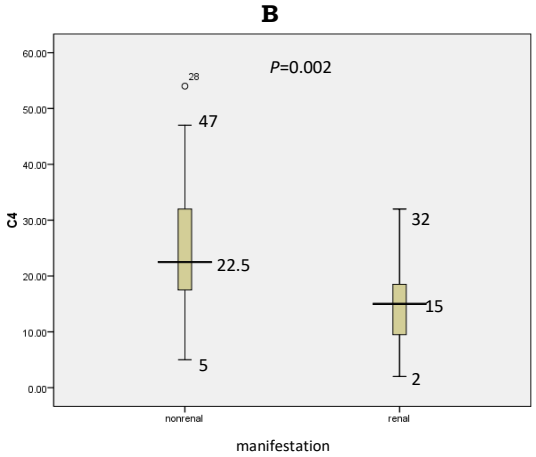
Values are the mean (±SD), median (25% quartile-75% quartile), or number (%) of patients.

Table 2. The levels of serum complement 3, complement 4 and anti-dsDNA in patients with renal and non-renal manifestations.

Parameters	Renal	Non-renal	*p*-value
Complement 3 in mg/dL; mean (± SD)	71.27 ± 32.65	94.47 ± 26.29	0.022
Complement 4 in mg/dL; mean (± SD)	14.55 ± 8.20	25.50 ± 11.05	0.002
Anti-dsDNA in IU/mL; mean (± SD)	242.7 ± 240.3	109.9 ± 166.11	0.014
Negative anti-dsDNA; n (%)	4 (3.36)	21 (65.62)	
Normal complement 3/complement 4; n (%)	3 (27.7)	17 (3.12)	
Low complement 3/normal complement 4; n (%)	5 (45.45)	14 (43.75)	
Normal complement 3/low complement 4; n (%)	-	-	
Low complement 3/complement 4; n (%)	3 (27.27)	1 (3.12)	

Values are the mean (± SD), median (25% quartile-75% quartile), or number (%) of patients.





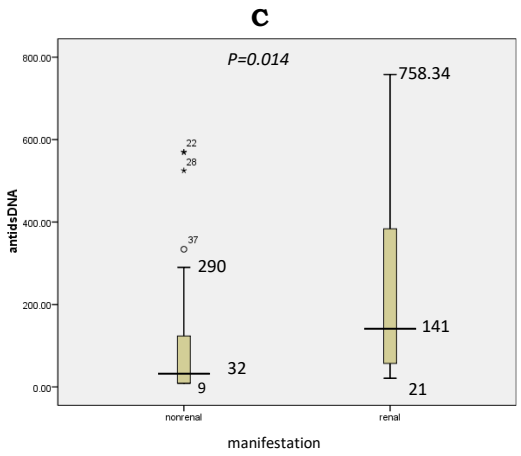


Figure 1. A) Serum complement 3 levels in patients with renal and non-renal manifestations. B) Serum complement 4 levels in patients with renal and non-renal manifestations. C) Serum anti-dsDNA levels in patients with renal and non-renal manifestations.

4. DISCUSSION

The results of this study indicate that the levels of serum complement 3 and complement 4 were significantly lower in SLE patients with renal manifestation compared to non-renal manifestations and show insights into the role of complements in renal manifestation of SLE. Immune complex (ICs)-mediated activation of complements in lupus nephritis is shown in experimental and study in human with SLE.[6] Increased accumulation of soluble ICs in renal manifestation was influenced highly by plasma filtration in glomerular capillaries,[11] fixed negative charges on the filtration barrier and physiochemical properties of ICs (charge, valence, size, antibody affinity, immunoglobulin class).[4] Interactions between fixed anionic sites and ICs could be an important factor in glomerular binding. Julkunen found that active nephritis was correlated significantly with low levels of complement 3 and complement 4.[5] Jacob demonstrated that the degree of renal pathology was consequent to the absence of glomerular complement 3 deposition.[12] Our study showed that not all patients with renal manifestation had low serum complement 3 and complement 4 levels, it might be due to the optimal treatment they received.

