

Plasma Antithrombin III Levels and its Correlation with Proteinuria in Pre-Eclamptic Females: A Study of 60 Cases

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The cause(s) of the low plasma antithrombin III (AT III) level in woman with pre-eclampsia (PE) is unknown. Decrease has been attributed to increased consumption, decreased synthesis or increased urinary loss. AT III levels correlate with maternal morbidity as revealed by hepato-renal damage. The purpose of this study was to evaluate the relationship of reduced plasma AT III level with proteinuria in pre-eclampsia. Sixty pre-eclamptic women (thirty with moderate and thirty patients of fulminant pre-eclampsia) were studied. Prospects of urinary loss as a cause of low AT III level in PE were intended to be examined as proteinuria was the best predictor of foetal outcome. However, there was a significant increase ($P < 0.001$) in proteinuria in PE. But correlation of proteinuria with AT III level in moderate PE ($r = -0.0083$) and fulminant PE ($r = -0.2028$) showed no significant correlation. These findings support the hypothesis that, though there is significant increase in proteinuria but it has got no significant correlation with low AT III level. Thus, it excludes proteinuria as a cause of reduced AT III level in PE. Monitoring of AT III level and proteinuria may be used for diagnosis and proper management of PE.

Key words: Antithrombin III, proteinuria, pre-eclampsia

Pre-eclampsia is defined as the presence of the triad of elevated blood pressure, proteinuria and oedema after 20 weeks of gestation and upto 6 weeks post-partum¹. Pre-eclampsia continues to be a major cause of maternal and perinatal mortality². Until now the exact pathophysiological and psychosocial basis has not been entirely elucidated³. The cause of the decline in antithrombin III (AT III) levels associated with pre-eclampsia (PE) is unknown. Though several possibilities exist. AT III is the principle inhibitor in vivo of thrombin generation and its functional reserve is reduced during pregnancy⁴ and the low level could represent increased consumption due to accelerated clotting cascade activity. Heparin accelerated the reaction largely by enhancing formation of thrombin-AT complexes⁵. Antithrombin III and low molecular weight heparin are useful in DIC⁶ The reduction might result from decreased hepatic synthesis and it could also reflect extrinsic loss such as that known to occur in non-pregnant patients with nephrotic syndrome⁷ The decline might be the product of enhanced catabolism unrelated to the coagulation system. It was also concluded that urinary loss appears to be the major mechanism of decreased AT III level in PE⁸, which has got association with thromboembolism⁹ The purpose of this study was to determine the degree of proteinuria and its correlation with the decreased levels of AT III with a view to establishing the fact of better planning for management of PE.

Material 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.

Plasma antithrombin III (AT III) level was determined by NOR-partigen AT III plates based upon the principle of Radial Immuno-diffusion (RID). The plates contained mono-specific antiserum to human AT III in agarose gel layer. The antiserum was obtained by immunization of rabbits (Behringwerke AG, Germany). Briefly, samples obtained by phlebotomy were anticoagulated with trisodium citrate. Nine volumes of blood were added to 1 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°C . The contents of the standard, control and test plasma were put in the equal sized wells. Wells are punched in the agarose gel in which mono-specific, anti-human AT 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 (Ag excess) with the antibody (Ab). These continued to diffuse outwards, binding more Ab, until an equivalence point was watched and the complexes precipitate 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. AT III levels below 0.244 g/l represented by 5.6 mm diameter 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 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 composed of 30 healthy pregnant women.

Plasma Antithrombin III Levels

2) *Patient (Group II)*: It consisted of 60 diagnosed cases of PE.

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

b) Proteinuria 1 to 5 g/l

c) Weight gain 1 kg/week

IIB 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 >5 g/l

c) Oedema Generalized

The control and patients were age, gestation and socioeconomically matched.

A commercial kit supplied by Randox, UK was used for the estimation of total urinary proteins (24 hours) on a clean catch specimen. Protein forms complex with pyrogallol red in acid medium containing molybdate ions and gives rise to blue coloured complex with maximum absorption at 600 nm optical density.

The collection of 24 hours urine was done in a clean, wide necked, dry leak-proof container to obtain clean-catch specimen. The first urine specimen, at a particular time, was discarded and on subsequent voiding, urine was collected till the next day at the same specified time. The last urine sample was included in the total volume. The 24 hours urine (refrigerated during collection) was well mixed and total volume was recorded. The required specimen was brought to room temperature and centrifuged to remove suspended matter for the determination of urine total proteins.

