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Investigation of the actin scavenging system in pre-eclampsia

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ABSTRACT

Objectives: Cell injury releases actin, the most abundant cell protein. Gelsolin and vitamin D binding protein (VDBP) together depolymerise and clear cell-free actin. Impaired actin clearance is associated with several diseases and correlates with clinical outcome. The actin scavenging system was investigated in pre-eclampsia (PE), a procoagulant and proinflammatory state with placental and vascular damage.

Study design: Plasma gelsolin and actin free VDBP (AFVDBP) were measured in PE (early onset <33 weeks; late onset ≥36 weeks), matched normal pregnant (normP) and non-pregnant (nonPr) women, using commercially available ELISAs. Longitudinal samples from normP and women who subsequently developed PE were also analysed.

Results: Plasma gelsolin fell during pregnancy ($p = 0.0002$), with a concomitant rise in actin-free VDBP ($p < 0.001$). Gelsolin concentrations were only significantly lower in established PE ($p < 0.05$) when compared to non-pregnant controls.

Conclusions: We have shown that the components of the actin clearance system, gelsolin and AFVDBP, are altered in normal pregnancy and further changes occur in established PE, suggesting depleted actin clearance in PE. Whether this is a cause or consequence of PE pathophysiology requires further investigation.

1. Introduction

Pre-eclampsia (PE) affects 3–5% of pregnancies, causing maternal and perinatal mortality or morbidity [1] and lasting health implications for both mother and baby [2]. It is characterised by a maternal systemic inflammatory response, endothelial cell dysfunction and increased coagulation, secondary to disordered placental function [1]. In PE, the physiological activation of coagulation, present in normal pregnancy, is exaggerated with excessive platelet activation and intervillous fibrin deposition in the placenta [3,4]. As the causative factor(s) originate from the placenta, removal of the placenta remains the only effective treatment.

Many placental derived factors are implicated in the maternal syndrome [5], with anti-angiogenic factors being the best characterised. Alternatives include 'danger' molecules released from damaged or dying cells that become toxic in the extracellular milieu, such as actin [6,7]. Extracellular actin can damage microvascular capillaries, activate platelets and impede clot lysis, all effects associated with the excessive procoagulation evident in PE [4,8,9]. An efficient system comprising gelsolin [10,11] and vitamin D binding protein (VDBP) works to cleave extracellular actin and inhibit repolymerisation, blocking its thrombotic effects [9,12,13]. Meanwhile, hypogelsolinemia is common in trauma and inflammatory diseases, with higher gelsolin levels correlating with improved mortality rates [14,15]. Restoring circulating gelsolin and VDBP levels may therefore avoid more serious complications and reduce mortality rates [16]. Gelsolin is also an attractive candidate for therapy as it modulates several proinflammatory pathways [14].

Cell-free actin levels are increased in normal pregnancy, shown by increased actin-VDBP complex levels [17]. In PE there is potential for a further increase in circulating actin due to increased placental apoptosis and necrosis and activated endothelial and immune cells [18,19]. It is not known how the components of the actin scavenging system change during normal pregnancy or PE. Therefore, the aim of this pilot study was to determine the circulating levels of gelsolin and actin-free VDBP (AFVDBP; to measure the actin binding capacity of VDBP) in normal pregnancy and PE, compared to non-pregnant women.

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Table 1
Clinical characteristics of participants recruited to a cross-sectional study investigating changes in plasma gelsolin and actin free vitamin D binding protein levels in normal pregnancy and pre-eclampsia (PE). A single plasma sample was collected from women diagnosed with either early onset (<33 weeks; $n = 10$) or late onset (≥36 weeks; $n = 10$) PE and matched non pregnant (NonPr; $n = 10$/group) and normal pregnant (NormP; $n = 10$/group) women. Data are shown as median (range).

	Age (yrs)	Gestation at sampling (wks^{+days})	Gestation at delivery (wks^{+days})	Nulli-parity	Blood pressure (mm Hg) (systolic) (diastolic)	Proteinuria (mg/24 h)	Birthweight (g)
Early onset PE matched group							
NonP ($n = 10$)	32 (21–38)	N/A	N/A	8/10	N/A	N/A	N/A
NormP ($n = 10$)	33 (23–37)	30^{+4} (24^{+2}–33^{+4})	40^{+5} (39^{+5}–42^{+0})	8/10	125/80 (120–140) (60–86)	None detected	3613.5 (3069–4002)
PE ($n = 10$)	30 (21–38)	29^{+2} (24^{+4}–32^{+5})	31^{+2}*** (25^{+4}–33^{+3})	8/10	187.5/120*** (170–215) (110–130)	3803.5 (940–9433)	1245.5*** (490–2009)
Late onset PE matched group							
NonP ($n = 10$)	31.5 (23–36)	N/A	N/A	6/10	N/A	N/A	N/A
NormP ($n = 10$)	33 (20–36)	37^{+1} (35^{+3}–38^{+3})	39^{+5} (38^{+5}–40^{+4})	6/10	130/79 (110–137) (70–88)	None detected	3480.5 (3022–3842)
PE ($n = 10$)	31 (21–39)	37^{+3} (35^{+5}–38^{+6})	38^{+0}*** (36^{+1}–38^{+6})	6/10	166/110*** (148–190) (100–120)	1022 (727–1687)	2911.5** (2241–3592)

NonPr, non-pregnant recruits; NormP, normal pregnancy and PE, pre-eclampsia.
** $p < 0.01$ compared to matched normP.
*** $p < 0.002$ compared to matched normP.

