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Stratégies d'exploration fonctionnelle et de suivi thérapeutique

# Pre-eclampsia: increased, unchanged, and decreased serum markers in comparison to healthy third trimester pregnancy. A synopsis

# Pré-éclampsie : marqueurs sériques augmentés, inchangés et abaissés en comparaison avec des grossesses normales au cours des trois derniers mois. Une vue d'ensemble

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**Abstract**

Inconsistent serum concentrations have been reported for several markers in women with pre-eclampsia, which can be explained by the complexity of the disease and resulting heterogeneity in the study groups. We have thus decided to compare a large number of established and new placental, non-placental, and mixed-origin markers in the same, small but well-defined group of late-onset pre-eclampsia sera, matched with an identical number (19) of controls. The proteins were quantified in one batch (microplate) using commercially available or in-house enzyme immunoassays. The most significant differences between cases and controls were observed for placenta growth factor (PLGF, strongest reduction) and activin A (strongest increase), while others were less strongly affected or did not significantly differ between the two groups. Serum levels of a number of markers were positively or negatively correlated to each other, but no general difference between placental or non-placental markers was observed.


**Résumé**

Des taux sériques très variables ont été publiés pour un bon nombre de marqueurs dans des cas de grossesses préeclamptiques, un fait aisément expliquable par la complexité de la pathologie et l'inhomogénéité des groupes d'échantillons qui en découle. Notre objectif a été donc de comparer plusieurs de ces marqueurs, d'origine placentaire ou non, dans un même petit groupe bien défini de sérums préeclamptiques, cela par rapport à un groupe de contrôle de taille et de caractéristiques cliniques identiques. Les marqueurs ont chacun été mesurés en une seule fois sur microplaques par des méthodes enzymo-immunoanalytiques, soit commerciales soit développées dans notre laboratoire. Les différences les plus marquées, entre pathologie et contrôle, ont été observées pour le facteur placentaire de croissance (*Placenta Growth Factor*, PLGF, réduction la plus prononcée) et l'activine A (augmentation la plus prononcée) tandis que d'autres marqueurs ont été modifiés de façon moins marquée ou non influencés d'une façon statistiquement significative par la présence de la préeclampsie. Des corrélations positives ou négatives ont été observées entre plusieurs de ces marqueurs, mais aucune différence généralisée n'a pu être mise en évidence entre les molécules d'origine placentaire ou de source maternelle.


*Keywords:* Pre-eclampsia; Serum markers; Placenta growth factor; Activin A

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## 1. Introduction

Pre-eclampsia is a poorly understood multisystem pregnancy disorder affecting 5–10% of pregnant women. The original underlying problem is thought to be an early placental disorder [45], with insufficient trophoblast invasion of the spiral arteries and consequent placental ischaemia [48]. The maternal symptoms of pre-eclampsia such as hypertension, proteinuria and oedema, appearing later in pregnancy are the consequence of endothelial activation and dysfunction [47]. The link between the early placental implantation failure, placental ischaemia and maternal vascular responses may be oxidative stress, the release of various placental/trophoblast substances such as placental debris, growth factors, placental hormones and pro-inflammatory cytokines leading to excessive maternal inflammatory response [14,44].

Due to the complexity of the mechanisms involved, proteins from different sources have been suggested to be useful as markers for the severity of the situation or for the study of the pathogenesis itself. We have recently confirmed the presence of elevated serum levels of the three placental products pregnancy-associated plasma protein A (PAPP-A), inhibin A, and activin A, and found a correlation with increased concentrations of soluble endothelial (sE-) selectin [9]. However, there are a number of serum proteins on which the literature is particularly inconsistent regarding the deviation of their serum levels in pre-eclampsia in comparison to healthy pregnant controls of identical gestational age. While placenta derived growth factor (PLGF) levels are significantly lower in pregnancies with pre-eclampsia [50], the structurally related vascular endothelial growth factor (VEGF) has been reported to be elevated in a number of studies [4,12,23,31,49]. More recently, however, several papers reporting reduced VEGF levels in pre-eclamptic pregnancies have appeared [34,35,46]. Reduced or less strongly increasing levels of macrophage colony-stimulating factor (M-CSF) between the first trimester and the time of labor have been associated with adverse pregnancy outcome and pre-eclampsia [26], while another study [21] found increased levels of this marker under similar clinical conditions. Insulin-like growth factor binding protein-1 (IGF-BP1) was found to be elevated in pre-eclampsia as a function of the severity of the disease in two reports [17,24], while in another study no such modification was observed [33].

