Changes in endothelial cell specific molecule 1 plasma levels during preeclamptic pregnancies compared to healthy pregnancies

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ABSTRACT

Objective: We aimed to assess the levels of endothelial cell specific molecule 1 (ESM-1) during pregnancy and preeclampsia.

Methods: Plasma and placental samples were collected from women with a control pregnancy, early- or late-onset preeclamptic women and non-pregnant women (experiment 1). Plasma samples were collected between weeks 12 and birth from pregnant women at high risk for developing preeclampsia (experiment 2). ESM-1 plasma levels were measured by ELISA and in the placenta mRNA and protein were detected by immunohistochemistry and qPCR.

Results: In the first experiment we observed lower concentrations of ESM-1 in pregnant women as compared to non-pregnant women and higher concentrations during early- and late-onset preeclampsia as compared to control pregnancies of the same gestational age. Early- and late-onset preeclamptic pregnancies were not different from their subsequent controls in ESM-1 mRNA or protein levels in placental tissue. The second experiment showed that in women who had a control pregnancy, plasma ESM-1 levels were decreased as compared to non-pregnant women from week 16 ± 2 until the end of pregnancy and returned to non-pregnant levels postpartum. In women who developed early- or late-onset preeclampsia, plasma ESM-1 was also decreased as compared to non-pregnant women from week 20 ± 2 until week 28 ± 2 of pregnancy. Then ESM-1 levels increased and were no longer different from levels in non-pregnant women on weeks 32 and 36.

Conclusions: Plasma ESM-1 levels are decreased during pregnancy and increased in early- and late-onset preeclampsia. The source of ESM-1 is probably not the placenta, but most likely maternal endothelial cells.

1. Introduction

Preeclampsia is one of the most serious complications that can occur during pregnancy, resulting in maternal and perinatal morbidity and mortality. The definition of preeclampsia was recently updated to the specification of a de novo hypertension (> 140/90 mm Hg) after 20 weeks of gestation accompanied by one or more of the following new onset conditions: proteinuria, other maternal dysfunction (renal insufficiency (creatinine > 90 µmol/L), liver involvement (elevated transaminases), neurological complications ( eclampsia, visual disturbance/blindness and, or headaches accompanied by hyperreflexia) or haematological complications (thrombocytopaenia, DIC, haemolysis)), or uteroplacental dysfunction (foetal growth restriction) [1]. Preeclampsia can occur as early-onset (birth before 34 weeks) or late-onset (birth after 34 weeks) preeclampsia [2] and risk factors, like diabetes mellitus, chronic hypertension, obesity and multiple gestation, increase the chance of developing preeclampsia [3].

The mechanisms underlying preeclampsia are still not well understood. Early onset preeclampsia is thought to develop following placental dysfunction, induced by impaired trophoblast invasion and abnormal spiral artery remodeling [4,5]. The dysfunctional placenta may release specific factors into the maternal circulation, such as proinflammatory cytokines, placental microvesicles, anti-angiogenic factors, such as sFlt-1 and sEndoglin [6,7] or ATP [8]. These factors further
agravate the normal occurring low grade systemic inflammatory re-
sponse during pregnancy [2,9]. They also activate endothelial cells
[7,10]. Finally, this proposed cascade of events may result in pre-
eclampsia.

Late-onset preeclampsia is hypothesized to be associated with re-
stricted villous perfusion, due to the increasing size of the placenta and
villus constraints [11]. This results in abnormal placental perfusion
and subsequent release of the same placent al factors in the maternal

circulation.

Endothelial cell specific molecule 1 (ESM-1) was described as an
inflammatory factor that was increased in cardiovascular disease, sepsis
and cancer [12–16]. This proteoglycan is produced by endothelial cells
and is involved in a wide range of biological processes, such as pro-
liferation, migration, cell adhesion and neovascularization. ESM-1 can
be found in plasma [17–20] and the production is increased in the
presence of proangiogenic molecules such as VEGF and FGF-2 [15].
The relation between these angiogenic factors and ESM-1 and the fact
that ESM-1 is an inflammatory factor [13], suggest that ESM-1 levels
might be increased during pregnancy and further increased in pre-
eclampsia.

