PLASMA ANTITHROMBIN LEVELS OF PAKISTANI WOMEN WITH PRE-ECLAMPSIA

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Plasma antithrombin HI level/activity was determined in the gravid Pakistani women. Levels of AT III were significantly decreased in pre-eclamptic women compared to normal pregnant controls. The degree of reduction in plasma A T III level was correlated with the severity of the disease. In no instance was low plasma AT III associated with normal pregnancy. The relation of decreased AT III level may he valuable as a tool in diagnosing pre-eclampsia as a screening test/predictor, and as an indicator of severity of disease.

INTRODUCTION

The syndrome termed pre-eclampsia (PE) is difficult to discern in the early stages. The diagnosis is evident when the classic pre-eclamptic triad of hypertension, proteinuria and pathologic oedema is present. However, when components of classic triad are absent, or when onset of the disease is fulminant, the diagnosis becomes uncertain. The varied presentation, clinical course and multi-system nature suggest that more than one disease entity may exist within the syndrome of PE.

major cause of maternal and foetal mortality and morbidity around the globe,[1] while 19% of Pakistani women suffer from PE [2].A myriad of biomechanical and biochemical tests have been applied diagnostically but none has found wide acceptance [3-8] AT 111 level may be useful in the hypertensive preterm woman in whom the diagnosis is unclear.[9]

A low level of AT III, a serine protease inhibitor, active against thrombin, factor Xa and VI1 has been reported in manifesting disseminated intravascular coagulation associated with severe PE. The coagulopathy could, successfully, be treated by the administration of AT HI concentrate.[10]

A low levels of AT III have been associated with deep vein thrombosis and pulmonary embolism[11-13] A deficiency in AT III may be found in families whose members have a high frequency of thromboembolic episodes.[14] Plasma AT III showed no significant change in the normal pregnant control.[15,16] The findings incidate that AT III level is significantly decreased in PE showing correlation with the severity of the disease.

MATERIALS AND METHODS

Samples of plasma were obtained from obstetric patients cared for at Lady Wellington Hospital, Sir Ganga Ram Hospital, Services Hospital, Jinnah Hospital and Lahore General Hospital, Lahore, Pakistan, during both outpatient and inpatient visits.

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Plasma antithrombin III level was determined by NOR-partigen AT III plates based upon the principal of Radial Immuno-diffusion (RID). The plates contained mono-specific antiserum to human antithrombin III in agarose gel layer. The antiserum was obtained by immunization of rabbits (Behrinwerke AG, Germany). Briefly, samples obtained by phlebotomy were anticoagulated with trisodium citrate. Nine volumes of blood were added to I volume of the anticoagulant solution and immediately well mixed and centrifuged at 1500 g for 15 minutes. Supernatant was removed and stored in a clean plastic tube at -20^{H}C for the estimation of AT III. Plasma was then thawed at 37°C. The contents of the standard, control and test plasma were put in the equal sized wells. Wells were punched in the agarose gel in which mono-specific, anti-human antithrombin III had been incorporated in uniform concentration. The volume required per well (5 ul) was dispensed with Behring dispenser. The antigen (Ag) diffused out of the wells to form soluble complexes (in Ag excess) with the antibody (Ab). These continued to diffuse outwards, binding more Ab, until an equivalence point was watched and the complexes precipitated in a ring after 48 hours' incubation at 37°C. The area within the precipitin ring, measured as ring diameter squared, was proportional to the Ag concentration. Unknowns were derived by interpolation from the standard table. AT HI levels below 0.244 g/1 represented by 5.6 mm diameter and 81% activity were considered as abnormal. The diameter of the precipitin ring was measured to accuracy by Behring lupe scale against a black background with lateral illumination. Plasma AT III level and activity was noted from the table published in the literature supplied by Behringwerke AG. Germany.

Analysis

A total of ninety subjects were divided into following groups:

1. Control (Group I): It comprised of 30 healthy pregnant women.
2. Patient (Group II): It consisted of 60 already diagnosed cases of PE.

The study group was further divided into two sub-groups;

II (A) Moderate Pre-eclampsia:
It included 30 patients. The criteria for the selection of the subjects was as follows:

a) Blood pressure 140/90 to 159/109
b) Proteinuria 1-5 g/l
c) Weight gain I kg/week

II (B) Severe/Fulminant Pre-eclampsia:
It consisted of 30 patients. The criteria for the selection of the subjects was as below;

a) Blood pressure >160/110 mmHg
b) Proteinuria > 5g/l
c) Oedema Generalized

RESULTS

Plasma AT III level in group I was 0.242±0.001 with a range of O. 192-0.407 g/l. The level in subgroup 11A was reduced i.e. 0.205±0.040 g/l with a range from 0.1 11-0.258 g/l. The AT III levels decreased even further in case of subgroup IIB i.e. 0.132-0.52 g/l with a range of 0.060-0.205 g/l. Statistically the decrease in AT III level was found to be highly significant (P<0.001) Table I).

