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Urine albumin is a superior predictor of preeclampsia compared to urine plasminogen in type-1 diabetes patients

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Title for the running head: Plasminogen in diabetic preeclampsia patients

Number of figures and tables: 1 table + 6 figures

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ABSTRACT

Context: Pregnant women with type 1 diabetes (T1DM) are at increased risk of developing preeclampsia (PE). Plasminogen is aberrantly filtrated from plasma into tubular fluid in PE patients and activated to plasmin. Plasmin activates the epithelial sodium channel (ENaC) in the collecting ducts potentially causing impaired sodium excretion, suppression of the renin-angiotensin-aldosterone system and hypertension in PE.

Objective: To test whether urinary total plasmin(ogen)/creatinine ratio and plasma concentration of aldosterone were better predictors of PE in pregnant women with T1DM compared to urine albumin and HbA1c.

Design: Longitudinal observational study of 88 pregnant T1DM patients at 2 Danish centers. Spot urine- and blood samples were collected at gestational weeks 12, 20, 28, 32 and 36.

Results: U-plasmin(ogen)/creatinine ratio increased during pregnancy. In gestational week 36 the ratio was significantly increased in the T1DM patients developing PE (P<0.05). P-aldosterone was significantly increased in gestational week 20 in the group developing PE (P<0.05). U-albumin/creatinine ratio was significantly increased and predicted PE at all tested gestational ages.

Conclusion: U-albumin/creatinine ratio had a stronger association with development of PE compared to u-total plasmin(ogen)/creatinine ratio and p-aldosterone. The positive association between u-total plasmin(ogen) and development of PE late in pregnancy is compatible with involvement in PE pathophysiology. The significance of albumin in urine emphasizes the importance of preventing renal complications when planning pregnancy in patients with type 1 diabetes.

Keywords: pregnancy, hypertension, aldosterone, proteinuria, diabetes.
INTRODUCTION

Preeclampsia (PE) is one of the most frequent complications in pregnancy (2-8%) occurring after gestational week 20. It is characterized by de novo hypertension and proteinuria. Pregnant women with pre-gestational type 1 diabetes mellitus (T1DM) are in a high risk group with a 2-7 fold increased risk of developing PE. Reliable predictors of PE are missing, both in healthy and diabetic pregnancies. Numerous biomarkers and clinical risk factors have been investigated and most recent data focus on the ratio between the soluble splice variant of the vascular endothelial growth factor (VEGF) receptor, the fms-like tyrosine kinase receptor-1 (sFlt-1) and placental growth factor (PIGF) (the sFlt-1/PIGF ratio). Established predictors that associate with the development of PE in women with T1DM are duration of diabetes, increased level of HbA1C, increased plasma level of sFlt-1, and albuminuria. Pre-gestational microalbuminuria predicts PE in women with T1DM. A 2016 review concluded that the strongest predictors of PE early in T1DM-pregnancies are HbA1C and increased levels of urinary (u-) albumin/protein.

The present study hypothesized that aberrant filtration of the zymogen plasminogen to pre-urine may be more strongly associated with and better predict the development of PE compared to established markers. This hypothesis is based on a putative mechanistic model coupling tubular proteolytic activation of the epithelial sodium channel ENaC and subsequent impaired renal sodium excretion causing suppressed aldosterone which in the end may secondarily attenuate placental growth (Fig. 1a).

In established PE patients, the degree of proteinuria correlates to disease severity and to perinatal morbidity and the highest risk (50-60%) of developing PE is seen in women with diabetic nephropathy. We hypothesized that proteinuria could be involved causally in the pathophysiological features of PE. We have shown significantly increased urinary excretion of the serine proteases plasmin and prostasin in non-pregnant diabetes patients and in PE patients without diabetes which correlated with u-albumin. Urine from PE- and diabetic nephropathy patients evoked significant amiloride-sensitive inward current in cortical collecting duct cells. Sensitivity to protease inhibitors indicated proteolytic activation of the epithelial sodium channel (ENaC) as the mechanism. The findings predict that aberrant filtration and activation of plasminogen to
plasmin confers proteolytic activity to pre-urine that may cause activation of ENaC in vivo and impair sodium excretion in PE. This, in turn, could explain the association with suppression of the renin angiotensin aldosterone system in PE previously observed\textsuperscript{20-22}. Aldosterone acts as a growth factor for placenta but preeclampsia is characterized by small placentas, with thrombotic areas which lead to intrauterine growth restrictions\textsuperscript{23,24}. Thus, early suppression of plasma aldosterone by NaCl retention could contribute to the placcental dysfunction (Fig. 1a).

