SERUM ALPHA-1- ANTITRYPSIN AND LUPUS ANTICOAGULANTS IN PREGNANCY INDUCED HYPERTENSION

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SUMMARY

Serum alpha-1- antitrypsin assay and lupus anticoagulant antibody detection were carried out in 24 cases of pregnancy induced hypertension (PIH) and 16 age and gestation period matched controls (normal pregancies). A high incidence of lupus anticoagulant antibodies were detected to the extent of 25% in PIH cases (6/24). No significant alteration in the levels of serum alpha-1- antitrypsin were seen between control and PIH.

INTRODUCTION

Recently antiphospholipid antibodies have been reported in PIH cases (Kilpatrick et al., 1989). Serum alpha-1-antitrypsin levels are also reported to be increased in PIH and not in chronic hypertension, (Espana et al., 1991).

Laboratory tests that have been used to detect lupus anticoagulants include activated thromboplastin time, Russel viper venom time, kaolin clotting time, tissue thromboplastin time (Dudley & Brameh., 1989). Activated partial thromboplastin time is widely used to detect lupus anticoagulants because of its ease and ready availability of kits and most physicians are familiar with the interpretation of the test (Branch et al., 1989.

In view of these observations the present study was undertaken with the following objectives

1) To evaluate the serum levels of alpha-1-antitrypsin as an acute phase marker in pregnancy induced hypertension cases.

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2) To study the incidence of lupus anticoagulants in these cases and correlate with serum alpha-1-antitrypsin.

MATERIALS AND METHODS

This study was carried out on selected patients from Govt. Maternity Hospital, Hyderabad. A total of 24 cases of pregnancy induced hypertension (PIH) along with 16 normal pregnancies as controls were included in this study. The age and gestational period of both the test and control groups were almost similar and majority of them were in their third trimester of pregnancy.

The following investigations were carried out on both test and control cases.

1) Serum alpha-1-antitrypsin assay. The enzymatic method of Albert et al., (1974) was adopted in this study.

2) Lupus anticoagulant activity (LAC). The method of Proctor and Rapaport (1961) was adopted in this study with some modifications. In the original methodology the phospholipid (APTT) reagent was used with a dilution of 1 in 5. However in the present study the phospholipid reagent supplied by Tulip laboratories was diluted 1 in 10. This modification was carried out suspecting a higher concentration of phospholpid in the reagent as it was giving inconsistent clotting times with 1 in 5 dilution.

Sample Collection :

Two types of blood samples were collected using plastic syringe from each patient or control subject:

(A) Clotted blood sample : 2 ml of blood was collected and allowed to clot. Serum was then separated after 1 hour and stored at -20 C until alpha-1-antitrypsin assay was performed. The assay was done either on the same day or before 48 hours after collection.

(B) Whole blood sample : Blood samples were anticoagulated with sodium citrate dihydrate (3.13% W/v in distilled water) in the ratio of 9:1 in plastic tubes. Plasma was separated immediately by centrifuging at 3000 rpm for 15 mts. The plasma samples thus collected were put to lupus anticoagulant activity detection by activated partial thromboplastin time test within one hour of collection. For each batch of test samples, pooled plasma collected from normal pregnancy cases were included to arrive at normal reference control clotting times (termed as reference control).

The general guide lines for the diagnosis of LAC suggested by the Kingston antiphospholipid antibody were followed (Dudley & Branch 1989). A patient's plasma was considered positive for LAC activity, when the following criteria were fulfilled.

(a) Prolongation of coagulation time in stage - I, (b) Consistant prolongation after mixing patient's plasma with equal volumes of normal control plasma in stage - II, (c) Inhibition of anticoagulant effect, thereby reversal to normal clotting time, when direct APTT reagent (excess phospholipid) was added in stage - III.

RESULTS

The analysed data by ANOVA (analysis of variance) were presented in Table I to III.

The mean normal level of clotting time obtained in reference controls (pooled plasmas) was 47.6 + 13.3 seconds (Table I). In PIH cases 6 patients revealed lupus anticoagulant antibodies (25%) in which prolongation of

Table I

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) RESULTS (IN SECONDS) IN LUPUS ANTICOAGULANT ANTIBODY (LAC) POSITIVE CASES OF PREGNANCY INDUCED HYPERTENSION (PIH) COMPARED TO REFERENCE CONTROLS (POOLED).

Total PIH cases - 24
LAC positive cases - 6
Controls cases - 13

Sl. No.	Stage I	Stage II	Stage III	Control
1.	48.5	51.0	35.5	31.0
2.	63.5	53.0	33.0	38.0
3.	99.0	74.0	55.0	55.0
4.	73.5	68.0	47.0	48.0
5.	114.0	81.5	42.0	33.0
6.	114.0	82.5	41.0	37.0
7.	-	-	-	42.0
8.	-	-	-	54.0
9.	-	-	-	79.0
10.	-	-	-	55.0
11.	-	-	-	54.0
12.	-	-	-	36.0
13.	-	-	-	57.0
	(a)	(a)	(b)	(b)
MEAN	85.4	68.3	42.2	47.6
S D	27.6	13.7	8.0	13.3
S E	11.3	5.6	3.3	3.7

Analyais of variance: (a) - significant ($p < 0.05$).
(b) - not significant.

