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The hemoglobin degradation pathway in patients with preeclampsia – Fetal hemoglobin, heme, heme oxygenase-1 and hemopexin – Potential diagnostic biomarkers?

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ABSTRACT

Objective: The aim of this study was to investigate how maternal cell-free fetal hemoglobin and heme impact the scavenger enzyme systems Hemopexin and Heme Oxygenase-1 in patients with preeclampsia (PE). The secondary aims were to evaluate these proteins as biomarkers for severity of the clinical manifestation i.e. hypertension, in early- and late onset PE.

Material and methods: Plasma samples taken within the last 24 h before delivery from 135 patients were analyzed, 89 PE and 46 normal pregnancies. All samples were analyzed for cell-free fetal hemoglobin (HbF), heme, hemopexin enzymatic activity (Hx activity), hemopexin concentration (Hx), and heme oxygenase 1 concentration (HO-1). Logistic regression analysis with ROC-curve analysis was performed to evaluate the possible use as biomarkers for preeclampsia.

Results: There were significantly higher levels of HbF ($p = 0.01$) and heme (0.01) but significantly lower Hx activity ($p = 0.02$), Hx ($p < 0.0001$) and HO-1 ($p = 0.03$) in PE plasma as compared to plasma of normal pregnancies. The Hx activity was significantly inversely correlated ($p = 0.04$) to the diastolic blood pressure. The HO-1 concentration was significantly inversely correlated to both the systolic and diastolic blood pressure ($p = 0.01$ and $p = 0.003$). ROC-curve analysis showed a combined detection rate for these biomarkers of 84% at 10% false positive rate.

Conclusions: Increased maternal plasma levels of heme and HbF in PE are associated with decreased HO-1 and hemopexin protein levels as well as reduced hemopexin activity. By measuring the consumption of the scavenger protein Hx, and the proteins in the Hb degradation system, clinical information about the dynamics of the disease can be obtained.

1. Introduction

Preeclampsia (PE) is a pregnancy related syndrome that causes major maternal and fetal morbidity and mortality worldwide [1]. It is estimated that PE causes up to 75,000 maternal and 500,000 fetal deaths worldwide each year, but especially in low and middle income countries [2]. The International Society for the Study of Hypertension in Pregnancy (ISSHP) defines PE by its clinical findings: *de novo* hypertension and proteinuria [7,8].

The pathogenesis underlying PE is still not fully understood but the two-stage model is up to date the most accepted way to describe how the syndrome progresses [3,4]. Early onset PE, defined by onset before 34 gestational weeks, is in general the more severe form of PE characterized by placental involvement and often intra-uterine growth restriction (IUGR). In early onset PE the first stage is initiated by impaired remodeling of the maternal spiral arteries, which induces oxidative stress in the placenta due to uneven blood flow [5]. Several placental factors have been suggested to leak over into the maternal blood circulation where inflammation causes vascular damage and vasoconstriction. The second stage of early onset PE is characterized by the maternal symptoms, hypertension and proteinuria [6]. In late onset PE, maternal constitutional risk factors such as body mass index (BMI) and other diseases, play an important role in the etiology. Also in late onset PE, in the second phase a diseased placenta produces factors into the maternal circulation inducing vascular damage and vasoconstriction.

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Increased synthesis and accumulation of cell free fetal hemoglobin (HbF) has been demonstrated in PE placentas [9]. The free HbF causes placental tissue damage and oxidative stress, which consequently leads to leakage of free HbF over the blood-placenta barrier into the maternal circulation [10,11]. In fact, increased concentrations of HbF have been shown in maternal plasma/serum in both early-[12,13] and late onset PE suggesting it to be an important etiological factor linking stage one and two of the two-stage model [11,14].

To prevent toxicity of free hemoglobin as well as its degradation metabolites heme and free iron, several scavenger proteins and degradation enzymes protect the body. Haptoglobin (Hp) is the most well described hemoglobin scavenging protein that binds free hemoglobin and transports it to macrophages and hepatocytes where the uptake is facilitated by CD163 receptor-mediated endocytosis [15]. In the intracellular compartment of primarily macrophages hemoglobin is degraded to heme in the lysosomes and then furthermore catabolized by the rate limiting enzyme heme oxygenase 1 (HO-1) to biliverdin, carbon monoxide (CO) and free iron [16]. Biliverdin is reduced to bilirubin which is excreted via the bile system. Carbon monoxide relaxes the smooth muscle layer of vessels causing a vasodilatating effect that lowers the blood pressure [17]. Yet another heme scavenger is α_1-microglobulin (A1M) a lipocalin protein, that in addition to heme also binds radicals and has enzymatic reducing capacity. Recently, A1M has been shown to be up-regulated in early [12,13] and late onset PE [11,14] as well as having therapeutic properties in several PE animal models [18].