The present observational study was designed to address the hypothesis that u-plasminogen and p-
aldosterone display an inverse relation and that urine plasminogen level predicts the development of preeclampsia in a cohort of pregnant T1DM patients with an a priori high risk of developing PE. By not including patients with type 2 diabetes and only selecting patients with T1DM, less confounding from differences in BMI, insulin need and peroral antidiabetic pharmacological treatment would be involved.

Secondary outcome/effect parameter was to assess the association of plasma aldosterone and the development of PE. To address the hypotheses, urine total plasminogen and plasma aldosterone concentrations were determined in a longitudinal study design throughout pregnancy in women with pre-gestational T1DM from 2 tertiary referral centers for a period of 2 years in Denmark. The association between levels of p-aldosterone and u-plasminogen and development of PE was compared with the established biomarkers: U-albumin and HbA\textsubscript{1C}. 
MATERIALS AND METHODS

The present study was an observational, longitudinal study conducted at 2 Danish centers: Department of Gynecology and Obstetrics at Aarhus University Hospital, Skejby, and Odense University Hospital. A total of 117 consecutive women with pre-gestational T1DM were eligible for the study before gestational week 13. Of these, 88 women (75%) were included. 14 patients (16%) developed PE between 32- and 37 weeks of gestation. In the following, women with T1DM and developing PE are referred to as the “PE group” and women with T1DM not developing PE are referred to as the “non-PE group”. Patients were enrolled between Aug. 2013 and Jan. 2015.

Inclusion and exclusion criteria

Singleton pregnant women above the age 18, diagnosed with pre-gestational T1DM were included. Patients with relevant comorbidity as pre-gestational hypertension, systemic lupus erythematosus, rheumatoid arthritis or other systemic diseases with kidney involvement were not invited to participate.

PE was defined as de novo hypertension, blood pressure (BP) ≥140/90 after 20th week of pregnancy, combined with >1 + for protein on a urine-dipstick in accordance with national and American guidelines (The American Society of Hypertension (ASH))

Study attendance

Blood and spot-urine samples were collected from each woman consecutively during pregnancy at a maximum of 6 times. Samples were collected as close as possible (but maximum ± 1 week) to gestational week 12, 20, 28, 32, 36 and 38 in connection with their routine antenatal visits.

All 88 pregnant women participated in antenatal visits in gestational week 12, 20, 28 and 32. 73 women participated in the antenatal visit in gestational week 36. 31 women participated in the antenatal visit...
arranged in gestational week 38. The participants not attending the last visits were mainly the women who
had given birth (Fig. 1b). Due to the scarce number of participants at the last visit in week 38, results from
that visit were excluded from most calculations and results were not included in the graphs.

The antenatal visit in gestational week 12 was considered as baseline.

Research procedure and analysis

Participants included in the study were managed in accordance with routine procedures and national
guidelines and seen at the obstetrical outpatient clinic every second week during the third trimester of
pregnancy. Diabetes regulation was managed by self-monitoring of glucose and insulin adjustments aiming
at capillary plasma glucose levels of 4-6 mmol/l pre-prandial and 4-8 mmol/l 1½ hour after meals.

Office BP was measured with the arm at heart level after 10 minutes of rest and in accordance with current
recommendations. At the Skejby Center, BP was measured with blood pressure monitor “microlife BP
a100 plus”, and at the Odense Center, standard device “AND Digital Blood Pressure Monitor, Model UA-
852” (A&D Company) was used. Both devices were validated.

