First trimester serum levels of the soluble transcobalamin receptor, holo-transcobalamin, and total transcobalamin in relation to preeclampsia risk

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First trimester serum levels of the soluble transcobalamin receptor, holo-transcobalamin, and total transcobalamin in relation to preeclampsia risk

Omar Abuyaman, Niels Torringle, Rima Obeib and Ebba Nexoa

ABSTRACT
Background: Human placenta expresses CD320, a receptor that ensures the uptake of holo-transcobalamin (holoTC). Soluble CD320 (sCD320) is present in the circulation and its concentration increases during pregnancy.
Aims: To investigate a possible association of sCD320, holoTC and total transcobalamin (TC) with the risk of subsequent preeclampsia using serum samples from asymptomatic first trimester pregnant women. Moreover, we aimed to establish reference intervals of the aforementioned biomarkers for first trimester pregnant women who remained healthy throughout pregnancy.
Study design: This study was a retrospective case-control study that we performed on biobank serum samples. Cases (n = 50) and controls (n = 198) (matched for gestational age and date of sample collection) were asymptomatic women in early pregnancy [median (range) gestational age = 10 (6–12) weeks]. Cases developed preeclampsia while the controls remained normotensive throughout pregnancy. We measured the serum concentration of sCD320, holoTC, and total TC by using in-house ELISA methods.
Results: First trimester median concentrations of sCD320, holoTC and total TC were not significantly different between cases and controls. The odd ratio for developing preeclampsia based on exposure to low or high levels of sCD320, holoTC or total TC at first trimester was not significant. The reference intervals (2.5–97.5% percentiles (median)) derived from the controls were 50–170 (90) pmol/L for holoTC and 560–1300 (810) pmol/L for total TC.
Conclusions: The risk of preeclampsia is not predicted by first trimester serum concentrations of sCD320, holoTC or total TC. The first trimester reference intervals for the three parameters is reported.

Introduction
Preeclampsia is a systemic vascular disorder [1] occurring in 2–8% of pregnant women [2]. The condition is characterized by new-onset hypertension and proteinuria at ≥20 weeks of gestation [3]. Preeclampsia is a main risk factor for maternal and fetal mortality and morbidity [4]. The disease can be classified according to the time of onset [early (<34 weeks) or late (≥34 weeks)] or according to the severity of the symptoms (mild or severe) [5].

The etiology of preeclampsia is poorly understood [6]. Among the suggested mechanisms are impaired placenta vascularization and endothelial dysfunction, which together is speculated to lead to an increased concentration of placenta-related parameters in the circulation. Currently the risk assessment (in the first trimester for subsequent development of the disease) involves a combination of maternal history, biochemical markers, and biophysical measurements. The biochemical markers include pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PIGF) and free β subunit of human chorionic gonadotropin (βhCG) [7–9]. Since no ideal marker has been identified there is a constant search for new biomarkers, notably biomarkers that at an early stage can identify women at risk. One such potential marker is sCD320, a receptor for the vitamin B12-transporter, TC.

Vitamin B12 is a coenzyme in metabolic processes essential for cells growth and division, and is of importance for fetal growth and development [10,11]. Dietary vitamin B12 is absorbed through a gastric intrinsic factor-mediated uptake in the ileal enterocytes [12]. After absorption, vitamin B12 is bound to TC. TC circulates in plasma saturated with vitamin B12 (holoTC) and in its free form (apoTC) [12]. Cellular uptake of vitamin B12 demands binding of the vitamin to TC and recognition of holoTC by the receptor, CD320 [13]. This receptor is highly expressed in human placenta [13] and also expressed in endothelial cells [14].

We recently identified a soluble form of CD320 (sCD320) in serum, urine and cerebrospinal fluid [15]. We speculate that more sCD320 may be released to serum at an early stage of placental or endothelial damage. Previous studies have shown increasing concentration of sCD320 during pregnancy [16] while total TC decreases and holoTC remains virtually unchanged or decrease slightly [11,17–19].
The concentration of holoTC, the ligand of CD320, correlates with sCD320 and total TC [20,21].