Hemopexin (Hx) is a circulating plasma glycoprotein mainly synthesized in the liver. It acts as an acute phase reactant and binds free heme with high affinity [19,20]. The heme affinity of Hx is affected by several factors, such as decreased pH, reducted state of the heme iron atom, binding of nitric oxide (NO) to the heme iron or the presence of chloride anions and divalent metal ions [17]. Sodium cations increase heme affinity to Hx [17]. The Hx-heme complex is transported to macrophages and hepatocytes expressing the LDL receptor-related protein 1 (LRP1), which facilitates uptake of the Hx-heme complex [21]. In this way Hx serves a backup system to Hp. When the Hp-system is overwhelmed, Hx clears the blood from free heme [17]. Hemopexin has in fact been shown to prevent endothelial damage in a mouse model [22].

Hemopexin also has serine protease activity [23]. This enzymatic activity can be measured by a method described by Bakker et al. [23,24]. Data from *in vitro* studies showed a down-regulating effect by Hx activity on the renin-angiotensin system (RAS). Hemopexin activity was shown to down-regulate the angiotensin II receptor in monocytes, endothelial cells, and rat aortic rings [25]. Therefore, the Hx activity has been suggested as a regulator of the vascular responsiveness to angiotensin II [26]. This suggestion is in line with the fact, that Hx activity in normal pregnancy is described to increase from 10 weeks of gestation onwards [25], and could be an explanation to the decreasing angiotensin II sensitivity in pregnancy. Moreover, during PE the Hx activity is decreased and the vascular angiotensin II sensitivity consequently increased [25].

The primary aim of the present study was to investigate the role of free HbF in relation to the hemoglobin/heme degrading pathways in subtypes of PE. The secondary aims were to evaluate how these scavenger proteins and their enzyme activities can be used as biomarkers to evaluate the severity of PE in early- and late onset PE.

2. Materials and methods

2.1. Patients and demographics

140 pregnant women were retrospectively included in the study. The patients were randomly selected from an on-going prospective cohort study, including both normal pregnancies and complicated pregnancies. The collection started 1999 at the delivery ward at Lund University Hospital, Sweden. All plasma samples were taken within the last 24 h before delivery. Exclusion criteria were gestational hypertension, essential hypertension or gestational diabetes. In total 5 cases were excluded due to pre-gestational diabetes or gestational diabetes. A total of 135 patients were included, 89 developed PE (cases) and 46 were normal pregnancies, used as controls. The controls were randomly selected normal pregnancies at term (37 + 0–42 + 0 weeks of gestation). A detailed patient demographic was previously described by Gram et al. [27].

2.2. Sample collection

The study was approved by the ethical committee review board for studies on human subjects at Lund University, Sweden. The patients signed informed consent after oral and written information. Maternal venous samples were taken in 6 ml EDTA Vacuette® plasma tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) within the last 24 h prior to delivery from patients admitted to the Department of Obstetrics and Gynecology, Lund University Hospital, Sweden. The samples were then centrifuged at 2000*g* for 20 min in room temperature. The plasma was then transferred into cryo tubes and stored in −80 °C until time of analysis. Pregnancy outcome for the patients were retrospectively obtained from their individual charts.

Aliquots from the samples were shipped on dry ice from Lund, Sweden to Groningen, The Netherlands for the Hx activity analysis. The samples were still frozen upon arrival.

Preeclampsia was defined as *de novo* hypertension after 20 weeks of gestation with 2 readings at least 4 h apart of blood pressure ≥ 140/90 mmHg and proteinuria ≥ 300 mg per 24 h according to the ISSHP definition [7]. Dipstick analysis was accepted if there was no quantification of proteinuria. Furthermore, the PE group was sub-classified into early-onset PE (diagnosis ≤ 34 + 0 weeks of gestation) and late onset PE (diagnosis > 34 + 0 weeks of gestation).

2.3. Hx activity

Plasma Hx activity was measured in EDTA plasma samples using the Hx-MCA substrate (synthesized by Pepscan, Lelystad, the Netherlands). The plasma samples (40 μl) were diluted 1:4 with the substrate solution (0.2 M Tris + 0.9% NaCl pH 7.6 (substrate concentration 80 μM/L) to a final volume of 200 μl. The emission was measured at 460 nm on a Varioskan spectrophotometer (Thermo Fisher) at 37 °C. The Hx activity, as measured by fluorescent intensity, was measured after 0 min, 30 min (Hx30), 60 min (Hx60) and 24 h. The value measured for Hx activity was on an arbitrary scale. If the value was < 5 after 24 h of incubation, the activity was considered *too low,* due to technical problems with either the assay or the samples, and the samples were expelled from further analysis. The area under the curve analysis was based on Hx30 and Hx60 measurements (HxAUC). The measures Hx30, Hx60 and HxAUC showed similar results; therefore, only the Hx30 data was used for further analysis and referred to as the Hx activity.