2. Materials and methods

To determine whether PE was associated with changes in circulating levels of plasma AFVDBP and gelsolin, a single blood sample was taken from women with PE (early onset (<33 weeks gestation; $n = 10$) and late onset PE (≥36 weeks gestation; $n = 10$)) and matched to normal pregnant women (age (±4 years), parity (0, 1–3, 4+), and gestational age (±13 days)) (normP) and non-pregnant (age and parity) (nonPr) controls (Table 1).

Longitudinal samples were collected to determine changes in plasma AFVDBP and gelsolin over the course of normal pregnancy (normP), prior to and subsequent to the onset of PE. These samples were collected from pregnant women recruited to the Oxford Pregnancy Biobank ($n = 10$) in the first (11–13 weeks gestation), second (20–22 weeks) and third (30–34 weeks) trimesters of pregnancy (Table 2). Blood samples from matched (age and parity) nonPr controls were also collected ($n = 10$). In those women who subsequently developed PE, 4 developed early onset PE (<33 weeks) and 11 late onset PE (≥35 weeks gestation).

All pre-eclamptic women were recruited in the hospital following a positive diagnosis. Matched normal pregnant and non-pregnant women were recruited during routine prenatal appointments and from the community. All blood samples were collected into EDTA tubes, plasma isolated and the samples stored at −80 °C until analysis. Due to the potential for cell stress and therefore release of actin, no samples were collected during labour. Normal pregnant women had a healthy singleton pregnancy and no history of chronic illness. Pre-eclampsia was defined as new hypertension (diastolic blood pressure ≥90 mm Hg on two consecutive occasions) and new proteinuria (24 h secretion of ≥500 mg). The Oxfordshire Research Ethics Committee C approved this study (ref. 09/H0606/10) and informed written consent was obtained from all recruits.

Gelsolin and AFVDBP concentrations were determined using commercially available ELISA kits (Gelsolin: USCN Life Sciences Inc.; VitDBP: Immundiagnostik AG) according to the manufacturers' instructions.

Kruskal–Wallis test with a Dunn's post hoc test and Mann–Whitney *U*-test were used to analyse study participants' clinical characteristics. Friedman test with Dunn's multiple comparison test was used to compare matched ELISA data. For non-parametric longitudinal data a Friedman repeated measures test was used to determine significance of changes over gestation in normP and PE. Statistical analyses were carried out using Prism software. $p < 0.05$ was considered statistically significant.

3. Results

Within the study groups, matching criteria did not differ. PE groups had high proteinuria and significantly higher blood pressure ($p < 0.002$), lower birthweights ($p < 0.05$–$p < 0.002$) and shorter gestations ($p < 0.002$) compared to the normP group (Tables 1 and 2).

In both the early onset and late onset PE groups, plasma AFVDBP levels were significantly higher in normP compared to nonPr ($p < 0.01$) (Fig. 1Ai). With established early onset PE, plasma AFVDBP levels tended to be lower than normP but not significantly. In both early onset and late onset PE, plasma gelsolin levels were significantly lower than nonPr controls ($p < 0.05$). Although there was a trend for gelsolin levels to be lower in normP than the nonPr group, this was not statistically significant (Fig. 1Aii).

Table 2
Clinical characteristics of matched non pregnant (NonPr), normal pregnant (NormP) and pre-eclamptic (PE) women recruited to a longitudinal study samples investigating changes in plasma gelsolin and actin free vitamin D binding protein levels during normal pregnancy and prior to and following development of pre-eclampsia (PE). Plasma samples were collected from each gestation and once PE had developed. Data are shown as median (range).

	Age (yrs)	Gestation at delivery (wks^{+days})	Nulli-parity	Blood pressure (mm Hg) (systolic) (diastolic)	Proteinuria (mg/24 h)	Birthweight (g)
Longi-tudinal group						
NonP ($n = 10$)	29 (20–33)	N/A	9/10	N/A	N/A	N/A
NormP ($n = 10$)	29 (19–34)	40^{+1} (37–41^{+5})	9/10	122/76.5 (116–136) (70–91)	None detected	3223 (2530–4000)
PE ($n = 10$)	29.5 (18–35)	37^{+2}*** (22^{+3}–40^{+0})	9/10	160/110*** (126–180) (94–128)	2570.5 (586–14645)	2597* (1560–5788)

NonPr, non-pregnant recruits; NormP, normal pregnancy and PE, pre-eclampsia.
* $p < 0.05$ compared to matched normP.
*** $p < 0.002$ compared to matched normP.