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Fetal hemoglobin, α_1-microglobulin and hemopexin are potential predictive first trimester biomarkers for preeclampsia

Ulrik Dolberg Anderson [a,b,*,1], Magnus Gram [c,1], Jonas Ranstam [d], Basky Thilaganathan [e], Bo Åkerström [c,1], Stefan R. Hansson [a,b,1]

[a] Section of Obstetrics and Gynecology, Department of Clinical Sciences Lund, Lund University, Sweden
[b] Skåne University Hospital, Malmö/Lund, Sweden
[c] Department of Clinical Sciences, Lund, Infection Medicine, Lund University, Sweden
[d] Department of Clinical Sciences, RC Syd, Lund University, Sweden
[e] Fetal Medicine Unit, St. George's University Hospital, London, United Kingdom

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ABSTRACT

Objective: Overproduction of cell-free fetal hemoglobin (HbF) in the preeclamptic placenta has been recently implicated as a new etiological factor of preeclampsia. In this study, maternal serum levels of HbF and the endogenous hemoglobin/heme scavenging systems were evaluated as predictive biomarkers for preeclampsia in combination with uterine artery Doppler ultrasound.

Study design: Case-control study including 433 women in early pregnancy (mean 13.7 weeks of gestation) of which 86 subsequently developed preeclampsia. The serum concentrations of HbF, total cell-free hemoglobin, hemopexin, haptoglobin and α_1-microglobulin were measured in maternal serum. All patients were examined with uterine artery Doppler ultrasound. Logistic regression models were developed, which included the biomarkers, ultrasound indices, and maternal risk factors.

Results: There were significantly higher serum concentrations of HbF and α_1-microglobulin and significantly lower serum concentrations of hemopexin in patients who later developed preeclampsia. The uterine artery Doppler ultrasound results showed significantly higher pulsatility index values in the preeclampsia group. The optimal prediction model was obtained by combining HbF, α_1-microglobulin and hemopexin in combination with the maternal characteristics parity, diabetes and pre-pregnancy hypertension. The optimal sensitivity for all preeclampsia was 60% at 95% specificity.

Conclusions: Overproduction of placentally derived HbF and depletion of hemoglobin/heme scavenging mechanisms are involved in the pathogenesis of preeclampsia. The combination of HbF and α_1-microglobulin and/or hemopexin may serve as a prediction model for preeclampsia in combination with maternal risk factors and/or uterine artery Doppler ultrasound.

1. Introduction

Preeclampsia (PE) is a pregnancy-related condition affecting up to 8% of pregnancies worldwide [1]. The incidence varies according to geographical, social, economic and racial differences [2]. It has been estimated that PE or complications of the condition account for more than 50,000 maternal deaths worldwide each year [1].

Clinical manifestations appear after 20 weeks of gestation. Although the diagnostic criteria are clear, it has been difficult to find a way to predict the condition in early pregnancy or to predict which women that will develop severe preeclampsia and/or eclampsia [3].

The details of the pathophysiology remain elusive but recent research has improved the understanding of the condition markedly. Preeclampsia is described to develop in two stages [4,5]. The first stage is characterized by a defect placentation [6]. The extravillous trophoblast cells do not remodel the spiral arteries in the maternal decidua properly and consequently fail to create a low-resistance even utero-placental blood flow [6,7]. Uneven perfusion leads to oxidative stress in the placenta that contributes to the damage of the blood–placenta barrier and subsequent leakage between the fetal and maternal circulation. In fact, cell-free fetal DNA [8,9], micro-particles [10] and cell-free fetal hemoglobin [11,12] have been described in the maternal circulation of women

* Corresponding author at: Department of Obstetrics and Gynecology, University Hospital Skåne, S-21466 Malmö, Sweden.
E-mail address: ulrik.dolberg_anderson@med.lu.se (U.D. Anderson).
[1] Contributed equally to the manuscript.

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with preeclampsia [13,14]. The second stage of PE is characterized by the clinical manifestations, e.g. increased blood pressure and proteinuria detected after 20 weeks of gestation [4,5].

The link between the stages 1 and 2 is still a main focus of modern PE research. Maternal constitutional factors such as obesity are important risk factors for late onset PE in particular. Furthermore a new focus area is the role of maternal cardiac strain in the development of PE [14–17].

Results from gene- and proteome profiling studies have shown an over-production and accumulation of cell-free fetal hemoglobin (HbF) in the preeclamptic placenta [18]. *Ex vivo* studies using the dual-placental perfusion model have shown that HbF causes damage to the blood–placenta barrier and leakage of cell-free hemoglobin into the maternal circulation [12,19,20]. Furthermore, cell-free fetal hemoglobin has been shown to appear in the maternal circulation early in pregnancy in women who subsequently develop PE. Therefore HbF has been suggested to be an important etiological factor and a potential biomarker for early detection of preeclampsia [11,12,21,22]. In term pregnancies HbF has been shown to correlate to the severity of the disease, *i.e.* blood pressure [23].