Haemoglobin levels in the group of patients with renal manifestation were significantly lower than those without renal manifestation. This could be caused by the effect of decreased kidney function on the synthesis of hemoglobin or by SLE itself due to anemia of chronic disease or hemolysis, but it was not examined further in this study. Many studies showed that complements played role in autoimmune hemolytic anemia (AIHA) in SLE.[13] In SLE patients with AIHA, CD55 and CD59, proteins play role as protection against complement-induced cell lysis on erythrocytes, were underexpressed.[14,15] Complement receptor 1 (CR1), a regulatory protein of complement, were also found lost on erythrocytes of SLE patients.[16,17] Moreover, complement-dependent autoantibodies were found to suppress bone marrow progenitor cells in SLE patients with aplastic anemia.[18-20] Study in animal model had the same result, that NZB lupus prone mice produced anti-erythrocyte autoantibodies.[21]

In this study, serum complement 3 levels were profoundly lower than complement 4 in patients with renal manifestations, suggested that the damage manifested as a renal disorder involved amplified complement 3 activation, and it mean predominantly the alternative pathway.[22] There are several explanations for this. complement 3 might be directly activated by urine pH or ammonia released from stressed epithelial cells or by convertase-like enzymes in the apical brush border of proximal tubule which deficient in complement regulatory proteins.[23-25] Disease severity is lower when an intact complement system is absent.[26] A different result from a study by Hussain in 2008, demonstrated that complement 4 was

depleted more than complement 3. They found that complement 4 levels were low in most of the lupus nephritis patients. Low complement 4 levels may be falsely regarded as partial defects in complement 4A or/and complement 4B which reduced total levels of complement 4. Decreased synthesis or increased catabolism of complement 4 without complement activation may also explain low complement 4 levels.[8] Other study found that low levels of complement 3 and complement 4 were specific for lupus nephritis, but had low sensitivity.[26]

This study also found that serum anti-dsDNA levels were elevated in patients with renal and non-renal manifestations. However, serum anti-dsDNA levels in renal manifestation were significantly higher than those of non-renal manifestations. Increased anti-dsDNA levels had been shown by many, but not all, studies to be the predictors of disease flares in SLE. In some cohorts, serum anti-dsDNA levels were correlated to nephritis with progression to end-stage renal disease.[27] Patients with lupus nephritis usually have antibodies against dsDNA and high avidity anti-DNA that activates complements strongly. High avidity anti-DNA also occurs in proliferative more than membranous lupus nephritis.[26]

In this study, 36.36% of patients with renal manifestation had negative serum anti-dsDNA. The possible cause is a delayed response in the early stages of renal manifestation.[28] Another possibility is the design of assay had significant influence on anti-dsDNA type, because of different nature and ability to detect subtypes of anti-dsDNA with distinct avidity. At least three factors are contributing to the pathogenicity of anti-dsDNA: the avidity, the cross-reactivity with alpha-actinin in renal and the specificity for individual DNA molecules.[28,29] Human IgG consists of four subclasses: IgG1, IgG2, IgG3 and IgG4, each has a different heavy chain.[30] Baudino found that IgG3 subclass of anti-dsDNA was highly pathogenic and induced lupus-like nephritis.[31] Although the occurrence of anti-dsDNA subtypes with difference pathogenicity is widely accepted, the mechanisms and conditions leading to a dominant synthesis of a subtype are still unknown.[28] Our findings may suggest that renal and non-renal SLE have difference pathomechanism, whereas complements activation and anti-dsDNA play a more prominent role in renal SLE than in non-renal SLE. Further understanding of the role of complements in renal manifestation requires a multivariate approach, with additional complement-related variables.

5. CONCLUSION

All cases of ASD in this study are multifactorial, in line with the theory. Both genetic and environmental factors related to the incidence of ASD. Further studies to analyze which risk factors play the most important part is needed.

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