PREVALENCE OF LUPUS ANTICOAGULANT IN RECURRENT MISCARRIAGES

ANITHA A. ● JYOTHY A. ● SOMAYAJULU G. ● REDDY P.P.

SUMMARY

Lupus anticoagulant levels were measured in a referred population of women with a history of two or more, first or second trimester miscarriages. Of the 102 women tested, 21 (20.6%) were found to be positive for LAC. An increased frequency was observed in the present study compared to earlier reports. This increase is attributed to the selection of cases and the methodology employed.

INTRODUCTION

The association between autoimmune disorders and recurrent pregnancy loss have been strenghthened with the discovery of antiphospholipid antibodies as an etiological factor in repeated pregnancy loss.

Lupus anticoagulant (LAC) is a circulating immunoglobulin which binds to negatively charged phospholipids. A laboratory characteristic of LAC is the inhibition of activated partial thromboplastin time and its derivative assays (Shapiro and Thiagarajan 1982). This in vitro laboratory phenomenon is a consequence of antibody binding to negatively charged phospholipid surface in which factor Xa and Va convert prothrombin to thrombin (Shapiro and Thaiagarajan 1982). In vivo lupus promotes thrombosis via a myriad of potential prothrombotic mechanisms. Several authors have reported an association between adverse pregnancy outcome and the presence of these antiphospholipid antibodies (Parazini et al 1991, Maclean et al 1994). These observations lend credence to the hypothesis that antiphospholipid antibodies are directly responsible for the associated pregnancy wastage. Related studies on the Indian population are very meagre. Therefore this study was aimed at determining the frequency of LAC in a referred population

Inst. of Genetics and Hospital for Genetic Diseases, Osmania Medical College, Hyderabad.

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of women with history of recurrent miscarriages.

MATERIALS AND METHODS

One hundred and sixteen women with a history of two or more first or second trimester miscarriages referred to our institute from different maternity hospitals were included in the study. Relative data and histories of these subjects were recorded in a special case proforma. Clinical examination, ultrasound, cytogenetic and routine biochemical investigations were performed to rule out the involvement of uterine, chromosomal, hormonal and other biochemical defects for the fetal loss. Among the selected subjects 14 women were found to have the above abnormalities and hence excluded from the study.

Blood samples were collected in citrated tubes by venipuncture and coagulation assay was carried out in the plasma that was separated on the same day from the patients as well as the control subjects. Lupus anticoagulant activity was measured by the activated partial thromboplastin time according to the method of Proctor and Rapaport (1961) with relevant modifications within 2 hrs of collection. For each batch of test samples, plasma collected from normal pregnant cases (pooled) were included to arrive at normal reference control clotting time. The test was carried out in 3 stages. The activated partial thromoboplastin supplied by Tulip was diluted (1 in 10 dilution). Patient's plasma 0.1 ml was added to 0.1 ml of the diluted activated partial thromboplastin and incubated for 2 minutes at 37°c. After incubation 0.1 ml of 0.025 M CaCl2 was added and the time to clot formation noted. Clotting times were measured in duplicates.

The general guidelines for the diagnosis of lupus anticoagulant suggested by the Kingston antiphospholipid antibody study group were followed (Dudley and Branch 1989). A patient's plasma was considered positive for LAC activity when the following criteria were fulfilled.

Stage I. Prolongation of coagulation time, Stage II. Consistent prolongation after mixing patient's plasma with equal volumes of normal control plasma, Stage III. Inhibition of anticoagulant affect, thereby reversal to normal aPTT, when direct aPTT reagent (excess phospholipid) was added.

RESULTS

Twenty one (20.6%) of 102 subjects with recurrent abortions revealed the presence of LAC. These 21 cases registered

Activated partial thromboplastin time in seconds in LAC antibody positive cases in recurrent miscarriages and control subjects.

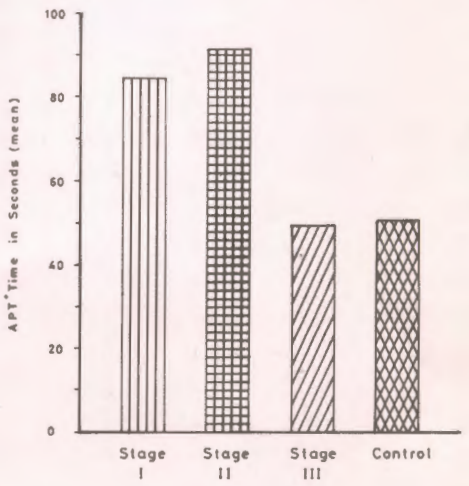

Fig. 1
The histogram shows the prolongation of aPPT in Stage I and Stage II of the patients and their reversal to the control time.

Table I

Shows the activated partial thromboplastin time in seconds in LAC antibody positive cases in recurrent miscarriages group and control subjects.

