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## Relationship of Heme Oxygenase-1 (HO-1) level with onset and severity in normotensive pregnancy and severe preeclampsia



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### ABSTRACT

**Background:** Preeclampsia still becomes a major problem in pregnancies. Various evidences showed that heme oxygenase-1 (HO-1) is very important in pregnancy. This study aims to understand the relationship of heme oxygenase-1 level with onset and severity in normotensive pregnancy and severe preeclampsia.

**Methods:** This is a cross sectional analytic comparative study, the subjects consisted of 26 patients with normotensive pregnancies and 26 patients with severe preeclampsia. Blood samples from women with < 34 / ≥ 34 weeks' normotensive pregnancies and women with severe preeclampsia were taken. HO-1 ELISA kit used to quantitate heme oxygenase-1 level in samples.

**Results:** The level of heme oxygenase-1 in normotensive pregnant women < 34 weeks lower than severe preeclampsia pregnant

women < 34 weeks (3.28 ± 0.46 ng/mL vs 4.20 ± 0.64 ng/mL, p=0.003, respectively). The median level of heme oxygenase-1 in normotensive pregnant women ≥ 34 weeks was 2.96 (2.41–4.39) ng/mL, while severe preeclampsia pregnant women ≥ 34 weeks was 3.52 (2.88–5.43) ng/mL, (p=0.040). The median level of heme oxygenase-1 in normotensive pregnant women was 3.04 (2.41–4.39) ng/mL, while severe preeclampsia pregnant women was 3.68 (2.88–5.67) ng/mL, (p=0.001).

**Conclusions:** There is correlation between the incidence of severe preeclampsia with heme oxygenase-1 level in < 34 and ≥ 34 weeks of pregnancy. There is a significant difference between the level of heme oxygenase-1 in pregnant women with severe preeclampsia and in women with normotensive pregnancy.

**Key words:** heme oxygenase-1 (HO-1), normotensive pregnancy, severe preeclampsia

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### INTRODUCTION

Preeclampsia is still an obstetric problem that cannot be solved completely. Preeclampsia occurs in approximately 1.8 to 16.7% of pregnancies, the figures varied between countries.<sup>1</sup> Based on a systematic review conducted by the WHO, 16% of maternal deaths in developed countries, including the United States, were caused by hypertension in pregnancy and its complication.

This figure surpassed other major causes including bleeding (13%), abortion (8%), and sepsis (2%).<sup>2</sup> In Indonesia, the average incidence of preeclampsia is 3-10%, with maternal mortality rate of approximately 4.91%, ranging from 8.739 to 170.725 deaths.<sup>3</sup>

Several factors have been associated with the incidence of preeclampsia. In general, these risk factors can be classified as maternal factors (e.g extreme age, parity, history of preeclampsia), medical risk factors (such as chronic hypertension, diabetes mellitus or renal disease) and factors of the placenta (e.g hyperplacentalosis on twin pregnancy and gestational trophoblastic diseases). Nevertheless, these factors are almost entirely a predisposing factor whose mechanism is unclear.<sup>4</sup>

In addition to the dominance of several risk factors, a number of theories have been developed to explain the path mechanism of preeclampsia, including, among others, genetic, immunological, oxidative stress, inflammation, hypoxia, angiogenic and hormonal factors. Although most theories tend to agree that the initial problem is the disruption of trophoblastic invasion, to date there are no theories with a satisfactory answer, with each factor being related to the others in a very complicated manner.

In recent years, a variety of important evidence has occurred, showing that heme oxygenase-1 (HO-1) is very important in pregnancy. Therefore, we need to understand the mechanisms underlying the protective effect of heme oxygenase-1 (HO-1).

These mechanisms can vary depending on the reproductive phase in which heme oxygenase-1 (HO-1) is involved. The microenvironment in which heme oxygenase-1 (HO-1) performs its action also greatly affects the mechanisms involved. Research on the mechanisms underlying the emergence of the effects of heme oxygenase-1 (HO-1), which positively affects the process of pregnancy is the release

of CO by heme oxygenase-1 (HO-1). This process does not only occur in the reproductive process,<sup>5,6</sup> but also occurs in several inflammatory processes.<sup>7,8</sup>

## MATERIALS AND METHODS

This is a cross sectional analytic comparative study, conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Sam Ratulangi/Prof. Dr. R. D. Kandou General Hospital Manado, from March 1 2015 to June 30 2015. Inclusion criteria includes: women with < 34 / ≥ 34 weeks' normotensive pregnancies and women with severe preeclampsia who had had filled informed consent and willing to participate in the study. Exclusion criteria include: pregnant women with diabetes mellitus, kidney disorders, heart conditions, chronic hypertension, premature rupture of membranes, women with clinical signs of infection, women with multiple pregnancy, in utero fetal death, and women who are not willing to participate in this study. HO-1 ELISA kit used to quantitate heme oxygenase-1 (HO-1) level in

samples. Non parametric Mann-Whitney test and T-test variances was used to analyze the relationship between the level of heme oxygenase-1 in normotensive pregnancy and in severe preeclampsia.