2.4. Measuring the potential biomarkers

The Hx concentration was measured with a Human Hemopexin ELISA Kit (Genway Biotech Inc). The analysis was performed according to manufacturer's instructions and the absorbance read at 450 nm with a Wallac 1420 Multilabel Counter. Cell free HbF was measured with a monoclonal Sandwich ELISA as previously described by Gram et al. [27]. Heme was measured with the QuantiChrom Heme Assay Kit (BioAssay Systems, Hayward, CA) according to the manufacturer's instructions. The HO-1 concentration was measured with ELISA (Enzo Lifesciences Inc., Farmingdale, New York) according to the manufacturer's instructions. The concentration of total cell-free Hb was measured with a Human Hb ELISA Quantification Kit (Genway Biotech Inc., San Diego, CA, USA). The analysis was performed according to the

Table 1
Description of pregnancies.

Outcome	Normal pregnancy – controls (n = 46)	Preeclampsia (n = 89)	Early-onset PE[1] (n = 17)	Late-onset PE[2] (n = 72)
Age	29 (28–30)	31** (30–32)	32 **NS** (30–34)	30 **NS** (29–32)
BMI (kg/m^2)	25.0 (23.7–26.3)	26.1 **NS** (25.1–27.0)	27.1 **NS** (24.3–29.9)	25.9 **NS** (24.9–26.9)
Parity (n)	0.2 (0.02–0.32)	0.5* (0.28–0.64)	0.82* (0.23–1.41)	0.37* (0.20–0.54)
Systolic BP[3] (mmHg)	123 (120–126)	161** (157–165)	176** (167–185)	157** (153–160)
Diastolic BP[4] (mmHg)	77 (75–79)	101** (99–103)	108** (103–112)	99** (97–101)
Proteinuria (g/L)	0.02 (0.00–0.04)	2.32** (2.02–2.61)	3.35** (2.68–4.02)	2.08** (1.77–2.39)
Gestational age at delivery (days)	282 (279–285)	256** (250–262)	212** (199–225)	269** (265–273)
Gestational age at sampling (days)	281 (278–284)	253** (247–260)	208** (196–220)	266** (262–270)

Patient demographics of PE cases and normal pregnancies (controls). Values are shown as mean (95% confidence interval) or number (%). Statistical comparison of the groups was performed with ANOVA. A p-value < 0.05 was considered significant.
NS: Not significant; *: $p = < 0.05$; **:$p = < 0.001$.
[1] Early-onset PE was defined as diagnosis before 34 + 0 weeks of gestation.
[2] Late-onset PE was defined as diagnosis after 34 + 0 weeks of gestation.
[3] Highest systolic blood pressure recorded within two weeks prior to delivery.
[4] Highest diastolic blood pressure recorded within two weeks prior to delivery.

manufacturer's instructions and the absorbance was red at 450 nm using a Wallac 1420 Multilabel Counter.

2.5. Correlation to previous findings

The same cohort has previously been used for HbF, A1M, Hp, Hx and CD163 measurements [27]. Previous data was used as complement to build the new algorithms presented in this study.

2.6. Statistical analysis

All statistical analysis was performed with the software Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) version 21 for Apple computers (Apple Inc., Cupertino, CA).

Mann-Whitney test was used to compare Hx activities, Hx, HO-1, heme, HbF and total Hb concentrations between PE and controls (Table 2). Subgroup-analyses were performed for early- and late onset PE.

2.7. Correlation analysis

Correlation between Hx activity and Hx concentration was calculated using the non-parametric Kendall's correlation coefficient. Furthermore, correlation analysis was performed between Hx activity and maternal blood pressure (defined as the highest measured blood pressure within 24 h before delivery). Correlation analyses were also done between cell-free Hb (HbF and Total Hb), heme, HO-1 and hemopexin concentrations. Furthermore, heme and HO-1 both were correlated to both systolic and diastolic blood pressure.

2.8. Logistic regression analysis

The detection rate was determined by ROC-curve analysis for each of the potential biomarkers. The detection rates were obtained at 10% and 20% false positive rates. The combined detection potential for the biomarkers was obtained by stepwise logistic regression analysis of the biomarkers and ROC-curve analysis.

