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Healthy and preeclamptic pregnancies show differences in Guanylate-Binding Protein-1 plasma levels

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ABSTRACT

The large interferon-inducible anti-angiogenic pro-inflammatory GTPase Guanylate Binding Protein-1 (GBP-1) is produced and secreted by activated endothelial cells and is highly induced by inflammatory cytokines and inhibited by angiogenic growth factors. During pregnancy a generalized mild inflammatory response is observed. During preeclampsia this generalized inflammatory response is even further activated and activation of the endothelium occurs. We hypothesized that GBP-1 is increased in healthy pregnancy and will be even further increased during preeclampsia.

In the first experiment, plasma and placentas were collected from healthy and preeclamptic pregnancies. Plasma was also collected from non-pregnant women. For the second experiment longitudinal blood samples from women with a healthy or preeclamptic pregnancy were collected from the end of the first trimester until birth and one sample postpartum. The plasma GBP-1 levels were measured by ELISA and GBP-1 mRNA and protein levels in the placenta were tested by qPCR and immunohistochemistry.

During pregnancy higher plasma concentrations of GBP-1 compared with non-pregnant women were observed. Surprisingly, during preeclampsia, plasma GBP-1 levels were lower than in control pregnancies and similar to the level of non-pregnant controls. Placental GBP-1 mRNA levels were not different between healthy and preeclamptic pregnancies and GBP-1 protein was virtually undetectable in the trophoblast by immunohistochemistry in placental tissue. Evaluation of longitudinal samples showed that plasma GBP-1 concentrations increased towards the end of pregnancy in healthy pregnancies, but not in preeclampsia. In line with our hypothesis, we found higher GBP-1 plasma levels during healthy pregnancy. However, plasma GBP-1 did not further increase during preeclampsia, but was stable. Further studies are needed to evaluate why GBP-1 does not increase during preeclampsia.

1. Introduction

Guanylate-Binding Protein-1 (GBP-1) is a large interferon-inducible anti-angiogenic and pro-inflammatory GTPase preferentially produced and secreted by endothelial cells [1–6]. The 67 kDa protein is formed by an alpha helical domain at the C-terminus and a globular domain at the N-terminus [7,8]. The protein is found to be induced by pro-inflammatory cytokines, like interleukin (IL)-1β, interferon (IFN)-γ and tumor necrosis factor (TNF)-α [5,9], and increased in various inflammatory diseases, such as inflammatory bowel diseases, bacterial infections and rheumatic autoimmune diseases, like rheumatoid arthritis and systemic lupus erythematosus, [2,10,11]. GBP-1 is thought to inhibit the effects of inflammatory cytokines on endothelial cell proliferation, migration and invasiveness [3,12–14] and thus protects endothelial cells during inflammation.

A healthy pregnancy is associated with adaptions in the immune response in order to accept the semi-allogeneic fetus [15–18]. This pro-inflammatory condition is associated with changes in the specific and

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non-specific immune system. Characteristic changes are the decrease in the Th1/Th2 ratio [19–21], an increased number of regulatory T cells [22,23] and an activated phenotype of monocytes and granulocytes compared to non-pregnant women [24–26]. Also elevated peripheral blood levels of IL-6, c-reactive protein, IFN- γ and TNF- α were found in pregnancy [27,28]. It is not exactly known how this inflammatory response is activated, but it is suggested that factors, such as hormones, cytokines and extracellular vesicles, released by the placenta into the maternal circulation, are involved [18].

Preeclampsia is a serious complication during pregnancy [29] and is characterized by new onset hypertension (greater than140/90 mmHg) after 20 weeks of gestation. This new onset hypertension should occur together with at least one or more new onset signs of organ dysfunction as described by Brown et al. [30]. Preeclampsia is associated with an even further activated inflammatory response as compared to a healthy pregnancy: there is further activation of monocytes and granulocytes [31–33] and a further increase in circulating inflammatory cytokines, like IFN-γ, IL-6 and IL-8 [28,34–36] as compared with a healthy pregnancy. It is suggested that the factors produced by the placenta are increased in number or different from healthy pregnancy resulting in an increased inflammatory response [37–39].

