ORIGINAL ARTICLE

# Alpha-1antitrypsin in pre-eclampsia; from a clinical perspective

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## Abstract

**Objective:** To assess serum Alpha-1 antitrypsin (AAT) levels in women with pre-ecclampsia and correlate them with other parameters of pre-eclampsia.

**Methods:** A case-control study was conducted at Al-Yarmouk Teaching Hospital, Baghdad from February 2019 to February 2020. Included were 85 pregnant women with 32-34 weeks of gestation. They were divided in 2 groups: pre-eclamptic cases 40/85, and normotensive controls (45/85). PE was defined based on NICE 2018 guidelines. Patients' age and blood pressure (BP) were recorded. We evaluated mean platelet volume (MPV), platelet distribution width (PDW), serum levels of uric acid, and AAT blood samples.

**Results:** AAT levels were significantly lower among preeclamptic cases (0.26) mg/dl versus controls (0.63) mg/dl. Negative correlations were found between levels of AAT and other variables (systolic BP, diastolic BP, MPV, PDW, and serum uric acid levels) with a coefficient of correlation ( r) -0.43,-0.43,-0.25,-0.25, and- 0.26 respectively. P-value <0.05 was estimated for all.

**Conclusion:** Significant lower levels of AAT in pre-eclamptic patients versus controls suggest that it contributes to pre-eclampsia development. The second finding was negative correlations of reduced AAT with the most common maternal parameters assessing pre-eclampsia severity. Taken together, these results indicate that AAT is intimately linked to the pathophysiology of PE development and progression. Further work is warranted to verify the AAT role in pre-eclampsia.

**Keywords:** Alpha-1 antitrypsin, Pre-eclampsia, Maternal platelets, Serum uric acid. (JPMA 71: S-53 [Suppl. 8]; 2021)

## Introduction

Pre-eclampsia (PE) is a unique pregnancy syndrome that affects 4%-8% of all pregnancies substantially influencing maternal and neonatal well-being.[1] The exact pathophysiology is undetermined.[2] Studies attribute maternal endothelial dysfunction as a cause of PE.[3] Therefore, various biomarkers were proposed to aid early screening and prediction.[4]

Alpha-1 antitrypsin (AAT) is a major liver-derived circulating protein, a component of the acute-phase inflammatory reaction, and a natural inhibitor of various protease enzymes. AAT is synthesized by the endoplasmic reticulum and secreted by the Golgi apparatus and is expressed in hepatic cells, neutrophils, placental cytotrophoblast, amnion cells, and pulmonary alveolar cells.[5,6] The liver's daily secreted levels are approximately 34mg/kg of body mass, with the normal plasma concentration being <3.5 mg/dl. Once the inflammatory acute phase response is triggered, AAT levels increase more than fourfold; orchestrating local and systemic inflammation.[6,7] As the neutrophil recruitment starts, serine proteinases are spilled, causing collateral tissue damage. This self-repeated cycle of inflammation and tissue damage continues if adequate protection; exerted by AAT is lost.[8,9]

Recently, AAT attracted attention to its crucial role in the immune system, immunomodulation, inflammation, and injured cell repair, especially during the COVID-19 pandemic where its extraordinary anti-inflammatory activity was suggested to protect against infection.[10] The biological nature of AAT molecules has contributed to significant variations in clinical manifestation. There were reports on therapeutic applications for various medical conditions as graft rejection, type 2 diabetes, and acute myocardial infarction cases as it limits the hypoxia-induced-cell apoptosis.[11]

AAT levels were notably high in women on oral contraceptive pills, suggesting an estrogen-mediated response.[12] Larsson et al.[13] showed that women during pregnancy have increased levels at a gestational age of 34 to 38 weeks, which tends to decrease in the puerperium. Furthermore, it enhances early implantation, increasing odds for a healthy pregnancy. Low levels were associated with spontaneous abortion and preterm labour.[9] Feng in 2012 was the first to describe the protective function of

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AAT in pre-eclampsia development.[14] Other researchers have discussed different mechanisms, pathways at the molecular and biochemical levels.[15,16] We investigated the relationship between serum AAT levels with clinical variables among patients with PE. Our goal was to evaluate AAT correlation to various clinical aspects of pre-eclampsia.