3. Results

3.1. The cohort

Description of the patient cohort is displayed in Table 1. In total 89 patients with PE were included in the study matched with 46 uncomplicated pregnancies as controls. Of the 89 PE cases, 17 were diagnosed with PE before 34 + 0 weeks of gestation, classified as early-onset PE. The groups were comparable concerning maternal age, BMI and parity.

The systolic and diastolic blood pressures along with proteinuria were significantly higher in the PE group. Due to increased incidence of

Table 2
Plasma concentrations.

	Controls N = 39	Preeclampsia n = 89	Early onset preeclampsia n = 17	Late onset preeclampsia n = 72
Hemopexin activity (mean)	0.80 (0.66–0.93)	0.59 (0.49–0.69) p = 0.019	0.81 (0.54–1.07) p = 0.96	0.54 (0.44–0.65) p = 0.004
Hemopexin plasma concentration[1] (mean)	0.93 (0.88–0.98)	0.69 (0.66–0.73) p < 0.0001	0.69 (0.61–0.77) p < 0.0001	0.69 (0.56–0.73) p < 0.0001
HbF[1] (mean)	3.85 (2.51–5.20)	15.26 (7.0–23.6) p = 0.01	18.72 (1.6–39.05) p = 0.006	14.60 (5.10–24.0) p = 0.17
Heme μM (mean)	59.86 (52.34–67.38)	75.03 (67.43–82.62) p = 0.01	69.54 (55.07–84.02) p = 0.26	77.55 (68.37–86.74) p = 0.02
HO -1 ng/ml (mean)	5.29 (4.69–5.9)	4.48 (4.04–4.93) p = 0.03	4.67 (3.37–5.97) p = 0.02	4.42 (4.69–5.89) p = 0.01

Table 2 shows the mean Hpx activity, hemopexin concentration, fetal hemoglobin (HbF), heme and heme oxygenase 1 in the different groups. All groups were compared with one-way ANOVA. $p \leq 0.05$ was considered statistically significant.
[1] Previously published by Gram et al. (13).

preterm labor in the PE group the blood sampling was consequently performed earlier in the PE group.

3.2. Hx activity

Eleven of the samples (8 controls and 3 PE) showed "too low value", indicating no activity after 24 h of incubation and were therefore excluded from further analysis based on technical problems with either the sample or the measurement.

The Hx activity was significantly lower in the PE groups compared to controls (p = 0.02, Table 2). However, when subdividing the PE patients into early- and late-onset, the early-onset group, the results showed similar values to the control group (0.81 vs 0.80) (Table 2). In contrast, the late onset group showed a clearer decrease in activity compared to the control group compared to all the PE patients (Hx activity = 0.54, p = 0.007) (Table 2).

3.3. Heme

The heme concentration was significantly higher in patients with PE compared to controls (75.03 μM vs. 59.86 μM p = 0.01). The concentration was higher in the early onset PE group (69.54 μM) than in the control group but this was not statistically significant (p = 0.26). In the late onset PE group, there was a statistically significantly higher concentration (77.55 μM, p = 0.02) (Table 2) as compared to the control group.

3.4. Heme oxygenase 1

The HO-1 concentration was significantly lower in the PE group compared to controls (4.48 ng/ml vs. 5.29 ng/ml p = 0.03). Both early- and late onset PE showed significantly lower HO-1 concentrations (4.67 ng/ml, p = 0.02 and 4.42 ng/ml, p = 0.01 respectively), compared to controls (table 2).

3.5. HbF and hemopexin protein concentration

There was a significantly higher HbF-concentration in all PE groups [27] compared to controls. The Hx concentration was significantly lower in the PE groups compared to controls as described in Gram et al. [27]. The total Hb was however not different between the groups (p = 0.53).

3.6. Correlation analysis

The Hx activity was not correlated to the Hx plasma concentration (p = 0.90), nor in early onset PE (p = 0.17) or late onset PE (p = 0.24).

The Hx activity was significantly inversely correlated to the diastolic blood pressure in all patients (p = 0.04). When the early onset PE patients were excluded from the analysis the correlation between the diastolic blood pressures and Hx activity was stronger (p = 0.009).

The heme concentration was not correlated to the HbF level (p = 0.31) but did significantly correlate to the total Hb concentration (Correlation coefficient = 0.18, p = 0.002). Furthermore, there were no correlations between heme and Hx activity (p = 0.82) or heme and HO-1 (p = 0.08).