## RESULTS

This study was conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Sam Ratulangi/Prof. Dr. R. D. Kandou General Hospital Manado, from March 1 2015 to June 30 2015. The subjects consisted of 26 patients with normotensive pregnancies and 26 patients with severe preeclampsia.

Based on the data presented in Table 1, most of the subjects are multiparous (65.38% in normotensive pregnancy and 69.23% in severe preeclampsia), with gestational age of ≥ 34 week (69.23% in normotensive pregnancy and 57.69% in severe preeclampsia), with senior high school education (88.46% in normotensive pregnancy and 57.69% in severe preeclampsia), and work as housewives (69.23% in normotensive pregnancy and 65.38% in severe preeclampsia).

Based on the data presented in Table 2, in 8 samples of normotensive pregnancy, the average level of heme oxygenase-1 (HO-1) is at 3.28 ng/mL (95% CI 2.89-3.66), with a standard deviation of 0.46 ng/mL. In 11 samples of severe preeclampsia, the average value of heme-oxygenase-1 (HO-1) level is 4.20 ng/mL (95% CI 3.77-4.63), with a standard deviation of 0.64 ng/mL. T-test variances by 2 means onset of heme oxygenase-1 (HO-1) level showed that p value: 0.003, meaning that there are significant differences of heme oxygenase-1 (HO-1) level between the normotensive group and the severe preeclampsia group in < 34 weeks of pregnancy. Based on the data presented in Table 3, in 18 samples of normotensive pregnancy, the median level of heme oxygenase-1 (HO-1) is at 2.96 (min-max 2.41-4.39) ng/mL. In 15 samples of severe preeclampsia, the median value of heme-oxygenase-1 (HO-1) level is 3.52 (min-max 2.88-5.43) ng/mL. Mann-Whitney test showed that p value: 0.040, meaning that there are significant differences of heme oxygenase-1 (HO-1) level between the normotensive group and the severe preeclampsia group in ≥ 34 weeks of pregnancy.

Based on the data presented in Figure 1, statistically significant correlation between the incidence of severe preeclampsia with heme oxygenase-1 (HO-1) level in < 34 and ≥ 34 weeks of pregnancy.

Based on the data presented in Table 4, in 26 samples of normotensive pregnancy, the median level of heme oxygenase-1 (HO-1) is at 3.04 (min-max 2.41-4.39) ng/mL. In 26 samples of severe preeclampsia, the median value of heme-oxygenase-1 (HO-1) level is 3.68 (min-max

**Table 1** Characteristics of subjects

Characteristics	N%	Normotensive		Severe Preeclampsia	
		N	%	N	%
Parity					
	Primiparous	9	34.61	8	30.76
	Multiparous	17	65.38	18	69.23
Gestational Age					
	< 34 weeks	8	30.76	11	42.30
	≥ 34 weeks	18	69.23	15	57.69
Education					
	Elementary school	1	3.84	4	15.38
	Junior high school	2	7.69	3	11.53
	Senior high school	23	88.46	15	57.69
	University	0	0	4	15.38
Occupation					
	Housewife	18	69.23	17	65.38
	Student	3	11.53	1	3.84
	Private company employee	5	19.23	4	15.38
	Government employee	0	0	4	15.38

