CLINICAL SIGNIFICANCE OF ANTICARDIOLIPIN ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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SUMMARY: We studied anticardiolipin antibody isotypes and VDRL in 45 systemic lupus erythematosus (SLE) patients. These patients were also screened for some other autoantibodies, such as anti-DNA, antimitochondrial (AMA) type 5 and antinuclear antibodies. Out of 45 patients, 55.5% were anticardiolipin (ACL) antibody positive. IgG class ACL antibodies were mainly detected in patients having thrombotic events and fetal losses. IgM class ACL antibody positivity was only associated with fetal losses. We also found a significant correlation between the presence of AMA type 5 and VDRL positivity with ACL-IgG and ACL-IgM antibodies.

Key Words: Anticardiolipin antibodies, fetal loss, antinuclear antibodies.

INTRODUCTION

SLE is one of the most common rheumatic autoimmune disorders and the diagnosis depends on both the clinical picture and presence of autoantibodies against nuclear and cytoplasmic elements of the cell (1-3). During the last decade, it became evident that besides other autoantibodies ACL antibodies were associated with SLE. Anticardiolipin antibodies were first noted in nonspecific syphilis test. Thereafter the antigen which bound to reagins in syphilitic sera was found to be cardiolipin (4). SLE patients may also have false positive floculation tests such as VDRL (Veneral Disease Research Laboratories) test (5).

ACL antibodies play an important role in the pathogenesis of coagulopathies, arterial and venous thromboses, recurrent abortions, thrombocytopenia and neurologic abnormalities which can be seen in most rhematic autoimmune disorders, especially in SLE (6-10).

ELISA and RIA techniques are more sensitive than standard syphilis tests and also have the advantage of defining immunoglobulin classes (7, 11-13).

In this study, ACL antibodies were investigated by ELISA and VDRL tests. The correlation of anticardiolipin antibody isotypes with clinical and serologic parameters were also evaluated.

MATERIALS AND METHODS

The patient group consisted of 45 SLE patients who admitted to Hacettepe University, department of Medicine between 1986-1988. Ages of the patients were between 18 and 64. Diagnosis of SLE was made according to the American Rheumatism Association (ARA) criteria. The control group consisted of 100 healthy hospital workers without any history of autoimmune disease and 100 blood donors admitted to the blood bank of the hospital.

ACL antibody detection was made by ELISA technique as described by Harris *et al.* but with some minor modifications (11,12,14). We first determined the mean and, standard deviation

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(SD) of optic density values of the 200 healthy controls sera. Then, 3 SD pulus the mean value was accepted as the "cut off" value. Concentrations above this level were considered as positive (14,15). The patient sera were studied twice and the mean of these two values was taken as the final value. (We used standard serum samples donated from Dr. Harris (U.K. Hammersmith Hospital).

Antinuclear antibodies against nuclear antigens were investigated by indirect fluorescent antibody technique (2). VDRL test was performed by a commercial microfloculation test kit (Behring). Anti-DNA antibodies were studied by a RIA test kit (Amersham) (16).

Statistical evaluation was made by Chisquare (χ^2) test and Fishers exact Chisquare (χ^2) test.

RESULTS

We studied ACL antibody classes and the correlation of these with clinical and other serologic data in 45 SLE patients.

ACL antibody positivity in SLE cases and control group are shown in Table 1. The positive antibody rates between the patient and control groups were significantly different ($p<0.05$). In Table 2, positive ACL results in patient and control groups are classified according to antibody classes. Out of 45 patients, 40% had IgG, 28.8% had IgM and 11% had IgA class ACL antibodies in their sera (Table 2). These results were statistically significant when compared with the control group ($p<0.05$). 6 SLE patients had both IgG and IgM, 1 patient had IgG and IgA and 2 patients had IgG, IgM and IgA classes of ACL antibodies.

Table 1: The incidence of ACL antibodies in patient and control groups.

Diagnosis	Total	Positivity	(%)	P
Patients	45	25	(55.5)	P< 0.05
Control group	200	17	(8.5)	P< 0.05

Table 2: The distribution of ACL-antibody classes in patient and control groups.

Diagnosis	Total	ACL-IGG		ACL-IgM		ACL-IgA	
		positivity	(%)	positivity	(%)	positivity	(%)
Patients	45	18	(40)	13	(28.8)	5	(11)
Controls	200	12	(6)	3	(1.5)	2	(1)

In Tables 3 and 4, correlation of IgG and IgM ACL antibody positivities with clinical and serological findings are evaluated. Correlation of ACL-IgG positivity with clinical findings was significant in thrombotic events and fetal losses ($p<0.05$; $p<0.08$). On the other hand ACL-IgM positivity showed significant correlation only with total losses ($p<0.05$). Statistical analysis related with ACL-IgA antibodies were omitted due to low numbers of positive results.

The association of antinuclear (ANA), anti-DNA, antimitochondrial (AMA) type 5 antibodies and VDRL tests with IgG and IgM ACL antibodies was also investigated. These findings are shown in Table 4. AMA type 5 antibodies and positive VDRL tests were present in only ACL-IgG and IgM positive cases. These relations were statistically significant ($p<0.05$).

