

## Research Article

## Heme Oxygenase-1 Level in Normotensive Pregnancy and Preeclampsia with Severe Features

### *Kadar Heme Oxygenase-1 pada Kehamilan Normotensi dan Preeklamsia dengan Karakteristik Perburukan*

Christian Homenta, John J Wantania, Juneke J Kaeng

*Department of Obstetrics and Gynecology  
Faculty of Medicine University of Sam Ratulangi/  
Prof. Dr. R. D. Kandou General Hospital  
Manado*

#### Abstract

**Objective:** To understand the relationship of heme oxygenase-1 (HO-1) level between normotensive pregnancy and preeclampsia with severe features.

**Method:** The cross sectional study was conducted in the Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sam Ratulangi/Prof. Dr. R. D. Kandou General Hospital Manado. The subjects consisted of 26 pregnant women with normal blood pressure and 26 women with severe features of preeclampsia. We took the patients' history, general physical examination, and laboratory assessment. The blood samples were taken from normotensive women more than 20 weeks of pregnancy and preeclamptic women with severe features more than 20 weeks of pregnancy. The data obtained was processed using SPSS 20.0 software. We did the non-parametric Mann-Whitney test to analyze the relationship between heme oxygenase-1 (HO-1) level in normotensive pregnancy and preeclampsia with severe features.

**Result:** The level of heme oxygenase-1 (HO-1) in normotensive pregnant women was at 3.24 (SD 0.58) ng/ml (95% CI 3.00-3.47), and the level of heme oxygenase-1 (HO-1) of preeclamptic women with severe features was 3.92 (SD 0.73) ng/ml (95% CI 3.62-4.21). The result of Mann-Whitney test showed p value of 0.001 which meant that there was significant difference in the level of heme oxygenase-1 (HO-1) between normotensive women and pre-eclamptic women with severe features.

**Conclusion:** There was the relationship between the level of heme oxygenase-1 (HO-1) and the incidence of preeclampsia with severe features.

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**Keywords:** heme oxygenase-1 (HO-1), normotensive pregnancy, preeclampsia with severe features

**Correspondence:** Christian Homenta. Obstetrics and Gynecology Department. Prof. Dr. R. D. Kandou General Hospital Jl. Raya Tanawangko, Manado 95115, North Sulawesi. Telp. (0431) 959178, 859177. E-mail: chris.nathan@gmail.com

#### Abstrak

**Tujuan:** Untuk memahami hubungan kadar heme oxygenase-1 (HO-1) pada kehamilan normotensi dan preeklamsia dengan karakteristik perburukan.

**Metode:** Penelitian ini merupakan studi potong lintang. Penelitian ini dilaksanakan di Bagian Obstetri dan Ginekologi FK Universitas Sam Ratulangi/BLU RSUP Prof. Dr. R. D. Kandou Manado. Subjek penelitian terdiri dari 26 pasien dengan kehamilan normotensi dan 26 pasien dengan kehamilan preeklamsia dengan karakteristik perburukan. Dilakukan anamnesis, pemeriksaan fisik umum dan kemudian dicatat dalam status penelitian, kemudian perempuan dengan kehamilan normotensi >20 minggu dan perempuan hamil >20 minggu dengan preeklamsia dengan karakteristik perburukan diambil sampel darah dan dibawa ke laboratorium. Data yang diperoleh diolah dengan komputer menggunakan perangkat lunak program SPSS 20,0. Uji non parametric Mann-Whitney digunakan untuk menganalisa hubungan antara kadar HO-1 pada kehamilan normotensi dan preeklamsia berat.

**Hasil:** Pasien hamil normotensi memiliki kadar HO-1 sebesar 3,24 (SD 0,58) ng/ml (95% IK 3,00-3,47), pasien hamil preeklamsia dengan karakteristik perburukan memiliki kadar HO-1 sebesar 3,92 (SD 0,73) ng/ml (95% IK 3,62-4,21). Uji Mann-Whitney menunjukkan bahwa  $p=0,001$  yang berarti bahwa terdapat perbedaan bermakna kadar heme oxygenase-1 (HO-1) pada pasien normotensi dan preeklamsia dengan karakteristik perburukan.

**Kesimpulan:** Terdapat hubungan bermakna antara kadar HO-1 dengan kejadian preeklamsia dengan karakteristik perburukan.