**Table 2** Descriptive analysis of the difference of heme oxygenase-1 (HO-1) level in < 34 weeks of pregnancy

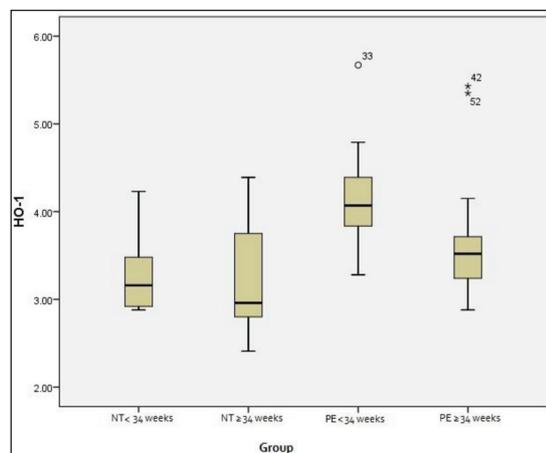
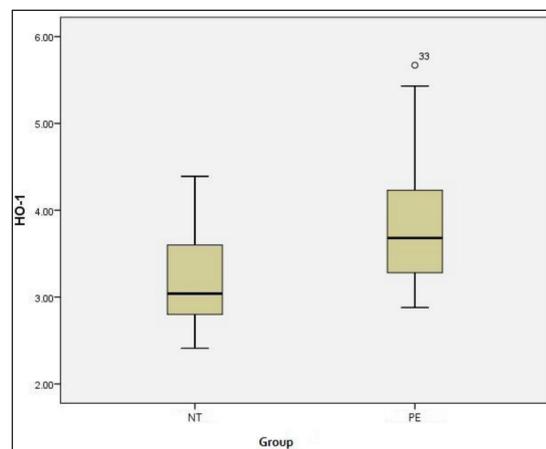
Variables	Normotensive, mean ± SD	Severe preeclampsia, mean ± SD	p
heme oxygenase-1 (HO-1), ng/mL	3.28 ± 0.46	4.20 ± 0.64	0.003

**Table 3** Descriptive analysis of the difference of heme oxygenase-1 (HO-1) level in  $\geq 34$  weeks of pregnancy

Variables	Normotensive, median (min-max)	Severe preeclampsia, median (min-max)	p
heme oxygenase-1 (HO-1), ng/mL	2.96 (2.41–4.39)	3.52 (2.88–5.43)	0.040

**Table 4** Descriptive analysis of the difference of heme oxygenase-1 (HO-1) level in normotensive pregnancy and severe preeclampsia

Variables	Normotensive, median (min-max)	Severe preeclampsia, median (min-max)	p
heme oxygenase-1 (HO-1), ng/mL	3.04 (2.41–4.39)	3.68 (2.88–5.67)	0.001

**Figure 1** Box and whisker plots analysis of the difference of heme oxygenase-1 (HO-1) level in  $< 34$  and  $\geq 34$  weeks of pregnancy**Figure 2** Box and whisker plots analysis of the difference of heme oxygenase-1 (HO-1) level in normotensive pregnancy and severe preeclampsia

2.88–5.67) ng/ mL. Mann-Whitney test showed that p value: 0.001, meaning that there are significant

differences in the levels of heme oxygenase-1 (HO-1) in normotensive patients and patients with severe preeclampsia.

Based on the data presented in Figure 2, statistically significant relationship was found between the levels of heme oxygenase-1 (HO-1) with the incidence of severe preeclampsia.

## DISCUSSION

This study shows that the level of heme oxygenase-1 (HO-1) in maternal serum during pregnancy were significantly higher in women with severe preeclampsia compared with women comprising the control group of normotensive pregnancy. Furthermore, this study shows a positive correlation between the levels of serum heme oxygenase-1 (HO-1) in women with severe preeclampsia compared with women comprising the control group of normotensive pregnancy.

HO is an important enzyme system in the human body. There are three isoforms of HO, inducible HO-1, constitutive HO-2, and HO-3 whose function is still unknown.<sup>9</sup> The importance of this enzyme and its catalytic product in the maintenance of normal pregnancy to term has recently been disclosed. HO catalyzes the oxidation of heme into carbon monoxide (CO), biliverdin and iron, as well as having a key role in tissue protection against oxidative stress.<sup>10</sup> Today, it is understood clearly that the HO-CO-biliverdin system is involved in the formation of a normal placenta and in the hemodynamic control of the placenta and fetal tissues. HO system regulation in the placenta is a complex process and partly dependent on the local concentration of glucose and oxygen.<sup>11,12</sup>

Most researchers found a decrease in the expression and / or activity of HO-1 in human placenta in pregnancy or in individuals with hypertensive disorders, while other researchers reported a decrease only in the levels of HO-2 and not on HO-1.<sup>13,14</sup>