The HO-1 level was significantly inversely correlated to both the systolic (p = 0.01, correlation coefficient = −0.15) and the diastolic blood pressure (p = 0.003, correlation coefficient = −0.25). The HO-1 concentration did not correlate to the Hx activity (p = 0.92).

3.7. Logistic regression analysis

The results from the logistic regression and ROC-curve analyses are presented in Table 3. The Hx activity showed a detection rate (DR) of 30% at a 10% false positive rate (FPR) with 0.66 AUC. The HO-1 concentration showed 21% DR at 10% FPR, AUC = 0.64 and the heme concentration showed 22% DR at 10% FPR and AUC = 0.63.

The combination of HbF, Hx activity, Hx concentration, heme and HO-1 together showed a DR of 84% at 10% FPR, AUC = 0.93 (Table 3, Fig. 1).

Table 3
Preeclampsia detection rates:

	10%	20%	Area under the ROC-curve
Hx activity	30%	44%	0.66
HO-1	21%	31%	0.64
Heme	22%	31%	0.63
Combination of biomarkers*	84%	87%	0.93

The results from the logistic regression analysis for PE vs. controls. Detection rates are displayed at fixed false positive rates (10% and 20%). All analyses were statistically significant p ≤ 0.05.

* Combination of HbF, Hemopexin activity, Hemopexin concentration, Heme and HO-1.

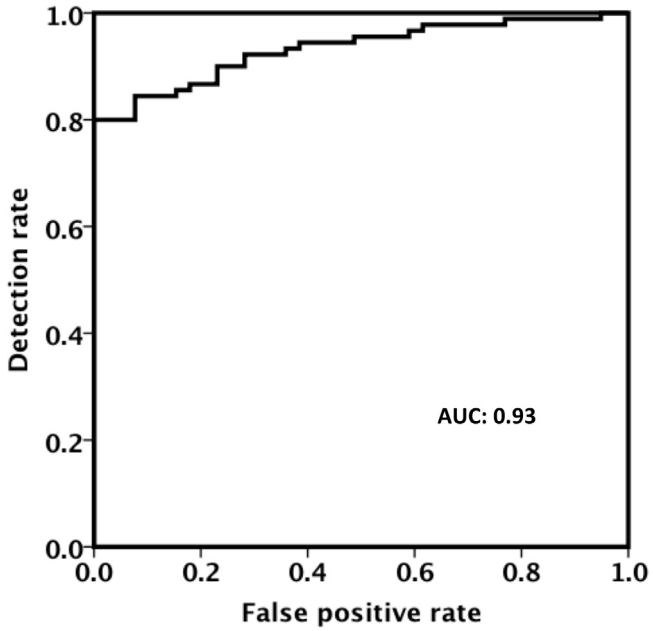

Fig. 1. Receiver operating characteristic curve (ROC-curve) for the combination of the biomarkers fetal hemoglobin, hemopexin activity, hemopexin concentration, heme and HO-1. The combination showed a detection rate of 84% at 10% false positive rate and an area under the ROC-curve of 0.93

4. Discussion

The main results showed that elevated levels of heme and HbF were associated with a decrease in hemopexin concentration indicating consumption of hemopexin. We also observed a decrease in both Hx- and HO-1 activity. Furthermore, the Hx activity and the HO-1 levels, correlated to maternal blood pressure, i.e. the severity of PE, indicating that the toxicity of free HbF may be an important etiological factor in PE. By monitoring the dynamics of these factors as biomarkers of the pathophysiological process, HbF, Hx activity, Hx concentration, heme and HO-1 could be particularly useful for prediction and diagnosis of late-onset PE. The ROC curve show a detection rate of 84% of all the PE patients at 10% FPR.

The results complement the recently published study in which decreased levels of haptoglobin were shown in PE patients. The heme scavenger A1M was in contrast, shown to be up-regulated in early onset [12,13] and late onset PE [11,14]. In concordance with previous findings, a decreased Hx activity has been shown in patients with manifest

PE [24]. Interestingly, the present result showed that the Hx activity was significantly decreased in patients with late-onset PE only, although previous studies have shown plasma Hx activity to be significantly decreased in early onset PE [24]. The main reason for this discrepancy could be the present study set-up; early onset patients were compared with term controls, while in the previous study, gestational age matched controls were used. If instead gestational age matched controls had been used the results might have been more in line with previous studies [24]. The set-up is clearly a limitation for the present study and will have to be confirmed in a study with controls matched for gestational age. In the perfect world, all samples should be transferred to multiples of the median (MOM) values. In the given study, however we did not have the needed number of controls to establish a median and normal range according to gestational age. The findings therefore need to be validated in a larger cohort.

