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RESEARCH

CORRELATION OF ANTINUCLEAR ANTIBODY (ANA) PROFILE WITH HEMATOLOGIC AND RENAL DISORDERS IN SYSTEMIC LUPUS ERYTHEMATOSUS

(Hubungan Antinuclear Antibody (ANA) Profile dengan Gangguan Hematologi dan Ginjal di Systemic Lupus Erythematosus)

Chelvi Wijaya, Asvin Nurulita, Ulung Bahrn

ABSTRAK

Systemic Lupus Erythematosus (SLE) adalah penyakit autoimun dan bersifat multi organ. Kelainan hematologi sering ditemukan di penyakit ini, begitu juga dengan kelainan ginjal yang merupakan salah satu faktor yang sangat berpengaruh. Uji ANA profile dapat mengetahui sub tipe antibodi antinuklear yang khas. Autoantibodi tersebut diduga berhubungan dengan manifestasi klinis. Penelitian ini merupakan penelitian analitik retrospektif di Laboratorium Patologi Klinik dan Instalasi Rekam Medik RSUP. Dr. Wahidin Sudirohusodo Makassar dengan mengambil data hasil ANA profile, darah rutin dan urinalisis pasien terduga SLE masa waktu Januari 2014–Juli 2016. Data dikelompokkan menjadi SLE dan nonSLE. Analisis statistik dengan uji Chi Kuadrat dan Fisher. Dari 72 sampel, 39 dengan diagnosa akhir SLE. Terdapat hubungan bermakna antara anti RNP/Sm, Sm, SS-A, Ro-52, dsDNA, Nucleosome, Histone, Ribosomal P dengan SLE ($p < 0,05$). Terdapat hubungan bermakna antara anti dsDNA ($p = 0,029$) dan anti nucleosome ($p = 0,037$) dengan anemia serta anti dsDNA ($p = 0,013$) dan anti nucleosome ($p = 0,036$) dengan gangguan ginjal. Tidak ditemukan hubungan bermakna antara autoantibodi dalam penelitian ini dengan leukopenia, limfopenia dan trombositopenia. Anti RNP/Sm, Sm, SS-A, Ro-52, dsDNA, nucleosome, Histones, Ribosomal P berhubungan dengan SLE. Anti dsDNA dan anti nucleosome berhubungan dengan anemia dan gangguan ginjal pada SLE, sehingga mungkin dapat digunakan untuk meramalkan kejadian tersebut, walaupun dibutuhkan penelitian lanjutan untuk membuktikannya. Tidak ditemukan autoantibodi yang dapat dihubungkan dengan leukopenia, limfopenia dan trombositopenia.

Kata kunci: Systemic lupus erythematosus, ANA profile, autoimun, autoantibodi

ABSTRACT

Systemic Lupus Erythematosus (SLE) is an autoimmune disease which affects multiple organs. Hematologic manifestation is common, as well as renal involvement, one of the very influential factors in SLE. Anti-Nuclear Antibody (ANA) Profile test can detect specific antinuclear antibodies. These autoantibodies are supposedly associated with the clinical manifestation. A retrospective analytical study was done in the Clinical Pathology Laboratory and Medical Record Installation of Dr. Wahidin Sudirohusodo Hospital Makassar by collecting the result of ANA profile, complete blood count and urinalysis test from suspected SLE patients during the period of January 2014–July 2016. Data were grouped into SLE and non-SLE. Statistical analysis was done by Chi Square and Fisher test. 72 samples were collected, 39 of them were SLE. There was a significant association between anti RNP/Sm, Sm, SS-A, Ro-52, dsDNA, Nucleosome, Histones, Ribosomal P with SLE ($p < 0.05$). There was a significant association between anti-dsDNA ($p = 0.029$) and anti-nucleosome ($p = 0.037$) with anemia and anti-dsDNA ($p = 0.013$) and anti-nucleosome ($p = 0.036$) with renal involvement. There was no significant association between autoantibodies in this study with leukopenia, lymphopenia and thrombocytopenia. Anti-RNP/Sm, Sm, SS-A, Ro-52, dsDNA, nucleosome, histones, Ribosomal P are associated with SLE. Anti-dsDNA and anti-nucleosome were associated with anemia and renal manifestation in SLE, so they may be used to predict this events, although further study is needed to prove it. There was no autoantibody that can be associated with leukopenia, lymphopenia and thrombocytopenia.

