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A SURVEY OF ANTIBODIES FOR THE EXTRACTABLE NUCLEAR ANTIGENS AND THEIR ROLE IN THE CLINICAL DIAGNOSIS AND PROGNOSIS OF SLE

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SUMMARY

Fifty-seven patients suffering from systemic lupus erythematosus with different clinical features were studied from various aspects of immunological abnormalities, including anti-nuclear antibodies; anti-DNA; anti-Sm (Smith's antigen); and, other extractable nuclear antigens.

The tests employed were microhemagglutination, counter immuno-electerphoresis, and radio immunoassay (RIA).

The comparison between the two techniques for detectiong anti-DNA titers showed a significant correlation between them.

There was also a significant positive correlation between the presence of anti-DNA and lupus nephritis ($P>0.05$). However, lupus nephritis has a negative association with anti-Sm.

We will discuss anti-Sm in SLE patients that may have an inhibitory effect on developing the lupus nephritis. The association of anti-Sm in SLE may help the physicians to prognose the disease and manipulate the treatment.

KEY WORDS: *Anti-Sm antigen; Deoxyribonucleic acid (DNA); Microhemagglutination; Systemic lupus erythematosus (SLE).*

INTRODUCTION

Osler first described *Erythema exudativum* as systemic lupus erythematosus (SLE) with variable visceral manifestations (1). After almost one century, although our knowledge about SLE (the main autoimmune disease) has been increased; however, a clear diagnostic procedure is not yet available (2).

Many sophisticated laboratory techniques have been developed to investigate the immunological abnormalities in patients, but the findings are not specific for SLE except: anti-DNA and anti-Sm antibodies evaluation (3-8).

Detection of anti-nuclear antibodies is the most useful tool for evaluating and diagnosing the disease (9-13), but the conventional assaywhich is immuno-flourescent anti-nuclear antibodies (FANA) is not considered to be the specific findings in SLE (14).

Looking for specific anti-nuclear antibodies which are not common in connective tissue disorders led to finding of so-called extractable nuclear antigens (ENA). There are a group of non-histone nuclear antigens, of which Sm's antibody is specifically associated with SLE (15-19).

In the present study, we have detected anti-Sm,ribonucleic protein (RNP) and some other ENA antibodies using counter immunoelectro--phoresis.

MATERIALS AND METHODS

1) **Sera**: Fifty-seven patients suffering from SLE were kindly referred from Collagenous Disease Clinic at Shariati Hospital.

Collected sera were kept at 70c̊ until being used. Fifty-six donors were tested as normal controls.

2) Reference anti-sera were purchased from AF CDC and ANAR Reference Laboratory in USA.

3) **Antigens:**

a= Soluble nuclear antigens (ENA) were extracted from calf's thymus by succharose gradient technique (2,15,20,21);

b= Isolation of Sm and RNP: ENA obtained by treatment of calf's thymus with different ammonium sulfate (30-60%), then dialysed through the dialysis bag. At the end, the samples were incubated with RNA ase (19,22);

c= Double stranded DNA (from Millipore Corporation).

4) **Tests:**

a= Microhemagglutination: The test was performed as described elsewhere (23), using human (O negative) blood group coated with DNA.

b= Counter immunoelecterophoresis was used to detect anti-Sm and anti-RNP antibodies. The applied technique was described by Rose (24).

RESULTS

Anti-DNA antibody titer in patients and controls sera have been compared in Table 1. Statistically, there is a significant difference between the two groups. $P<0.0001$ and $K^2=22.964$.

GMRT in patient= 1:224 and in control= 0.

Table 2 shows the frequency of anti-Sm antibody in patient and control group. $P<0.02$ and $K^2=6.254$.

GMRT in patient= 1:81 and in control= 1:2

Table 3 demostrates the comparison between the various anti-nuclear antibodies in SLE patients and control groups. The specificity of anti-Sm is 98% and for anti-DNA is 100%. These two antibodies have been compared in Table 4.

To evaluate the relationship between the presence of auto-antibodies and clinical manifestations, the SLE patients with different clinical symptoms are shown in Table 5. The anti-DNA and anti-Sm have been compared. Results indicate that:

a= renal involvement is associated with the presence of anti-DNA antibody $P< 0.005$ and $K^2=0.229$;

b= there was no significant difference in patients with neural, hematological complications and the patients without the above symptoms.

c= there was a negative correlation between anti-Sm antibody and kidney disorder.

Fisher Test has been used to confirm the above findings (Table 6).