In vitro studies suggest that the increase in Hx activity seen in normal pregnancy may contribute to the down-regulation of the vascular expression of Angiotensin II receptor 1 thereby promoting a relaxed and dilated maternal vascular bed [25]. It has further been suggested that the decreased Hx activity seen in PE may increase the maternal blood pressure, since less Angiotensin II receptor 1 is expressed in the vascular endothelium [24]. The data in this study show a significantly inverse correlation between Hx activity and the diastolic blood pressure supporting this mechanism.

Several factors may influence the Hx activity in the PE patients, but *in vitro* studies indicate that extra-cellular adenosine triphosphate (ATP) is an important inhibitor of Hx activity [23]. Although both Hx concentration and Hx activity were decreased in PE patients, the current data show that the Hx activity did not correlate to the Hx concentration suggesting that the activity is not solely dependent on its concentration. The decreased Hx concentration seen in PE may be a consequence of the elevated HbF concentrations as cell-free HbF is degraded by HO-1 causing elevated levels of heme that in turn consume Hx in PE [11,14,27].

Reduced circulating HO-1 levels have previously been described in patients with PE [28]. Furthermore, HO-1 has also been shown to be protective against inflammation, apoptosis, and to be involved in regulation of angiogenesis [28]. In line with previous studies, the present results indicate that the HO-1 concentration is reduced in PE, particularly in the late onset PE. As a result, the HO-1 enzyme may be gradually depleted, therefore lower in late onset PE compared to early onset PE. It is well described that HO-1 also has anti-inflammatory properties [29]. Reduced circulating levels of HO-1 could therefore aggravate the maternal inflammatory response contributing to the characteristic maternal endotheliosis and increasing blood pressure [29]. Furthermore, degradation of heme by HO-1 produces CO, a gas that has potent vasodilatory effects. Diminished levels of HO-1 consequently lead to decreased degradation of heme and thereby less production of CO. Free heme in turn, binds to the endothelial derived NO which contributes to vasoconstriction [30]. The net effect is an increased vasoconstriction leading to the main PE manifestation – hypertension.

The plasma heme concentration was elevated in late onset PE and obviously correlated well with total Hb concentration. Previously published studies indicated that the increased levels of HbF in PE slowly put a strain on the Hb- and heme scavenging systems [11,12,14,27]. A constant placental over-production of HbF cause damage to the blood-placenta barrier, eventually leading to damage to the maternal endothelium [11,12,14,27]. The strength of the maternal scavenger and enzyme systems may be important constitutional factors that determine how and when the clinical manifestations of PE present, early or late. The more the maternal protective systems are strained and/or depleted, the earlier and more severe the clinical symptoms will become.

5. Conclusions

The results from this study indicate that increased maternal plasma levels of heme and HbF in PE consume the natural protective scavenger proteins causing decreased levels of HO-1 and hemopexin. It might even reduce hemopexin activity. By measuring components in the Hb metabolism pathway as potential biomarkers, an indication and support of the PE diagnosis could be made. Future studies are needed to evaluate the role of these biomarkers as predictive biomarkers for maternal and neonatal outcome and as first trimester predictive biomarkers for PE, particularly late-onset PE.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.preghy.2018.02.005.

References

[1] B. Sibai, G. Dekker, M. Kupferminc, Pre-eclampsia, Epub 2005/03/0doi: S0140-6736(05)17987-2 [pii] 10.1016/S0140-6736(05)17987-2. PubMed PMID: 15733721Epub 2005/03/0doi: S0140-6736(05)17987-2 [pii] 10.1016/S0140-6736(05)17987-2. PubMed PMID: 15733721, Lancet 365 (9461) (2005) 785–799.

[2] WHO, The World Health Report 2005: Make every mother and child count, 2005.

[3] A.C. Staff, S.J. Benton, P. von Dadelszen, J.M. Roberts, R.N. Taylor, R.W. Powers, et al., Redefining preeclampsia using placenta-derived biomarkers, Epub 2013/03/06. doi: 10.1161/HYPERTENSIONAHA.111.00250. PubMed PMID: 23460278, Hypertension 61 (5) (2013) 932–942.

[4] J.M. Roberts, C.A. Hubel, The two stage model of preeclampsia: variations on the theme, Epub 2008/12/17. doi: 10.1016/j.placenta.2008.11.009. PubMed PMID: 19070896; PubMed Central PMCID: PMC2680383, Placenta 30 (Suppl A) (2009) S32–S37.

[5] C.W. Redman, I.L. Sargent, Immunology of pre-eclampsia, Epub 2010/03/2doi: 10.1111/j.1600-0897.2010.00831.x. PubMed PMID: 20331588, Am. J. Reprod. Immunol. 63 (6) (2010) 534–543.