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**Kata kunci:** heme oxygenase-1 (HO-1), kehamilan normotensi, preeklamsia dengan karakteristik perburukan

## INTRODUCTION

Preeclampsia as one of the most devastating obstetric problems is still not solved completely. Preeclampsia was occurred in approximately 1.8 to 16.7% of pregnancies, whereas the incidence of preeclampsia was varied among countries.<sup>1</sup> Based on the systematic review conducted by the WHO,

about 16% of maternal deaths in developed countries, including the United States, were caused by hypertension in pregnancy and its complications. This complication passed through other major causes, such as bleeding (13%), abortion (8%), and sepsis (2%).<sup>2</sup> In Indonesia itself, the average incidence of preeclampsia was 3-10% and the maternal mortality rate (MMR) was 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 (extreme age, parity, history of preeclampsia), medical risk factors (chronic hypertension, diabetes mellitus or renal disease) and factors of the placenta (hyperplacentalosis on twin pregnancy and gestational trophoblastic diseases). Nevertheless, these predisposing factors cannot be explained with clear pathogenesis.<sup>4</sup>

In addition to the dominance of several risk factors, a lot of theories have been developed to explain the pathogenesis of preeclampsia, including genetic, immunological, oxidative stress, inflammation, hypoxia, angiogenic, and hormonal factors. Many theories tend to agree that the initial problem is the disruption of trophoblastic invasion, unfortunately, there are still no theories with satisfactory answers which correlating among each factors to the complication of preeclampsia.

In recent years, various evidences show that heme oxygenase-1 (HO-1) is very important in pregnancy. Therefore, we need to understand the mechanisms underlying the protective effect of HO-1. These mechanisms can be varied depending on the reproductive phase of the HO-1 involvement. The microenvironment in which HO-1 performs its action also greatly affects the protective mechanisms. Study on the mechanisms underlying the

emergence of HO-1 effects to the process of pregnancy is through the release of CO by HO-1. This process not only occurs in the reproductive process,<sup>5,6</sup> but also in several inflammatory processes.<sup>7,8</sup> This study aims to understand the relationship of HO-1 level between normotensive pregnancy and preeclampsia with severe features.

## METHODS

This was a cross sectional study which conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sam Ratulangi/ Prof. Dr. R. D. Kandou General Hospital Manado, from March 1<sup>st</sup> to May 31<sup>st</sup> 2015. The inclusion criteria include pregnant women with more than 20 weeks of gestational age, having normal blood pressure or preeclampsia with severe features, they would like to fill the informed consent and participate in the study. Meanwhile, the exclusion criteria consist of pregnant women with diabetes mellitus, kidney disorders, heart diseases, chronic hypertension, premature rupture of membranes (PROM), clinical signs of infection, multiple pregnancy, in utero fetal death (IUID), and also not willing to participate in this study. The data was processed by computer using SPSS 20.0 software. Non parametric Mann-Whitney test was used to analyze the relationship between the level of HO-1 in normotensive pregnancy and preeclampsia with severe features.

**Table 1.** Characteristics of Subjects

Characteristics	Normal Blood Pressure		Preeclampsia with Severe Features	
	N	%	N	%
Parity				
Primiparous	9	34.61	8	30.76
Multiparous	17	65.38	18	69.23
Gestational Age				
20-28 week	6	23.07	6	23.07
29-36 week	10	38.46	10	38.46
≥ 37 week	10	38.46	10	38.46
Education				
Elementary	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 Heme Oxygenase-1 (HO-1) Level Differences between Normotensive Pregnancy and Preeclampsia with Severe Features.

Study Variables	Normal Blood Pressure	Preeclampsia with Severe Features	p
Heme oxygenase-1 (HO-1)			
Mean	3.2388	3.9154	0.001
95% Confidence Interval	3.00 - 3.48	3.62 - 4.21	
Minimum	2.41	2.88	
Maximum	4.39	5.67	
Standard Deviation	0.58	0.73	

## RESULTS

We got 26 pregnant women with normal blood pressure and the other 26 women with preeclampsia with severe features. Based on the data presented in Table 1, most of the subjects are multiparous (65.38% in normotensive pregnancy and 69.23% in preeclampsia with severe features). The subjects with 29-36 weeks and  $\geq 37$  weeks of gestational age had the same percentage between women with normal blood pressure and preeclampsia with severe features (38.46% for each). Most of them were graduated from senior high school (88.46% in normotensive pregnancy and 57.69% in preeclampsia with severe features) and they worked as housewives (69.23% in normotensive pregnancy and 65.38% in preeclampsia with severe features).

Table 2 depicted the differences of HO-1 level in normotensive pregnancy and preeclampsia with severe features women. Of 26 samples in normotensive pregnancy, the average level of HO-1 was at 3.24 ng/ml (95% CI 3.00-3.48), with a standard deviation of 0.58 ng/ml. Meanwhile, of 26 samples in preeclampsia with severe features, the average value of HO-1 level was 3.92 ng/ml (95% CI 3.62-4.21), with a standard deviation of 0.73 ng/ml. The statistical analysis described there was significant relationship between the levels of HO-1 and the incidence of preeclampsia with severe features.