Treatment of PE followed national guidelines and antihypertensive treatment was initiated at a blood
pressure of 135/85 mmHg. Blood-samples were collected by venipuncture after at least 15 minutes of rest in
a seated position. Blood-samples for plasma aldosterone analyses were collected in EDTA tubes on
crushed ice and within one hour centrifuged at 4°C, 10 min, 1500xG (Rotina 420 R, Hettich zentrifugen,
Switzerland). Samples were then stored at -80°C until analysis.

Aldosterone was analyzed in plasma by a competitive binding ELISA (MS E-5200, Labor Diagnostika Nord
GmbH & Co. KG, Germany), EDTA-plasma (50 µL) incubated with Aldosterone-HRP conjugate for 1 hour
as described by the manufacturer. For inter-assay variation, aliquots from the same human EDTA-plasma
pool were used as an intern standard. Values were 75 ± 12 pg/ml. Between-assay coefficient of variation was
9.5%.
Total plasmin(ogen) (plasmin, plasminogen, immunogenic fragments and active plasmin bound to inhibitors) in urine was analyzed by ELISA Kit (Human Plasminogen Total Antigen, IHPLGKT-TOT, Innovative Research, Inc., Novi, Michigan, U.S.A), a sandwich ELISA. Urine (100 µL) was added to a plate coated with capture antibody for human plasminogen, and polyclonal anti-human plasminogen primary antibody was added, which binds to the captured protein. Then secondary antibody conjugated to horseradish peroxidase was added, allowing for detection with substrate developing color readable at 450nm. Assay procedure performed as described by the manufacture. Human EDTA-plasma, diluted 1:10.000 was used as an internal standard (121.4 ± 20.4 microg/ml). Between-assay coefficient of variation was ~10 %, The detection limit in the assay U-plasmin(ogen) was 0.11 ng/ml. All values below that range were set to 0.11. All samples were collected before analyses began. All samples from one participant were run on the same ELISA plate. The clinical outcome PE/non PE was not known to the technician performing the analyses. Samples were run over some weeks and in the order of sampling. Electrolyte analysis and HbA$_1c$ were performed by the Department of Biochemistry and Clinical Pharmacology, Aarhus University Hospital, Skejby. Analyses were performed according to their general practice. The detection limit in the urine albumin essay was 3 mg/l. All the values below that range were set to 2 mg/l, chosen after having evaluated graphs with a limit set to respectively 1 and 3 mg/l. There was very little difference between graphs, but 2 mg/l was an acceptable intermediate. Postpartum details were registered.

**Study ethics**

The study was approved by the local Scientific research ethics committee (Region of Central Denmark with Project ID: 1-10-72-1-13) and by the Data Protection agency (ID: 1-16-02-69-13). No active intervention was performed but an outcome was recorded and the study was registered at [www.Clinicaltrials.gov](http://www.Clinicaltrials.gov) with identification number NCT01821053. The study conformed to the Declaration of Helsinki and all participants gave written informed consent.

**Statistical Evaluation**
The sample size was based on results from a study on albumin as a predictor for PE in a cohort of women with diabetes where 136 participants were enrolled and a highly significant relation was found. Since plasminogen relates directly to albumin in urine, a population size ~100 was aimed at in the present study with no formal power calculation. Of 117 eligible, 100 participants were enrolled in a period of 2.5 years (Fig 1b).

Student’s t-test was used for comparisons of continuous data between the two groups. If standard deviations differed significantly and appeared proportional to the mean, data were log-transformed prior to analysis to comply with the assumption of a normal distribution and the results were then presented in semi-logarithmic diagrams. In tables data are presented as absolute numbers ±SD, but p-values are derived from the log-transformed data.

For categorical data comparisons between the two groups were performed by Fisher’s exact test. The predictive values of the variables were derived from a logistic regression analysis and expressed as odds ratios. For continuous variables the OR represented the risk associated with a 10-fold increase of the variable.

For categorical variables the OR represented the increased risk relative to that in the reference category.

Correlations are expressed as correlation coefficients (r) and evaluated statistically by linear regression analysis.

P-values less than 0.05 were considered significant.