In view of the generalized inflammatory response in healthy pregnancy and the even further activated inflammatory response in preeclampsia, we hypothesized that GBP-1 is increased in the maternal circulation in a healthy pregnancy and even further increased in preeclamptic women. If so, GBP-1 could be helpful in preeclampsia diagnosis and prognosis in the future.

2. Material and methods

2.1. Study population

Medical ethical approval (application number NL25930.042.08) was given by the Medical Ethical committee of the University Medical Center Groningen and from all patients written consent was obtained. During categorization of the samples we used the definition from the International Society for the Study of Hypertension in Pregnancy (ISSHP) as defined in Brown et al. in 2008 [40]. Preeclampsia was defined as new onset diastolic blood pressure ≥ 90 mmHg two times or more, measured more than 4 h apart, and proteinuria of ≥ 300 mg/24 h. Giving birth before and in or after 34 weeks defined the preeclampsia as being either early- or late-onset preeclampsia.

For the first experiment we used the Li-Heparin sample from the same study samples in our biobank as described in Schuitemaker et al. [41]. Patient numbers and characteristics are shown in Table 1. In this set of samples the control samples were collected from healthy pregnancies visiting the antenatal ward. These were healthy women, with no known diseases. These control women had an uncomplicated pregnancy and were matched for gestational age at the time of sampling with the preeclamptic group. For all groups exclusion criteria were pre-existing hypertension, diabetes mellitus, vasculitis, renal disease, autoimmune disease, malignancies or women who had recent infection, trauma or surgery. Just before determining the GBP-1 concentration, the samples, coded and evaluated blinded for outcome, were thawed and diluted to be used in the GBP-1 specific ELISA (GBP-1 ELISA kit, CUSABIO Biotech Co., Houston, TX) according to the protocol supplied with the kit. Both intra-assay variation and inter-assay variation were ≤ 10%.

Placenta material was collected from twenty-seven early onset (EO) preeclamptic and twelve late onset (LO) preeclamptic women to stain for placental GBP-1 protein and measure mRNA expression. For both EO and LO placentas, we collected control placentas that were delivery mode matched, respectively, caesarean and vaginal deliveries. Fifteen placentas delivered by caesarean section for other reasons than preeclampsia, for instance breech presentation, were collected as control to the early-onset preeclamptic placentas. Fourteen placentas from vaginally delivered control pregnant were collected as controls to the LO preeclamptic placentas, since all these patients delivered vaginally. Table 2 shows the relevant patient data. As described in Schuitemaker et al. [41], placental biopsies were randomly collected, avoiding infarcted areas. Five 1X1 cm tissue samples were taken from the chorionic villi and fixed in formaldehyde, paraffin embedded and stored at room temperature. For mRNA isolation, we pooled five randomly taken

Table 2
Patient characteristics of control pregnant 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 age. For all other parameters the mean with standard deviation are shown.

	Pregnant (control)	Preeclampsia (early-onset)	Pregnant (control)	Preeclampsia (late-onset)
Mode of delivery	Caesarean section	caesarean section	vaginal delivery	vaginal delivery
Number of women	15	27	14	12
Systolic blood pressure (mmHg)	127 (±11)	176 (±21)*	117 (±16)	144 (±20)*
Diastolic blood pressure (mmHg)	81 (±4)	111 (±10)*	74 (±10)	102 (±8)*
Proteinuria (g/24hrs)	NR	3.0 (±3.0)	NR	0.9 (±0.6)
Gestational age at delivery (weeks)	39 (38-41)	29 (26-34)*	39 (37-41)	37 (36-40)
Birth weight (g)	3782 (±577)	1037 (±318)*	3544 (±541)	3324 (±1169)

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 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.

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

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

biopsies from the chorionic villi. These biopsies were snap frozen and stored at −80 °C until the moment of mRNA isolation.

