in vivo *25*: 445-450 (2011)

Antepartum and Postpartum Serum Heme Oxygenase-1 Levels in Preeclamptic and Normotensive Pregnant Women

NIKOLAOS VITORATOS, KATERINA PAPAKONSTANTINOU, AIKATERINI DELIVELIOTOU, EMMANUEL ECONOMOU, CONSTANTINOS PANOULIS, DIMITRIOS HASSIAKOS and GEORGE K. CREATSAS

Second Department of Obstetrics and Gynecology, Aretaieion University Hospital, Athens, Greece

Abstract. *Aim: To determine antepartum and postpartum serum heme oxygenase-1 (HO-1) levels in pre-eclamptic (PE) and normotensive pregnant women and to investigate the relationship between HO-1 levels and severity of PE. Patients and Methods: Ten normotensive women were compared to 9 women with mild PE and 12 women with severe PE. Serum HO-1 levels were measured at 30-34 gestational weeks and 12-14 weeks postpartum. Results: The severe PE group had significantly higher serum HO-1 levels antepartum compared to the mild PE and normotensive groups (5.50±1.54 vs. 3.04±0.72 ng/ml, p=0.0003, and 5.50±1.54 vs. 3.12±1.57 ng/ml, p=0.002, respectively). Serum HO-1 levels decreased significantly postpartum in the normotensive group only (3.12±1.57 vs. 2.00±0.97 ng/ml, p=0.0005). In the severe PE group, HO-1 levels antepartum were positively correlated to mean blood pressure (r=+0.79, p=0.004). Conclusion: Severe PE is associated with elevated serum HO-1 levels both antepartum and postpartum, suggesting a key role of chronic oxidative stress in the pathogenesis of PE and the endothelial dysfunction of these patients later in their life.*

Pre-eclampsia (PE) is a severe obstetric complication which usually manifests by maternal hypertension and proteinuria, progressing to a systemic hypoperfusion of multiple maternal organs and often accompanied by subnormal fetal growth (1, 2). PE contributes significantly to maternal and perinatal morbidity and mortality. Maternal and perinatal outcomes depend on the gestational age at time of the disease, severity of disease, quality of management and presence of pre-existing medical disorders (3-5).

Correspondence to: Aikaterini Deliveliotou, MD, Second Department of Obstetrics and Gynecology, Aretaieion Hospital, 76 Vas. Sofias Avenue, GR-11528, Athens, Greece. Tel: +30 210 7286353, Fax: +30 2107233330, e-mail: kdeliveliotou@hotmail.com

Key Words: Heme oxygenase-1, pre-eclampsia, mean blood pressure.

Women in normal pregnancy have an increase in oxidative stress and lipid peroxidation, when compared with non-pregnant women. However, increased serum antioxidant capacity and a gradual favoring of antioxidative activity over oxidative stress and lipid peroxidation occur as normal pregnancy advances (6). Pre-eclamptic women have an excessive increase in lipid peroxidation (7) and many studies reported that the levels of several important antioxidants are markedly increased (8-10).

Recently a new enzymatic substance, the enzyme heme oxygenase (HO-1) has been implicated as contributing factor to the antioxidant capabilities in several organ systems. The HO-1 protein is localised in placental tissue and has been shown to be involved in the maintenance of uterine quiescence throughout gestation, regulation of hemodynamic control in uterus and placenta, regulation of the apoptotic and inflammation cascades in trophoblast cells and the maintenance of a balance of the oxidant–antioxidant status within the placental tissues (11).

The potential role of HO-1 in the pathogenesis of PE has been studied, but the results of these studies are conflicting. Both an increase (12) and a reduction (13-15) of HO-1 protein expression in placentas from PE have been reported. Given that the level of HO-1 in maternal circulation has not been studied, limited knowledge exists regarding serum activation of HO-1 in preeclamptic women and whether or not this activity is altered after parturition.

The aim of the present study was to determine maternal serum HO-1 levels during pregnancy and postpartum, both in normal women, as well as in women with PE, and to investigate the relationship between maternal serum HO-1 levels and severity of PE.

