Prevalence of anti–C-reactive protein autoantibody and its correlation with disease activity in systemic lupus erythematosus patients at Cipto Mangunkusumo General Hospital

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease with various underlying mechanisms characterized by autoantibody overproduction. It has been known that mortality and morbidity of SLE was higher in Asian patients compared with white patients. Several studies had showed that C-reactive protein (CRP) has the ability to suspend the progression of SLE through regulatory and clearance pathway, and low level of CRP and high level of anti-CRP antibody has been detected in SLE patients. A question raise whether mortality and morbidity in Asian SLE patients are associated with anti-CRP antibody.

Objective: To study the prevalence of anti-CRP antibody and its relationship with disease activity in SLE patients at Cipto Mangunkusumo General Hospital, Jakarta.

Methods: This is a cross-sectional study conducted at Cipto Mangunkusumo General Hospital from December 2009 until May 2010. Subjects were SLE patients who were diagnosed based on the 1982 American College of Rheumatology criteria. Disease activity was measured using the Mexican SLE Disease Activity Index scoring system. Anti-CRP antibody assay was performed using the Western blot analysis. Correlation between the presence of anti-CRP antibody and disease activity was evaluated using the T-test and multivariate logistic regression analysis.

Result: Forty SLE patients with a mean age of 31.65 (SD 8.84) were enrolled in the study, 33 of which (82.5%) had positive autoantibody to CRP pentamer. The anti-CRP antibody was significantly correlated ($p = 0.024$) with disease activity.

Conclusions: There was a relatively large proportion of patients with positive anti-CRP antibody among SLE patients in Cipto Mangunkusumo General Hospital. There was also a significant correlation between anti-CRP antibody and the disease activity.

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by autoantibody overproduction, with a very wide clinical symptoms and various etiopathology that are yet to be discovered.[1,2] Many interesting researches have been done to investigate the mechanism that lies underneath this unique and dynamic disease. We now know several autoantibodies involved in this disease, including autoantibody against nuclear antigens, circulating immune complex, activated complement, and extracelullar antigens (including plasma protein such as C-reactive protein (CRP) and sphingolipid activator protein).

The prevalence of SLE is 3 times higher in Asian compared with white population.[3,4] A study that reviewed medical journals published between 1950–2006 found a wide variations of incidence and prevalence of SLE in many countries. This variation described the differences among different populations, geography, race, and time. It also provide important clues concerning the etiology of this disease,[5] and suggest the possibility of a specific SLE etiopathology in Asian race.

There are many factors that contribute to the pathogenesis of SLE, including genetic, sex, environmental exposure, certain diseases, disorder of the immune system, and autoantibody formation.[4] Recent studies revealed that besides the common autoantibodies in SLE patients, antibody to C-reactive protein also played some roles in the pathogenesis of SLE. C-reactive protein is believed to possess the ability to limit SLE disease progression through clearance and regulatory pathways.

There have been several researches, with varying results, regarding the presence of anti-CRP antibody and its relationship with disease activity. Bell et al[6] in 1998 reported no relationship between anti-CRP antibody and disease activity. However, Sjowall et al[7] in 2004 reported contrasting results. Regarding this fact, a question raises whether any relationship exist between higher mortality and morbidity in Asian SLE patients and formation of anti-CRP antibody. Fischbacher et al[8] in 2003 had found that autoantibody formation was higher in Asian patients in comparison with white patients (13.5 g/dL vs. 7.4 g/dL, respectively).

Based on those findings, we would like to investigate the prevelance of antibody to CRP among SLE patients in Cipto Mangunkusumo General Hospital and its association with disease activity.