The placental GBP-1 mRNA expression was analyzed using real-time RT PCR. This set consisted of seven control placentas delivered by caesarean section (as control for the EO preeclamptic placentas), nine EO placentas, six control placentas vaginally delivered (as control for the LO preeclamptic placentas) and seven LO preeclamptic placentas. mRNA isolation, reverse transcription to cDNA and PCRs were performed as described in Schuitemaker et al. [41]. Analysis of GBP-1 and PSMD-4 (proteasome non-ATPase regulatory subunit 4) (reference gene), was done using Taqman primer-probes (Hs00977005_m1 and Hs00356654_m1, Life Technologies). GPB-1 mRNA levels were evaluated by calculating the ΔCt (Ct GPB-1 – Ct PSDM4) value and visualized as $2^{-\Delta Ct}$ values.

Immunohistochemical (IHC) staining of paraffin embedded placental sections was performed in line with Lubeseder-Martellato et al. [5]. The anti-GBP-1 (mAb 1B1) was kindly provided by Michael Stürzl, Erlangen, Germany). Control sections, stained without primary antibody, were consistently negative. Slides were scanned with the Aperio TMAscanner (Aperio, Vista, USA) and GBP-1 protein expression was evaluated by Aperio ImageScope (Aperio).

For a second experiment, we used the published cohort from Wong et al. [42], in which longitudinal samples were collected from high risk pregnant women. High risk was defined as having preeclampsia or a history of Hemolysis, Elevated Liver enzymes and Low Platelets syndrome (HELLP syndrome) in a previous pregnancy or having diabetes mellitus, obesity, chronic hypertension, multiple pregnancy or autoimmune diseases. From this cohort, 16 women developed preeclampsia. The samples of these women were used for our preeclamptic groups. The other women in this cohort did not develop preeclampsia. From these women, we selected 21 women as control. These control pregnancies, which were uncomplicated pregnancies, were matched as much as possible for risk factors with the preeclamptic pregnancies. Exclusion criteria for all groups were women who had recent infection, trauma or surgery. Samples were taken between week 12 until birth with a four-week interval and one sample was collected 1 to 3 months postpartum. From this cohort we selected the following women (clinical characteristics in Table 3): preeclamptic women (N = 16) and control pregnant women (N = 21), matched for risk factors. Half of the women with preeclampsia had EO preeclampsia (N = 8) and the other half LO preeclampsia (N = 8). Samples were classified into the following gestational ages: 12 (±2) weeks, 16 (±2) weeks, 20 (±2) weeks, 24 (±2) weeks, 28 (±2) weeks, 32 (±2) weeks, 36 (±2) weeks and at 40 (±2) weeks and a postpartum sample. Regretfully we have not been able to collect all samples for all women. From 14 of the 21 control pregnant women, we collected samples from each gestational age, but not from week 40 ± 2 (only 9 samples). From 5 early-onset preeclamptic women we collected samples from all gestational ages, although from week 12 ± 2 we had only samples from 3 women. From none of the LO preeclamptic women we had samples of all gestational ages. We collected at least 6 of the 9 blood samples from these women, but not from weeks 36 ± 2 (4 samples) and 40 ± 2 (1 sample). Postpartum samples (1 to 3 months after delivery) were collected from most of the women with a healthy pregnancy (n = 13), from all EO preeclamptic women (n = 8) and most of the LO preeclamptic women (n = 5). EDTA blood samples were used and processed as described above and GBP-1 levels were determined by ELISA.

2.2. Statistical analysis

For both sets of experiments, we used Mann Whitney U tests to evaluate the differences in patient characteristics.

For the first experiment, we tested differences in GBP-1 plasma levels with a Kruskall-Wallis test followed by Dunns Multiple Comparison Test after a Grubbs' test to determine outliers. For the second experiment, the differences in GBP-1 plasma levels of the control, EO or LO preeclamptic women were compared to postpartum samples of the respective groups. Unfortunately, several sets of samples collected throughout the pregnancy for the 2nd experiment were not complete and this prevented us from doing a paired statistical analysis between samples from the same group or statistical analysis per gestational age cluster between the groups. Analysis on the available samples was done using the Kruskall-Wallis test followed by the Dunns after a Grubbs' test to determine outliers.

We considered differences with a $p \leq 0.05$ to be significant. GraphPad Prism (GraphPad Software, La Jolla, CA) was used to do the statistical analysis.

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 smoking women is indicated and for all other parameters the means with standard deviations are shown.