RESULTS

Baseline patient characteristics (12 weeks of gestation)
At baseline, the PE group was similar to the non-PE group with respect to age, gestational age at baseline, pre-pregnancy BMI and weight, duration of diabetes, weight and blood pressure (Table 1). Urine albumin/creatinine ratio was significantly higher (p<0.01) at baseline in the PE group with a mean value of 56.4 mg/g which is compatible with clinical microalbuminuria (normal range< 30 mg/g) compared to the non-PE group (Table 1). A total of 5 patients displayed >1+ for protein on the urine dipstick at baseline. 3 out of those 5 developed PE. At baseline, urine total plasmin(ogen) - and creatinine concentrations and urine total plasmin(ogen)/creatinine ratios were not different between groups. Likewise, values of HbA1c and plasma concentrations of sodium and potassium were similar. Mean plasma aldosterone concentration and blood pressure were not significantly different between groups (Table 1).

Neonatal outcome

Significantly more women in the PE group delivered preterm (< 37 weeks) compared to the non-PE group (respectively 57 % vs. 26%; p<0.05). There were no differences in birth- or placenta-weight between the groups, when adjusted for gestational age at delivery.

Urine total plasmin(ogen)/creatinine ratio and prediction of preeclampsia

Urine total plasmin(ogen)/creatinine (U-plg/crea) ratio increased significantly during pregnancy regardless of development of preeclampsia (PE p<0.001; non-PE p<0.001). No difference in increase during pregnancy (delta values) was found between groups (Fig. 2). At gestational week 36, the mean u-plg/crea ratio was significantly higher in the PE group compared to the mean value in the non-PE group (55770 (CI: 11836-262773) vs. 9043 (CI: 4898-16697) p<0.05) and the odds of being diagnosed with PE was significantly increased when comparing the two groups in gestational week 36 (OR= 2.49; p=0.048). In total, (all visits included) 44 u-total plasmin(ogen) measurements were below range, and two of those were from patients with preeclampsia.
Plasma aldosterone concentration and association with development of preeclampsia

P-aldosterone increased significantly during pregnancy from 12 weeks of gestation to 36 weeks of gestation in both groups (PE group: p<0.01; non-PE group: p<0.001). P-aldosterone increased significantly more in the non-PE group compared to the PE group (mean change: 129 pg/ml (CI: 109;148) in the non-PE group vs. 98 pg/ml (CI: 4-191) in the PE group; p=0.01, Fig. 3). The mean value of P-aldosterone concentration was significantly higher in gestational week 20 in the PE group compared to the non-PE group (Fig.3).

U-albumin/creatinine ratio and association with development of preeclampsia

U-albumin/creatinine ratio increased significantly during pregnancy from 12 weeks of gestation to 36 weeks of gestation in both groups (PE group: p<0.001; non-PE group: p<0.001). U-albumin/creatinine ratio increased significantly more in the PE group compared to the non-PE group (mean change PE: 358.31 mg/g, mean change non-PE: 11.34 mg/g; p<0.01) (Fig. 5a). The U-albumin/creatinine ratios were higher at all visits throughout pregnancy in the PE-group compared to the non-PE group. Increased U-albumin/creatinine ratios were associated with the development of PE. In gestational week 12: OR=3.92 (p=0.02), in 20 weeks of gestation: OR=3.43 (p=0.02) and in 28 weeks of gestation: OR=3.58 (p=0.02), in week 32: OR=6.15 (p<0.01) and in week 36: OR=17.94 (p<0.01) when comparing the two groups.