AT III activity in group I was 81.06±14.36% with a range of 64-136% with a range of 64-136% of normal. The activity in subgroup 11A was 68.70±13.63 with a range of 37-86%. Further reduction was observed in subgroup IIB i.e. 44.03H7.45 with a range from 20-73% of the normal. The reduction was observed to be highly significant (P<0.00) in patients of pre-eclampsia (Table 2).

No case of clinically diagnosed thromboembolism was observed in moderate preeclampsia group. Two subjects out of thirty, had thromboembolism (6.6%) with decreased AT III level/activity in fulminant pre-eclampsia. Deep vein thrombosis was found in 1(3.3%) patient with AT III levels/activity of 0.080 g/l (27%). Same was the number for pulmonary embolism i.e. 1(3.3%). However, the level of AT III was 0.060 g/l (20%) (Table 3).

DISCUSSION

The present study is reflective of the fact of significantly decreased levels of AT III in gravid Pakistani women with pre-eclampsia. The reduction was found to be even pronounced in severe/fulminant pre-eclampsia. The results are in conformity with the results of many research workers.[17, 22] However, Gow et al[23] were unable to show alteration and Beller et al[24] found no significant change in AT III levels. Plasma AT III level showed no change in the normal pregnant control, the finding was consistent with the observations of Weiner and Brandt[15] and Xu et al[16].

Pre-eclampsia is called a disease of theories. No definite cause has been identified. [25] But association of thromboembolism with reduced AT III levels have been documented.[17,27,31] However, the thromboembolic complications were observed in 6.6% of the patients of fulminant pre-eclampsia. Plasma III level/activity was also reduced in these females with pulmonary embolism and deep vein thrombosis indicating relation of decreased level of AT III with thromboembolism. However, the difference in incidence is probably based on the tendency to report clinical events, and therefore asymptomatic individuals who have AT III defects detectable by laboratory testing, may well be under represented.[31] Recognition of point mutation in coagulation factor V and a metabolic disorder, homocytonaemia. can substantially, improve the diagnosis of venous and arterial thrombosis. [32]

It has been observed that there is definite decrease of AT III levels showing substantial correlation with the severity of pre-eclampsia. Radial immuno-diffusion (RID) technique exhibits a wide, uniform assay range from 0.060 g/l (20%) to 0.906 g/l (302%). The range in terms of precipitin ring diameter is 3.0 to 9.3 mm. The degree of reduction of AT 111 level determines the severity and grouping of the patients i.e. moderate and severe/fulminant preeclampsia. More marked the decrease, the more severe PE. [34,35]

Plasma AT III level/activity starts decreasing as much as 13 weeks prior to the development of clinical manifestations. The minimum level of AT III in different studies was reported, 15%°, 22.33% [36] and 27.73%.[37] Therefore, it was concluded that a pathologically significant drop would range between 15 and 30%.[28] It has been suggested that infusion of AT III concentrate should be tried when plasma AT III is significantly decreased in pre-eclampsia.[35] The variation in the plasma AT III activity found by different workers and in the present study may be due to selection of patients at different stages of disease process, variable sample size, and the criteria for the selection of the subjects.

We conclude that, in our patients, comprising of Pakistani women with pre-eclampsia, there was a definite decrease of AT III levels. The reduction correlated well with the severity of the disease. The patients with symptom less prodromal condition detected by the clinician at antenatal booking should be considered as a "potential candidate" for the development of pre-eclampsia at a later stage of pregnancy. Such women should be screened at this stage for early diagnosis and better management of mother and baby. RID method seems to provide a simple, feasible and reproducible method of testing AT III levels in our set-up.

Table-1: Plasma Antithrombin III (AT III) Levels (g/I) In The Controls and Patients of Pre-eclampsia (PE).

Plasma AT 111 (g/1)	Control (Group 1)	Moderate PE (Subgroup IIA)	Fulminant PE (Subgroup IIB)
Mean	0.242±0.001	0.205±0.040	0.132±0.052
Range	0.192±0.0.407	0.1 11±0.258	0.060±0.0.2051
Number of subjects	30	30	30

P Value:

I vs HA P>0.00l (Highly significant)
I vs HB P<0. 001 (Highly significant)
HA vs HB P<0.001 (Highly significant)
SD Standard deviation

Table-2: Plasma (%) of Normal Activity of Antithrombin III (AT III) in the Control and Patients of Pre-eclampsia (PE).

AT III (% of normal)	Control (Group 1)	Moderate PE (Subgroup IIA)	Fulminant PE (Subgroup IIB)
Mean	81.06±14.36	68.70±13.63	44.03±17.46
Ramie	64-136	37-86	20-73
Number of subjects	30	30	30

P Value:

I vs HA P>0.00l (Highly significant)
I vs HB P<0. 001 (Highly significant)
HA vs HB P<0.001 (Highly significant)
SD Standard deviation

Table-3: Thromboembolism and Pre-eclampsia (PE).

	Moderate PE (Subgroup IIA)		Fulminant PE (Subgroup IIB)	
	Pulmonary embolism	Deep vein thrombosis	Pulmonary embolism	Deep vein thrombosis
AT HI (.4/1)	-	-	0.060	0.080
AT 111 activity(%)	-	-	20	27
Number of patients	-	-	1(3.3%)	1(3.3%)
No. of total subjects	30		30	

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