	Pregnant controls	Preeclampsia (early-onset)	Preeclampsia (late-onset)
Number of women	21	8	8
Maternal BMI (kg/m^2)	27.8 (5.8) #	27.9 (6.3)	30.7 (5.2)
Smoking	1 (4.7%)	0 (0%)	0 (0%)
Highest Systolic blood pressure (mmHg)	138 (±5)	175 (±17)*	167 (±11)*
Highest Diastolic blood pressure (mmHg)	89 (±7)	112 (±6)*	108 (±10)*
Proteinuria (g/24 h)	NR	1.8 (±2.4)*	0.75 (±1.8)*
Pre-existing risk factors			
Diabetes	2	0	2
Obesity	2	3	0
(treated) Hypertension	6	1	1
Obesity & (treated) Hypertension	4	1	2
Preeclampsia in earlier pregnancy	7	3	3
Gestational age at delivery (weeks)	40 (37–42)	31 (28–33)**	37 (34–39)
Birth weight (g)	3575 (±492)	1313 (±293)**	2742 (±688)*

NR: routinely measured and within normal ranges, but not routinely recorded. # from 3 women in the control group the BMI was not recorded. * $p \leq 0.05$: Mann Whitney *U* test, significantly different compared to corresponding healthy pregnant group. ** $p < 0.01$: Mann Whitney *U* test, significantly different compared to corresponding healthy pregnant group.

3. Results

In the first experiment, both groups of preeclamptic women show a higher blood pressure compared to control pregnant women (Table 1). Birth weight was significantly decreased for preeclamptic women with both EO and LO compared with controls. For EO preeclamptic pregnancies also the gestational age at delivery is significantly decreased as compared to the group of control pregnant women. All other baseline characteristics were comparable (Table 1).

In the group with healthy pregnancies, the plasma levels of GBP-1 are increased versus non-pregnant women. Plasma concentrations of GBP-1 of pregnant women (median 100 pg/ml, 95%CI 89–142 pg/ml and median 110 pg/ml, 95%CI 92–138 pg/ml; respectively) were significantly higher than of non-pregnant women (median 49 pg/ml, 95%CI 37–56 pg/ml) ($p < 0.001$) (Fig. 1). During EO preeclampsia significantly lower GBP-1 plasma levels (median 49 pg/ml, 95%CI 39–57 pg/ml) compared to gestational aged matched healthy pregnant controls were observed ($p < 0.0001$). Similarly, GBP-1 plasma concentrations of women with LO preeclampsia (median 56 pg/ml, 95%CI 37–72 pg/ml) were decreased as compared to their gestational age matched group of healthy pregnant controls ($p < 0.01$). The GBP-1 plasma concentrations of the women with EO and LO preeclampsia were not significantly different from plasma levels of non-pregnant women.

The placental slides stained for GBP-1 did hardly show any staining

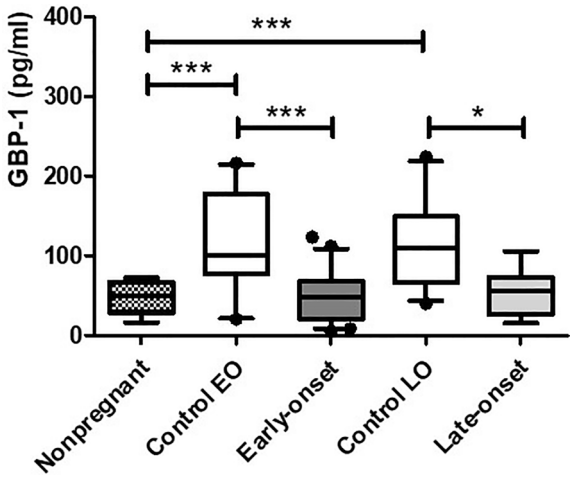

Fig. 1. GBP-1 plasma concentrations of non-pregnant, pregnant and pre-eclamptic women. Whisker plots with median plasma GBP-1 concentrations (5–95% percentile) of non-pregnant women (n = 21), healthy pregnant (control early-onset)(n = 24) matched for gestational age with early-onset preeclampsia pregnancies, early-onset preeclampsia (n = 23), healthy pregnant (control late-onset)(n = 27) matched for gestational age with late-onset preeclampsia pregnancies, and late-onset preeclampsia (n = 15). For each of the groups the median is shown as horizontal line. ** = $p < 0.01$; *** = $p < 0.001$: Dunns Multiple Comparison Test after Kruskall-Wallis Test.