Comparing the predictive value of the u-plg/crea ratio and the albumin/creatinine ratio

A ROC curve comparing the predictive value of the u-plg/crea ratio and the albumin/creatinine ratio in gestational week 36 (Fig. 4), showed a ROC area-under-the-curve (AUC) of 0.74 and 0.91 for u-plg/crea ratio and u-albumin/creatinine ratio respectively. Besides week 36, there was no predictive value of u-plg/crea ratio at any gestational week (ROC AUC week 12: 0.50 (CI: 0.34-0.66) and 0.68 (CI: 0.51-0.84) respectively).
HbA\textsubscript{1C} and association with development of preeclampsia

P-HbA\textsubscript{1C} decreased significantly during pregnancy from 12- to 36 weeks of gestation in the non-PE group (p=0.001) but not in the PE group (p=0.69). At baseline (week 12) there was no significant difference in HbA\textsubscript{1C} values between the two groups (Table 1 and Fig. 5b) but from week 20 and through the rest of the pregnancy, the HbA\textsubscript{1C} value was significantly lower in the non-PE group (week 20: p<0.05; week 28: p<0.01; week 32: p<0.01; week 36: p<0.01). Increased HbA\textsubscript{1C}-levels were associated with increased odds of getting PE. In gestational week 20: OR=1.08 (p<0.03) for each 1% increase in HbA\textsubscript{1C} and in 28 weeks of gestation: OR=1.09 (p<0.05), in week 32: OR=6.15 (p<0.01) and in week 36: OR=17.94 (p<0.01) when comparing the two groups.

Blood pressure and association with development of preeclampsia

Despite antihypertensive treatment, BP was registered as a secondary outcome. Systolic blood pressure did not change significantly during pregnancy, from week 12 to 36; whereas the diastolic blood pressure increased significantly in both groups (Fig. 5c). From gestational week 28 and through the pregnancy the systolic BP was significantly higher in the PE group compared to the non-PE group whereas the diastolic BP was significantly higher from gestational week 20 (Fig. 5c). In pregnancy week 36 the mean value of both systolic blood pressure and diastolic blood pressure was significantly higher in the PE group (mean systolic BP: 137 mmHg in the PE group versus 121 mmHg in the non-PE group; p<0.001) (mean diastolic BP: 90 mmHg in the PE group versus 79 mmHg in the non-PE group; p<0.001) (Fig. 5c).

Increased systolic BP in gestational week 28 was associated with PE with an OR=1.08 (p<0.05) and at week 32: OR=1.09 (p<0.01) and in week 36: OR=1.13 (p<0.01) when comparing the two groups. Increased diastolic BP in gestational week 20 was associated with development of PE with an OR=1.20 (p<0.01) and
through the rest of the pregnancy. At 28 weeks of gestation: OR=1.18 (p<0.01), in week 32: OR=1.18
(p<0.01) and in week 36: OR=1.20 (p<0.01) when comparing the two groups.

Correlation analyses

A priori hypotheses (u-total plasmin(ogen) is aberrantly filtered; u-total plasmin(ogen) promotes suppression
of aldosterone; aldosterone promotes placental growth) were evaluated by correlation analyses.

In week 20 there was a positive correlation between u-albumin and u-plasmin(ogen) in the PE group (r=0.60;
p<0.01). In GA week 28, the positive correlation was not significant (r=0.49; p=0.11) but in week 32 and 36
the positive correlation was significant again and only in the PE group (r=0.7; p=0.02 and r= 0.78; p= 0.02
respectively).

The correlation between p-aldosterone and u-total plasmin(ogen) was significant only in week 36, where a
positive relation was observed (data not shown). P-aldosterone concentration displayed a positive correlation
with the placenta weight at all times, when the groups were analyzed together (Week 12: r=0.24; p=0.04;
week 20: r=0.29; p=0.01, week 28: r=0.24; p= 0.04, week 32: r=0.24; p= 0.03, week 36: r= 0.29; p=0.02)
(Fig. 6). There was no significant relation between the change in p-aldosterone during pregnancy (from week
12-36) and the placenta weight at delivery.

DISCUSSION

Suppressed circulating levels of the renin-angiotensin-aldosterone system (RAAS) and impaired renal NaCl
excretion are associated with preeclampsia (PE) compared to normal pregnancy \(^{33-35}\). The present study was
designed to test the concept that aberrant filtration of the zymogen plasminogen to tubular fluid may predict
PE better than established markers. This was based on the putative mechanistic coupling to tubular
proteolytic activation of the epithelial sodium channel ENaC and subsequent impaired renal sodium
excretion and suppressed aldosterone that may secondarily attenuate placental growth \textsuperscript{12,13}. An observational design was adopted and applied to a high-risk population of women with pre-gestational type 1 diabetes in order to evaluate the predictive value of u-plg/crea ratio and development of preeclampsia. The association between p-aldosterone and development of preeclampsia was also evaluated and compared to the established predictors, u-albumin and HbA\textsubscript{1C}.