Patients and Methods

Study groups. The study population consisted of 31 *primigravidas* women between 30 and 34 weeks of gestation. All women were normotensive at the booking visit and signed an informed consent form prior to inclusion into the study. Group A consisted of 10 healthy pregnant women who remained normotensive throughout their

0258-851X/2011 $2.00+.40

Table I. *Demographic characteristics of normal controls (Group A) and women with mild (Group B), and severe PE (Group C). Comparisons are presented comparing Groups B and C with Group A. Data are expressed as mean±(SD). *p<0.05, **p<0.001.*

Demographics	Group A (n=10)	Group B (n=9)	Group C (n=12)
Age (years)	32.0 (2.2)	33.0 (4.1)	34.0 (3.8)
Gestational age at sampling (weeks, range)	31.6 (30-33)	32.8 (30-34)	31.2 (30-34)
Gestational age at delivery (weeks, range)	38.5 (38-39)	36.1 (36-37)	31.2 (30-34)**
Systolic blood pressure (mmHg)	117.8±8.6	149.2±5.6*	179.2±15.0**
Diastolic blood pressure (mmHg)	71.8±7.6	100.7±1.5**	109.2±6.7**
Mean blood pressure (mm Hg)	85.6±5.0	116.9±4.0**	132.5±8.0**
Maternal serum HO-1 (ng/ml) (pregnancy)	3.12±1.57	3.04±0.72	5.50±1.54*
Maternal serum HO-1 (ng/ml) (postnanally)	2.00±0.97	3.50±1.95*	5.00±1.37**
Maternal hematocrit (%)	36.4 (0.4)	35.8 (0.8)	36.7 (0.5)

gestation. Group B consisted of 9 pregnant women who subsequently developed mild PE, and group C consisted of 12 pregnant women who subsequently developed severe PE. The diagnosis of mild and severe PE was established according to the criteria of International Society for the Study of Hypertension in pregnancy (16): mild PE was diagnosed if systolic blood pressure (SBP) was greater than 140 mmHg with a rise of ≥30 mmHg, and/or a diastolic blood pressure (DBP) was greater than 90 mmHg with a rise of ≥15 mm Hg, measured on two or more occasions, after 20 weeks of gestation, with coexisting proteinuria. Proteinuria was defined as more than 300 mg of protein per 24 hour urine collection, without the presence of urinary tract infection. Severe PE was diagnosed if SBP was greater than 160 mmHg and/or the DBP was greater than 110 mmHg, and/or more than 2 g of protein in 24-hour urine collection, and/or one or more of the following symptoms, such as blurred vision or blindness, epigastric or right upper-quadrant pain, nausea or vomiting. Blood pressure was measured after positioning of the arm at heart level and calibration of equipment. Mean blood pressure (MBP) was calculated according to the formula (SBP + (DBP × 2))/3.

None of these women had any previous history of PE, pregnancy induced hypertension, diabetes or glucose intolerance, or any other significant endocrine disorder in the current pregnancy or in the past. All women had a similar socioeconomic status and were non-smokers. None of them used drugs or had any previous history of metabolic or renal disease. All women had received ferrous sulphate during their pregnancy and none had haemoglobin levels less than 10.5 g/ml.

HO-1 serum levels were measured at 30-34 gestational weeks at the time of clinical diagnosis of PE, as well as at 12 to 14 weeks postpartum.

Blood samples and assays. Briefly, 15 ml of blood was collected from the untecubital vein into citrated vacutainers. The tubes remained at 4˚C for 30 min and the blood samples were then centrifuged. The supernatant was collected and stored at –70˚C for subsequent analysis. For the measurement of HO-1, a commercially available assay kit (EKS-800; StressXpress, Pennsylvania, USA) was used, with an inter-assay and intra-assay variability of less than 10% and a minimum detection level of 0.78 ng/ml. No significant cross-reactivity with human HO-2 or HO-3, which are the other two known heme-oxygenase isoforms, has been noted with this assay. All assays were performed without knowledge of the case control status, in duplicate.

Statistics. For the statistical analysis, a commercially available statistical package (SPSS v 13.0; Chicago, IL, USA) was used. For normally distributed variables an independent samples Student's *t*-test was used to compare healthy pregnant women to those with PE. To compare changes in HO-1 levels before and after delivery for each group, a paired *t*-test was used. A Pearson's product-moment correlation coefficient was used to evaluate relationships between continuous variables. All continuous variables are expressed a mean±standard deviation (SD). All reported confidence interval (CI) values are calculated at the 95% level. All reported *p*-values are two-tailed and a *p* level of less than 0.05 was considered significant.