The aim of the current study was to explore maternal serum concentrations of sCD320, holoTC and total TC in first trimester pregnant women in relation to future risk of preeclampsia and to report the first trimester reference intervals for the three parameters.

**Subjects and methods**

**Study design and settings**

This was a retrospective archive-based case-control study that used samples and data collected during the prenatal screening program at the department of clinical biochemistry, Aarhus University Hospital from 2011–2014. The exclusion criteria were: active smoking, non-Caucasian ethnic origin, missing key information or insufficient sample volume, pregnancy complication other than preeclampsia, multiple births, or non-spontaneous conception. We retrieved serum samples from 50 asymptomatic pregnant women (gestational age 8–12 weeks) who later developed preeclampsia (cases) and samples matched for gestational age and sampling times from 198 pregnant women who remained asymptomatic throughout pregnancy (controls).

The Danish Central Biomedical Research Ethics Committee, and Data Protection Agency in Denmark reviewed the study protocol and approved using the samples and relevant data to investigate potential biomarkers for preeclampsia prediction (Approval No. 1-10-72-265-13). Samples and data were coded and were anonymous for the investigators.

**Preeclampsia diagnosis**

Mild preeclampsia was defined as de novo hypertension or systolic/diastolic blood pressure ≥140/90 mmHg at rest, measured twice at 15 min. interval and proteinuria developed after 20 weeks of gestation as measured by dipstick (1+). Severe preeclampsia was defined as de novo hypertension (>160/110 mmHg), proteinuria (2+), and by clinical symptoms [20]. The diagnosis of preeclampsia was confirmed by an obstetrician.

**Data and samples collection**

Complementary data related to study scope were retrieved from the central health database. The extracted data included birthdate, date of delivery, maternal weight, smoking status, ethnicity, type of conception, gestational age at the time of blood sampling. The gestational age was defined based on the crown-rump length measurements [16]. Subjects’ characteristics are described in Table 1. Blood samples were collected into plain tubes and aliquots of serum were subsequently stored at −80°C in the hospital biobank until analysis.

<table>
<thead>
<tr>
<th>Table 1. Basic characteristics of the first trimester pregnant women included in the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
</tr>
</tbody>
</table>

Data are median (range). p values are according to Mann-Whitney test. Cases are pregnant women who later developed preeclampsia. Controls are pregnant women who remained free of preeclampsia.

**Biochemical analysis**

The samples were analyzed blinded to the clinical outcomes. Levels of sCD320 were quantified by using an in-house sandwich ELISA (inter-assay imprecision of 4.0–8.0% and an intra-assay imprecision of 3.5–4.3%) as described [15,22]. Total TC was measured by an in-house sandwich ELISA (inter-assay imprecision of 4.0–6.0% and an intra-assay imprecision of 3.0%) [23]. HoloTC was measured by the TC-ELISA after removal of the apoTC (i.e. unsaturated TC) with vitamin B12-coated beads (inter-assay imprecision of 8.0% and an intra-assay imprecision of 4.0%) [24,25]. All values are rounded to the nearest 10.

**Statistical analysis**

Study variables did not follow a normal distribution (using Kolmogorov-Smirnov test). Thus, non-parametric statistical tests were used and measurements were reported as medians and range. Spearman’s rank test was used to investigate the correlations between study variables. Mann-Whitney U test was applied for testing the medians difference. Reference interval was calculated as the 95% central interval (the interval between the 2.5% and the 97.5% percentiles). The odds ratios for preeclampsia according to sCD320, holoTC and total TC levels in the first trimester were calculated using binary logistic regression analyses. The cut-off values for the three markers were chosen to be the upper limit of the lowest quartile of the marker calculated in the 198 controls samples; p values <.05 were considered statistically significant. Statistical analyses were performed using SPSS statistical computer software (version 20, IBM Inc.).

**Results**

We present data on serum sCD320, holoTC and total TC in asymptomatic first trimester pregnant women who subsequently develop preeclampsia (cases) and in asymptomatic pregnant women who remained healthy throughout pregnancy (controls).