We found that u-plg/crea ratio increased in both groups but the relation to u-albumin reached significance only in the PE group and late in pregnancy which is in agreement with aberrant filtration of plasminogen in established PE as in other diseases with defect filtration barrier\textsuperscript{19,36}. The present data revealed no added value of using urinary total plasmin(ogen) concentrations as a tool in the prediction of PE early in pregnancy (GA 12 and 20) compared to e.g. albumin. In gestational week 36, u-plg/crea ratio was significantly increased in the PE group suggesting an association between u-plg/crea ratio and late stages of PE. The feasibility of u-plg/crea ratio as a predictor/severity marker of PE in week 36 is of minor clinical relevance as other disease biomarkers (symptoms, s-urate, platelets, liver enzymes etc.) are more relevant at that time.

The association between u-plg/crea ratio and development of PE only in late stages of PE might be due to the difference size of molecules with earlier escape of 66 kDa albumin than ~100kDa plasminogen precursor across the glomerular barrier. It could also relate to the fact that the ELISA assay provides total plasminogen and not the essential parameter for the hypothesis, namely urine plasmin enzyme activity which is the biological effector in the putative cascade. The ROC curve confirmed that u-albumin/creatinine ratio remained a stronger predictor of PE development relevant early in pregnancy and throughout pregnancy\textsuperscript{8-10,32}. A negative relation between u-total plasmin(ogen) and p-aldosterone was predicted late in pregnancy but was not observed. The positive association between p-aldosterone and development of PE was borderline significant in predicting PE, especially early in pregnancy. A significantly lower dynamic increase in p-aldosterone was observed in those individuals developing PE and a positive relation with placenta weight was observed. The present measurements suggest a higher aldosterone level, at least early in pregnancy, in T1DM patients as the reason for less dynamic change. It is interesting that aldosterone displayed a highly significant correlation with placental weight which is in agreement with data from various other species, e.g.
mice with deficient aldosterone synthase that have smaller litters and grossly impaired placenta morphology.

In general, T1DM patients display an activated RAAS. In pregnancies with GDM and T1DM, data have shown higher aldosterone levels in late pregnancy compared to pregnant controls without diabetes. In a study by Ringholm et al., the associations between the development of PE and pro-renin, renin, angiotensinogen and ACE (but not aldosterone) in pregnant women with T1DM was investigated. Higher concentrations of pro-renin in early pregnancy related directly to the development of PE. Thus it appears that both pro-renin and aldosterone are elevated in those T1DM patients that develop PE.

From the present study, suppressed plasma aldosterone concentration in early pregnancy is less likely an explanation for later development of PE. Suppressed plasma aldosterone is more likely a consequence of the pathophysiology of established PE which was also the conclusion reached in the study with mice deficient of aldosterone synthase. The tendency that elevated levels of aldosterone in plasma related to development of PE in patients with diabetes must be tested in larger cohorts since the present study lacked power to reach level of significance.

In the present study HbA1C decreased significantly during pregnancy in the non-PE group but not in the group of patients developing PE. An association between glycemic control and PE previously has been reported by others. The difference in HbA1C between groups became significant between gestational weeks 12 and 20. These findings are in accordance with results from other studies and highlight the importance of glycemic control. In patients with diabetes, hyperglycemia caused abnormalities in blood flow and increased vascular permeability reflecting decreased activity of vasodilators (as nitric oxide), hypoxia and increased activity of vasoconstrictors (as Ang II and endothelin 1). These vascular changes are quite similar to changes observed in PE and therefore it appears feasible that increased levels of HbA1C are associated with PE development.