Several other studies have shown that there is no difference of HO-1 protein levels between pregnancies with mild preeclampsia and uncomplicated pregnancy.<sup>10</sup> However, all these studies were conducted on the levels of HO in the placenta, and only few data are available regarding the changes in maternal serum HO-1 level in normal pregnancies and pregnancies complicated by preeclampsia. Our results show that HO-1 level is increased in plasma of women with severe preeclampsia compared with women with normal pregnancies. To our knowledge, there are only two studies, namely the research conducted Eide et al and Vitoratos et al, whose findings are in line with our findings, i.e. increased

level of HO-1 in serum and decidual tissue, accompanied by changes in the expression of the decidua, supporting the role of these substances in oxidative stress and excessive inflammatory responses in the pathogenesis of maternal preeclampsia.<sup>15,16</sup>

Reactive oxygen species (ROS) will be sequestered by antioxidants, which may have non-structural proteins, such as vitamin E, C and A, as well as by metabolites such as glutathione, ubiquinone and uric acid. Protein based antioxidants include catalase, HO, glutathione peroxidase and thioredoxin peroxidase.<sup>17</sup> Normal pregnancy is characterized by a transient increase in the production of ROS partially neutralized by the induction of antioxidant defense mechanisms.<sup>18</sup> Preeclampsia is associated with increased oxidative stress not in the placenta but scattered in the maternal circulation, and is thought to be part of a systemic inflammatory response.<sup>19,20</sup> Increased oxidative stress occurs as a result of excessive ROS production or due to interference in antioxidant capacity,<sup>17</sup> but furthermore, oxidative stress is closely related to the severity of preeclampsia clinically.<sup>21</sup> HO-1 enzyme experiences rapid upregulation by oxidative stress and the induction of HO-1 may protect the cells by binding metaloporphyrin catalyze pro-oxidants, such as heme, bile pigments (biliverdin, bilirubin) that functions as free radicals.<sup>22</sup> We found an increased serum HO-1 in severe preeclampsia compared to normal pregnancy. Previous report showed that the level of oxygen radical absorbance based on the direct neutralization of free radicals is unchanged in women with mild preeclampsia.<sup>23</sup>

In contrast, free irons and particularly carbon monoxide are produced from HO-1 mediated heme catabolism.<sup>24</sup> Previous report observed an increase in serum iron concentration and carboxy-hemoglobin in preeclampsia, which reflects an increased heme and erythrocyte turnover and demonstrate that this endogenous production can change the maternal and fetal oxygenation.<sup>25</sup> Thus, there may be a potential link between HO-1 and the severity of preeclampsia. Indeed, based on our results, the serum levels of HO-1 seem to be related to the severity of the disease in women with severe preeclampsia, which also showed a positive correlation between the levels of HO-1 enzyme. Our findings are consistent with that of Eide and Vitoratos et al, considering that there is a similar parallel relationship between serum levels of HO-1 in the cases of preeclampsia.<sup>15,16</sup> Eide et al reported that HO-1 levels were significantly higher among cases compared to controls (3.1+/-1.3 versus 1.9+/-0.5 ng/ml, p=0.008) and Vitoratos et al reported the severe preeclampsia group had significantly higher serum HO-1 levels antepartum compared to the mild preeclampsia 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). Although in those study the serum levels of HO-1 were positively correlated with both the overall study population and in women with preeclampsia, we cannot show such a correlation in our control group. In addition, Vitoratos et al study have shown for the first time that the increased activity of HO-1 serum in severe preeclampsia will persist long after childbirth, showing the key role of persistent oxidative stress, increased vascular resistance and the chronic excessive maternal inflammatory response in the pathophysiology of preeclampsia.<sup>16</sup> However, further clinical trial with larger sample and repeated measurement during pregnancy is required to properly evaluate the exact role of HO-1 both in normal pregnancy and in preeclampsia.

There are several limitations to our study. The limited number of patients can reduce the strength of this study and increase the chances of error. As a result, a significant correlation may be missed. Measurement of HO-1 level was only performed once during pregnancy. Repeated measurements of maternal serum HO-1 level must be performed every week to allow a better assessment of the temporal changes and may explain the interaction further.

## CONCLUSION

In conclusion, this study examined the levels of maternal serum HO-1 in severe preeclampsia as well as in normal pregnancy. Increased levels of HO-1 were found in severe preeclampsia compared with normal pregnancy, reflecting the significant correlation between HO-1 levels with the severity of preeclampsia. Statistical analysis found a significant correlation between heme oxygenase-1 (HO-1) level and the incidence of severe preeclampsia in < 34 and ≥ 34 weeks of pregnancy.

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