ANTICARDIOLIPIN ANTIBODIES IN RECURRENT FETAL LOSS

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SUMMARY: Antiphospholipid antibodies can be detected both in some autoimmune disorders and in women with habitual abortion without evidence of a clinical autoimmune disease. Anticardiolipin antibodies and lupus anticoagulant factor are the two antiphospolipid antibodies which seem to have a role in habitual and spontaneous abortions, fetal growth retardation and intrauterine fetal deaths. The cause of the fetoplacental pathology remains unknown but it is likely that the thrombotic tendency observed in these patients also involves the decidual and placental blood vessels. A possible explanation for this thrombotic tendency has been the inhibition of vascular prostacyclin synthesis with resultant thromboxane dominance. We studied the incidence of anticardiolipin antibodies and lupus anticoagulant factor in habitual and spontaneous abortions and intrauterine fetal deaths in this report. An increased incidence of anticardiolipin antibodies was found in women with unexplained recurrent fetal loss.

Key Words: Anticardiolipin Antibodies, Lupus Anticoagulant Factor, Habitual Abortion.

INTRODUCTION

The incidence of habitual abortion is in the range of 0.4-0.8 % and in approximately half of these cases a specific etiologic factor can be found (1). 3-5% of habitual abortion eases are thought to be due to autoimmune abnormalities (2). Anticardiolipin antibodies (ACA) and lupus anticoagulant factor (LAF) are the two antiphospholipid antibodies which have a role in this situation. Negatively charged phospholipids are present in most of the mammalian tissues (e.g. endothelial cell membrane, platelets). Thus it is not surprising that antibodies against these negatively charged phospholipids can cause various kinds of disorders.

Cardiolipin is a negatively charged phospholipid which is obtained from beef heart with alcoholic extraction. This is the basis of the floculation test, VDRL, which has been used in the diagnosis of syphilis until recent years. The tissue damage in syphilis results in formation of some auto antibodies which react with cardiolipin and some other phospholipids. False positive VDRL tests can be observed in habitual abortions as well as in some patients with autoimmune disorders (3,4,5).

In 1952 Conley and Hartman observed hemorrhagic tendency in two SLE patients and ascribed this fact to a coagulation inhibitor (6). This factor was later named "the lupus anticoagulant factor" but contrary to the initial observations its presence was seen to be associated with throbotic events. To day, LAF and ACA are found in many disorders characterized by thrombosis, arterial and venous thromboses, habitual abortion, intrauterine growth retardation and intrauterine fetal death (7). LAF is also an antiphospholipid antibody. 30% of LAF (+) cases have also false positive VDRL tests (5).

Most sensitive tests for the detection of anticardiolipin antibodies are the solid phase RIA and ELISA techniques in which microplates covered with cardiolipin antigens react with anticardiolipin antibodies (8). LAF is detected indirectly by prolongation of activated partial thromboplastin time test (APTT). It can also be detected by several other coagulation tests (Kaolin Clotting time, Tissue Thromboplastin inhibition test) (9).

In this study we determined the incidences of LAF with APTT and ACA with ELISA test.

PATIENTS AND METHODS

63 patients who referred to Dr. Zekai Tahir Burak Women's Hospital in 1987-1988 because of pregnancy wastage were included in the study. Habitual aborters had 3 or more consecutive abortions and were also evaluated for other specific etiologic factors. Spontaneous abortion group consisted of patients who failed to fulfill habitual abortion criteria. Late intrauterine fetal deaths are defined as deaths occurring in the third trimester i.e. after the fetus has gained

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viability. 35 patients had habitual abortion, 22 had spontaneous abortions and 6 had late intrauterine fetal deaths.

VDRL, ELISA-ACA, ANA and APTT were performed in the sera of these 63 patients. The control group consisted of an age-matched group of 27 women who had successful reproductive histories.

LABORATORY TESTS

1. Anticardiolipin antibodies - Both IgG and IgM anticardiolipin antibodies were determined by the ELISA technique as described by Harris *et al.* (8). The cut off value was determined by adding 3SD to the mean optic density of 27 healthy controls. The values above this level were accepted positive. The cut off value was accepted as 3 arbitrary Units and positive tests were evaluated accordingly.

2. Lupus anticoagulant factor- LAF was detected by the activated partial thromboplastin time test (9). To avoid false positivity due to deficiencies of coagulation factors, test sample was mixed in a ratio of 1;1 in cases of prolonged APTT's. If it did not return to normal levels the patient was said to have the LAF.

Normal values of APTT in our laboratory are 25-50 seconds. Actin activated cephalophastin reagent (Dade) and manual filt tube methods were used.

3. VDRL - This floculation test was performed with the VDRL antigen (Behring). It consisted of 0.3% cardiolipin, 9% cholesterol and 2.1% lecithin. Antigen was diluted in a ratio of 1:10 before the test.