The diastolic BP increased significantly during pregnancy in both groups. In the PE group the diastolic BP was significantly higher compared to the non-PE group already from gestational week 20, confirming the
importance of blood pressure controls in pregnant T1DM patients, and not to underestimate the importance of the diastolic blood pressure.

At baseline the urine albumin/creatinine ratio was significantly higher in the PE group which is in accordance with findings by others demonstrating that urine albumin early in pregnancy is a predictor of PE development.\cite{8-10} From gestational week 20 there was a positive correlation between u-albumin and u-plasmin(ogen) in the PE group as we expected due to our hypothesis of aberrant filtration of plasmin(ogen) as the filtration barrier is affected. However, it can only be speculated why a positive correlation was not found already at baseline and moreover we did not found u-plasmin(ogen) to be a superior predictor of preeclampsia compared to urine albumin.

**Strengths and limitations**

Variance in aldosterone concentrations are likely since salt intake was not controlled for and patients were not fasting. Plasma aldosterone displays diurnal variation and samples were not collected at the exact same time of the day (between 9 a.m. and 2 p.m. after at least 15 minutes of rest). Only 2 out of 14 women were diagnosed with PE before week 34, indicating that results are most relevant in the context of late incident PE. Fewer participants than aimed for were enrolled in the study and thus a lack of power could have resulted in failure to reach significant levels (e.g. the association of aldosterone, u-total plasminogen difference from controls). Ethnicity was solely Caucasian which will limit extrapolation of the findings to other populations.

**Conclusion**

In summary, the present study was designed to test whether levels of u-total plasmin(ogen) and plasma aldosterone, based on a pathophysiological activation of the ENaC channel, could be superior predictors of incident preeclampsia in a high-risk population of T1DM patients. U-total plasmin(ogen) and p-aldosterone were not superior to well-established u-albumin, but the association of u-total plasmin(ogen) and development of PE late in pregnancy was compatible with involvement in PE pathophysiology. Low plasma
concentrations of aldosterone were not associated with PE, but PE was associated with less dynamic change in aldosterone suggesting that low plasma aldosterone is a likely consequence but not cause of preeclampsia.

The significant association of HbA1C, u-albumin and PE-development emphasizes the importance of glycemic control and prevention of diabetic renal complications when planning pregnancy in T1DM.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
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REFERENCES


Table 1 Baseline patient characteristic

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<th>PE</th>
<th>non-PE</th>
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<td>Age (years)</td>
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<td>Gestational age at visit 1 (days)</td>
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<td>BMI before pregnancy (kg/m(^2))</td>
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<td>Diabetes duration (years)</td>
<td>16.2±7.5</td>
<td>13.3±8.9</td>
<td>0.25</td>
</tr>
<tr>
<td>Weight, pregestational (kg)</td>
<td>78.5±13.9</td>
<td>72.8±14.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Weight, baseline (kg)</td>
<td>81.5±13.4</td>
<td>74.7±14.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125±7</td>
<td>120±10</td>
<td>0.13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77±7</td>
<td>76±7</td>
<td>0.62</td>
</tr>
<tr>
<td>Urine albumin /creatinine ratio (mg/g)</td>
<td>56.4±149.3</td>
<td>9.9±37.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Urine total plasmin(ogen) (ng/ml)</td>
<td>5.9±8.6</td>
<td>8.3±16.6</td>
<td>0.69</td>
</tr>
<tr>
<td>Urine total plasmin(ogen)/creatinine ratio (ng/g)</td>
<td>6045±7830</td>
<td>11549±25680</td>
<td>0.91</td>
</tr>
<tr>
<td>Urine creatinine concentration ( mmol/l)</td>
<td>10.3±5.7</td>
<td>8.6±4.5</td>
<td>0.20</td>
</tr>
<tr>
<td>HbA(_{1C}) (%)</td>
<td>6.9±0.7</td>
<td>6.6±0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Plasma aldosterone concentration (pg/ml)</td>
<td>179.6±127.9</td>
<td>133.1±82.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Plasma sodium concentration (mmol/l)</td>
<td>135.8±1.4</td>
<td>135.7±1.6</td>
<td>0.96</td>
</tr>
<tr>
<td>Plasma potassium concentration (mmol/l)</td>
<td>4.0±0.4</td>
<td>3.8±0.3</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Table 1 Baseline clinical- and laboratory characteristics of patients in the 2 groups, at inclusion, in gestational week 12.