Extracellular hemoglobin is in general toxic to tissues and organs [24,25]. Hemoglobin and its metabolites heme and free iron are particularly reactive and generate free radicals, which can cause cell- and tissue damage, oxidative stress, inflammation, and vascular endothelial damage [25]. The human system has evolved several different scavenging proteins that protect the body from these metabolites. Haptoglobin, the most well investigated human hemoglobin clearance system, binds cell-free hemoglobin in the blood [25,26]. The hemoglobin–haptoglobin complex subsequently binds to its receptor CD163, which eliminates the complex from the blood [27]. Hemopexin is a circulating plasma protein and is the major scavenger of free heme [28]. The hemopexin–heme complex is taken up by cells such as macrophages and hepatocytes, expressing the CD91 receptor, thus facilitating the heme clearance heme from the blood [24]. Previous *in vitro* results have indicated that decreased hemopexin *activity* may regulate blood pressure through the renin–angiotensin-system in patients with preeclampsia [29,30].

α_1-Microglobulin (A1M) is a plasma- and extravascular protein that provides protection through its ability to bind and neutralize free heme and radicals [31–33]. Several *in vitro* and *in vivo* studies have shown that A1M protects cells and tissues in conditions with increased concentrations of extracellular hemoglobin, heme and reactive oxidative species [23]. *In-vitro* studies using liver- and placenta cells, have shown that A1M expression is up-regulated following exposure to hemoglobin, heme, and reactive oxygen species [23,34]. Furthermore, the serum concentration of A1M has been shown to be significantly elevated in maternal blood in the first trimester in patients who subsequently developed PE [21].

The aim of this study was to analyze the serum concentrations of HbF and the hemoglobin- and heme scavenging systems; haptoglobin, hemopexin, and A1M in a larger cohort of uncomplicated pregnancies and pregnancies with subsequent development of early-, late-, and term onset PE.

2. Materials and methods

2.1. Patients and samples

The study was approved by the local ethical committees at St Georges University Hospitals, London, UK. All participants signed a written informed consent prior to inclusion. Women attending a routine antenatal care visit at St. Georges Hospitals' Obstetric Unit were recruited from 2006 to 2008. First trimester uterine artery Doppler screening at St. Georges Hospitals is offered routinely to all nullipara and any multipara who have had a previously affected pregnancy with either PE or IUGR.

The gestational length was calculated from the last menstrual period and confirmed by ultrasound crown-rump-length measurement. Uterine artery Doppler ultrasound was measured as previously described [35]. A maternal venous blood sample was collected at 6–20 weeks of gestation (mean 13.7 weeks) in a 5 mL vacutainer tube (Becton Dickinson, Franklin Lakes, NJ, USA) without additives. After clotting, the samples were centrifuged at 2000*g* at room temperature (RT) for 10 min and serum was separated and stored at -80 °C until further analysis.

All pregnancy-outcome data was obtained from the main delivery suite database and checked for each individual patient. Preeclampsia was defined according to ISSHP definitions as: 2 readings of blood pressure $\geq$140/90 mmHg at least 4 h apart, and proteinuria $\geq$300 mg in 24 h, or 2 readings of at least +2 on dipstick analysis of midstream or catheter urine specimens if no 24-h urine collection was available [3]. Early onset PE was defined as PE with clinical manifestations before 34 + 0 weeks of gestation and late onset PE was defined as clinical manifestations after 34 + 0 weeks of gestation. Term PE was defined as PE with clinical manifestations between 37 + 0 to 42 + 0 weeks of gestation. Normal uncomplicated pregnancy was defined as delivery at or after 37 + 0 weeks of gestation with normal blood pressure. The uncomplicated pregnancy samples (controls) included in this study were randomly selected from samples collected during the same period. Uncomplicated pregnancy was confirmed after delivery.

2.2. Measurement of HbF, A1M, cell-free total hemoglobin, haptoglobin, and hemopexin

The HbF concentrations in the serum samples were measured with a sandwich ELISA as previously described [23]. Briefly, 96-wells plates were coated with affinity-purified rabbit anti-HbF (4 μg/ml in PBS) overnight at RT. In the second step, a standard series of HbF or the patient samples diluted in incubation buffer were incubated for 2 h at RT. In the third step, HRP-conjugated affinity-purified rabbit anti-HbA antibodies, were added and incubated for 2 h at RT. Finally, a ready-to-use 3,3′,5,5′-tetramethylbenzidine (TMB, Life Technologies, Stockholm, Sweden) substrate solution was added. The reaction was stopped after 30 min using 1.0 M HCl and the absorbance was read at 450 nm using a Wallac 1420 Multilabel Counter (Perkin Elmer Life Sciences, Waltham, MA, USA).

The A1M concentrations were determined by a radioimmunoassay (RIA) as previously described [23]. Briefly, RIA was performed by mixing goat antiserum against human A1M ("Halvan"; diluted 1:6000) with ^{125}I-labelled α_1-microglobulin (appr. 0.05 pg/ml) and unknown patient samples or calibrator A1M-concentrations. After incubating overnight at RT antibody-bound antigen was precipitated after which the ^{125}I-activity of the pellets was measured in a Wallac Wizard 1470 gamma counter (Perkin Elmer Life Sciences).

The concentration of total hemoglobin was determined with the Human Hemoglobin ELISA Quantification Kit from Genway Biotech Inc. (San Diego, CA, USA). The analysis was performed according to the manufacturer's instructions and the absorbance was read at 450 nm using a Wallac 1420 Multilabel Counter.

The concentrations of haptoglobin in serum samples were determined using the Human Haptoglobin ELISA Quantification Kit as described by the manufacturer (Genway Biotech Inc.). The serum concentrations of hemopexin were determined using a Human Hemopexin ELISA Kit as described by the manufacturer (Genway Biotech Inc.).