Results

Demographic characteristics of the three groups are shown in Table I. All women were Caucasian and married (data not shown). There were no differences between the groups in maternal age and gestational age at sampling. Maternal serum HO-1 levels during pregnancy were significantly higher in women with severe PE than those with mild PE and those in the control group (5.50±1.54 ng/ml *vs*. 3.04±0.72 ng/ml, $p=0.0003$, and 5.50±1.54 ng/ml *vs*. 3.12±1.57 ng/ml, $p=0.002$, respectively), as shown in Figure 1. In contrary, no statistically significant difference was found between serum HO-1 levels of women with mild PE during pregnancy and controls (3.04±0.72 ng/ml *vs*. 3.12±1.57 ng/ml, $p=0.9$). Postpartum severe pre-eclamptic women again had significantly higher serum HO-1 levels as compared to those with mild PE and the control group respectively (5.00±1.37 ng/ml *vs*. 3.50±1.95 ng/ml, $p=0.05$, and 5.00±1.37 ng/ml *vs*. 2.00±0.97 ng/ml, $p=0.00003$, respectively).

A paired samples *t*-test was used to evaluate changes in maternal serum HO-1 levels during pregnancy and 12-14 weeks after delivery. There was a statistically significant reduction in serum HO-1 levels only in the control group (3.12±1.57 ng/ml *vs*. 2.00±0.97 ng/ml, $p=0.0005$). Conversely, serum HO-1 levels remained unchanged both in women with severe as well as in those with mild PE (5.50±1.54 ng/ml *vs*. 5.00±1.37 ng/ml, $p=0.65$, and 3.04±0.72 ng/ml *vs*. 3.50±1.95 ng/ml, $p=0.51$, respectively), as shown in Figure 1.

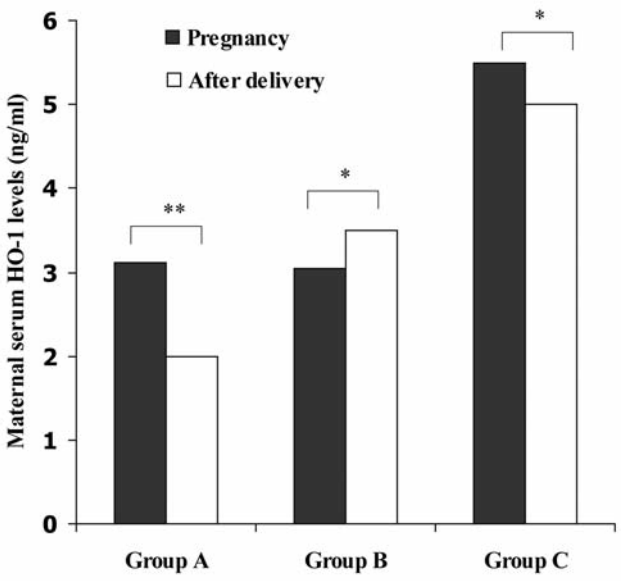

Figure 1. *Maternal serum HO-1 levels before and 12-14 weeks after delivery in normal controls and in women with mild or severe PE (Groups A, B and C, respectively). A significant reduction in serum HO-1 levels was noted in normal controls (**p=0.005), but not in women with mild or severe PE (*p=0.51 and p=0.65, respectively).*

The relationships between maternal serum HO-1 levels during pregnancy and MBP were investigated using Pearson product-moment correlation coefficient. A preliminary data analysis was performed to ensure that there was no violation of the assumptions of normality, linearity and homoscedasticity. There was a significant positive correlation between maternal serum HO-1 levels and MBP in the severe PE group (r=0.79, p=0.004), with higher levels of HO-1 levels associated with higher MBP (Figure 2). On the contrary, a weak positive correlation between HO-1 levels and MBP was observed in mild PE and control groups (r=0.2, p=0.06 and r=0.05, p=0.32, respectively) which did not reach statistical significance.