METHODS

Study design

This is a cross-sectional study conducted at rheumatology, hematology and allergy-immunology clinic at Cipto Mangunkusumo General Hospital, Jakarta from December 2009 until February 2010. The sample size was calculated using formula for proportion estimation of a population and coefficient correlation for single sample. The dependent variable in this study was the positivity of anti-CRP antibody, while the independent variable was disease activity according to Mexican Systemic Lupus Erythematosus Disease Activity Index (Mex-SLEDAI) scoring system. Guzman et al[9] in a study that compared three clinical indices (Lupus Activity Criteria Count, SLE Disease Activity Index (SLEDAI), and Mex-SLEDAI) in measuring disease activity in SLE patients found that all those indices showed significant association with experts' judgment, managing physician's opinion, changes in treatment and clinical course, with good convergent validity ($rs = 0.76$ to 0.79, $p<0.0001$), and responsiveness. The study also concluded that Mex-SLEDAI was the least expensive instrument.

Subjects

Inclusion criterion were native Indonesian patients who fulfilled the 1982 revised American College of Rheumatology criteria for SLE. The study protocol had been approved by the appropriate local ethical board. All patients were then asked to give informed consent.

Measurement

Blood and urine sample collection was performed to acquire necessary data to measure disease activity according to Mex-SLEDAI (complete blood count, differential count, reticulocyte count, serum creatinine, creatine kinase, urinalysis, and 24-hour quantitative protein) as well as level of CRP and anti-CRP antibody. Anti-CRP antibody was bound with alkaline-phosphatase-conjugated anti-human IgG (Dako, Glostrup, Denmark). Human CRP (Sigma Chemical Co., St. Louis, USA) was used for negative marker. We also examined factors contributing to the increase in IgG autoantibody: statin and/or steroid use, smoking, alcohol consumption, body mass index, duration of illness, ethnicity, and number of involved organ/organ system.

Anti-CRP autoantibody assay

Anti-CRP autoantibody was quantified using the mini Westerblot assay. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) procedure was carried out by making 5 mL of 8% separating gel, which was left overnight at room temperature, followed by preparation of 2 mL of 5% stacking gel. After 45 minutes, CRP + reducing sample buffer (RSB) (each contain 10 µg of CRP) and marker were poured in each appropriate wells. Running the SDS-PAGE procedure until the protein move at the ± 2 mm bottom glass plate. And then run the transfer procedure using polyvinylidene fluoride (PVDF) and filter paper with voltage set for about 1.5 hours in 150 mV. After the transfer process was done, the PVDF paper was blocked by 5% of phosphate-buffered saline with skim milk and was stored overnight at temperature of 4°C. In the following day, the PVDF paper was washed three times with phosphate-buffered saline Tween 20 (PBST) and was cut into 10 lanes/well. Each lane was mixed with appropriate sample serum at a concentration of 1:500 and was stirred for about 1 hour at room temperature. The PVDF was then washed with PBST for three times, each take as long as 5 minutes. Each PVDF was added with secondary autoantibody at a concentration of 1:2000 and left for 1 hour at room temperature, then washed three times with PBST. PVDF will be set in the hypercassete and added with electrochemiluminescence solution and then exposed in the dark room to get the result.

Statisitical analysis

In this study we analyzed the correlation of demographic and clinical characteristics with the positivity of anti-CRP antibody. We used bivariate analysis for correlation test using unpaired T-test (or Fischer's exact test for abnormally distributed data), then continued with multivariate logistic regression analysis.

RESULTS

A total of 40 SLE patients were recruited, with the following characteristics: all were women, ranging from 20 to 57 years old; 60% had medium educational level, 57.5% were married, 47.5% worked as homemakers, 60% belonged to the low income level group, and the majority (42.5%) were Javanese. The demographic and clinical characteristics of the patients are elaborated in table 1 and 2.

Table 1 Demographic characteristics of patients (N = 40)

Characteristics	n (%)*
Sex	
Male	0 (0)
Female	40 (100.0)
Age, years, mean (SD)	31.65 (8.84)
13–45 years old	35 (87.5)
>45 years old	5 (12.5)
Educational level	
Low (none–elementary school)	2 (5.0)
Medium (junior high school–senior high school)	24 (60.0)
High (diploma, bachelor, master degree, or higher)	14 (35.0)
Marital status	
Single	17 (42.5)
Married	23 (57.5)
Job	
Homemaker	19 (47.5)
Civil servant	4 (10.0)
Entrepreneur	4 (10.0)
Student	3 (7.5)
Employee	4 (10.0)
Unemployed	6 (15.0)
Income level	
Low (<1 million rupiah/month)	24 (60.0)
Middle (1–5 million rupiah/month)	13 (32.5)
Middle–high (>5 million rupiah/month)	3 (7.5)
Ethnicity	
Javanese	17 (42.5)
Sundanese	5 (12.5)
Betawi	10 (25.0)
Batak	4 (10.0)
Minang	3 (7.5)
Lampung	1 (2.5)

*Unless otherwise specified.