4. Autoantibodies - Other autoantibodies were detected by indirect immunoiorescence in rat liver, stomach and kidneys.

All sera were test for antinuclear, anti-partietal, anti-smooth muscle and anti-mitochrondrial antibodies (10).

RESULTS

The results of immunological test in ACA positive cases are shown in Table 1. The incidence of anticardiolipin antibodies was 33.3% (21/63). 14 patients had IgG, 13 patients has IgM class ACA and 6 patients had both classes of antibodies.

4 patients (6.3%) were VDRL positive and 2 patients (3.1 %) had prolonged APTT's. In 5 patients (7.9%) other autoantibodies were detected. In patient no. 1, all tests studied were positive. This patient has SLE with renal, cerebral involvement, livedo reticularis and is on treatment. In all patients with VDRL positivity, prolonged APTT and other autoantibodies, ACA results were also positive. The positive autoantibodies were antinuclear antibodies in two patients, antimitochondrial antibodies in two patients and antiparietal antibodies in one patient.

Table II and III show the results of IgG and IgM anticardiolipin antibodies in our patient groups. The patients with habitual abortions had a significantly higher rate of ACA positivity of both classes than the control group (IgG; $p=0.0348$ $p<0.05$, IgM; $p= 0.0136$ $p<0.05$). In Table IV, rates of positive tests are given. Although habitual abortion group had a higher rate of ACA positivity (34.2%) than the spontaneous abortion group (27.2%), the difference was not significant.

Table 1: The Results of VDRL, APTT and Other Autoantibody Tests in Abortion and Intrauterine Death Cases with Positive ACA's.

PATIENTS	ACA* IgG	ACA IgM	VDRL	APTT (sec.)	AUTOANTIBODY
1. 11 SA	+(5U)	-	+	90	ANA**
2. 3 SA	+(4.5U)	-	-	46	-
3. 3 SA	-	+(3.6U)	-	36	-
4. 3 SA	+(6U)	+(6U)	-	37	-
5. 4 SA	+(3.4U)	+(4U)	-	32	-
6. 9 SA	+(3U)	-	-	40	-
7. 4 SA	-	+(3U)	-	42	-
8. 3 SA, 2IUEX	+(3U)	+(4U)	-	31	-
9. 5 SA, 1IUEx	+(3.(U)	+(3.7U)	+	50	-
10. 4 SA, 2IUEx	+(4U)	-	-	32	-
11. 3 IUEx	-	+(3.7U)	-	38	Mitochondrial
12. 3 IUEx	+(4.3U)	-	-	30	-
13. 2 SA	+(7U)	+(4.2U)	-	35	ANA
14. 2 SA	-	+(3.4U)	-	42	-
15. 1 SA	+(4U)	+(4U)	-	30	-
16. 2 SA	+(3.5U)	-	-	28	-
17. 2 SA	+(5U)	-	-	42	-
18. 4 SA, 1 IUEx	-	+(3.9U)	-	43	Mitochondrial
19. 1 Late IUEx	-	+(4.2U)	+	34	Parietal
20. 2 Late IUEx	+(4U)	-	+	31	-
21. 3 Late IUEx	-	+(4.3U)	-	32	-

*Anticardiolipin antibody, **Antinuclearantibody

Table 2: ELISA-ACA IgG Results in Habitual Spontaneous Abortions and Late Intrauterine Deaths.

PATIENTS	IgG (+)	IgG (-)	TOTAL
Habitual Abortion	9	26	35 (P=0.0348, p < 0.05)
Spontaneous abortion +			
Intrauterine Death	5	23	28 (P=0.0282, p < 0.05)
Control group	0	27	27

Table 3: ELISA-ACA IgM Results in Habitual Spontaneous Abortions and Late Intrauterine Deaths.

PATIENTS	IgM (+)	IgM (-)	TOTAL
Habitual Abortion	7	28	35 (P=0.0136, p < 0.05)
Spontaneous abortion +			
Intrauterine Death	6	22	28 (P=0.0129, p < 0.05)
Control group	0	27	27

DISCUSSION

Recent studies have demonstrated an increased rate of fetal wastage in patients having serological autoimmune abnormalities. In SLE and some other autoimmune disorders, increased incidence of fetal wastage is encountered. Most of these patients have antinuclear antibodies, lupus anticoagulant factor, false positive VDRL tests and positive ELISA-ACA tests (3,4,5,11).