## Patients and Methods

A prospective case-control study conducted in the Department of Obstetrics and Gynaecology at Al-Yarmouk Teaching Hospital in Baghdad, Iraq which lasted one year from February 2019 to 2020. Ethical approval for the study was provided by the hospital's Ethics Committee. All participants gave informed consent before enrollment. A total of 85 pregnant Iraqi women were enrolled. Two groups were defined: the controls normotensive pregnant women comprised (45/85), and pre-eclampsia cases comprised (40/85). Pre-eclampsia was defined based on NICE guidelines[1] as increased blood pressure in an initially normotensive woman measured on two occasions in 4-6 hours apart after 20 weeks of pregnancy and the presence of proteinuria 1+ in dipstick testing. Alternatively, in the absence of proteinuria, a recently detected hypertension along with thrombocytopenia (<100,000/μL), renal dysfunction (serum creatinine concentrations >1.1 mg/dL), liver dysfunction (elevated concentrations of hepatic enzymes double the normal concentration), pulmonary oedema, or cerebral or visual disturbances[1]. At a gestational age of 32-34 weeks, all pregnant women with a viable singleton foetus were enrolled. Full medical history, general examination, vital signs, and obstetric examination were performed. We excluded all pre-eclamptic women in a gestational age <32 weeks or those having a preexisting medical illness (hepatic, renal, and thyroid), diabetes (personal or family history), cardiovascular, or neuronal disorders, blood disorders, personal history of chronic hypertension, and smokers were an exclusion. Maternal parameters recorded for each participant included systolic and diastolic blood pressure, estimated albumin/creatinine ratio from a randomly collected urine sample. The mean platelet volume and platelet distribution width, serum uric acid, and AAT were estimated from blood samples.

On the examination day, 5 mL of blood samples were taken; serum was separated and frozen. Serum uric acid levels were assessed using Chemistry Analyzer AU480 (Beckman Coulter Sn2018011735). According to the manufacturer's instructions, a 2-mL serum was used to estimate AAT levels by human enzyme-linked immunosorbent assay. Blood in EDTA vials used for platelet indices estimation (MPV and PDW) using an automated haematology analyzer DxH520 (Beckman Coulter Sn BC010420).

The Shapiro-Wilk test determined the normality of the observations. Since the data were not normally distributed, results were presented as median and interquartile range IQR for the non-normally distributed parameters. Spearman correlation test assessed the correlation between AAT as the independent variables, while other study parameters were the dependent variables. The Receiver operating characteristic curve (ROC) was used to evaluate the cut-off value for AAT and its highest specificity and sensitivity between the controls and study groups. The analysis was performed using MedCalc and SAS software (2010). Significance was set at P< than 0.05.

## Results

A total of 85 women in 32-34 weeks of pregnancy were recruited, in two groups: the normotensive (45/85) and the pre-eclampsia cases (40/85) cases. Upon comparison, systolic and diastolic blood pressure, platelet MPV and PDW, and serum uric acid were significantly lower in healthy controls versus pre-eclampsia cases, P < 0.0001. Maternal serum levels of AAT were significantly higher among the controls versus pre-eclampsia cases (0.63 versus 0.26) mg/dl, P < 0.0001. Table-1 correlated maternal serum AAT as a primary independent variable with other study variables via the median non-parametric Spearman correlation test. The outcome was a negative correlation between maternal AAT against all other variables, P <0.0001.

In Table-2, the ROC evaluated maternal serum AAT's cut-off value associated with the highest sensitivity and specificity among the pre-eclampsia cases versus the controls. The cut-off value was >0.05mg/dl, with

**Table-1:** Spearman correlation coefficient of maternal serum AAT levels versus maternal parameters.

| Variable | Coefficient of correlation r | P-value | Variable | Coefficient of correlation r |
|---|---|---|---|---|
| Systolic blood pressure (mm/Hg) | -0.43 | <0.0001 | Systolic blood pressure (ml/Hg) | -0.43 |
| Diastolic blood pressure (mm/Hg) | -0.43 | <0.0001 | Diastolic blood pressure (ml/Hg) | -0.43 |
| MPV(fL) | -0.25 | 0.04 | MPV(fL) | -0.25 |

MPV: mean platelet volume; and PDW: platelet distribution width.

**Table-2:** The validity of markers used in diagnosing pre-eclampsia with the associated cut-off values calculated by the ROC curve.

| Variable | Cut-off | Sensitivity | Specificity | AUC | SE a | 95% CI b |
|---|---|---|---|---|---|---|
| MPV(fL) | >8.5 | 66.7 | 100 | 0.895 | 0.038 | 0.79 to 0.96 |
| PDW(fL) | >16.5 | 75.6 | 95 | 0.9 | 0.037 | 0.80 to 0.96 |
| Uric acid(mg/dl) | >6.33 | 100 | 95 | 0.99 | 0.011 | 0.93 to 1.00 |
| AAT*(mg/dl) | >0.05 | 100 | 100 | 1.00* | 0.00 | 0.95 to 1.0 |

MPV, mean platelet volume, PDW platelets distribution width, *AAT alpha-1 antitrypsin mg/dl was the most significant, AUC area under the curve, SE a standard error, 95% CI; confidence interval.

sensitivity 100% and specificity 100%.

