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# Assessment of annexin A5 and annexin A2 levels as biomarkers for pre-eclampsia: A pilot study

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ABSTRACT

Deficient anticoagulant activity of annexin A5 and deficient profibrinolytic activity of annexin A2 have been linked to increased risk of thrombotic events. Placental dysfunction due to fibrin deposition/microthrombi has been implicated in the pathogenesis of pre-eclampsia (PE). In this study, we aimed to assess serum levels of annexin A5 and annexin A2 in a cohort of PE patients and investigate their role as biomarkers for the development of the disease. We examined 80 women in total; 40 healthy pregnant women and 40 pregnant women with PE after 20 weeks of pregnancy. Women were subjected to full clinical assessment, ultrasonography, and laboratory testing including complete blood picture, liver and kidney function tests and assessment of serum and urine proteins. Annexin A5 and annexin A2 were analyzed using enzyme-linked immunosorbent assay. The study showed serum annexin A2 but not annexin A5 was significantly reduced ($P = 0.029$) in women with PE (total and severe cases) compared to those with normal pregnancy. The ROC analysis of annexin A2 level for the prediction of development of PE showed an area under the curve of 0.64 ($P = 0.029$), and the best cut-off value was 0.89 ng/ml with a sensitivity of 70.0% and a specificity of 70.0%. Univariate analysis showed annexin A2 of <0.89 ng/ml, proteinuria, lower platelet count and higher BP were associated with significantly higher risk to develop PE. Based on this pilot study, serum annexin A2 levels may be a useful biomarker for pre-eclampsia. However, a larger study is required before a final conclusion is made.



## 1. Introduction

Pre-eclampsia (PE) is a multifactorial disease affecting 2–8% of pregnancies and remains a leading cause for maternal and perinatal morbidity and mortality worldwide [1]. The exact mechanisms, which lead to PE, are not clear but several factors appear to help predict who will develop this disease. These factors include family history, age and parity. The primary established pathophysiology in PE resides in the abnormal trophoblastic implantation with reduced placental perfusion. The secondary pathology appears to be endothelial cell injury, oxidative stress and activation of the coagulation system [2]. Placental dysfunction has been reported to play a major role in the pathogenesis of PE as well as modulate the disease outcome. Fibrin deposition and microthrombi are known histopathologic alterations in placental dysfunction in preeclampsia [2,3]. The mechanisms of these thrombotic changes are not fully understood. Most studies have focused on the clotting activities and little is known about the changes in the fibrinolytic system in pregnancies complicated with this disease [4,5].

Annexin A5 belongs to the family of annexins, which are highly homologous phospholipid binding proteins. It can be found in various cell types such as platelets and endothelial cells and has also been detected in placental villi [6]. It is an anionic phospholipid-binding protein with potent anticoagulant activity. It inhibits prothrombin activation and is able to prevent thrombus formation under normal venous and arterial blood flow conditions [7]. Several studies have suggested that annexin A5 may protect the syncytial surface against clot formation [8,9] and lower amounts of

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annexin A5 expression on trophoblasts has been noted in pre-eclamptic patients compared to healthy pregnant [10–12].

Annexin A2, a member of annexin family, is a calcium-regulated phospholipid binding protein which is expressed on a number of cell types including endothelial cells, macrophages, a variety of tumor cells, and also on the brush-border membrane of placental syncytiotrophoblast [13,14]. It is a cell surface co-receptor for plasminogen and its activator; tPA and its binding promotes significantly cell surface plasmin generation [15]. In peripheral maternal blood, there was an association between low levels of annexin A2 and PE and low fetal birth weight. Levels of expression of annexin A2 were inversely correlated with the severity of placental thrombin generation [5].

Few studies have investigated the role of annexin A5 [11,12,16] and annexin A2 [5,17] in pre-eclampsia. These studies have either explored one or the other. Moreover; they mostly focused on the local expression of these proteins in the placenta tissue rather than examining blood levels of these proteins. In the current study, the role of serum annexins A5 & A2 as biomarkers of PE is carefully examined.

## 2. Material and methods

### 2.1. Patients' recruitment and classification

This pilot study examined a total of 80 women; 40 were healthy pregnant women with an average age of 27 years (range:19–37) and 40 women diagnosed with pre-eclampsia (after 20 weeks of gestation) with an average age of 28 years (range:18–43). The patients were selected from the outpatient clinic of Obstetric and Gynecological Department in Mansoura University Hospital. Clinical characteristics of the study population are shown in Table 1. Diagnosis of pre-eclampsia and determination of severity were defined according to the criteria established by the International Society of Hypertension in Pregnancy [18]. Mild pre-eclampsia is characterized by hypertension with a systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg, and proteinuria with a urine dipstick of $\geq$1 + or $\geq$0.3 g per 24 h, after 20 weeks' gestation in a previously normotensive woman. Severe pre-eclampsia is characterized by a systolic blood pressure $\geq$160 mmHg or diastolic blood pressure $\geq$110 mmHg, proteinuria $\geq$3 g per 24 h. The pre-eclamptic women group was divided, according to the above criteria, into 2 groups: mild PE group (18 patients) and severe PE group (22 patients).