The aim of the present study was therefore to test the hypothesis
that ESM-1 is increased during pregnancy and further increased during
preeclampsia.

2. Material and Methods

2.1. Study population

This study has been approved by the medical ethics committee of
the University Medical Center Groningen. Written informed consents
were obtained from all patients.

Preeclampsia was defined according to the definition of the
International Society for the Study of Hypertension in Pregnancy
(ISSHIP): diastolic blood pressure of ≥90 mmHg on two or more occa-
sions, more than 4 h apart, and proteinuria of ≥300 mg/24 h published
by Tranquilli et al. [1,21]. Early-onset preeclampsia was defined as
giving birth before 34 weeks; late-onset preeclampsia was defined as
giving birth in week 34 or later. In all our cases, preeclampsia was
classified as severe (blood pressure higher or equal to 160 mmHg sys-
tolic or 110 mmHg diastolic). Experiment 1: For this experiment ex-
clusion criteria for all groups were pre-existing hypertension, diabetes
mellitus, vasculitis, renal disease, autoimmune disease, malignancies or
manifestations of the medical condition (130

Belgium). Plasma samples were processed immediately by centrifuga-
tion. EDTA-plasma collection tubes (Venoject, Terumo Europe NV, Leuven,
Belgium) were thawed just before dilution for use in the assay. To determine
the plasma ESM-1 concentration, the enzyme-linked immunosorbent assay
(ELISA) supplied with the ELISA (Pregnostic®-PE伊) (IQ Products BV, Groningen,
The Netherlands). The plasma samples were coded and evaluated
blinded for outcome.

For the analysis of placent al ESM-1 protein and mRNA expression,
biospies were collected from early-onset preeclamptic (N = 27) and late-onset preeclamptic women (N = 12). Since all early-onset
preeclamptic women delivered by caesarean section, control placentas
were collected from women (N = 15) who delivered by caesarean
section for other reasons than preeclampsia, for instance breech pre-

tation. Women with late-onset preeclampsia delivered vaginally,
therefore control placentas were collected from control pregnant
women (N = 14) who delivered vaginally. Patient characteristics are
shown in Table 2. Placental biospies were randomly collected by taking
1 by 1 cm tissue samples from the chorionic villi (after removal of the
decidua), avoiding infarcted areas, and snap frozen in liquid nitrogen
and stored at −80 °C until further processing. For mRNA isolation 5
small biospies of the chorionic villi (after removal of the decidua) were
taken randomly (avoiding infarcted areas), pooled, snap frozen and
stored at −80 °C until further processing.

Immunohistochemical staining of placental sections: Placental
cryostat sections (4 µm) were cut. After drying, 10 min acetone fixation
and drying again, the sections were stained with the primary antibody
mouse anti-human ESM-1 monoclonal MEP14 (1 µg/ml) (IQ Products
BV, Groningen, The Netherlands). Control sections were stained
without the use of a primary antibody. After washing with phosphate
buffered saline (PBS), exogenous peroxidase was blocked by incubation
for 30 min in 0.25% H2O2 in PBS. After washing, the slides were incu-
bated with biotin-conjugated goat-anti-mouse (Southern Biotech,
Birmingham, AL, USA) for 60 min, washed again and incubated with
horseradish peroxidase conjugated streptavidin (Dako, Heverlee,
Belgium) in PBS for 30 min. The presence of ESM-1 was visualized with
3-amino-9-ethyl-carbazole, followed by hematoxylin staining. All of the
incubation steps were carried out at room temperature and all sections
were stained in 1 procedure. After staining, all slides were scanned with
the Aperio TMA scanner (Aperio, Vista, USA). The control sections were
consistently negative. Quantification of the ESM-1 protein expression in
placenta was done using Aperio ImageScope (Aperio, Vista, CA, USA).
We determined the amount of positivity using the ‘positive pixel count
v9’. This was done in at least 3–5 tissue areas per section. Of each
placent al section the mean number of positive pixels per tissue area was
used for evaluation.