## Discussion

Alpha-1 antitrypsin, a serine proteinase inhibitor, was significantly lower in pre-eclamptic women than normotensive groups. We enrolled two groups, healthy controls and pre-eclampsia cases at 32-34 weeks of pregnancy. Feng et al.[14] compared three groups: a control group and 2 study groups; early-onset PE and late-onset PE with gestational ages of 34 weeks as a divider. In this study, maternal serum AAT was negatively correlated with systolic and diastolic BP, $P < 0.0001$, and a correlation coefficient $r= -0.43$ in line with Feng study.[14]

Analyses showed that PDW and MPV were higher among PE cases versus healthy controls. This is in accordance with Karateke et al. and Dhakre et al's. study.[17,18]

An inverse correlation was found between platelets indices PDW, MPV, and maternal serum AAT with a correlation coefficient $r= -0.25$, $P < 0.04$.

Kumara et al.[19] examined the role of serum uric acid in maternal and foetal outcomes. He describes a better prediction for the foetal outcome rather than maternal outcome.[19] The result obtained in this study confirms a negative correlation $r= -0.26$, $P < 0.03$ with maternal serum AAT.

Feng et al.[14] reported maternal serum AAT levels of 1.22 mg/dl, 0.72 mg/dl for the early and late-onset PE group, respectively Twina et al. 20 reported a maternal serum level of AAT among women with PE as 3.85 mg/dl versus 7.39 mg/dl in normotensive pregnant females at 24-42 weeks of gestation. In this study, the maternal serum AAT level was 0.26 mg/dl in pre-eclamptic cases versus 0.63 mg/dl in a normotensive group at 32-34 weeks. Differences in the inclusion criteria, gestational age, and sample size have contributed to variations in the estimated maternal serum values of AAT.

The ROC assessed the cut-off value for AAT in the PE diagnosis; >0.05mg/dL (95% CI 0.95 to 1.0), $P<0.05$ associated sensitivity and specificity were 100% and 100% respectively. Alpha-1 Antitrypsin was correlated with pre-eclampsia's clinical aspects, including systolic and diastolic BP, platelet indices, and serum uric acid levels. Earlier reports considered the molecular and bio-physiological aspects of that relationship.[14,16,20]

Feng separated AAT among other protamine's related to PE from the placenta. He spotted the lowest level in endothelial cells, trophoblast cells, and chorionic cells. He proved the defense protein action by injecting it into animal models deemed by PE. Interestingly, the animal reverted to lower blood pressure reading and protein excretion in urine.[14,15]

Feng et al. and Jonigk et al.[21,22] adapted oxidative stress theory as a platform to explain the action of AAT in pre-eclampsia. Oxidative stress initiates injury into endothelial cells, which represents the core of PE. Consequently, a cascade of inflammatory response is initiated if this continues without suppression and renders the path to PE progression.[23,24]

The protective role of AAT was proposed through a family of proteins called Smad family proteins and Id family proteins.[23] They control the inflammatory reaction and endothelial cell function. AAT directly regulates the activity of Ids via Smads, empowers the antioxidant defense system and lowers apoptosis. Preeclampsia contributes to the suppression of Id4 and Smad2 whereas AAT can set the balance.[21,23]

Another pathway for the antioxidant effect is the suppression of the P38 mitogen-activated protein kinase signaling pathway; P38 MAPK is a member of the MAPK superfamily, which regulates diverse cellular events, needed in placental organogenesis besides trophoblastic invasion and growth, AAT located in the trophoblast will encourage its proliferation, migration, and suppress apoptosis. Its deficiency manifests as endothelial dysfunction and will trigger PE cascade syndrome.[14,25]

The present study has its strength as it correlates maternal serum levels of AAT with pre-eclamptic women's clinical aspects. The study has its limitations also. The participants could not be followed until term. Neither could the effect of the estimated serum AAT levels on maternal and foetal outcomes be estimated. There is a paucity of data

regarding both the mother and infant outcomes, which warrant further work.

## Conclusion

Maternal serum AAT levels were significantly lower in pre-eclamptic cases than controls, suggesting its involvement in pre-eclampsia's pathophysiology. The inverse correlation with pre-eclampsia's clinical aspects indicates that it can be of value in severity assessment. Identification of rapid and reliable prognostic markers can improve survival and guide the obstetrician.

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