Table 1

Antibody titer	Patients		Controls	
	No.	%	No.	%
Negative	36	63.1	56	100
1	–	–	–	–
1:2	–	–	–	–
1:4	–	–	–	–
1:8	1	1.7	–	–
1:16	2	3.5	–	–
1:32	1	1.7	–	–
1:64	1	1.7	–	–
1:128	1	1.7	–	–
1:256	4	7.1	–	–
1:512	7	12.3	–	–
1:1024	4	7.1	–	–
Total	57	100	56	100

Absolute and partial frequency distribution of anti-DNA and SLE patients, and controls
$P < 0.0001$ and $K^2 = 22.964$
Geometric mean of reciprocal titer (GMRT) in patients= 1:224 and controls= 0

Table 2

Antibody titer	Patients		Controls	
	No.	%	No.	%
Negative	40	74.1	33	9
1	–	–	–	–
1:2	–	–	1	
1:4	–	–	–	–
1:8	1	1.8	–	–
1:16	2	3.7	–	–
1:32	4	7.4	–	–
1:64	3	5.6	–	–
1:128	4	7.4	–	–
Total	54	100	34	

GMRT in: Absolute and partial frequency distribution of anti-Sm in SLE patients and controls $P = 0.02$ and $K^2 = 6.254$, patients= 1:18 and controls= 1:2

Table 3

Test	Correlation	K^2	P	Specificity%	Sensitivity%	RR%
Anti-DNA	Positive	22.994	0.0001	37	100	33
Anti-RNA	Positive	5.923	0.02	55	70	0-1
Anti-Sm	Positive	6.245	0.02	26	97	2-7

The comparison of anti-nuclear antibodies in SLE and control groups according to specificity of relative risk (RR), sensitivity, K^2, P, and coefficient of correlation.

Table 4

Population / Anti-DNA antibody	Control (40 Cases)				Patients(32)	
	Positive		Negative		Positive	
	No.	%	No.	%	No.	%
Positive	0	0	1	2.5	3	9.4
Negative	0	0	39	97.5	8	25

Absolute and partial frequency distribution of anti-DNA and anti-Sm antibodies in patients and control groups

Table 5

Antibody		Anti DNA				Anti Sm			
		Positive		Negative		Positive		Negative	
Clinical manifestation		No.	%	No.	%	No.	%	No.	%
Renal involvement	+	20	80	5	20	5	19.2	21	80.8
	–	7	31.8	15	68.2	5	26.3	14	73.7
Neural involvement	+	5	55.6	4	44.4	2	22.2	7	77.8
	–	12	30	28	70	11	27.5	29	72.5
Hematological	+	11	64.7	7	41.3	4	22.2	14	77.8
abnormalities	–	9	29	22	71	7	22.6	24	77.4

Asolute and partial frequency distribution of anti-DNA and anti-Sm antibodies in SLE patients with various clinical symptoms

Table 6

Clinical symptoms / Anti-DNA antibody / Anti-Sm antibody	Neural involvement				Renal disorders				Hematological abnormalities			
	Yes		No		Yes		No		Yes		No	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Positive	0	22.2	5	22.5	3.7	18.5	5.3	21	0	22	6.5	16.1
Negative	55.6	22.2	25	47.5	13	8	21	52.6	61.7	66.7	22.6	54.8

Absolute and partial frequency distibution of anti-DNA and anti-Sm antibodies in SLE patients with different clinical symptoms according to Fisher Test.

DISCUSSION

Systemic lupus erythematosus (SLE) the main autoimmune disease is diagnosed through several criteria. Among laboratory findings auto-antibodies are the most helpful.

Anti-Sm and anti double stranded DNA are the two antibodies which are specific for SLE and not common in other connective tissue disorders.

We have employed *Microhemagglutination Test* for evaluating anti-DNA antibody. The technique is sensitive and comparable to RIA Method, and it also has both time and money saving advantage especially in our country. To identify the extractable nuclear antigens (ENA) as Sm and RNP, the *counter Immunoelecterophoresis (CIE) Method* was performed. The technique is very specific and reproducible in any laboratory. Antigens were achieved from calf's thymus using the extraction method (16,26). Anti-DNA antibodies are specific and helpful to demonstrate the acute phase of disease (27). The presence of anti-DNA may also predict the prognosis of the disease. According to our data (Table 6) anti-DNA (80%) is accompanied by nephrotic lupus. In other words, this antibody is responsible for kidney damage via immune complex deposition. Anti-Sm antibody could be found in 30% of SLE patients. It is specified on the basis of our data which is comparable to other reports (27), anti-Sm antibody is not present in other connective tissue disorders. In contrast to anti-DNA, anti-Sm antibody is a protective antibody. The anti-Sm antibody, though confirms the clinical SLE features, is also helpful since it may protect the patient from kidney complications.

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