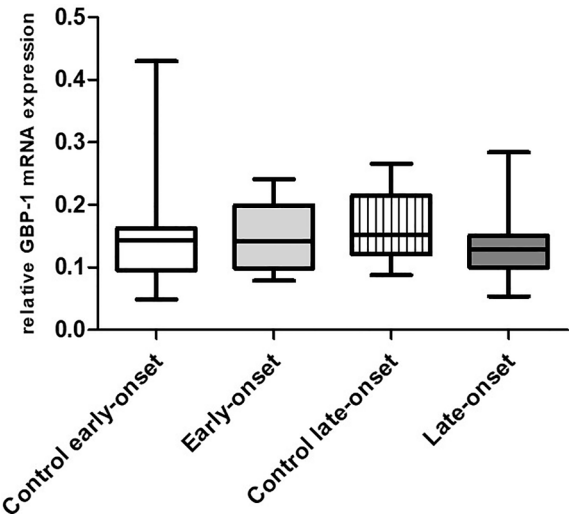

Fig. 3. GBP-1 mRNA expression in placental biopsies. GBP-1 mRNA expression (corrected for PMSD4 ($2^{-\Delta Ct}$ value)) from control early-onset (n = 7) and early-onset preeclampsia (n = 9) placentas, both delivered by caesarean section, and control late-onset (n = 6) and late-onset preeclampsia (n = 6) placentas, both delivered vaginally, are plotted with for each of the groups interquartile range boxes with min–max whiskers and median (horizontal line).

for GBP-1. No GBP-1 staining was observed in the syncytiotrophoblasts, stromal cells or fetal blood vessel endothelial cells in the villi. In areas of the decidua positive cells, which might be immune cells (Fig. 2), and maternal endothelial cells (not shown), were observed.

Placenta material was also tested for the presence of GBP-1 mRNA. GBP-1 mRNA was not different between the preeclamptic groups and their respective controls (Fig. 3). The two modes of delivery (control) groups didn't show a significant difference either.

Table 3 shows that, as expected, preeclamptic women have a higher blood pressure and proteinuria compared to the women with healthy pregnancies. In these preeclamptic pregnancies, gestational age at birth (EO preeclampsia) as well as birth weight (EO and LO preeclampsia) were significantly lower as compared to the healthy pregnancies. There was no difference between the groups with respect to risk factors for preeclampsia (Table 3).

During a healthy pregnancy, the plasma GBP-1 concentration increased from 149 pg/ml (95%CI 113–198 pg/ml) at week 12 ± 2 to 289 pg/ml (95%CI 195–398 pg/ml) at week 40 ± 2 (Fig. 4). GBP-1 levels in these samples were higher as compared to samples from the first experiment, due to the fact that the samples in this second experiment were EDTA samples, while in the first experiment we collected Li-heparin samples. Compared to postpartum samples (median 114 pg/ml, 95%CI 97–169 pg/ml), the GBP-1 plasma concentration of women with an healthy pregnancy was significantly increased ($p < 0.004$) at week 24 ± 2 (median 167 pg/ml, 95%CI 135–218 pg/ml) and at weeks 32 ± 2 (median 205 pg/ml, 95%CI 175–273 pg/ml) until week 40 ± 2 (median 289 pg/ml, 95%CI 195–398 pg/ml) (Fig. 4A).

The GBP-1 plasma concentrations of women who developed LO preeclampsia (Fig. 4B) did not increase during pregnancy and did not significantly differ from postpartum values (median 135 pg/ml, 95%CI 64–215 pg/ml) at any moment during pregnancy. Also, the plasma GBP-1 concentrations of pregnant women with EO preeclampsia did not increase during pregnancy and did not significantly differ from postpartum values (median 132 pg/ml, 95%CI 95–305 pg/ml) (Fig. 4C).