[6] J.M. Roberts, C. Escudero, The placenta in preeclampsia, Epub 2012/06/30. doi: 10.1016/j.preghy.2012.01.001. PubMed PMID: 22745921; PubMed Central PMCID: PMC3381433, Pregnancy Hypertens. 2 (2) (2012) 72–83.

[7] M.A. Brown, M.D. Lindheimer, M. de Swiet, A. Van Assche, J.M. Moutquin, The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP), PubMed PMID: 12044323, Hypertens. Pregnancy 20 (1) (2001) IX–XIV, http://dx.doi.org/10.1081/PRG-100104165.

[8] A.L. Tranquilli, G. Dekker, L. Magee, J.M. Roberts, B. Sibai, W. Steyn, et al., The Classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP, Pregnancy Hypertens. 4 (2014) 97–104.

[9] M. Centlow, P. Carninci, K. Nemeth, E. Mezey, M. Brownstein, S.R. Hansson, Placental expression profiling in preeclampsia: local overproduction of hemoglobin may drive pathological changes, Epub 2008/01/02. doi: 10.1016/j.fertnstert.2007.09.030. PubMed PMID: 18166190; PubMed Central PMCID: PMC2628488, Fertil Steril. 90 (5) (2008) 1834–1843.

[10] K. May, L. Rosenlof, M.G. Olsson, M. Centlow, M. Morgelin, I. Larsson, et al., Perfusion of human placenta with hemoglobin introduces preeclampsia-like injuries that are prevented by alpha1-microglobulin, Epub 2011/03/02. doi: 10.1016/j.placenta.2011.01.017. PubMed PMID: 21356557. Placenta 32 (4) (2011) 323–332.

[11] M. Gram, U.D. Anderson, M.E. Johansson, A. Edstrom-Hagerwall, I. Larsson, M. Jalmby, et al., The human endogenous protection system against cell-free hemoglobin and heme is overwhelmed in preeclampsia and provides potential biomarkers and clinical indicators, PLoS One 10 (9) (2015) e0138111, http://dx.doi.org/10.1371/journal.pone.0138111. PubMed PMID: 26368565; PubMed Central PMCID: PMCPMC4569570.

[12] U.D. Anderson, M.G. Olsson, S. Rutardottir, M. Centlow, K.H. Kristensen, P.E. Isberg, et al., Fetal hemoglobin and alpha1-microglobulin as first- and early second-trimester predictive biomarkers for preeclampsia, Am. J. Obstet Gynecol. 204 (6) (2011) 520 e1–e5. Epub 2011/03/29. doi: 10.1016/j.ajog.2011.01.058. PubMed PMID: 21439542.

[13] U.D. Anderson, M. Gram, J. Ranstam, B. Thilaganathan, B. Kerstrom, S.R. Hansson, Fetal hemoglobin, alpha1-microglobulin and hemopexin are potential predictive first trimester biomarkers for preeclampsia, PubMed PMID: 27155336, Pregnancy Hypertens. 6 (2) (2016) 103–109, http://dx.doi.org/10.1016/j.preghy.2016.02.003.

[14] M.G. Olsson, M. Centlow, S. Rutardottir, I. Stenfors, J. Larsson, B. Hosseini-Maaf, et al., Increased levels of cell-free hemoglobin, oxidation markers, and the antioxidative heme scavenger alpha(1)-microglobulin in preeclampsia, Epub 2009/11/03. doi: S0891-5849(09)00697-2 [pii] 10.1016/j.freeradbiomed.2009.10.052. PubMed PMID: 19879940. Free Radic. Biol. Med. 48 (2) (2010) 284–291.

[15] D. Chiabrando, F. Vinchi, V. Fiorito, E. Tolosano, Haptoglobin and hemopexin in heme detoxification and iron recycling, acute phase proteins – regulation and functions of acute phase proteins, Prof. Francisco Veas (Ed.) (2011), http://dx.doi.