APTT was seen in stage I (85 + 28 seconds) and stage II (68 + 14 seconds). The abnormal clotting time observed in stage I & II returned to normal in stage III (42 + seconds). Analysis of variance showed signiificance between reference control clottting times and stage I & II times of the 6 patients ($P < 0.05$). No control group revealed lupus antibodies.

Table II

SERUM ALPHA-1-ANTITRYPSIN (A1AT) LEVELS (UMOL/MT/ML) ALONG WITH LUPUS ANTICOAGULANT (LAC) FINDINGS AND GESTATIONAL PERIOD IN CONTROL & PIH GROUPS.

Total control cases - 16
Total PIH cases - 24

Sl. No.	Control			PIH Group			
	Age (yrs)	A1AT	Gestational (Trimester)	Age (yrs)	A1AT	Gestational (Trimester)	LAC
1.	30	4.5	3	25	5.51	3	+ve
2.	20	4.4	3	35	5.78	3	+ve
3.	25	2.9	2	20	5.87	3	-ve
4.	25	4.5	3	18	4.42	3	-ve
5.	30	3.6	2	20	4.50	3	-ve
6.	22	4.5	3	22	4.68	3	-ve
7.	20	4.6	2	18	4.68	3	-ve
8.	25	4.3	3	20	4.68	3	-ve
9.	25	4.5	3	25	4.41	3	-ve
10.	30	4.6	3	21	4.68	3	-ve
11.	25	4.6	3	22	4.20	3	-ve
12.	20	4.2	3	21	4.50	3	+ve
13.	26	4.6	3	20	4.40	3	-ve
14.	22	4.4	3	20	4.60	3	-ve
15.	22	4.4	3	28	4.70	2	+ve
16.	27	4.3	3	25	4.40	3	-ve
17.	—	—	-	21	4.30	3	-ve
18.	—	—	-	22	4.70	3	+ve
19.	—	—	-	20	4.10	3	-ve
20.	—	—	-	19	4.10	3	-ve
21.	—	—	-	35	3.90	3	-ve
22.	—	—	-	19	4.10	3	-ve
23.	—	—	-	18	4.60	3	-ve
24.	—	—	-	20	4.70	2	+ve
MEAN	25	4.31	-	22	4.60	-	-
S D	3	0.45	-	5	0.49	-	-
S E	0.9	0.11	-	0.9	0.10	-	-

ANOVA = Not Significant

Table III

SERUM ALPHA-1-ANTITRYPSIN LEVELS (UMOL/MT/ML) IN LAC POSITIVE AND NEGATIVE CASES IN PIH GROUP.

Total PIH cases - 24
LAC Positive - 6
LAC Negative - 18

Sl. No.	Serum alpha-1-antitrypsin level	
	LAC positives	LAC negatives
1.	5.51	5.87
2.	5.78	4.42
3.	4.50	4.50
4.	4.70	4.68
5.	4.70	4.68
6.	4.70	4.68
7.	-	4.41
8.	-	4.68
9.	-	4.20
10.	-	4.40
11.	-	4.60
12.	-	4.40
13.	-	4.30
14.	-	4.10
15.	-	4.10
16.	-	3.90
17.	-	4.10
18.	-	4.60
MEAN	4.98	4.49
S D	0.53	0.42
S E	0.22	0.10

ANOVA : Not Significant

The mean serum alpha-1-antitrypsin (umol/mt/ml) level obtained in control group was 4.3 + 0.45 (Table ii). There was predominance of third trimester gestation in both control (81%) and PIH group (92%). The mean ages of these two groups were

almost similar. The serum alpha-1-antitrypsin level in PIH group was 4.6 + 0.49 and no significance was seen by ANOVA studies with control level.

The levels of serum alpha-1-antitrypsin studied from the point of LAC findings were depicted in Table III. Despite slight elevation in the mean level in LAC positives of PIH group, no statistical significance could be seen between these LAC postitives and negatives.

DISCUSSION

The mean serum alpha-1-antitrypsin level in pregnant control (4.31 + 0.45 umol/mt/ml) was higher than the non-pregnant level obtained (3.43 +0.49 umol/mt/ml) for females as mentioned in chapter five. This was due to the hormone status in pregnancy. It was clearly established that high estrogen levels in pregnancy states boost up serum alpha-1-antitrypsin and mechanisms that induce enhanced synthesis rates are not known (Ganrot 1972).

Autoimmune mechanisms appear to be significant in PIH. This study revealed a high percentage of lupus anticoagulant antibodies in PIH (25%). Branch et al (1989) reported 16% incidence of antiphospholipid antobodies in PIH and 14% of this had lupus antibodies also. It is surmised that the modifications adopted in the present study could have increased the sensitivity in the detection limits of lupus antibodies.

Despite higher incidence of LAC antibodies in PIH, the serum levels of alpha-1-antitrypsin did not register any significant deviation from control cases. Unlike the observations of Espana et al., (1991), no significant elevations of serum alpha-1-antitrypsin were recorded in PIH group and hence it is not a good marker of acute phase states in these cases.

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