4. Discussion

In the present study we described GBP-1 concentrations in plasma as well as GBP-1 placental expression in healthy pregnant women and in women with preeclampsia (both EO and LO) as well as in non-pregnant women. In line with our hypothesis, GBP-1 plasma levels were increased in women with healthy pregnancies as compared to non-pregnant women. However, in contrast to our hypothesis, in women suffering from either EO or LO preeclampsia, the GBP-1 levels were lower as

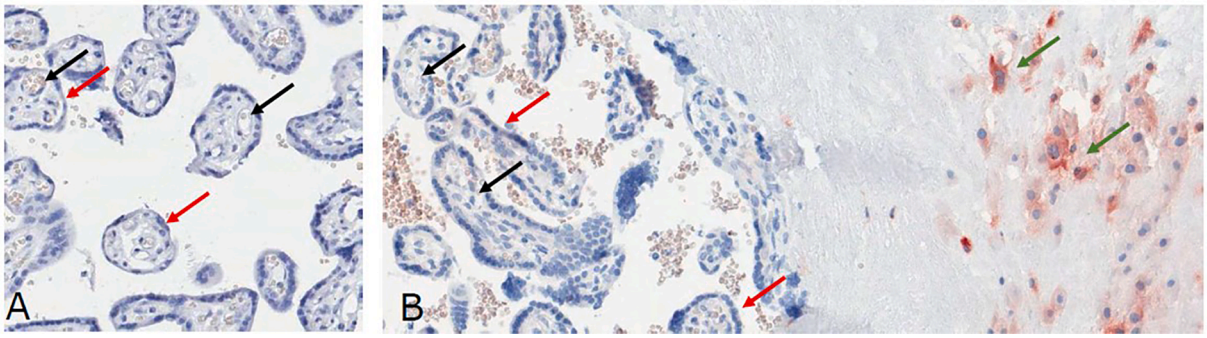

Fig. 2. GBP-1 protein expression in placental biopsies. The placental GBP-1 protein expression after immunohistochemical staining. Representative photographs of GBP-1 expression are shown from a control (A) and early-onset (B) placental biopsy. The red arrows indicate the syncytiotrophoblast and the black arrows indicate the fetal endothelial cells both negative for GBP-1. (C) Representative area of positively stained cells in decidual tissue.

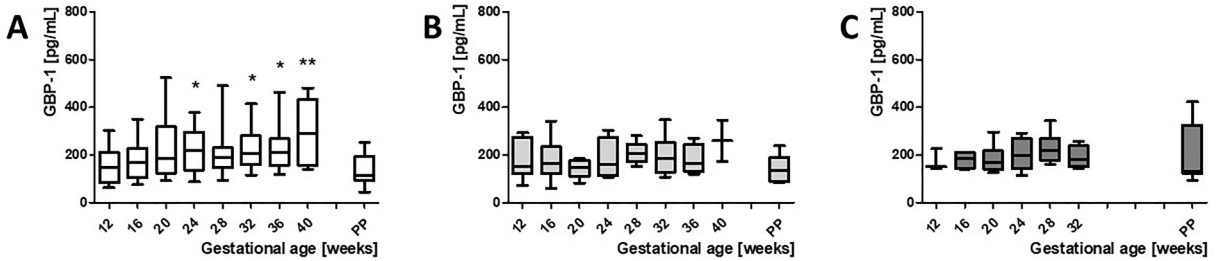

Fig. 4. GBP-1 plasma concentrations during pregnancy between the end of the first trimester and birth, postpartum and compared to non-pregnant women. Whisker plots with median plasma GBP-1 concentrations (5%-95% percentile) during pregnancy (week 12 ± 2 until birth and postpartum (PP)) of healthy pregnant women in A., late-onset preeclamptic women in B. and early-onset preeclamptic women in C.. * $p < 0.05$, ** $p < 0.01$ significantly different as compared to postpartum (Dunns Multiple Comparison Test after Kruskall-Wallis Test).

compared to the pregnant controls matched for gestational age and did not differ from non-pregnant women. Similar results were found in our second experiment, in which we showed that in healthy pregnant women GBP-1 levels were increased as compared to postpartum levels, while this was not the case in women with either EO or LO preeclampsia.