Placental ESM-1 mRNA expression was analyzed using real-time RT
PCR from a random selection of the placentas used for im-
munohistochemistry. This set consisted of nine early-onset pre-
eclamptic placentas and of seven control placentas from women who
delivered by caesarean section and six late-onset preeclamptic placentas
and six control placentas from women who delivered vaginally.
mRNA isolation was done using TRIzol (Life Technologies Europe,
Bleswijk, the Netherlands) followed by the determination of the 260/
280 ratio with use of the Nanodrop spectrophotometer (Thermo Fisher
Scientific, DE, USA), to determine the purity and quality of the mRNA.
All samples had an optimal 260/280 ratio of about 2.0, indicating
the purity of the mRNA. Reverse transcription to cDNA was done using
Superscript II reverse transcriptase (Eurogentec, Maastricht, The
Netherlands). ESM-1 and proteasome non-ATPase regulatory subunit 4
(PSMD-4), used as a reference gene, analysis was done using prede-
signed gene expression assay Taqman primer-probe combinations
(Hs00199831_m1 and Hs00356654_m1, Life Technologies). PCRs were
performed in a reaction volume of 25 µl with Absolute QPCR ROX mix
(Life Technologies) and 40 cycles on a ABI Prism 7900HT Sequence
Detection System (Life Technologies). The analysis of the qPCR data
was performed using the software of the ABI Prism 7900HT platform.
ESM-1 mRNA levels were corrected for PSMD-4 expression by calcu-
lating the ΔΔCt (Ct ESM-1 – Ct PSMD4) value and plotted as 2-ΔΔCt va-

ues.

Experiment 2: In the second set of experiments, we tested the course
of ESM-1 concentration in plasma during pregnancy and preeclampsia.
Therefore, a different set of plasma samples was collected between
week 12 ± 2 until birth from pregnant women at high risk for devel-
opng preeclampsia. Samples were used from the cohort described by
Wong et al [22]. In this cohort a total of 103 pregnant women were
included. They were in their first trimester and presented with one of

the following risk factors to develop preeclampsia: previous history of preeclampsia, chronic hypertension, previous history of HELLP syndrome, diabetes mellitus, multiple pregnancy, obesity, or autoimmune disease [22]. For the present study samples from those patients defined as having early-onset (n = 8) or late-onset preeclampsia (n = 8), stratified according to characteristics indicated before, were used. The characteristics of these women can be found in Table 3. Control pregnant samples have been collected from the same cohort of women (n = 21), with the difference that these women did not develop preeclampsia or pregnancy-induced hypertension. The controls included non-pregnant samples were used from experiment 1. Blood samples from the pregnant women were taken when they had an appointment with their obstetrician. Samples were categorized into the following risk factors to develop preeclampsia: previous history of HELLP syndrome, diabetes mellitus, multiple pregnancy, obesity, or autoimmune disease [22]. For the present study samples from those patients defined as having early-onset (n = 8) or late-onset preeclampsia (n = 8), stratified according to characteristics indicated before, were used. The characteristics of these women can be found in Table 3. Control pregnant samples have been collected from the same cohort of women (n = 21), with the difference that these women did not develop preeclampsia or pregnancy-induced hypertension. The controls included non-pregnant samples were used from experiment 1. Blood samples from the pregnant women were taken when they had an appointment with their obstetrician. Samples were categorized into the following

Table 1
Patient characteristics of patients from which blood samples were drawn in experiment 1. Means with max and min values are shown for the gestational ages; means with standard deviations are shown for the other parameters.