Exclusion criteria were history of hypertension, hepatic or renal disease, cardiac disease, diabetes mellitus, established atherosclerosis, malignancy, autoimmune disease, systemic infection, recent major surgery or trauma, alcohol consumption, and cigarette smoking.

### 2.2. Laboratory investigations

Following careful clinical assessment and ultrasonography, the following laboratory testing were conducted: complete blood picture using automated hematology analyzer KX-21N (Sysmex, Japan), liver and kidney function tests using automated chemistry analyser (Hitashi 912, Japan) including: serum albumin, ALT and AST, serum creatinine, and urine proteins using dipstick urinalysis (Meditest, Germany).

### 2.3. Blood collection protocol

Peripheral venous blood was aspirated by venipuncture with minimal stasis using a 21-gauge needle. The aspirated blood was collected in a K2 EDTA tube (Becton Dickinson, Franklin Lakes, New Jersey, USA) for blood counts and in additive-free tubes to obtain serum for the assessment of liver and kidney function and annexin levels. Blood samples were allowed to clot for two hours at room temperature before centrifugation for 20 min at 1000g, and stored at −80 °C for later use. The samples were thawed only once.

### 2.4. Measurement of annexin A5 and annexin A2

Annexins were assessed using enzyme-linked immunosorbent assay (ELISA). Serum annexin A5 was measured using Bender Med Systems (BMS252), eBioscience, Vienna, Austria, with

**Table 1**
Clinical characteristics of patients' groups compared with pregnant controls.

| Parameters | | Controls (n = 40) | Total PE (n = 40) | Mild PE (n = 18) | Severe PE (n = 22) | $P^1$ | $P^2$ | $P^3$ | $P^4$ |
|---|---|---|---|---|---|---|---|---|---|
| Maternal age (years) | Mean ±SD | 26.60 ±5.15 | 28.20 ±6.30 | 27.5 ±6.72 | 28.77 ±6.03 | 0.217 | 0.578 | 0.140 | 0.532 |
| Gestational age (weeks) | Mean ±SD | 36.02 ±3.52 | 33.68 ±3.07 | 33.78 ±3.38 | 33.59 ±2.86 | 0.002* | 0.006* | 0.001* | 0.851 |
| *Gravidity* | | | | | | | | | |
| | Mean ±SD | 2.05 ±0.99 | 2.22 ±1.07 | 2.0 ±1.03 | 2.41 ±1.1 | 0.450 | 0.861 | 0.193 | 0.450 |
| Primigravida No. (%) | | 15 (37.5) | 11 (27.5) | 7 (38.9) | 4 (18.2) | 0.340 | 0.920 | 0.114 | 0.173 |
| Multigravida No. (%) | | 25 (62.5) | 29 (72.5) | 11 (61.1) | 18 (81.8) | | | | |
| *Parity* | | | | | | | | | |
| | Mean ±SD | 1.00 ±0.96 | 1.10 ±1.06 | 0.83 ±0.92 | 1.32 ±1.13 | 0.659 | 0.539 | 0.246 | 0.659 |
| Nullipara No. (%) | | 15 (37.5) | 13 (32.5) | 8 (44.4) | 5 (22.7) | 0.639 | 0.617 | 0.234 | 0.145 |
| Multipara No. (%) | | 25 (62.5) | 27 (67.5) | 10 (55.6) | 17 (77.3) | | | | |
| Systolic blood pressure (mmHg) | Median (Range) | 110 (100–120) | 140 (130–180) | 140 (130–150) | 155 (140–180) | <0.00* | <0.00* | <0.00* | <0.00* |
| Diastolic blood pressure (mmHg) | Median (Range) | 70 (60–80) | 100 (80–110) | 90 (90–100) | 100 (80–110) | <0.00* | <0.00* | <0.00* | <0.00* |

***p*$^1$**: significance between total cases versus controls. ***P*$^2$**: significance between mild PE versus controls. ***P*$^3$**: significance between severe PE versus controls. ***P*$^4$**:significance between mild versus severe PE.
PE: preeclampsia. *: ***p*** is significant.

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