Table 2 Clinical characteristics of patients (N = 40)

Characteristics	n (%)*
Anti–C-reactive protein	
Positive	33 (8.25)
Negative	7 (17.5)
Mex-SLEDAI score	
<5	11 (27.5)
≥5	29 (72.5)
CRP level, mg	
<5	31 (77.5)
≥5	9 (22.5)
Steroid (prednisone-equivalent) dose, mg/day	
<11	28 (70)
11–40	11 (27.5)
41–100	1 (2.5)
Body mass index, mean (SD)	22.32 (4.39)
Alcohol consumption**	0 (0)
Cigarette smoking	1 (2.5)
Statin use	4 (10.0)
Renal disorder	24 (60)
Number of involved organ/organ system, mean (SD)	3.4 (1.39)

*Unless otherwise specified.
**Defined as alcohol consumption of >2 servings/day (male) or >1 serving/day (female).
Mex-SLEDAI, Mexican Systemic Lupus Erythematosus Disease Activity Index.

Anti-CRP antibody was found in 33 out of 40 patients (82.5%). Mex-SLEDAI score ranged from 0 to 22, with median value of 6. The mean number of involved organ/organ system was 3.40 with standard deviation of 1.39. Arthritis is the most common (62.5%) organ involvement, followed by mucocutaneous disorder (42.5%), fever (35%), hemolysis (25%), lymphopenia (25%), renal disorder (22.5%), serositis (15%), and vasculitis (2.5%). This study has a sensitivity and specificity of 91% and 100%, respectively.

Statistical analysis showed that positivity of anti-CRP antibody had significant correlation with the disease activity, but it had no significant correlation with most of the other variables (the demographic and clinical characteristics), except for two variables: renal disorder and number of involved organ/organ system (table 3). On subsequent multivariate analysis of these 3 variables we found that the Mex-SLEDAI score had significant correlation with anti-CRP (OR = 0.739, 95% CI 0.557–0.981) (table 4).

Table 3 Bivariate analysis of several clinical characteristics with anti–C-reactive protein (anti-CRP) antibody

	Anti-CRP		
Variables	Positive	Negative	p Value
Mex-SLEDAI score, mean (SD)	8.33 (4.88)	3.57 (4.79)	**0.024**
CRP level, n (%)			
<5 mg	26 (83.9)	5 (16.1)	0.645*
≥5 mg	7 (77.8)	2 (22.2)	
Renal disorder, n (%)			
Yes	22 (91.7)	2 (8.3)	**0.094***
No	11 (68.8)	5 (31.3)	
Number of involved organ/ organ system, mean (SD)	3.52 (1.48)	2.86 (0.69)	**0.088**

Bold indicates significance.
*Data have abnormal distribution; statistical analysis was performed using Fischer's exact test.
Mex-SLEDAI, Mexican Systemic Lupus Erythematosus Disease Activity Index.

Table 4 Multivariate analysis of several variables with significant correlation with anti–C-reactive protein

Variables	OR (95% CI)	p Value
Mex-SLEDAI score	0.739 (0.557–0.981)	**0.036**
Renal disorder	0.912 (0.449–1.852)	0.799
Number of involved organ	2.156 (0.290–16.019)	0.453

Bold indicates significance.
Mex-SLEDAI, Mexican systemic lupus erythematosus disease activity index.