Discussion

The present study demonstrates that maternal serum HO-1 levels during pregnancy are significantly higher in women with severe PE compared to these with mild PE and normal controls. In addition, we have observed for the first time no significant change in serum HO-1 levels in pre-eclamptic women 12-14 weeks postpartum, while a statistically significant reduction was noticed in normal pregnancies. Moreover, our study demonstrated a positive correlation between serum HO-1 levels and severity of PE as expressed by MBP.

HO is an important enzymatic system within the human body. There are three isoforms of HO, inducible HO-1, constitutive HO-2 and HO-3 with unknown function (11). The importance of this enzyme and its catalytic products in the maintenance and progression of a healthy pregnany to term have recently come to light. HO catalyzes the oxidation of heme to carbon monoxide (CO), biliverdin and iron and is thought to play a key role in protecting tissues from oxidative stress (14). It is now well understood that the HO-CO-biliverdin system is involved in normal placentation, hemodynamic control within the placenta and regulation of the antioxidant status within the placental and fetal tissues (11). The regulation of the HO system in the placenta is complex and is partially reliant upon local glucose and oxygen concentrations (17, 18). However, the role of HO-1 in placental level is still unclear.

Some authors found a decrease in HO-1 expression and/or activity in human placenta in hypertensive disorders of pregnancy (11), others reported a reduction only in HO-2 and not in HO-1 (13, 15), while others yet demonstrated no difference in HO-1 protein levels between uncomplicated and mild pre-eclamptic pregnancies (14). However, while all these studies referred to HO expression in placenta, there is limited data regarding the alteration of maternal serum HO-1 levels in normal and PE-complicated pregnancies. Our results showed that HO-1 levels are increased in the plasma of severe as compared to mildly pre-eclamptic women as well as in normal pregnant women. To the best of our knowledge, there is only one study conducted by Eide *et al*. that in agreement with our findings reported increased decidual and serum HO-1 levels, together with altered decidual expression, supporting the role of oxidative stress and excessive maternal inflammatory response in the pathogenesis of PE (19).

Reactive oxygen species (ROS) are sequestered by anti-oxidants, that may be non-protein based, such as vitamin E, C and A, or metabolites such as glutathione, ubiquinone and uric acid. Protein-based anti-oxidant enzymes include catalase, HO, glutathione peroxidase and thioredoxin peroxidase (20). Normal pregnancy is characterized by a transient increase of ROS production that is partially counteracted by an induction of anti-oxidant defense mechanisms (21). PE is associated with increased oxidative stress which is not localized to the placenta but disseminated in the maternal circulation, and is an expected part of the systemic inflammatory response (22, 23). The increased oxidative stress is due to excessive generation of ROS or deficient anti-oxidant capacity (20), but, furthermore, oxidative stress is closely related to the clinical severity of PE (24). The enzyme HO-1 is rapidly up-regulated by oxidative stress and induction of HO-1 may protect cells by catalyzing pro-oxidant metalloporphyrins, such as heme, to bile pigments (biliverdin, bilirubin) with free radical scavenging capabilities (25). We found elevated serum HO-1 levels in cases with severe PE compared to those with mild PE, as well as in healthy pregnant women. Previous reports indicated that the oxygen radical absorbance based on direct