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant PE</th>
<th>Pregnant (controls for early-onset PE)</th>
<th>Preeclampsia (early-onset)</th>
<th>Pregnant (controls for late-onset PE)</th>
<th>Preeclampsia (late-onset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>21</td>
<td>24</td>
<td>23</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>Blood sampling (week of gestation)</td>
<td>NA</td>
<td>30 (28-31)</td>
<td>30 (23-31)</td>
<td>36 (34-38)</td>
<td>36 (33-38)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>NR</td>
<td>NR</td>
<td>172 (± 40)</td>
<td>NR</td>
<td>157 (± 13)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>NR</td>
<td>NR</td>
<td>110 (± 10)</td>
<td>NR</td>
<td>100 (± 6)</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>NR</td>
<td>NR</td>
<td>3.1 (± 2.7)</td>
<td>NR</td>
<td>2.1 (± 1.8)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>NA</td>
<td>40 (39-42)</td>
<td>39 (23-34)</td>
<td>39 (38-41)</td>
<td>36 (34-39)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>NA</td>
<td>3539 (± 337)</td>
<td>1085 (± 387)*</td>
<td>3499 (± 410)</td>
<td>2738 (± 468)*</td>
</tr>
</tbody>
</table>

NA: not applicable. NR: routinely measured and within normal range confirmed for each individual, but not routinely recorded. * p < 0.05: Mann Whitney test, significantly different compared to corresponding control pregnant group (controls), matched on the basis of gestational age.

Table 2
Patient characteristics of control pregnant control women matched for mode of delivery with early- and late-onset preeclamptic women from which placental tissue was obtained. Means with min and max values are shown for the gestational ages; means with standard deviations are shown for the other parameters.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant (control)</th>
<th>Preeclampsia (early-onset)</th>
<th>Pregnant (control)</th>
<th>Preeclampsia (late-onset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of delivery</td>
<td>caesarean section</td>
<td>caesarean section</td>
<td>vaginal delivery</td>
<td>vaginal delivery</td>
</tr>
<tr>
<td>Number of women</td>
<td>15</td>
<td>27</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127 (± 11)</td>
<td>176 (± 21)*</td>
<td>117 (± 16)</td>
<td>144 (± 20)*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 (± 4)</td>
<td>111 (± 10)*</td>
<td>74 (± 10)</td>
<td>102 (± 4)*</td>
</tr>
<tr>
<td>Proteinuria (g/24 hrs)</td>
<td>NR</td>
<td>3.0 (± 3.0)</td>
<td>NR</td>
<td>0.9 (± 0.6)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39 (38-41)</td>
<td>29 (26-34)*</td>
<td>39 (37-41)</td>
<td>37 (36-40)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3782 (± 577)</td>
<td>1037 (± 318)*</td>
<td>3544 (± 541)</td>
<td>3324 (± 1169)</td>
</tr>
</tbody>
</table>

NR: routinely measured and within normal ranges, but not routinely recorded. * p < 0.05: Mann Whitney U test, significantly different compared to corresponding control pregnant group, matched on the basis of mode of delivery.

Table 3
Patient characteristics of patients from which blood samples were drawn for experiment 2. Medians with max and min values are shown for the gestational ages. The number and percentage of parity and smoking women is indicated. The number of women in each group with a certain risk factor is indicated and for all other parameters the means with standard deviation are shown.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant controls</th>
<th>Preeclampsia (early-onset)</th>
<th>Preeclampsia (late-onset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>21</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>27.8 (5.8)*</td>
<td>27.9 (6.3)</td>
<td>30.7 (5.2)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>9 (43%)</td>
<td>2 (25%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Second</td>
<td>12 (52%)</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Third</td>
<td>0</td>
<td>2 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>Smoking</td>
<td>1 (4.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Highest Systolic blood pressure (mmHg)</td>
<td>138 (± 5)</td>
<td>175 (± 17)*</td>
<td>167 (± 11)*</td>
</tr>
<tr>
<td>Highest Diastolic blood pressure (mmHg)</td>
<td>89 (± 7)</td>
<td>112 (± 6)</td>
<td>108 (± 10)</td>
</tr>
<tr>
<td>Proteinuria (g/24h)</td>
<td>NR</td>
<td>1.8 (± 2.4)*</td>
<td>0.75 (± 1.8)*</td>
</tr>
<tr>
<td>Pre-existing risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Obesity</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>(treated) Hypertension</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Obesity &amp; (treated) Hypertension</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Preeclampsia in earlier pregnancy</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>40 (37-42)</td>
<td>31 (28-33)*</td>
<td>37 (34-39)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3575 (± 492)</td>
<td>1313 (± 293)*</td>
<td>2742 (± 688)*</td>
</tr>
</tbody>
</table>