Key words: Systemic lupus erythematosus, ANA profile, autoimmune, autoantibody

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by the presence of autoantibodies against cell nuclei, and involving many organ systems in the body. Clinical manifestation of this disease is various, such as polyarthritis, oral ulcers, skin rashes, hematologic, kidney and brain disorders. SLE is generally more common in female with a ratio of 12: 1 and death is usually caused by kidney failure.¹⁻⁴

This disease, moreover, has a characteristic of multiple autoantibody production. Although the presence of autoantibodies in SLE has been known since more than 60 years ago, until now the role of autoantibodies in the pathogenesis, diagnosis, and prognosis still continues to be investigated. Anti-Nuclear Antibody (ANA) is a specific autoantibody that has an ability to bind to and destroy the structures in the cell and subcellular nucleus as well as organelles, including cell surface, cytoplasm, nucleus and nucleolus. This autoantibody helps in the diagnosis and prognosis of an autoimmune disease.^{5,6}

Antinuclear antibody, furthermore, has a low specificity in the diagnosis of SLE as found in most systemic autoimmune diseases and even in healthy people. Antinuclear antibody can also be considered as a screening test, and if the result of the test is positive, ANA profile test can be performed to determine the specific subtype of antibodies.^{7,8} It means that autoantibodies can be used for diagnosis and monitoring of SLE disease activity.

In addition, renal disorder is a very influential factor in SLE. Prognosis even can be improved by diagnosis and early treatment. A previous research showed that patients with positive anti-dsDNA tend to suffer more from lupus nephritis than those with negative anti-dsDNA.^{9,10}

Hematologic abnormalities, such as anemia, leukopenia, lymphopenia, and thrombocytopenia are also often found in patients with SLE. Patients who have anti-Ro will have significantly lower neutrophils, than those who do not have anti-Ro.¹¹ Similarly, a research on the pattern of antinuclear antibodies in SLE also found that a homogeneous ANA pattern is associated with anemia and leukopenia.^{5,11,12}

Unfortunately, there is still no research on ANA profile in SLE patients in Indonesia, particularly in Makassar. Therefore, this research aimed to determine the correlation of ANA profile with hematologic and kidney disorders in SLE.

METHODS

This research was a retrospective analytical research conducted at the Laboratory of Clinical Pathology and the Unit of Medical Records in the Dr. Wahidin Sudirohusodo Hospital in Makassar. Data needed were taken from ANA profiles of patients with SLE from January 2014 to July 2016. The data were then classified into SLE and non-SLE based on diagnosis of the patients. Data of routine blood and urinalysis in the SLE group were also used to determine the hematologic disorders suffered, such as anemia, leukopenia, lymphopenia, thrombocytopenia, and renal disorders (proteinuria or urinary cylinder).

Next, the correlation of ANA profile and hematologic and renal disorders in the SLE group were analyzed using Chi Square test. Meanwhile, data not qualified for the Chi-square test were tested using Fisher test. Statistical analysis then were processed using a computer program with a p value less than 0.05.

RESULTS AND DISCUSSION

The number of patients who suffered from SLE and had ANA profile from January 2014 to July 2016 was 72. The number of patients with the final diagnosis of SLE was 39 people. The number of female patients was higher (97%) than males with a ratio of 38: 1. The

Table 1. Characteristics of the samples

Variables	SLE (n=39)	Non SLE (n=33)
Sex		
Males	1 (2.6%)	7 (21.2%)
Females	38 (97.4%)	26 (78.8%)
Age (years)		
10-19 years	12 (30.8%)	9 (27.3%)
20-29 years	18 (46.2%)	8 (24.2%)
30-39 years	5 (12.8%)	6 (18.2%)
>40 years		
Diagnosis		10 (30.3%)
Renal disorders		11 (33.3%)
Hematological disorders		7 (21.2%)
Respiratory system diseases		6 (18.2%)
Musculoskeletal system diseases		5 (15.2%)
Skin diseases		2 (6.1%)
Nervous system diseases		1 (3%)
Gastrointestinal system diseases		1 (3%)

Source: Secondary data

largest age range of those patients was 20–29 years (see Table 1).