The increased plasma GBP-1 concentration during pregnancy is most likely the result of the generalized pro-inflammatory condition, which is found during pregnancy [26,35]. We hypothesize that in response to this pro-inflammatory condition, endothelial cells start producing GBP-1. The main source of plasma GBP-1 in non-pregnant individuals are endothelial cells [1–5] and it is likely that also during pregnancy endothelial cells are the most important GBP-1 production site, too. Although low GBP-1 mRNA levels were present in the placenta, hardly any protein expression was observed in the trophoblast in the placentas.

This suggests that it is unlikely that the placenta is the source of GBP-1 in the maternal circulation during pregnancy. We hypothesize that the physiological role for GBP-1 in healthy pregnancy may be to protect the endothelial cells from the effects of the inflammatory response, since it has been suggested that GBP-1 may induce a transient and potentially reversible non-proliferating endothelial cell phenotype in response to inflammation [12,13,43].

In contrast to our hypothesis, the stronger pro-inflammatory state of preeclampsia is not related to a further plasma GBP-1 increase in preeclampsia as compared to a healthy pregnancy. In several inflammatory disorders, like rheumatism [2] and psoriasis [5], GBP-1 is clearly increased. Why GBP-1 is not increased in preeclampsia is unclear. Our data may, however, suggest that in preeclampsia a factor or factors inhibit the production of GBP-1.

It could be suggested that such a factor or factors may be angiogenic factors, since these factors, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) have been described to inhibit the expression of GBP-1 [13]. However, free VEGF levels appear to be very low during preeclampsia and difficult to measure with the current assays [44–46]. This is most likely due to the presence of high levels of soluble fms-like tyrosine kinase-1 (sFlt-1), which binds VEGF [45,47]. We suggest that the binding of sFlt-1 to VEGF, resulting in low VEGF levels, may induce endothelial dysfunction [48,49]. This endothelial dysfunction may inhibit the production of GBP-1.

Plasma levels of bFGF have been found significantly increased in women with pregnancy induced hypertension and preeclampsia [50–52]. This may suggest that these increased bFGF levels in preeclampsia may inhibit GBP-1 production. Unfortunately, we did not measure bFGF in the present samples and we are working on studies to show a potential relation between GBP-1 and bFGF during pregnancy and preeclampsia. However, data of sFlt-1, sEng and placental growth factor were available for the samples of the third trimester (data not shown) and a Pearson correlation analysis showed that there was no correlation between GBP-1 and any of these three factors in EO or LO preeclampsia, nor in healthy pregnancies. This indicates that these factors probably did not directly affect GBP-1 production. Why GBP-1 is not increased in preeclampsia is not clear and further studies into the effects of inflammatory cytokines and angiogenic factors on GBP-1 are necessary.

In the second experiment, GBP-1 concentrations were increased during healthy pregnancy as compared to the postpartum concentration. In contrast, GBP-1 levels were not increased in women later developing preeclampsia as compared to postpartum concentrations. Our observations have been made in a relative limited number of women with risk factors for preeclampsia. Unfortunately, despite the fact that control and preeclamptic women were matched for risk factors, there were slightly more women with pre-existing hypertension in the control group. It remains, therefore, to be established whether GBP-1 concentrations will show the same pattern in women without risk factors for preeclampsia and in a larger cohort.

In summary, we demonstrated that the GBP-1 concentration is increased during a healthy pregnancy as compared to non-pregnant women, while GBP-1 concentrations are not increased in EO and LO preeclampsia as compared with non-pregnant women. The increase in GBP-1 during healthy pregnancies is likely due to an increased endothelial production of GBP-1, which may be related to the generalized pro-inflammatory condition during healthy pregnancy. Whether the lack of increase of GBP-1 in the maternal circulation during preeclampsia reflects an increased dysfunctional endothelium, possibly secondary to an angiogenic disbalance, remains to be investigated further.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JHNS and RHJB are employed by IQ Products. JHNS and TIFHC are shareholders of IQ Products. The other authors have no conflict of interest.

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