org/10.5772/18241.
[16] R. Tenhunen, H.S. Marver, R. Schmid, The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase, Proc. Natl. Acad. Sci. U.S.A. 61 (2) (1968) 748–755 Epub 1968/10/01. PubMed PMID: 4386763; PubMed Central PMCID: PMC225223.
[17] E. Tolosano, S. Fagoonee, N. Morello, F. Vinchi, V. Fiorito, Heme scavenging and the other facets of hemopexin, PubMed PMID: 19650691, Antioxid. Redox Signal. 12 (2) (2010) 305–320, http://dx.doi.org/10.1089/ars.2009.2787.
[18] L. Erlandsson, A. Naav, A. Hennessy, D. Vaiman, M. Gram, B. Akerstrom, et al., Inventory of novel animal models addressing etiology of preeclampsia in the development of new therapeutic/intervention opportunities, PubMed PMID: 26685057, Am. J. Reprod. Immunol. 75 (3) (2016) 402–410, http://dx.doi.org/10.1111/aji.12460.
[19] W.T. Morgan, A. Smith, Binding and transport of iron-porphyrins by hemopexin, Adv. Inorg. Chem. 51 (2001) 205–241.
[20] S. Immenschuh, D.X. Song, H. Satoh, U. Muller-Eberhard, The type II hemopexin interleukin-6 response element predominates the transcriptional regulation of the hemopexin acute phase responsiveness, Biochem. Biophys. Res. Commun. 207 (1) (1995) 202–208 Epub 1995/02/06 PubMed PMID: 7857266.
[21] D.J. Schaer, A.I. Alayash, Clearance and control mechanisms of hemoglobin from cradle to grave, Epub 2009/10/01. doi: 10.1089/ars.2009.2923. PubMed PMID: 19788393, Antioxid. Redox Signaling 12 (2) (2010) 181–184.
[22] F. Vinchi, S. Gastaldi, L. Silengo, F. Altruda, E. Tolosano, Hemopexin prevents endothelial damage and liver congestion in a mouse model of heme overload, Epub 2008/06/17. doi: 10.2353/ajpath.2008.071130. PubMed PMID: 18556779; PubMed Central PMCID: PMC2438305, Am. J. Pathol. 173 (1) (2008) 289–299.
[23] W.W. Bakker, T. Borghuis, M.C. Harmsen, A. van den Berg, I.P. Kema, K.E. Niezen, et al., Protease activity of plasma hemopexin, Epub 2005/07/15. doi: 10.1111/j.1523-1755.2005.00438.x. PubMed PMID: 16014037, Kidney Int. 68 (2) (2005) 603–610.
[24] W.W. Bakker, R.B. Donker, A. Timmer, M.G. van Pampus, W.J. van Son, J.G. Aarnoudse, et al., Plasma hemopexin activity in pregnancy and preeclampsia, Epub 2007/05/01. doi: 10.1080/10641950701274896. PubMed PMID: 17469012, Hypertens Pregnancy 26 (2) (2007) 227–239.
[25] W.W. Bakker, R.H. Henning, W.J. van Son, M.G. van Pampus, J.G. Aarnoudse, K.E. Niezen-Koning, et al., Vascular contraction and preeclampsia: downregulation of the Angiotensin receptor 1 by hemopexin in vitro, Epub 2009/05/06. doi: 10.1161/HYPERTENSIONAHA.108.127951. PubMed PMID: 19414647, Hypertension 53 (6) (2009) 959–964.
[26] W.W. Bakker, F. Spaans, L. el Bakkali, T. Borghuis, H. van Goor, E. van Dijk, et al., Plasma hemopexin as a potential regulator of vascular responsiveness to angiotensin II, Epub 2012/05/19. doi: 10.1177/1933719112446081. PubMed PMID: 22598486, Reprod. Sci. 20 (3) (2013) 234–237.
[27] M. Gram, U.D. Anderson, M.E. Johansson, A. Edström, I. Larsson, M. Jälmby, et al., The human endogenous protection system against cell-free hemoglobin and heme is overwhelmed in preeclampsia: Valuable clinical indicators and potential biomarkers? PLOS One (2015) Manuscript in press.
[28] M. Ozen, H. Zhao, D.B. Lewis, R.J. Wong, D.K. Stevenson, Heme oxygenase and the immune system in normal and pathological pregnancies, Epub 2015/05/13. doi: 10.3389/fphar.2015.00084. PubMed PMID: 25964759; PubMed Central PMCID: PMC4408852, Front. Pharmacol. 6 (2015) 84.
[29] M.P. Soares, I. Marguti, A. Cunha, R. Larsen, Immunoregulatory effects of HO-1: how does it work? Epub 2009/07/10. doi: 10.1016/j.coph.2009.05.008. PubMed PMID: 19586801, Curr. Opin. Pharmacol. 9 (4) (2009) 482–489.
[30] S.R. Hansson, A. Naav, L. Erlandsson, Oxidative stress in preeclampsia and the role of free fetal hemoglobin, Epub 2015/01/doi: 10.3389/fphys.2014.00516. PubMed PMID: 25628568; PubMed Central PMCID: PMC4292435, Front. Physiol. 5 (2014) 516.