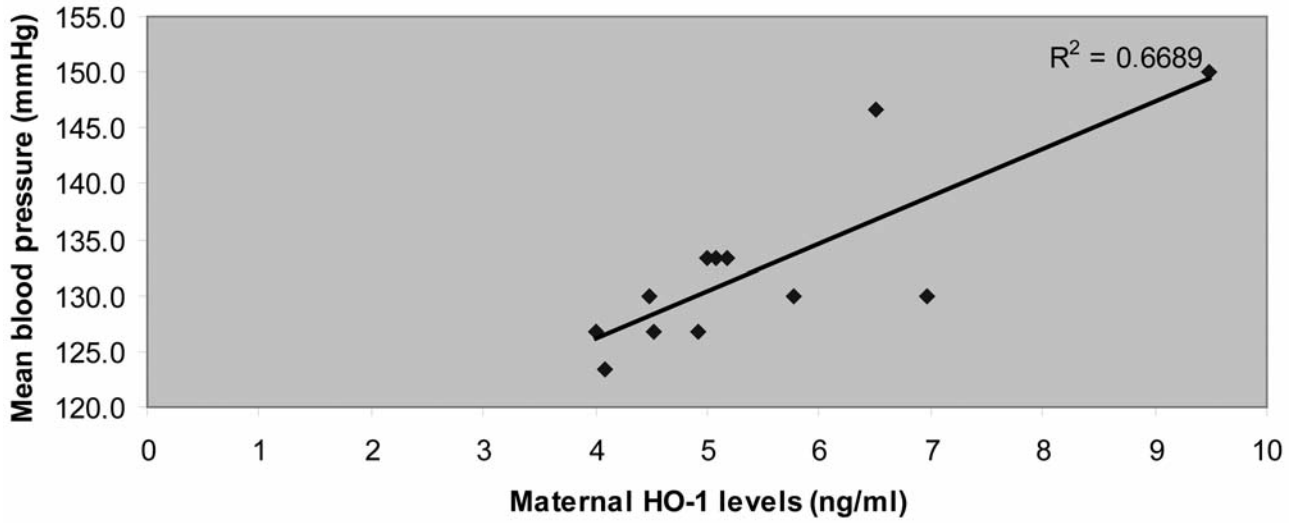

Figure 2. *Maternal mean blood pressure was plotted against the serum HO-1 levels in women with severe PE. The line indicates the fitted regression line. Maternal mean blood pressure was positively correlated to serum HO-1 levels.*

quenching of free radicals were unchanged in women with mild PE (26). This may explain the similar serum levels of HO-1 between healthy and mild pre-eclamptic pregnant women found in our study.

Conversely, free iron and particularly carbon monoxide, are generated from HO-1-mediated heme catabolism (27). Previous reports have observed elevation in carboxyhemoglobin and serum iron concentrations in PE, reflecting an increase in heme and red cell turnover and suggested that these endogenous productions could alter maternal and fetal oxygenation (28). Thus, it is possible that a potential relationship between HO-1 enzyme and the severity of PE exist. Indeed, according to our results, serum HO-1 levels seem to correlate with the severity of the disease in severely pre-eclamptic women, showing a positive relationship between HO-1 enzyme concentrations and MBP. Our findings are in agreement with those of Eide *et al*., considering the similar parallel correlation between serum HO-1 levels and MBP in PE cases (19). Although in the above study, levels of serum HO-1 correlated positively with MBP, both in the total study population and among PE cases, we failed to demonstrate such a significant correlation in mild PE and control groups.

Furthermore, no data exists concerning serum HO-1 levels during the postnatal period, when the harmful effects of the hypertensive disorders on various organs are minimal or totally absent. Long after delivery, we have observed a decrease in serum HO-1 levels which was pronounced and reached statistical significance only in healthy pregnant women. On the contrary, there were no changes in serum HO-1 levels long after delivery in severely and mild pre-eclamptic pregnant women, so that the latter still had statistically higher HO-1 levels as compared to the control group. It is difficult to explain why a precipitous drop in HO-1 levels was observed only in healthy women and not in the PE groups. Our findings probably indicate that postnatally, both the oxidative damage and anti-oxidants levels return to preconception levels in healthy women, as Cikot *et al*. have emphasized (29). On the other hand, it is possible that the oxidative stress and the maternal inflammatory reaction remains for some time after delivery in pre-eclamptic women, while the harmful effect mediated by the increased presence of HO-1 levels in the serum remains unchanged. There is strong supporting data that chronic oxidative stress and persistent inflammatory reaction is responsible for vascular endothelial dysfunction seen in those patients later in their life (30, 31).

It is well known that PE-complicated pregnancies are frequently delivered preterm. This was true in our study too, as the mean gestational age at delivery for the severe PE group was nearly 7 weeks shorter than that for the controls (Table I). Since the placental expression of HO-1 has been reported to increase towards term (32), comparison of serum HO-1 levels between PE pregnancies and term controls should be biased and lead to perplexing results. For this reason, in order to rule out possible gestational age-related changes, although sampling of gestational age-matched healthy controls is difficult, in our study all the study population consisted of women between 30 and 34 weeks of gestation.

Several drawbacks in this study should also be reported. The limited number of patients could underpower the study and increase the chances of type-beta error. As a result, potentially

significant correlations could remain unnoticed. Measurements of HO-1 levels were performed only once during gestation. Serial measurements of maternal serum HO-1 levels on a weekly basis could possibly provide a much better assessment of the temporal changes and possibly elucidate further interactions throughout the course of pregnancy.