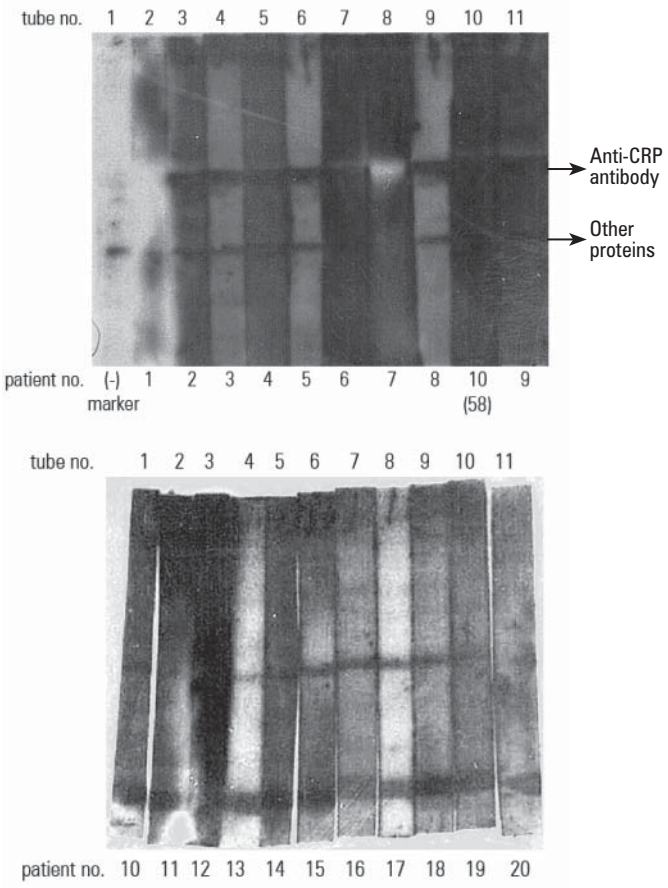

Figure 1 Result of the Western blot assay to detect anti–C-reactive protein antibody. There were two bands of protein, each containing protein with molecular weight ranging from 96 to 123 kDa and 40 to 60 kDa, respectively (molecular markers were not shown). Anti-CRP antibody, which has a molecular weight of 105 kDa, was contained in the first band.

DISCUSSION

It is already known that SLE has higher incidence and prevalence among Asian patients, with higher morbidity and mortality.[7,10,11] Related to the role of anti-CRP antibody in SLE, in this study we aimed to investigate the prevalence of anti-CRP antibody and its correlation with disease activity in Indonesian SLE patients at Cipto Mangunkusumo General Hospital, Jakarta. Because this study only included native Indonesian patient, we hope to be able to represent the diversity of different ethnicity in Indonesia.

Subjects in this study had a mean age of 31.65, with standard deviation of 8.84. This result differs from the result of a study by Juariah[12] conducted at rheumatology outpatient

clinic at Cipto Mangunkusumo General Hospital in 2008, which reported a mean age of 27.48. This discrepancy may be due to the relatively small sample size of this study. However, the mean age of patients in this study is relatively similar to those by Ndiaye et al[13] in Dakar (mean age 34 years old) and Doria et al[14] in Italy (mean age 36 years old). These findings emphasize the importance and influence of factors such as geography, methods and aim of the study, and genetics of the subjects on the result of a study.

We attempted to study the correlation of the demographic and clinical characteristics of our patients with the positivity of anti-CRP antibody; however, the statistical analysis revealed no significant correlation. This may be due to the fact that this study was conducted in one hospital with limited samples. A larger sample size may be needed for those variables to show a more significant correlation. Further studies should also take into consideration the fact that Indonesia consists of various ethnicity with very wide genetic difference.

In this study we used the Western blot analysis to measure anti-CRP antibody. The decision to use this qualitative method instead of the ideal enzyme-linked immunosorbent assay (ELISA) was made after previous attempts to use the method had failed due to optimization failure. The kappa value of the Western blot analysis was 0.7.