Results are presented as mean with standard deviation. P stands for significance difference and r for correlation. Students "t" test was used.

Results

The total urinary proteins (g/24 hour) in group I was 0.13 ± 0.10 g/24 hour with a range of 0.04-0.27 g/24 hour. In subgroup IIA, the total urinary proteins increased to 3.12 ± 1.36 g/24 hour showing range of 1.3-5 g/24 hour and in subgroup IIB, it further increased to 7.93 ± 2.62 g/24 hour with a range of 5.0-13.8 g/24 hour. Total urinary proteins demonstrated highly significant ($P < 0.001$) increase in patients of PE (Table 1).

Plasma AT III mean level in group I was 0.242 ± 0.001 with a range of 0.192-0.407 g/l. The level in subgroup IIA was found to be reduced i.e. 0.205 ± 0.040 g/l with a range from 0.111-0.258 g/l. The AT III level decreased even further in case of subgroup IIB i.e. 0.132 ± 0.052 g/l with a range from 0.060-0.205 g/l. Statistically, the decrease in AT III level was found to be highly significant i.e. $P < 0.001$ (Table 2).

Table 1 Total urinary protein (g/24 hour) in the control and patients of pre-eclampsia (PE) (Expressed as mean \pm SD)

Protein (g/24 hour)	Control (Group. I)	Moderate PE (Subgroup IIA)	Fulminant PE (Subgroups IIB)
Mean	0.13 ± 0.10	3.12 ± 1.36	7.93 ± 2.62
Range	0.04-0.27	1.3-5	5.0-13.8
Number of subjects	30	30	30

P value:
 I Vs IIA $P > 0.001$ (highly significant)
 I Vs IIB $P < 0.001$ (highly significant)
 IIA Vs IIB $P < 0.001$ (highly significant)
 SD Standard deviation

Table 2 Plasma antithrombin III (AT III) levels (g/l) in the control and patients of pre-eclampsia (PE) (Expressed as mean \pm SD)

Plasma AT III (g/l)	Control (Group. I)	Moderate PE (Subgroup IIA)	Fulminant PE (Subgroups IIB)
Mean	0.242 ± 0.001	0.205 ± 0.040	0.132 ± 0.052
Range	0.192-0.407	0.111-0.258	0.060-0.205
Number of subjects	30	30	30

P value:
 I vs IIA $P > 0.001$ (Highly significant)
 I vs IIB $P < 0.001$ (Highly significant)
 IIA vs IIB $P < 0.001$ (Highly significant)
 SD Standard deviation

There was no significant correlation between total urinary proteins and plasma AT III ($r = -0.2028$) in fulminant PE group. In case of moderate PE, the relationship showed no significance i.e. $r = -0.0083$ (Table 3).

Table 3 Correlation and significance of total urinary proteins with antithrombin III levels in patients of pre-eclampsia (PE)

Statistical Analysis	Moderate PE (Subgroup IIA)	Fulminant PE (Subgroups IIB)
R	-0.0083	-0.2028
P value	0.965	0.282
Significance	NS	NS
Number of Subjects	30	30

Key:

NS = Not significant

r = Correlation co-efficient

P = Probability of chance

Discussion

The present study clearly proves the fact of significantly decreased levels of AT III in PE. The reduction was found to be even pronounced in fulminant PE. The results are in conformity with the results of a number of research workers¹⁰⁻¹⁵ However, Gow et al¹⁶ was unable to show alteration and Beller et al¹⁷ 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 Brandt¹⁸ and Xu et al¹⁹. The degree of proteinuria was significant in patients in comparison to the control group. Tayyab and Salam²⁰ stated that protein present in the urine is too low to be detected by the routine urine analysis methods. The presence of proteinuria in the present study correlated well with the findings of Sibai²¹, Davey²² and Crombleholme and Evans²³ where it was further concluded that the enlargement of glomeruli; thickening of the glomerular tuft; vacuoles in tuft epithelium etc. are the salient features in pre-eclampsia. The relationship between proteinuria and AT III level was not significant. The finding was in accordance with the conclusion of Weenink et al²⁴ and Weiner²⁵.

We conclude that, in our patients, the level of AT III was significantly decreased. The reduction was more pronounced in fulminant PE. The degree of proteinuria was significant but correlation of proteinuria with decreased AT III level showed no significant relationship. The findings ruled out proteinuria/urinary loss as a cause of reduced levels of AT III in PE. Based up the findings, further studies on different population groups, could be designed for the right-time diagnosis and proper management of pre-eclampsia.

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