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First trimester serum afamin concentrations are associated with the development of pre-eclampsia and gestational diabetes mellitus in pregnant women

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ABSTRACT

Objective: Aim of this study was to assess the prognostic capability of afamin to predict pregnancy complications.
Method: First-trimester screening was consecutively performed in 4948 pregnant women, of whom 474 women developed pregnancy complications [gestational hypertension (n = 84), pre-eclampsia (n = 30), intrauterine growth restriction (n = 107), preterm birth (n = 44), and gestational diabetes mellitus (n = 209)]. To each woman with pregnancy complications an uncomplicated pregnancy was matched for body mass index. Afamin serum concentrations were measured in 948 pregnant women at the first-trimester screening.
Results: Median afamin concentrations were significantly higher in women developing pre-eclampsia or gestational diabetes mellitus when compared to women with uncomplicated pregnancies (76 mg/L vs. 65 mg/L, $p = 0.001$ and 80 mg/L vs. 69 mg/L, $p < 0.001$). There was no difference in median afamin values between all other pregnancy complications and their matched controls. Increased afamin (i.e. > 65 mg/L) was a strong and independent predictor for the development of pre-eclampsia (risk ratio, 24.58; 95%CI, 2.82–214.12; $p = 0.004$) as well as gestational diabetes mellitus (risk ratio, 2.07; 95%CI, 1.33–3.22; $p = 0.001$).
Conclusion: In this large nested case-control study increased afamin concentrations were a strong and independent predictor for pre-eclampsia and gestational diabetes mellitus, suggesting a potential role of afamin as predictive marker for pregnancy-related metabolic disorders.

1. Introduction

Pregnancy complications such as hypertensive disorders like pre-eclampsia (PE) and metabolic disorders like gestational diabetes mellitus (GDM), are multi-systemic conditions of pregnancy, associated with vascular insufficiency, and the leading cause of fetal and maternal morbidity and mortality [1,2]. The current understanding of mechanisms causing PE underlies a multifactorial etiology leading to impaired placentation with placental ischemia and placental release of soluble factors that cause generalized endothelial dysfunction [3,4]. Moreover, pathological transformation of the utero-placental circulation produces cytokines and reactive oxygen species (ROS) to trigger a systemic inflammatory and oxidative state, crucial for cardiovascular health later in life [5–7]. Furthermore, metabolic changes including obesity, diabetes mellitus (DM) and hyperlipidemia contribute to an increased additive risk for potential cardiovascular diseases (CVD). Thus, PE and pregnancy-related hypertensive or metabolic disorders have been discussed as the „metabolic syndrome of pregnancy “[8].

The plasma glycoprotein afamin was previously found to be associated with the prevalence and development of insulin resistance and metabolic syndrome in the general population and in polycystic ovary syndrome [9,10]. Latest findings demonstrated afamin concentrations to increase linearly in maternal serum during the course of uncomplicated pregnancy and showed in a small pilot study higher serum concentrations of afamin in women with pregnancy complications associated with vascular insufficiency and multi-systemic conditions of

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pregnancy [11].

The aim of this study was to assess the association between first-trimester serum afamin concentrations and the development of pregnancy complications in a large nested case-control study and to evaluate the prognostic capability of afamin for the prediction of pregnancy complications.

2. Materials and methods

2.1. Study design and subjects

For this nested case-control study, all pregnant women who registered within a period of almost four years (January 2010–November 2013) for delivery at the Department of Gynecology and Obstetrics, Danube Hospital, Vienna, Austria, were routinely screened receiving a "combined test" between the 11th and 14th week of pregnancy. Exclusion criteria were multiple pregnancies, fetal malformations as well as missing data. Venous blood was collected, serum obtained by low-speed centrifugation and aliquots were frozen and kept at −70 °C for quantification of afamin.

All women were followed up until delivery and the following pathological pregnancy outcomes were recorded:

- **Gestational hypertension (GH)**: The clinical diagnosis of GH was defined by the new onset of hypertension at > 20 weeks of gestation in the absence of proteinuria or new signs of end-organ dysfunction [12], according to the guidelines of the International Society for the Study of Hypertension in Pregnancy (ISSHP) [13].
- **Pre-eclampsia (PE)** is a multi-system disorder characterized by the new onset of hypertension and proteinuria or end-organ dysfunction or both in the last half of pregnancy [14]. The diagnosis was made according to the guidelines of the ISSHP [13].
- **Intrauterine growth restriction (IUGR)**: IUGR neonates were defined as those with birth weight below the 3rd percentile as the sensitivity of estimated fetal weight for predicting associated adverse outcome is highest for infants with severe growth restriction (birth weight < 3rd percentile) [15,16].
- **Preterm birth (PB)** was defined as spontaneous preterm delivery with spontaneous onset of labor or preterm pre-labor rupture of the membranes occurring before the 34th completed week of pregnancy [17].
- **Gestational diabetes mellitus (GDM)** is defined as onset of first recognition of abnormal glucose tolerance during pregnancy. The diagnosis was made according to the guidelines of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) [18]. GDM was subdivided according to therapeutic requirement of the disease into GDM managed by dietary change and GDM managed by insulin therapy.