Sr.No.	Activated partial thromboplastin time in seconds			
	Stage I	Stage II	Stage III	Control
1.	137.0	143.0	87.0	79.0
2.	78.0	87.0	46.0	48.0
3.	101.0	104.0	51.4	49.0
4.	95.0	99.0	47.5	42.0
5.	84.0	93.0	50.0	53.0
6.	64.5	73.0	43.0	47.5
7.	56.0	81.0	33.0	32.0
8.	91.0	103.5	61.0	59.0
9.	72.0	73.0	37.5	36.0
10.	74.0	83.0	41.0	43.0
11.	81.0	87.5	49.5	52.0
12.	89.0	91.0	51.0	54.0
13.	82.5	88.0	42.5	47.0
14.	76.0	74.0	37.0	38.0
15.	74.0	86.0	49.0	51.0
16.	77.0	89.0	51.0	54.0
17.	88.0	90.0	53.0	53.5
18.	110.0	114.0	64.0	58.0
19.	79.0	85.0	50.0	54.0
20.	88.0	93.0	49.0	48.0
21.	71.0	84.0	51.0	53.0
22.	—	—	—	53.0
23.	—	—	—	55.0
24.	—	—	—	56.0
25.	—	—	—	58.0
26.	—	—	—	52.0
27.	—	—	—	33.0
28.	—	—	—	54.0
29.	—	—	—	33.0
30.	—	—	—	53.5
31.	—	—	—	55.0
32.	—	—	—	54.5

Sr.No.	Stage I	Stage II	Stage III	Control
33.	—	—	—	53.5
34.	—	—	—	54.0
35.	—	—	—	56.5
36.	—	—	—	53.5
37.	—	—	—	50.0.
38.	—	—	—	52.5
39.	—	—	—	49.0
40.	—	—	—	51.5
41.	—	—	—	52.0
42.	—	—	—	51.0
43.	—	—	—	54.5
44.	—	—	—	51.0
45.	—	—	—	37.5
46.	—	—	—	53.0
	(a)	(a)	(b)	(b)
MEAN	84.19	91.48	49.71	50.61
Standard Deviation	16.70	15.24	10.95	7.99
Standard Error	3.64	3.32	2.40	1.17

Analysis of variance (a) = significant ($p < 0.05$)
(b) = not significant

significant prolongations of aPTT in stage I (84.2 + 3.64) and in stage II (91.5 + 3.4), which later returned to normal reference control timing (49.7 + 2.4) (fig.1). The results obtained were found to be statistically significant. The details are shown in Table I.

DISCUSSION

LAC was first identified in systemic lupus erythematosus patients (Conley and Hartmann, 1952). It was in this group of patients that an increased rate of recurrent abortion and fetal death has been observed (Lockshin et al, 1985). This led to the investigation of lupus antibodies

in women without an apparent cause for repeated pregnancy wastage.

LAC is an immunoglobulin usually IgG but may be IgM (Maclean et al, 1994). It acts by prolonging phospholipid dependent coagulation tests (Hougie, 1985). Various laboratory tests have been proposed (Dudley and Branch, 1989) but we have used the activated partial thromboplastin time because it is readily available and most physicians are familiar with the interpretation of the test.

The important clinical implication of lupus antibodies is its association with systemic and placental vascular thrombosis with decidual vasculopathy leading to placental infarction (Brow, 1991). It is also associated with other obstetric problems (Branch et al, 1989) which are attributed to the hypercoaguable state associated with LAC.

Various studies have found an increased number of women positive for LAC with a history of pregnancy loss. Parazzini et al (1991) found lupus antibody (LA) in 7% of 220 women with a history of 2 or more miscarriages. Maclean et al (1994) found LA in 6.6% of their subjects. A study by Das et al (1991) revealed 10% incidence for lupus antibodies in recurrent abortions. Our study revealed an increased frequency of 20.6% positivity for lupus antibodies in women with two or more first or second trimester miscarriages. It is surmised that the modification adopted in the present study could have increased the sensitivity in the detection limits of lupus antibody. According to original methodology (Proctor and Rapaport 1961), one in five dilution of activated partial thromboplastin showed inconsistent clotting times. Hence suspecting a higher concentration of phospholipid in the reagent supplied by Tulip, further dilution was done according to the principle (the more dilute the phospholipid portion the more sensitive is the test).

The distinguishing feature of LAC associated pregnancy loss is the high incidene of early fetal loss. It has been shown that in untreated LAC positive women a successful pregnancy with a normal grown foetus at term is an unusual event. These women are at an increased risk of repeated early miscarriages and second or third trimester intrauterine deaths. Examination of placenta has shown thrombosis of decidual and placental vessels and multiple placental infarcts (Lubbe et al, 1984). Thus thrombosis might be one of the various mechanisms involved in the etiology of pregnancy loss, emphasising the need for a treatment protocol.

Various therapeutic assays have been proposed, low dose aspirin 75-85 mg/day and prednisone 40-60 mg/day have been adminstered during pregnancy to women with antiphospholipid antibodies (Lubbe et al, 1983) in one such assay. Aspirin inhibits thromboxane synthetase and reduces the risk of vascular thrombosis, where as steriods suppress LA activity (Hadi and Treadwell, 1990). In one of the study (Scott et al, 1987) 36 patients with LAC who had 93% fetal loss rate before therapy achieved 70% fetal viability. Despite this some studies have failed to demonstrate an improved fetal outcome with prednisone alone (Lockshin et al, 1989). Anticoagulants such as heparin and other immunosuppressive agents such as

azathioprine or cyclophosphamide have also been used.

In conclusion, this study further confirms that there is a relation between lupus antibodies and recurrent miscarriages. The fact that in 21 out of 102 women with recurrent miscarriages, we found a 'Lupus' anticoagulant that might offer an explanation for some obstetric problems, suggests that screening for antiphospholipid antibodies must be carried out as a part of routine investigation. On the presence of these antibodies proper treatment should be commenced so that the overall incidence of recurrent fetal loss can be successfully reduced.

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