All these studies have logically been performed on different populations of patients, which may have differed in the severity of pre-eclampsia (affected organ system), gestation, proportions of growth-restricted fetuses and parity. All these parameters are definitely or supposedly influencing the blood levels of the substances studied in pre-eclampsia. We have thus decided to study the three growth factors with the most inconsistent results in the literature (VEGF, M-CSF, and IGF-BP1) in the same groups of pre-eclampsia patients and carefully matched controls which, moreover, have already been used in a previous investigation focusing on placentally derived products [9]. This approach is short-circuiting the patient-to-patient variations, which is making the comparisons between published studies difficult. The aim of this paper is thus the comparison between a large panel of serum proteins (placental or maternal) regarding their difference in serum concentration between healthy and pre-eclamptic pregnancies rather than the investigation of the biological role of these substances. We are presenting data on the growth factors PLGF, VEGF, M-CSF, IGF-BP1, epithelial neutrophil activating peptide-78 (ENA-78, on which no pre-eclampsia related information is available in the literature to date), endothelial selectin, the placental markers pregnancy-specific $\beta_1$-glycoprotein (SP1) and placental lactogen (hPL), the partially placental markers PAPP-A, inhibin A, activin A, the inflammation marker C-reactive peptide (CRP), and leptin which is also linked to inflammation [38] and partially produced by the placenta [40]. The literature is inconsistent; in pre-eclampsia, increased [3,5], but also unchanged [39] or even reduced [32] leptin levels have been reported, and this independently of the assay method used, RIA [5,32] or ELISA [3,39]. Adiponectin is another protein of adipocyte origin with anti-inflammatory properties and for which placental production in the human has been suggested [13] but not confirmed [15]. Increased serum levels have, however, been consistently observed in pre-eclampsia [41,43] and we have included this recently described marker in our comparisons.

This synopsis therefore compares the extent of the deviation between the serum levels of 14 marker proteins in pre-eclampsia and matched controls, and shows the correlations between them.

## 2. Materials and methods

### 2.1. Patients and controls

Blood samples were obtained from 19 patients with pre-eclampsia and 19 women in normal pregnancies matched for parity, age and gestational age at the John Radcliffe Maternity Hospital in Oxford as approved by the Central Oxford Research Ethics Committee. The definition for pre-eclampsia used has been described previously in [9]. The clinical description of the two study groups is shown in Table 1. Serum was obtained by centrifugation after clotting, and stored at –40 °C until the assays were performed in batches.

### 2.2. Analytical procedures

All assays were non-isotopic double-antibody immunometric tests (ELISA) based on 96-well microplate technology. The methods for PAPP-A, SP1, inhibin A and activin A from

Table 1
Clinical and pregnancy parameters of the case and control groups. Values are mean ± S.D. (and/or range)

| | Pre-eclampsia | Controls |
|---|---|---|
| Maternal age (years) | 29.2 ± 4.0 (18–37) | 28.4 ± 4.8 (19–36) |
| Weight at booking (kg) | 66.0 ± 10.3 | 67.1 ± 10.3 |
| Nulliparous/total (*N*) | 13/19 | 13/19 |
| Gestation at sampling (weeks) | 31.8 ± 4.1 (25–39) | 31.2 ± 3.8 (25–39) |
| Gestation at delivery (weeks) | 32.4 ± 3.4 (26–39) | 39.6 ± 3.2 (28–42) |
| Birth weight (g) median, range | 1298 (559–3869) | 3645 (2771–4935) |