NR: routinely measured and within normal ranges, but not routinely recorded. # from 3 women in the control group the BMI was not recorded (N = 18). * p < 0.05: Mann Whitney U test, significantly different compared to corresponding control pregnant group. ** p < 0.01: Mann Whitney U test, significantly different compared to corresponding control pregnant group.
Multiple Comparison Test. Di centa were tested using Kruskall-Wallis test followed by Dunns Plasma levels, ESM-1 protein and mRNA expression levels in the pla centa were tested using Kruskall-Wallis test followed by the Dunns postpartum samples (1–3 months postpartum) from part of the women with a control pregnancy (n = 16), early-onset preeclampsia (n = 8) and late-onset pregnancies (n = 5). Blood samples were drawn from the antecubital vein into 10 mL tubes containing EDTA (BD Biosciences, Breda, the Netherlands). Blood samples were immediately centrifuged twice (130g, 10 min. at 4 °C followed by 700g, 10 min. at 4 °C), and plasma was frozen at −80 °C until further analysis. ESM-1 levels were determined in plasma as described above.

2.2. Statistical analysis

Experiment 1: Differences in patient characteristics of each of the groups from which blood and/or placental tissue was obtained were evaluated using Mann Whitney U tests. The differences in ESM-1 plasma levels, ESM-1 protein and mRNA expression levels in the placenta were tested using Kruskall-Wallis test followed by the Dunns Multiple Comparison Test. Differences were considered to be significant when p ≤ 0.05.

Experiment 2: The differences in patient characteristics of each of the groups from which blood was collected were evaluated using Mann Whitney U tests. Differences in ESM-1 plasma levels of early or late onset preeclamptic women vs control pregnant women between the different gestational age categories were tested by Kruskall-Wallis followed by Dunns Multiple Comparison Test. Differences were considered to be significant when p ≤ 0.05. Statistical analysis was done using GraphPad Prism (GraphPad Software, La Jolla, CA).

3. Results

Experiment 1: The baseline characteristics of non-pregnant women, the women who developed preeclampsia as well as those of the control pregnant women are shown in Table 1. This table shows that blood pressure is higher in both groups of preeclamptic women (in line with the definition of preeclampsia [1]) as compared to control pregnant women. Also gestational age at delivery and birth weight are decreased in early-onset preeclamptic women as compared with control pregnant women. For late-onset preeclamptic pregnancies only the birth weight is significantly decreased as compared with control pregnant women. Plasma levels of ESM-1 in these groups of women are shown in Fig. 1. Plasma ESM-1 concentrations were significantly decreased in both groups of control pregnant women (266 pg/ml median, 95% CI 191–741 pg/ml, for the early-onset control group and 173 pg/ml median, 95% CI 136–571 pg/ml) for the late-onset control group) as compared with non-pregnant women 1607 pg/ml (median, 95% CI 1277–2566 pg/ml) (Dunns Multiple Comparison Test after Kruskall-Wallis test, p < 0.0001) (Fig. 1). There was no significant difference between the two control pregnant groups. During early-onset preeclampsia, significantly higher levels of plasma ESM-1 (899 pg/ml median, 95% CI 1105–2310 pg/ml) compared to control pregnancies of the same gestational age were observed (266 pg/ml median, 95% CI 191–741 pg/ml) (Dunns Multiple Comparison Test after Kruskall-Wallis, p < 0.0001). The plasma concentration of ESM-1 in women with late-onset preeclampsia (422 pg/ml median, 95% CI 368–877 pg/ml) was also higher as compared to the gestational age matched control pregnant individuals (173 pg/ml median, 95% CI 136–571 pg/ml) (Dunns Multiple Comparison Test after Kruskall-Wallis, p ≤ 0.05).