Nilson *et al.* were the first to suggest the relationship between lupus anticoagulant factor and fetal wastage (12). In 1980, Firkin *et al.* detected LAF in 4 patients with recurrent fetal loss (13). Lockshin *et al.* stated that ELISA-ACA test had a higher sensitivity than other antiphospholipid antibody assays in predicting fetal outcome (11). Lubbe *et al.* compared LAF and ELISA-ACA in 30 patients with recurrent fetal losses and suggested that LAF tests were more sensitive than the ELISA-ACA tests although some patients had normal pregnancies with a very high titre of LAF (14). These reports demonstrated with certainly that antiphospholipid antibodies could be found in patients with recurrent fetal loss without any clinical on laboratory evidence of an autoimmune disease. Therefore anticardiolipin antibody testing is suggested in screening habitual aborters. It should also be mentioned that the presence of these autoantibodies can be the first sign of a future autoimmune disease which may be subclinical at present (5).

Although the precise mechanism of fetal death remains unknown, some explanation for the role of antiphospholipid antibodies in this situation have been proposed: 1) Inhibition of prostacyclin production by vascular tissues. This causes a relative increase of thromboxane and a tendency to thrombosis and infarction results. 2) It has been postulated that lupus anticoagulants damage platelets and increase their adhesiveness initiating thrombosis (7). 3) Inhibition of prekallikrein activation. 4) Inhibition of protein C activation invivo results in hypercoagulability and vasoconstriction. Production of prostacyclin by the endothelial cells in considered an important mechanism that protets the vascular wall from the deposition of platelet aggregates and subsequent thrombosis. The success with a prostaglandin inhibitor, aspirin, also supports this hypothesis. Pathological studies of the placenta have shown multiple infarctions in these patients with second and third trimester losses (14,15,16).

In this study 35 habitual abortion, 22 spontaneous abortion and 6 late intrauterine fetal death cases were primarily investigated for autoantibodies. The low incidence of autoantibodies could be explained by the lack of autoimmune histories or clinical signs except for one patient.

ELISA-ACA test has detected antiphospholipid antibodies with a higher frequency than VDRL (33.3% vs. 6.3 %) and APTT (33.3 vs. 3.1%). These findings are consistent with the literature. In a study of 40 habitual aborters, 9 intrauterine fetal death and 9 intrauterine growth retardation cases, Lockwood *et al.* observed a positvity rate of 27% (15/55) with ELISA-ACA test (17). They were able to detect LAF in 4 (7%) patients with APTT. As also mentioned above, Lockshin *et al.* compared the sensitivities of ELISA-ACA and LAF in 50 patients with SLE. In their series 6 of 12 patients with prolonged APTT's and 10 of 13 patients with positive ELISA-ACA tests had fetal loss.

Although all of these tests (ELISA-APTT, VDRL) detect antiphospholipid antibodies, many authorities noticed discorddances between them. Lockshin *et al.* detected a discordance rate of 35% between ELISA-ACA and LAF tests, and 50% between ELISA-ACA and VDRL tests (18). These discordances have been attributed to the complex immunochemistry of phospholipid molecules. Cross-reactions between different phospholipid molecules in the tissues are frequent. VDRL antigen is a mixture of cardiolipin, phosphatidylcholine and cholesterol. Some investigators have suggested that antibodies formed in syphilis changed the configuration of cardiolipin molecule and this facilitated their interaction with reagins. In the other hand anticardiolipin antibodies formed in

Table 4: ACA, VDRL, APTT and Other Autoantibody Results in Habitual Abortion - Spontaneous Abortion, Late Intrauterine Death and Controls. (x = 11.479, p < 0.05 significant)

PATIENTS	ACL	VDRL	APTT	OTHER AUTOANTIBODIES
	IgG - IgM			
Habitual abortion	12/35 (34.2 %)	2/35 (5.7 %)	2/35 (5.7 %)	2/35 (5.7 %)
Spontaneous abortion	6/22 (27.2 %)	0/22 (0 %)	0/22 (0 %)	0/22 (9 %)
Late Intrauterine Death	3/6	2/6	0/6	1/6
TOTAL	21/63 (33.3 %)	4/63 (6.3 %)	2/63 (3.1 %)	5/63 (7.9 %)
CONTROL	0/27 (0 %)	0/27 (0 %)	0/27 (0 %)	0/27 (0 %)

autoimmune diseases react more easily with pure cardiolipin molecule.

The discordances in LAF tests can be explained in part technical difficulties and variations. It should also be noted that LAF is more easily bound to phosphatidylserine of the phospholipid group. The different tests used in different laboratories and whether a plasma without platelets and phospholipid is used or not can also account for the variations in results (4).

Successful treatment regimens in patients with habitual abortion and antiphospholipid antibodies have been reported (18,19). Although the underlying disorder can not be totally eliminated it can be suppressed with corticosteroids (Prednisolone 40-60 mg/d) and low dose aspirin (80 mg/d). These pregnancies should be monitored very closely as these patients are prone to complications such as preeclampsia, intrauterine fetal growth retardation and death (20).

In conclusion it can be said that in unexplained recurrent fetal losses, the presence of anticardiolipin antibodies can explain the poor reproductive performance and be the only sing of a subclinical autoimmune disorder.

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