From the Western blot analysis we obtained two bands of proteins (figure 1). One contained molecules with weight ranging from 96 to 123 kDa. The anti-CRP antibody, which has a molecular weight of 105 kDa, was contained in this band. The other protein band contained molecules with weight ranging from 40 to 60 kDa. Autoantibodies to protein kinase-C, guanine nucleotide-binding protein Gi3, insulin-like growth factor–binding protein, actin-protein, ovalbumin, bovine serum albumin, dan Hsp60 are molecules that may be contained in this band.[15] The emergence of the latter protein band may result from the impurity of the reagents or the low specificity of the IgG reagents used for the detection of anti-CRP antibody.

There was a relatively larger proportion (82.5%) of positive anti-CRP antibody compared with a previous study by Sjowal et al[16] who obtained a proportion of 40% (out of 10 subjects). The disparity may be explained by the different technique of anti-CRP measurement. Sjowal et al in his study used ELISA technique with normal control, while in this study we only utilized the qualitative Western blot analysis. Besides, the difference in sample size may also play a role.

The proportion of positive anti-CRP in this study is relatively similar to the study by Bell et al[7], which showed 78% positive anti-CRP. The study also detailed that they found autoantibody to m-CRP. CRP has two different conformational form: native CRP and the denaturated form called modified CRP (m-CRP). Hee Gu Lee et al[17] conducted a research in the purification of CRP and found that pentamer CRP (naive CRP) had molecular weight of 118 kDa, and the sub unit (m-CRP) had molecular weight of 23.6 kDa. We reviewed several studies and found that molecular weight of pentamer CRP varies between 105 and 123 kDa.[6,15,17] In this study we found IgG autoantibody formation to pentamer (native) CRP.

The mechanism of anti-CRP antibody formation in SLE is yet to be fully inderstood. Abnormality of innate and adaptive immune system may explain this phenomenon. Immune system imbalance in SLE is marked by an increase in B lymphocyte activity, which results in formation of a large number of autoantibodies, and decreased in vitro and in vivo cellular immune response. Dysfunction of T-helper lymphocytes and antigen-presenting cells could also play a role. Interleukin (IL)-10 is the most potent inductor of B lymphocyte differentiation and regulator of T-helper lymphocyte; thus overproduction of IL-10 or immune cells hypersensitivity to this cytokine may result in immune system imbalance. Recent studies have also found increased production of IL-10 by peripheral blood mononuclear cells (PBMC), which spontaneously release a large number of IL-10 in untreated patients. It was also stated that IL-6 plays an important role in the induction of autoimmunity in SLE, and at least half of the autoantibody production depends on IL-6.[18]

Another theory stated that SLE patient have low level of regulatory T cells that express CD4, IL-2 receptor chain α (CD25), and CD45RO. Barreto et al[19] found that there were decreased number of CD4+CD25+CD45RO+ T cells in SLE patient due to defect in conversion of FOXP3+CD25- to FOXP3+CD25+ cells. Frequency of CD4+CD25+CD45RO cells had a negative correlation with disease activity and level of anti–double-stranded DNA in patient and control groups.

In this study we found a significant correlation between anti-CRP antibody and disease activity, with an odds ratio of 0.739 (95% CI 0.557–0.981). It seems from this result that high disease activity "protects" the incidence of anti-CRP antibody formation. We propose several explanations for this contradictory result. Firstly, this study did not separate subjects according to disease activity. Such result may occur if the number of patients in remission were higher or almost equal with patients in active state of disease. Secondly, more than half of the patients (28 out of 40) were receiving corticosteroid therapy, with a dose equivalent to <11 mg/day of prednisone, which would suppress autoantibody formation.

Although statistical analysis showed significant correlation between anti-CRP antibody and disease activity, it is important to consider that the measurement of anti-CRP antibody was only done at one point and this is the limitation of this study. A larger, multi-centered, and multi-ethnic study using longitudinal design would provide a more reliable result concerning the association between anti-CRP antibody and disease activity.

CONCLUSIONS

We found a high prevalence of anti-CRP antibody in SLE patient in Cipto Mangunkusumo General Hospital, Jakarta. There was also a significant correlation between anti-CRP antibody and disease activity. Further studies, ideally with larger sample size that may better encompass the diverse ethnic groups and genetic difference in Indonesia is needed to obtain better understanding of the various mechanisms involved in the pathogenesis of SLE.

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