To each woman with a pregnancy complication a woman with an uncomplicated pregnancy was matched in a 1:1 design regarding body mass index (BMI) (± 0.1 points). Control women were selected on the basis of the first match from our chronologic database of consecutively screened pregnant women.

The study was approved by the local ethics committee (Magistrat der Stadt Wien, Magistratsabteilung 15 – Gesundheitsdienst der Stadt Wien) in accordance with the Declaration of Helsinki.

2.2. Analysis and assessment of study parameters

Demographic (i.e. age, parity, BMI) and pregnancy outcome data (i.e. gestational age at delivery, fetal birth weight, percentile for gestational age, occurrence of pregnancy complications: GH, PE, GDM, IUGR, and PB) were retrieved from medical records.

Human chorionic gonadotropin (HCG) and pregnancy-associated plasma protein-A (PAPP-A) were routinely measured on the BRAHMS Kryptor analyzer.

Afamin serum concentrations were measured with a previously described custom-made double-antibody sandwich ELISA test (Microcoat Biotechnologie, Bernried, Germany), using a biotinylated affinity-purified polyclonal antibody for binding to streptavidin-coated microtiter plates and the peroxidase-conjugated monoclonal antibody N13 for detection [19]. The intra-assay and inter-assay coefficients of variation were 3.3% and 6.2%, respectively, at a mean afamin concentration of 73 mg/L.

2.3. Statistical analyses

Data were analyzed with SPSS 13.0.0 software (SPSS Inc., Chicago, IL). Continuous variables were presented as median (25th–75th percentiles). Univariate comparisons between groups were performed with the Mann-Whitney *U* test for continuous variables (respective *P* values were not adjusted for multiple comparisons and are therefore descriptive only). To assess the prognostic value of first trimester afamin serum concentrations for the prediction of pregnancy complications univariate and multivariate logistic regression analyses were performed using a continuous and a dichotomized approach. For the continuous approach afamin was entered per 1 standard derivation increase in the log transformed values, and for the dichotomized approach afamin was dichotomized according to a cut-off of 65 mg/L, which was previously established as median reference value of a women without pregnancy complications at first trimester screening [11].

3. Results

During the recruitment period of almost four years first trimester screening was performed in 4948 pregnant women. All women were followed up until delivery and pathological pregnancy outcomes were recorded for 668 women. Of these pregnant women, 49 were excluded because of multiple pregnancies or fetal malformations. Further, data (i.e. medical record details, blood samples, pregnancy outcome data) was missing in 145 pregnant women. Thus, 474 pregnant women with first trimester screening were eligible for the present study and developed a pregnancy complication [GH (n = 84), PE (n = 30), IUGR (n = 107), PB (n = 44), and GDM (n = 209)]. To each woman with pregnancy complications an uncomplicated pregnancy was matched by BMI. Therefore, 948 pregnant women (i.e. 474 women with pregnancy complications and 474 matched women without pregnancy complications) were finally included in the present study (Fig. 1.)

Baseline demographic and delivery characteristics of 474 women with pregnancy complications and 474 matched women without pregnancy complications are displayed in Table 1. Maternal anamnestic data such as age and BMI was assessed between the 11th and 14th week of pregnancy. Fetal pregnancy outcome data of gestational age at delivery, fetal birth weight and percentile for gestational age was documented at the time of delivery. All baseline demographic data of women with pregnancy complications, divided into different pregnancy outcomes (GH, PE, IUGR, PB and GDM respectively), and healthy women was comparable.

Differences of first trimester serum concentrations of HCG, PAPP-A and afamin between women with pregnancy complications and those without pregnancy complications are presented in Table 2. Median concentrations of HCG, PAPP-A and afamin (25%, 75%-ile) of all women were assessed between the 11th and 14th week of pregnancy. There were significant differences in serum afamin concentrations between women with PE (P = 0.001), GDM ($P \leq 0.001$) and women without pregnancy complications. There was no difference in serum afamin concentrations between all other pregnancy complications and women without pregnancy complications. The results showed that afamin differed significantly between women developing PE or GDM during pregnancy and women with uncomplicated pregnancies, whereas HCG and PAPP-A did not show an overall difference in terms of