No difference was observed for age or weight between cases and controls (Table 1). The medians (range) concentration in pmol/L for sCD320, holoTC and total TC in cases were 80 (10–770), 70 (30–140), and 810 (580–1740), respectively. These concentrations were not significantly different from sCD320, holoTC and total TC levels of the controls (p-values = .209, .187, .686, respectively) (Table 2). sCD320, holoTC and total TC concentrations were not different according to the severity
of preeclampsia (mild versus severe) (Table 2). Comparing cases subsets to the controls (severe cases versus controls and mild cases versus controls) showed no difference in medians (data not shown). In addition, there was no change in the risk of developing preeclampsia based on exposure to low or high levels of sCD320, holoTC and total TC (cut-off values were defined by the lower quartile calculated using the 198 controls samples) (Table 3).

In agreement with previous data [21] we found a positive correlation between sCD320 and its ligand, holoTC (Spearman’s rank correlation = 0.349, p < .001, n = 248) and between sCD320 and total TC (Spearman’s rank correlation = 0.264, p < .001, n = 248).

Our data allowed us to report first trimester reference intervals for sCD320, holoTC and total TC based on the 198 pregnant women who remained healthy throughout pregnancy. No significant differences between sCD320, holoTC and total TC medians were observed upon dividing the control subjects to tertiles based on age, weight or gestational age (data not shown) and because of that we computed a common reference interval (95% interval (median), n = 198) for sCD320 (50–170 (90) pmol/L, for holoTC is 20–140 (70) pmol/L and for total TC is 560–1300 (810) pmol/L).

Discussion

We report no predictive value of first trimester serum sCD320, holoTC and total TC for subsequent development of preeclampsia. Previous studies have indicated the same to be the case for total serum vitamin B12 [26,27]. The findings are in agreement with reported correlations between the B12 related parameters (B12, holoTC, total TC and sCD320) [15,21,22].

Acknowledgements

We appreciate the excellent technical assistance offered by Inger Marie Jensen and Jette Fisker Petersen.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Table 2. Serum concentrations of sCD320, holoTC, and total TC in first trimester pregnant women.

<table>
<thead>
<tr>
<th>Number</th>
<th>Controls</th>
<th>Cases</th>
<th>p-value</th>
<th>Cases – Severe</th>
<th>Cases – Mild</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD320 (pmol/L)</td>
<td>198</td>
<td>50</td>
<td>.209</td>
<td>80 (60–130)</td>
<td>80 (10–770)</td>
<td>.752</td>
</tr>
<tr>
<td>HoloTC (pmol/L)</td>
<td>70 (10–330)</td>
<td>70 (30–140)</td>
<td>.187</td>
<td>70 (50–120)</td>
<td>70 (30–140)</td>
<td>.942</td>
</tr>
<tr>
<td>Total TC (pmol/L)</td>
<td>810 (520–1410)</td>
<td>810 (580–1740)</td>
<td>.686</td>
<td>810 (580–1740)</td>
<td>810 (600–1430)</td>
<td>.990</td>
</tr>
</tbody>
</table>

Table 3. Odds ratio for preeclampsia based on exposure to low or high levels of sCD320, holoTC and total TC in blood samples collected during first trimester pregnancy.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Range, pmol/L</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD320</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>&lt;77</td>
<td>15</td>
<td>49</td>
<td>1.3 (0.7–2.6)</td>
</tr>
<tr>
<td>High</td>
<td>≥77</td>
<td>35</td>
<td>149</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>HoloTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>&lt;49</td>
<td>8</td>
<td>48</td>
<td>0.6 (0.3–1.4)</td>
</tr>
<tr>
<td>High</td>
<td>≥49</td>
<td>42</td>
<td>150</td>
<td>1.0 (Reference)</td>
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<tr>
<td>Total TC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>&lt;715</td>
<td>8</td>
<td>47</td>
<td>0.6 (0.3–1.4)</td>
</tr>
<tr>
<td>High</td>
<td>≥715</td>
<td>42</td>
<td>151</td>
<td>1.0 (Reference)</td>
</tr>
</tbody>
</table>

Data are median (range). p-values are according to Mann-Whitney test. Cases are pregnant women who later developed preeclampsia. Controls are pregnant women who remained free of preeclampsia.
Funding

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References