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Increased urinary orosomucoid excretion predicts preeclampsia in pregnant women with pregestational type 1 diabetes☆

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ABSTRACT

Aims: We evaluated the urinary orosomucoid excretion (UOE) as a biomarker of preeclampsia and preterm delivery in pregnant women with type 1 diabetes.
Methods: Singleton pregnant women with pregestational type 1 diabetes were included provided one urine sample had been collected before 17 gestational weeks. Serum and urinary orosomucoid were analysed by immunoturbidimetry. Primary outcome measurements were development of preeclampsia (blood pressure > 140/90 mmHg and proteinuria) and preterm delivery before 37 weeks.
Results: In total 173 women were included. The UOE increased during pregnancy. Preeclampsia developed in 20 women and 65 women delivered preterm. Using logistic regression analysis we found that UOE > 1.37 mg/l (OR: 6.85 (95% CI: 1.97–23.88; $p < 0.003$)), nulliparity (3.88 (1.10–13.72); $p < 0.04$), systolic blood pressure > 120 mmHg (4.12 (1.35–12.59); $p < 0.02$) and duration of diabetes > 20 years (3.69 (1.18–11.52); $p < 0.03$) independently predicted the development of preeclampsia. Independent predictors of preterm delivery were duration of diabetes and HbA1c > 7%. The remaining covariates included in the regression models were BMI, serum creatinine, smoking and microalbuminuria.
Conclusions: Increased UOE early in pregnancy predicted preeclampsia in women with pregestational type 1 diabetes independently of albuminuria and other known risk factors. No association to preterm delivery was found.

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1. Introduction

Pregnant women with pregestational type 1 diabetes have a 2–7-fold increased risk of developing preeclampsia [1–4] and increased risk of preterm delivery (4–7-fold) [2–4]. Both preeclampsia and preterm delivery are associated with increased perinatal morbidity and mortality [1–3]. In daily clinical practice it would be of great value to be able to identify women with a specifically increased risk of these complications, e.g. by the use of biomarkers.

In a nationwide Danish study of women with type 1 diabetes compared with the background population the increase in neonatal deaths were primarily associated with preterm deliveries and not associated to preeclampsia [3]. In a smaller study preterm delivery was primarily caused by preeclampsia [6].

The pathophysiology of preeclampsia is not fully understood; however, signs of endothelial dysfunction and activation of the inflammatory system have been found among preeclamptic patients [5], and inflammation is probably an essential part of the condition. In diabetic women proteinuria and microalbuminuria before or in early pregnancy increases the risk of preeclampsia [6,7].

Orosomucoid is an inflammatory protein produced in the liver. Urinary orosomucoid excretion (UOE) increases along with albuminuria in both type 1 and type 2 diabetes [8,9]. UOE has been shown to predict cardiovascular mortality independently of cardiovascular risk markers including albuminuria in patients with type 2 diabetes [8]. Among patients with type 1 DM, increased UOE has been associated with increased mortality; however, UOE was not an independent predictor in type 1 diabetes [8]. Furthermore UOE has been related to markers of inflammation and endothelial dysfunction in patients with type 2 diabetes without signs or history of cardiovascular disease [10]. In non-diabetic pregnancies the increase of UOE has been demonstrated to precede the increase of albuminuria and UOE has been associated with the development of preeclampsia [11].

The aim of the present study was to evaluate the use of UOE early in pregnancy as a biomarker for subsequent development of preeclampsia and preterm delivery in women with pregestational type 1 diabetes.

2. Subjects, materials and methods

2.1. Subjects

Details of inclusion of patients and classification according to urinary albumin excretion have been described earlier [6]. All women with pregestational type 1 diabetes and singleton pregnancies admitted to the Center for Pregnant Women with Diabetes at Rigshospitalet, Copenhagen were consecutively included in the study if they had minimum one urine sample collected before 17 weeks. The inclusion period was from January 1996 to July 2002. Women with macroalbuminuria (urinary albumin excretion $>$ 300 mg/24 h) (n = 3) or abortion (before 22 weeks) (n = 13) were excluded from the study. Normal urinary albumin was defined as urinary albumin excretion $<$ 30 mg/24 h; microalbuminuria was defined as 30–300 mg/24 h. Classification of urinary albumin excretion was based on pregestational values when available; if unavailable, samples from the first trimester were used instead (n = 23) [6].

Informed written consent was given from all participants. The study was approved by the ethics committee in Copenhagen (KF01-095/95) and the study was carried out in accordance with the Helsinki Declaration 2000.

2.2. Analytical methods

All women collected 24-h urine samples that were delivered immediately or kept frozen at home until delivery at the hospital. Urinary albumin during pregnancy was analysed by ELISA or immunoturbidimetry using the same antibodies and buffers. All urine samples were hereafter kept frozen at −20°C until analysis of orosomucoid. Samples used were collected before 17 weeks, at 17–27 weeks and after 27 weeks together with serum samples. Urinary orosomucoid was analysed using a particle-enhanced immunoturbidimetric assay [12] on Cobas Mira and Cobas Integra 700 (Roche, Basel, Switzerland). Frozen samples were thawed at 37 °C in a heating cupboard to give the highest possible recovery [12]. Previous investigations of long term storage (300 days) at −20 °C gave a mean recovery of 69%. Reference values of fresh samples were used as the cut off value for urinary orosomucoid: the 97.5 percentile of the normal population was used (1.98 mg/l), and subsequently subtracted 31% due to long term storage, giving 1.37 mg/l [12].

Serum samples were kept frozen at −20 °C until analysis of orosomucoid (reference range: 0.45–1.17 g/l) and was determined using immunoturbidimetry. For serum creatinine the reference range was 40–110 μmol/l. Haemoglobin A1c (HbA1c) was analysed using high-performance liquid chromatography or using an immunoassay: normal range outside pregnancy 4.7–6.3%; in early pregnancy 4.5–5.7% and in late pregnancy 4.4–5.6% [13].

Diagnosis of preeclampsia was based on office blood pressure $>$ 140/90 mmHg (three measurements) accompanied by proteinuria $>$ 0.3 g/24 h (two urine samples) later than 20 weeks of gestation. Preterm delivery was defined as delivery before 37 gestational weeks.

2.3. Statistical analyses

Statistical analyses were performed using SAS version 9.1 for Windows. Two-sample T-tests were used for the comparison of continuous variables with log-transformed values of positive skewed variables. Chi-square test values were used for the comparison of dichotomous variables. Backward stepwise logistic regression analysis was used for the determination of predictors in early pregnancy of the primary outcome [14].

Clearance of orosomucoid was calculated as: (urine orosomucoid concentration × urine volume)/(serum orosomucoid concentration × urine collection time). Values of urinary orosomucoid below the detection limit ($<$0.07 mg/l) were assigned the value 0.06 mg/l. A p-value of $<$0.05 was considered statistically significant.