Systemic lupus erythematosus disease is actually more common in females with a ratio of 9: 1 to 15: 1 with the age range of 20–40 years, considered as productive ages. The increasing incidence of SLE in females at the productive ages may be due to hormonal influences. Excessive estrogen activity and inadequate androgen hormones contribute to changes in the immune response.^{1,13,14}

Examination of ANA profile in this research involved 15 antibodies, namely antibodies against RNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, Centromere, PCNA, dsDNA, Nucleosome, Histones, Ribosomal P-Protein (RIB), and AMA-M2. The results of ANA profile on Scl-70 and Jo-1 were negative in all patients with SLE (see Table 2).

Scl-70 antibody is specific in Scleroderma disease, while Jo-1 antibody is a myositis-specific autoantibody found in polymyositis and dermatomyositis diseases.^{15,16}

Based on the results of this research, there were significant correlations between antinuclear antibodies of RNP/Sm, Sm, SS-A, Ro-52, dsDNA, Nucleosome, histones, and Ribosomal P with Systemic Lupus Erythematosus (see Table 2). Anti-RNP can be found in SLE and other systemic autoimmune diseases, whereas anti-Sm only in SLE. Anti-SS-A/Ro, moreover, are antibodies against an antigen, which consists of two cellular proteins with the molecular weight of 52 and 60 kD, namely Ro-52 and Ro-60. Anti-SS-A/Ro are found in Sjögren's syndrome, SLE and other autoimmune diseases. Anti-dsDNA has a high specificity (95%) for SLE. Anti-nucleosome plays an important role in the pathogenesis of SLE. Anti-histones is usually found in drug-induced lupus and SLE. Anti-Ribosomal P are antibodies against components of the ribosome and found in over 40% of patients with SLE.^{1,6,17}

Anti-Sm and anti-dsDNA, furthermore, have been used in the diagnostic criteria for SLE according to the revised ARA in 1997, whereas other antibodies have not been included. However, some researches have questioned the validity of these diagnostic criteria. For instance, a research on meta-analysis showed that anti-nucleosome as an antibody had a better diagnostic value than anti-dsDNA. Another research showed that anti-Ribosomal P had the same sensitivity and specificity with anti-Sm so that it is possible to replace the anti-Sm in ARA criteria.⁶

Hematological disorders in SLE may include anemia, leukopenia, lymphopenia, and thrombocytopenia.

Table 2. Correlation of ANA Profile and Systemic Lupus Erythematosus

ANA Profile	non SLE (n=33)	SLE (n=39)	P
RNP/Sm			
Negative	33	22	<0.001*
Positive	0	17	
Sm			
Negative	33	28	0.003*
Positive	0	11	
SS-A			
Negative	32	23	<0.001*
Positive	1	16	
Ro-52			
Negative	33	23	<0.001*
Positive	0	16	
SS-B			
Negative	32	33	0.116**
Positive	1	6	
Scl-70			
Negative	32	39	0.458**
Positive	1	0	
PM-Scl100			
Negative	33	38	1.000**
Positive	0	1	
Jo-1			
Negative	32	39	0.458**
Positive	1	0	
Centromere			
Negative	31	38	0.590**
Positive	2	1	
PCNA			
Negative	33	36	0.245**
Positive	0	3	
dsDNA			
Negative	31	17	<0.001*
Positive	2	12	
Nucleosome			
Negative	31	18	<0.001*
Positive	2	11	
Histones			
Negative	32	29	0.020*
Positive	1	10	
Ribosomal P			
Negative	32	25	0.002*
Positive	1	14	
AMA-M2			
Negative	33	34	0.058**
Positive	0	5	

* Chi Square Test ** Fisher Test

The results of this research showed that there was a significant correlation between anti-dsDNA ($p=0.029$) and anti-nucleosome ($p=0.037$) with anemia in SLE (see Table 3). But, there no significant correlation between autoantibodies was found in this research with leukopenia, lymphopenia and thrombocytopenia.