In summary, this is the first study to examine maternal serum HO-1 levels both during pregnancy and postpartum in PE and normal pregnancies. We speculate that increased levels of HO-1 in severe but not in mild PE-complicated pregnancies could reflect a significant relationship between HO-1 levels and the severity of PE, as this was documented by a significant correlation between serum HO-1 levels and MBP during pregnancy. In addition, our study has demonstrated for the first time that elevated serum HO-1 activity in severe PE remains long after delivery, suggesting a key role of persistent oxidative stress, increased vascular resistance and chronic excessive maternal inflammatory response in the pathophysiology of PE. However, further clinical studies with a larger number of patients and serial measurements during the course of the gestation are necessary to evaluate the exact role of HO-1 in both healthy pregnancies and those complicated by PE.

References

1 Sibai B, Dekker G and Kupferminc M: Pre-eclampsia. Lancet *365*: 785-799, 2005.

2 Lain KY and Roberts JM: Contemporary concepts of the pathogenesis and management of preeclampsia. JAMA *287*: 3183-3186, 2002.

3 Sibai B: Diagnosis and management of gestational hypertension and preeclampsia. Obstet Gynecol *102*: 181-192, 2003.

4 Buchbinder A, Sibai BM, Caritis S, Macpherson C, Hauth J, Lindheimer MD, Klebanoff M, Vandorsten P, Landon M, Paul R, Miodovnik M, Meis P and Thurnau G: Adverse perinatal outcomes are significantly higher in severe gestational hypertension than in mild pre-eclampsia. Am J Obstet Gynecol *186*: 66-71, 2002.

5 Hnat MD, Sibai BM, Caritis S, Hauth J, Lindheimer MD, MacPherson C, VanDorsten JP, Landon M, Miodovnik M, Paul R, Meis P, Thurnau G and Dombrowski M: Perinatal outcome in women with recurrent preeclampsia compared with women who develop pre-eclampsia as nulliparas. Am J Obstet Gynecol 186: 422-426, 2002.

6 Wang YP, Walsh SW, Guo JD and Zhang JY: Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy. Am J Obstet Gynecol *165*: 1690-1694, 1991.

7 Gupta S, Agarwal A and Sharma RK: The role of placental oxidative stress and lipid peroxidation in pre-eclampsia. Obstet Gynecol Surv *60*: 807-816, 2005.

8 Mikhail MS, Anyaegbunam A, Garfinkel D, Palan PR, Basu J and Romney SL: Pre-eclampsia and antioxidant nutrients: decreased plasma levels of reduced ascorbic acid, alpha-tocopherol, and beta-carotene in women with pre-eclampsia. Am J Obstet Gynecol *171*: 150-157, 1994.

9 Vitoratos N, Salamalekis E, Dalamaga N, Kassanos D and Creatsas G: Defective antioxidant mechanisms *via* changes in serum ceruloplasmin and total iron binding capacity of serum in women with pre-eclampsia. Eur J Obstet Gynecol Reprod Biol *84*: 63-67, 1999.

10 Hung JH: Oxidative stress and antioxidants in pre-eclampsia. J Chin Med Assoc *70*: 430-432, 2007.

11 Bainbridge SA and Smith GN: HO in pregnancy. Free Radic Biol Med *38*: 979-988, 2005.

12 McLaughlin BE, Lash GE, Smith GN, Marks GS, Nakatsu K, Graham CH and Brien JF: Heme oxygenase expression in selected regions of term human placenta. Exp Biol Med (Maywood) 228: 564-567, 2003.

13 Barber A, Robson SC, Myatt L, Bulmer JN and Lyall F: Heme oxygenase expression in human placenta and placental bed: reduced expression of placenta endothelial HO-2 in pre-eclampsia and fetal growth restriction. FASEB J *15*: 1158-1168, 2001.

14 Lash GE, McLaughlin BE, MacDonald-Goodfellow SK, Smith GN, Brien JF, Marks GS, Nakatsu K and Graham CH: Relationship between tissue damage and heme oxygenase expression in chorionic villi of term human placenta. Am J Physiol Heart Circ Physiol *284*: H160-167, 2003.