## DISCUSSION

This study showed that the level of HO-1 in maternal serum during pregnancy were significantly higher in preeclampsia women with severe features compared with normal blood pressure women. Further more, this study showed a positive correlation between the levels of serum HO-1 and the severity of preeclampsia.

Actually, heme oxygenase is an important enzyme system in the human body. There are three isoforms of heme oxygenase, consisting of inducible HO-1, constitutive HO-2, and HO-3 which function are 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. Heme oxygenase catalyzes the oxidation of heme into carbon monoxide (CO), biliverdin, and iron, as well as it has a key role in tissue protection against oxidative stress.<sup>10</sup> Today, it is clearly understood that the HO-CO-biliverdin system is involved in the formation of normal placenta and in the hemodynamic control of placenta and fetal tissues. Heme oxygenase system regulation in the placenta is a complex process and it partly depends on the local concentration of glucose and oxygen.<sup>11,12</sup>

Some studies found a decrease in the expression and/or activity of HO-1 in human placenta in pregnancy or in individuals with hypertensive disorders;<sup>9</sup> while, other studies reported a decrease occurred only in the level of HO-2 and not in the level of HO-1.<sup>13,14</sup> Several other studies had shown that there was no difference of HO-1 protein level 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 were available regarding the changes in maternal serum of HO-1 level in normal pregnancies and pregnancies complicated by preeclampsia. Our results showed that HO-1 level was increased in plasma of preeclampsia women with severe features compared with women with normal blood pressure. There were only two studies conducted by Eide, et al. and Vitoratos, et al. which resulted similar to our study. They found the increased level of HO-1 in serum and decidual tissue, accompanied by changes in the expression

of the decidua. This result supported 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. Antioxidants based protein include catalase, heme oxygenase, glutathione peroxidase and thioredoxin peroxidase.<sup>17</sup> Normal pregnancies are characterized by a transient increase ROS production which neutralized by the induction of antioxidant defense mechanisms.<sup>18</sup> Preeclampsia is associated with the increased of oxidative stress not only in the placenta, but also scattered in the maternal circulation. It 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> however, oxidative stress is closely related to the severity of preeclampsia clinically.<sup>21</sup> Heme oxygenase-1 enzyme experiences rapid up-regulation through oxidative stress and the induction of HO-1 may protect the cells through binding metaloporphyrin catalyze pro-oxidants, such as heme, bile pigments (biliverdin, bilirubin) that has function as free radicals.<sup>22</sup> We found an increased serum HO-1 in preeclampsia with severe features compared with normal blood pressure group. Previous report showed that the level of oxygen radical absorbance based on the direct neutralization of free radicals was unchanged in women with mild preeclampsia.<sup>23</sup>

In contrast, free irons and particularly carbon monoxide are produced from HO-1 which is mediated by heme catabolism.<sup>24</sup> Previous study explained an increase of serum iron and carboxy-hemoglobin concentration in preeclampsia, which reflected the raising of heme and erythrocyte turnover; also it demonstrated that this endogenous production could change the maternal and fetal oxygenation.<sup>25</sup> Thus, there might be a potential link between HO-1 and the severity of preeclampsia. Indeed, based on our results, the serum level of HO-1 seemed to be related to the severity of the disease in preeclamptic women with severe features. 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 could not describe the correlation in control group. Vitoratos, et al. through their study showed

that the increased activity of HO-1 serum in severe preeclampsia would persist long after childbirth. It explained the key role of persistent oxidative stress, the increased vascular resistance, and the chronic excessive maternal inflammatory response in the pathophysiology of preeclampsia.<sup>16</sup> Therefore, further clinical trial with larger samples and repeated measurement during pregnancy is required to properly evaluate the exact role of HO-1 both in normal pregnancy and preeclampsia.

There are several limitations to our study. The limited number of patients could reduce the strength of this study and increased the chances of error. As a result, a significant correlation might be missed. The measurement of HO-1 level was only performed once during pregnancy. It would be more accurate if the repeated measurement of maternal serum HO-1 level was performed every week to allow a better assessment of the temporal changes and explain the further interaction.

## CONCLUSION

This study assesses the level of maternal serum HO-1 in preeclampsia with severe features as well as in normal pregnancy. Increased level of HO-1 is found in preeclampsia with severe features compared with normal pregnancy, reflecting the significant correlation between HO-1 level and the severity of preeclampsia.

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