A research conducted by Desoiky *et al.*⁹ showed that anti-nucleosome levels were significantly higher in patients with hematological disorders than in those without hematological disorders. It indicated

Table 3. Correlation of ANA Profile and hematological disorder in SLE patients

ANA Profile	Anemia		Leukopenia		Lymphopenia		Thrombocytopenia	
	(n=39)	p	(n=39)	P	(n=39)	P	(n=39)	p
RNP/Sm								
Negative	20/22	1.000**	4/22	0.158*	14/22	0.288**	1/22	1.000**
Positive	15/17		7/17		14/17		1/17	
Sm								
Negative	24/28	0.309**	8/28	1.000**	18/28	0.130**	2/28	0.309**
Positive	11/11		3/11		10/11		0/11	
SS-A								
Negative	20/23	0.631**	6/23	0.734**	15/23	0.471**	1/23	1.000**
Positive	15/16		5/16		13/16		1/16	
Ro-52								
Negative	20/23	0.631**	7/23	1.000**	15/23	0.471**	1/23	1.000**
Positive	15/17		4/16		13/16		1/16	
dsDNA								
Negative	13/17	0.029**	4/17	0.725*	11/17	0.482**	1/17	1.000**
Positive	22/22		7/22		17/22		1/22	
Nucleosome								
Negative	14/18	0.037**	6/22	0.510*	13/18	1.000*	0/18	0.490**
Positive	21/21		5/21		15/21		2/21	
Histones								
Negative	25/29	0.556**	8/29	1.000**	21/29	1.000**	1/29	0.452**
Positive	10/10		3/10		17/20		1/10	
Ribosomal P								
Negative	21/25	0.277**	6/25	0.478**	17/25	0.713**	2/25	0.528**
Positive	14/14		5/14		11/14		1/15	

* Chi Square Test** Fisher Test

that hemoglobin levels were significantly negatively correlated with anti-nucleosome levels. Similarly, a research conducted by Ghrahani *et al.*⁵ found that children suffering from SLE with ANA test results in the form of a homogeneous pattern (anti-dsDNA, nucleosome and histones) were more at risk of developing anemia and leukopenia since anemia in SLE could be caused by immune and non-immune mechanisms. The most common causes of anemia in this disease were chronic anemia disease, iron deficiency anemia, autoimmune hemolytic anemia and renal anemia.^{5,9,12}

Based on the data obtained in this research, there was a significant correlation between anti-dsDNA (p=0.013) and anti-nucleosome (p=0.036) with kidney disorders in SLE (see Table 4).

In addition, based on the results of this research, anti-dsDNA had a good correlation with renal disorders in SLE. Immune complex of anti-DNA- DNA can be deposited in mesangium matrix and stimulate complement activation resulting in inflammation and nephritis. Recent researches estimated a possibility that these antibodies bind to nucleosome. Nucleosome is released during apoptosis, and in SLE, cleaning interference of apoptotic cells is stimulated

Table 4. Correlation of ANA Profile and kidney disorders in SLE patients

ANA Profile	Kidney Disorders (n=36)	P
RNP/Sm		
Negative	11/21	0.257*
Positive	5/15	
Sm		
Negative	11/25	1.000**
Positive	5/11	
SS-A		
Negative	9/20	0.940*
Positive	7/16	
Ro-52		
Negative	10/20	0.453*
Positive	6/16	
dsDNA		
Negative	3/15	0.013*
Positive	13/21	
Nucleosome		
Negative	4/16	0.036*
Positive	12/20	
Histones		
Negative	10/27	0.146*
Positive	6/9	
Ribosomal P		
Negative	11/22	0.400*
Positive	5/14	

* Chi Square Test ** Fisher Test

by macrophages, resulting in the production of anti-nucleosome/DNA. Anti-nucleosome then will be bound to the glomerular basement membrane and become a bridge connecting nucleosome of antibodies and kidney tissue. Similarly, a research on cohort conducted by Adrienzen *et al.*¹⁸ concluded that anti-nucleosome was more sensitive than anti-dsDNA in assessing the activity of lupus nephritis.^{1,6,18,19}

CONCLUSIONS AND SUGGESTIONS

Based on the results of this research, it may be concluded that there was a significant correlation between the antinuclear antibodies of RNP / Sm, Sm, SS-A, Ro-52, dsDNA, Nucleosome, Histones, Ribosomal P with Systemic Lupus Erythematosus (SLE). It is also known that Anti-dsDNA and anti-nucleosome are associated with anemia and renal disorders in SLE, so it may be useful to predict these diseases. Thus, further researches are needed to be conducted. Nevertheless, there are no autoantibodies that may be associated with leukopenia, lymphopenia and thrombocytopenia.

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