15 Zenclussen AC, Lim E, Knoeller S, Knackstedt M, Hertwig K, Hagen E, Klapp BF and Arck PC: Heme oxygenases in pregnancy II: HO-2 is down-regulated in human pathologic pregnancies. Am J Reprod Immunol *50*: 66-76, 2003.

16 Davey DA and MacGillivray I: The classification and definition of the hypertensive disorders of pregnancy. Am J Obstet Gynecol *158*: 892-898, 1988.

17 Appleton SD, Marks GS, Nakatsu K, Brien JF, Smith GN and Graham CH: Heme oxygenase activity in placenta: direct dependence on oxygen availability. Am J Physiol Heart Circ Physiol *282*: H2055-2059, 2002.

18 Appleton SD, Lash GE, Marks GS, Nakatsu K, Brien JF, Smith GN and Graham CH: Effect of glucose and oxygen deprivation on heme oxygenase expression in human chorionic villi explants and immortalized trophoblast cells. Am J Physiol Regul Integr Comp Physiol *285*: R1453-1460, 2003.

19 Eide IP, Isaksen CV, Salvesen KA, Langaas M, Schønberg SA and Austgulen R: Decidual expression and maternal serum levels of heme oxygenase 1 are increased in pre-eclampsia. Acta Obstet Gynecol Scand *87*: 272-279, 2008.

20 Vanderlelie J, Venardos K, Clifton VL, Gude NM, Clarke FM and Perkins AV: Increased biological oxidation and reduced anti-oxidant enzyme activity in pre-eclamptic placentae. Placenta. 26: 53-58, 2005.

21 Chappell LC, Seed PT, Briley A, Kelly FJ, Hunt BJ, Charnock-Jones DS, Mallet AI and Poston L: A longitudinal study of biochemical variables in women at risk of pre-eclampsia. Am J Obstet Gynecol *187*: 127-136, 2002.

22 Jauniaux E, Poston L and Burton GJ: Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update *12*: 747-755, 2006.

23 Lyall F and Belfort M: Ed Pre-eclampsia etiology and clinical practice. Cambridge, University Press, New York, p. 110, 2007.

24 Hung JH: Oxidative stress and antioxidants in pre-eclampsia. J Chin Med Assoc *70*: 430-432, 2007.

25 Stocker R, Yamamoto Y, McDonagh AF, Glazer AN and Ames BN: Bilirubin is an antioxidant of possible physiological importance. Science *235(4792)*: 1043-1046, 1987.

26 Zusterzeel PL, Steegers-Theunissen RP, Harren FJ, Stekkinger E, Kateman H, Timmerman BH, Berkelmans R, Nieuwenhuizen A, Peters WH, Raijmakers MT and Steegers EA: Ethene and other biomarkers of oxidative stress in hypertensive disorders of pregnancy. Hypertens Pregnancy *21*: 39-49, 2002.

27 Schipper HM, Chertkow H, Mehindate K, Frankel D, Melmed C and Bergman H: Evaluation of heme oxygenase-1 as a systemic biological marker of sporadic AD. Neurology *54*: 1297-1304, 2000.

28 Entman SS, Kambam JR, Bradley CA and Cousar JB: Increased levels of carboxyhemoglobin and serum iron as an indicator of increased red cell turnover in preeclampsia. Am J Obstet Gynecol *156*: 1169-1173, 1987.

29 Cikot RJ, Steegers-Theunissen RP, Thomas CM, de Boo TM, Merkus HM and Steegers EA: Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr *85*: 49-58, 2001.

30 Callaway LK, Lawlor DA and McIntyre HD: Hypertensive disorders of pregnancy and long-term prognosis. Am J Obstet Gynecol *199*: e20; author reply e20-21, 2008.

31 Bellamy L, Casas JP, Hingorani AD and Williams DJ: Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ *335(7627)*: 974, 2007.

32 Ahmed A, Rahman M, Zhang X, Acevedo CH, Nijjar S, Rushton I *et al*: Induction of placental heme oxygenase-1 is protective against TNFalpha-induced cytotoxicity and promotes vessel relaxation. Mol Med *6*: 391-409, 2000.

Received January 25, 2011
Revised March 7, 2011
Accepted March 11, 2011