DISCUSSION

Many investigators have noticed an increased incidence of antibodies against negatively charged phospholipids-especially cardiolipin- in rheumatic autoimmune disorders. It was suggested that these autoantibodies could have an important role in the etiology and pathogenisis of these disorders for all cell membranes have phospholipid components.

SLE is one of the most intensely investigated disorders related to the incidence and pathogenetic roles of ACL antibodies. In most of these studies, a correlation between ACL antibodies and certain clinical findings such as thrombosis, recurrent fetal loss thrombocytopenia and neurologic involvement was demonstrated (6,8,17). However, correlation of ACL antibodies with other serologic findings such as ANA, anti-DNA, AMA type 5 antibodies and VDRL has been controversial (11,18,19).

In our study, 55.5% of 45 SLE patients were found to be ACL antibody positive. ACL-IgG, IgM and IgA antibodies were present in 40%, 28.8% and 11% of these 46 patients respectively. ACL-IgG antibodies showed a relation with thrombotic events and fetal losses, whereas ACL-IgM antibodies were only associated with recurrent fetal losses. Correlation of ACL antibodies with serologic parameters such as VDRL positivity and AMA type 5 antibodies was also statistically significant.

Using the RIA technique, Harris *et al.* (11) found an ACL positivity of 81% in 65 SLE patients (ACL-IgG 54%, ACL-IgM 41%, ACL-IgA 18%). They also reported corre-

Table 3: Correlation of clinical findings with positive ACL IgG and IgM antibodies.

Clinical findings	ACL-IgG postive patients		ACL-IgG postive patients		ACL-IgM postive patients		ACL-IgM postive patients	
	(n=18)	%	(n=27)	%	(n=13)	%	(n=32)	%
Skin lesions	12	(67)	17	(63)	10	(76)	19	(59)
Renal involvement	7	(38)	5	(19)	2	(15)	10	(31)
Neurologic findings	3	(17)	3	(11)	3	(23)	3	(9)
Thrombosis	4	(22)	0	(0) p<0.05	2	(15)	2	(6)
Fetal loss	5*	(55)	3*	(18) p<0.08	5	(71)	3	(17) p<0.05
Corticosteroid therapy	12	(66.6)	10	(37)	4	(30.4)	10	(31.2)

*These findings were evaluated only for married women.

ACL- IgG positive n=9

ACL- IgG negative n=16

ACL- IgM positive n=7

ACL- IgM negative n=16

Table 4: The relation of IgG and IgM ACL antibodies with other serological findings in SLE patients.

Serological findings	ACL-IgG postive cases		ACL-IgG postive cases		ACL-IgM postive cases		ACL-IgM postive cases	
	(n=18)	%	(n=27)	%	(n=13)	%	(n=32)	%
A N A	16	(89)	23	(85)	11	(85)	28	(88)
Anti - DNA	12	(67)	13	(48)	8	(62)	17	(53)
AMA - type 5	5	(27.7)	0	(0)	3	(23)	0	(0) p<0.05
V D R L	5	(27.7)	0	(0)	3	(23)	0	(0) p<0.05

lation between ACL-IgG positivity and thrombocytopenia, arterial and venous thromboses. Among the serologic tests, only VDRL positivity showed correlation with ACL antibody results in their study.

Norberg *et al.* (20) found an ACL antibody incidence of 47.5% in SLE patients with the ELISA technique. They found an increased incidence of recurrent abortions and thromboembolic phenomena in patients with a high ACL antibody titre.

Our ACL antibody positivity rate (55.5%) is consistent with other studies (11,20,21). However the distribution of ACL isotypes are different from some other studies (21). These differences may be due to difference in the "cut off" values of RIA or ELISA tests and to different populations.

The correlation of ACL antibodies with thrombosis, fetal loss, VDRL and AMA type 5 positivity in our study is consistent with other reports (11,20,21). Like many other studies, we found that only a small number of ELISA positive cases were also VDRL positive. Harris *et al.* (22), divided antiphospholipid antibodies into "syphilitic" and "autoimmune" groups. They suggest that the syphilitic group antibodies are specific to the cardiolipin antigen in the VDRL test. The "autoimmune" group is said to have cross reactions with other negatively changed phospholipids.

We were unable to show any correlation with ACL antibodies and serologic findings such as ANA and anti-DNA antibodies which are important diagnostic parameters in SLE. These findings are also consistent with the literature (23,24). Inhibition studies have shown that cross reactions between cardiolipin and DNA antibodies are infrequent but anti-DNA antibodies with low avidities can cross react with cardiolipin (25).

AMA type 5 antibodies are also thought to be correlated with anticardiolipin antibodies in SLE patients. We found a significant correlation between these autoantibodies and ACL IgG and IgM antibodies. As cardiolipin is one of the components of mitochondrial membrane, it is suggested that it may have cross reactions with other mitochondrial autoantibodies. AMA type 5 positive cases also had thrombotic events and this finding is consistent with the literature (19).

We found no difference in ACL positivity rate in SLE patients under corticosteroid therapy but it is difficult to make a conclusive statement about the effect of therapy because the duration of therapies could not be determined precisely.

In conclusion our results have shown that ACL antibody positivity is associated with thrombotic events, fetal losses, VDRL and AMA type 5 positivity in SLE patients. It can be stated that ACL antibody studies are helpful in evaluation and treatment of clinical complications in SLE.

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