The baseline characteristics of the pregnant women from which placenta samples were taken are shown in Table 2. In accordance with the definition of preeclampsia, we observed significantly higher blood pressures in the preeclamptic women as compared with the control pregnant women. The gestational age at delivery and birth weight were lower for the early-onset preeclampsia compared to the control.

Immunohistochemical staining of placental tissue showed positive staining of syncytiotrophoblasts (red arrows), but also positive cells in the connective tissue of the villi (blue arrows) (Fig. 2). The fetal endothelial cells, lining the fetal blood vessels in the villi, did not stain for ESM-1 (black arrows). Quantitative analysis of placental staining for ESM-1 did not show differences in expression of ESM-1 in the placenta of early and late onset preeclamptic pregnancies versus their respective control control placentas (Fig. 3).

The levels of ESM-1 mRNA in the placental biopsies were low. No significant difference was observed between placental ESM-1 mRNA expression in preeclamptic placentas vs controls (Fig. 4). A significant difference (Dunns Multiple Comparison Test after Kruskall-Wallis, p ≤ 0.05), however, was found for placental ESM-1 mRNA expression levels between early- and late-onset preeclamptic tissue.

Experiment 2: The baseline characteristics of the pregnant women are shown in Table 3. As expected, the blood pressure and proteinuria differed between the control and the preeclamptic pregnancies at the moment of diagnosis of preeclampsia. Moreover, gestational age (early-onset preeclampsia) and birth weight (early- and late-onset preeclampsia) were significantly lower in preeclamptic women. Presence of risk factors for preeclampsia did not differ between the groups (Table 1).

The ESM-1 plasma concentration significantly decreased during a control pregnancy from 1607 pg/ml (median, 95% CI 1277–2566 pg/ml) in non-pregnant women to 216 pg/ml (median, 95% CI 166–356 pg/ml) at week 20 ± 2 (p < 0.0001) in control pregnant women. As of week 16 ± 2 the ESM-1 concentration significantly differed (p < 0.0001) from the non-pregnant controls and remained
significantly decreased until the end of pregnancy (week 40 ± 2). Postpartum ESM-1 concentrations (median 1956 pg/ml, 95%CI 1564–2480 pg/ml) control were not different from ESM-1 concentrations in non-pregnant women (Fig. 5A).

In women who will later develop late-onset preeclampsia, ESM-1 plasma concentrations were significantly decreased (p < 0.01) as compared to non-pregnant controls from week 20 ± 2 until week 28 ± 2 (Fig. 5B). At week 32 ± 2, the ESM-1 concentration (median 995 pg/ml, 95%CI 409–1526 pg/ml) increased and was not different compared with non-pregnant controls anymore. The postpartum ESM-1 concentration (median 995 pg/ml, 95%CI 409–1526 pg/ml) was not different from non-pregnant women.

In pregnant women who later developed early-onset preeclampsia, plasma ESM-1 concentrations were decreased as compared to non-pregnant ESM-1 concentrations from week 20 ± 2 until week 28 ± 2 (Fig. 5C)(p ≤ 0.05). At week 32 ± 2 the ESM-1 concentration (median 983 pg/ml, 95%CI –36–3186 pg/ml) increased and was not significantly different anymore from ESM-1 concentrations in non-pregnant women. Postpartum ESM-1 concentrations in these women (median 1200 pg/ml, 95%CI 693–1576 pg/ml) were not different from ESM-1 concentrations in non-pregnant women, but they were significantly lower than the postpartum concentrations of those women that had a control pregnancy (p ≤ 0.05).