PE: pregnant women with pre-gestational type 1 diabetes that developed preeclampsia; non-PE: pregnant women with pre-gestational type 1 diabetes that did not develop preeclampsia.

Baseline data were complete in 12 cases in the PE group, and in 68 cases from the non-PE group. This was not caused by exclusion of data or patients.
Legends

Figure 1

A. Proposed scheme of the hypothesized pathophysiological mechanism involved in preeclampsia.

Activation of the epithelial sodium channel (ENaC) by plasmin in PE patients with glomerular filtration barrier injury and proteinuria. Plasminogen in tubular fluid is activated to plasmin likely by urokinase-type plasminogen activator. Plasmin activates ENaC through proteolytic cleavage of the $\gamma$-subunit which causes channel activation and impaired Na$^+$ excretion. The renin-angiotensin aldosterone system is subsequently suppressed and lower circulating plasma levels of aldosterone in PE may potentially further aggravate placental dysfunction.

B. CONSORT flow chart illustrating the numbers of persons eligible, patients included, dropouts and numbers attending each of the antenatal visits in gestational weeks 12, 20, 28, 32, 36 and 38 allocated in the two groups.

Figure 2

Concentrations of urine total plasmin(ogen)/creatinin ratio measured in the PE group and the non-PE group in gestational weeks 12, 20, 28, 32 and 36. PE group: Week 12-32, n=14. Week 36, n= 8. Non-PE group: Week 12-32, n= 74. Week 36, n=65. Data were log-normally distributed, and the mean values are presented with standard errors (SE). *$P < 0.05$.

Figure 3.

The figure shows plasma aldosterone concentration measured in the PE group and the non-PE group in gestational weeks 12, 20, 28, 32 and 36.
PE group: Week 12-32, n=14. Week 36, n= 8. Non-PE group: Week 12-32, n= 74. Week 36, n=65. Data were
log-normally distributed. Observations are illustrated as means ± SE. *P < 0.05.

**Figure 4.**

ROC curve comparing the area under the curve (AUC) for urine total plasmin(ogen)/creatinin ratio
(illustrated by the black line with black dots) and urine albumin/creatinine ratio (illustrated by the black line
with open, grey diamonds) in gestational week 36. Urine total plasmin(ogen)/creatinin ratio had a ROC
AUC of 0.74 (CI: 0.55-0.93) and urine albumin/creatinine ratio had a ROC AUC of 0.91 (CI: 0.79-1.00).

**Figure 5.**

A, urine albumin/creatinin ratio B, HbA1c values (%) C, Systolic and diastolic blood pressure measured in the
PE group and the non-PE group in gestational week 12, 20, 28, 32 and 36.

Observations are illustrated as means ± SE. *P < 0.05, **P < 0.01, *** P < 0.001.

**Figure 6.**

Correlation between p-aldosterone in gestational week 20 and placenta weight at delivery in the two groups
joined together (r=0.29; p=0.01).
Aberrant glomerular filtration

Plasminogen

\[ \gamma\text{-ENaC} \]

Plasmin

Activated \[ \gamma\text{-ENaC} \]

\[ \text{Na}^+ \text{ retention} \]

\[ \text{Suppressed aldosterone} \]

\[ \text{Placental dysfunction} \]

Fig. 1a
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Fig. 1b
Fig 2
Fig 3
Fig 5
Fig. 6
**Highlights**

- U-plasmin(ogen)/creatinine ratio increased during pregnancy in pregnant T1DM patients from gestational week 12 to 36.
- There was a positive association between u-total plasmin(ogen) and development of preeclampsia late in pregnancy.
- U-albumin/creatinine ratio was significantly increased and associated with development of preeclampsia at all tested gestational ages.
- U-albumin/creatinine ratio had a stronger association with development of preeclampsia compared to u-total plasmin(ogen)/creatinine ratio and p-aldosterone.