4. Comments

In the present study we report ESM-1 plasma concentrations and placental ESM-1 expression in pregnancy and preeclampsia; in contrast
that in control pregnancies plasma ESM-1 decreased from the end of the first trimester until the end of pregnancy. In women who later develop early- or late-onset preeclampsia plasma ESM-1 levels first decreased, but increased during or just before preeclampsia.

The question arises why the plasma ESM-1 levels decrease and what the source of ESM-1 during pregnancy is. It is well-known that ESM-1 is produced by endothelial cells [13,20,25,26]. It seems therefore most likely that these cells are also the source of plasma ESM-1 during pregnancy and preeclampsia, because previous studies have indicated that the placenta does not express ESM-1 mRNA [13]. This appears to be in line with our data, in which we found very little ESM-1 mRNA expression in the placenta, with no differences between control pregnancy and preeclampsia, although data may be biased, since placentas from early onset preeclamptic women were of an earlier gestational age as compared with their controls. Moreover, a decreased ESM-1 concentration during the course of a control pregnancy in the face of increased placental mass, does not suggest the placenta as a source of ESM-1. The fact that the mRNA expression is slightly increased in those biopsies obtained from control term placentas, which were all delivered vaginally, as compared with preterm placenta’s, which were all delivered by cesarean section, might be explained by placental stress preceding or during the process of a vaginal delivery [27,28] or by differences in gestational age between the two groups. However, we did find ESM-1 protein expression in the placenta, specifically in the syncytiotrophoblast lining the villi. These cells might contribute to the maternal ESM-1 plasma concentration. On the other hand, we cannot exclude that, with the immunohistochemical staining, syncytiotrophoblast cells were positive due to binding of circulating ESM-1, which was originally produced by maternal cells. Also cells in the connective tissue of the villi, which appear to be Hofbauer cells, are positive for ESM-1.

During preeclampsia, ESM-1 levels were increased as compared to control pregnancy, in both early and late onset preeclampsia. As, in our study, ESM-1 mRNA and protein expression were not increased in preeclamptic placentas, we hypothesize that during preeclampsia, maternal endothelial cells are the main source of plasma ESM-1. The ESM-1 production can be stimulated by a number of factors, such as proinflammatory factors, like TNFα, but also by angiogenic factors, such as FGF-2, HGF and VEGF [20,25,29,30]. Since during preeclampsia these factors are elevated in maternal plasma [31], these factors could have induced higher ESM-1 concentrations in the maternal circulation during preeclampsia.

Our second experiment confirmed our findings of the first experiment, since also in this study we demonstrated that ESM-1 concentrations are increased just before or during early- and late-onset preeclampsia and were not different from non-pregnant women anymore. Moreover, this experiment showed that ESM-1 levels started to decrease at the end of the first trimester and were significantly decreased as of week 16 ± 2 of pregnancy until the end of pregnancy in control pregnant women. A similar pattern was observed in women who later developed preeclampsia (early- or late-onset), with the differences in gestational age between the 2 groups. However, we did not find ESM-1 protein expression in the placenta, specifically in the syncytiotrophoblast lining the villi, which appear to be Hofbauer cells were positive due to binding of circulating ESM-1, which was originally produced by maternal cells. Also cells in the connective tissue of the villi, which appear to be Hofbauer cells, are positive for ESM-1.

The postpartum samples showed that the ESM-1 concentrations returned back to levels of non-pregnant controls in all groups within 3 months delivery, suggesting a quick return to normal after pregnancy and preeclampsia.

In summary, we demonstrated that the ESM-1 concentrations decreased during control pregnancies compared to non-pregnant women and that this decrease was observed from week 16 onward. During both early- and late-onset preeclampsia, the concentration of ESM-1 was increased as compared to control pregnancies. The increase in ESM-1 concentration during preeclampsia is most likely due to the endothelial activation in this syndrome.
Conflict of interest

J.S. is employed by IQ Products. The remaining authors report no conflict of interest.

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