

## RESEARCH ARTICLE

# Comparison of Serum Interleukin-6 (IL-6) Levels Between Patients with HELLP Syndrome Versus Normotensive Pregnant

Eugenius Phywai Ganap<sup>1\*</sup>, Sulchan Sofoewan<sup>1</sup>, Risanto Siswosudarmo<sup>1,2</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta, Indonesia

<sup>2</sup>Center for Health Policy and Management, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta, Indonesia

\*corresponding author, email: [phywai\\_ku@yahoo.com](mailto:phywai_ku@yahoo.com)

### ABSTRACT

**Background:** Interleukin-6 (IL-6) has been known associated with oxidative stress and endothelial dysfunction, and also has important roles in pathogenesis of preeclampsia. Several studies have demonstrated that there was a significant increase of serum IL-6 levels in preeclampsia compared to normotensive pregnant women. However, study to evaluate serum IL-6 level in pregnant women with HELLP (hemolysis, elevated liver enzyme, and low platelet) syndrome is not well documented yet. **Objective:** This study aims to evaluate serum IL-6 levels between women with HELLP syndrome compared to normotensive pregnant.

**Methods:** The research design is observational cross-sectional study. Samples were recruited consecutively using inclusion and exclusion criteria from emergency department and inpatient wards at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. Serum IL-6 were collected from venous blood sample and measured by enzyme-linked immunoassay (ELISA) method. Independent-samples t-test or Mann-Whitney test was used to compare serum IL-6 values between women with HELLP syndrome and normotensive pregnancy. A statistical measurement conducted using SPSS IBM Statistics 19® and considered significant when  $p < 0,05$ .

**Results:** There were 46 subjects, consisted of 23 women with HELLP syndrome and 23 women with normotensive pregnancy. Serum IL-6 level was a mean of  $0,11 \pm 0,08$  pg/mL and  $0,15 \pm 0,20$  pg/mL in women with HELLP syndrome compared to normotensive pregnancy respectively. There was no significant statistical difference between both groups ( $p = 0,17$ ).

**Conclusion:** The result of this study has shown that there is no significant difference of serum IL-6 level in HELLP syndrome women compared to normotensive pregnant women.

**Keywords:** Interleukin-6, HELLP syndrome, Preeclampsia, normotensive, hypertensive disorders

### ABSTRAK

**Latar Belakang:** Interleukin-6 (IL-6) telah diketahui berhubungan dengan stres oksidatif, disfungsi endotel, dan memiliki peranan dalam pathogenesis preeclampsia. Beberapa penelitian sebelumnya membuktikan bahwa terjadi peningkatan kadar IL-6 serum yang signifikan pada wanita preeclampsia dibandingkan dengan wanita hamil normotensif. Namun, hingga saat ini penelitian untuk mengetahui kadar IL-6 serum pada wanita dengan sindroma HELLP (hemolysis, elevated liver enzyme, and low platelet) belum banyak dilakukan. **Tujuan:** untuk mengetahui kadar IL-6 serum pada wanita dengan sindroma HELLP dibandingkan wanita hamil normotensive.

**Metode:** Desain penelitian yang digunakan adalah studi observasional potong lintang. Subyek penelitian direkrut dengan metode consecutive sampling yang memenuhi kriteria inklusi dan eksklusi dari instalasi gawat darurat (IGD) dan bangsal rawat inap di RSUP Dr. Sardjito, Yogyakarta, Indonesia. Pengukuran kadar IL-6 serum dilakukan dengan pengambilan darah sampel vena diikuti dengan pemeriksaan metode enzyme-linked immunosorbent assay (ELISA). Perbandingan hasil rerata kadar IL-6 serum pada kedua kelompok diuji menggunakan independent-samples t-test atau Mann-Whitney test dengan SPSS IBM Statistics 19®. Hasil dianggap signifikan apabila  $p < 0,05$ .

**Hasil:** Didapatkan sebanyak 46 subyek penelitian yang terdiri dari 23 wanita hamil dengan sindroma HELLP dan 23 wanita hamil normotensif. Pada penelitian ini tidak ditemukan adanya perbedaan rerata kadar IL-6 serum yang signifikan (mean $\pm$ SD) pada kelompok dengan sindroma HELLP ( $0,11 \pm 0,08$  pg/mL) dibandingkan kelompok control ( $0,15 \pm 0,20$  pg/mL) ( $p = 0,17$ ).

**Kesimpulan:** Hasil dari penelitian ini menunjukkan tidak adanya perbedaan yang signifikan antara kadar IL-6 wanita hamil dengan sindroma HELLP dibandingkan wanita hamil normotensif.

**Kata Kunci:** Interleukin-6, HELLP syndrome, Preeclampsia, normotensif, gangguan hipertensif

### INTRODUCTION

Endothelial dysfunction and increasing endothelial permeability are the key characteristics

of the pathogenesis of preeclampsia. One of the most important cytokine that plays significant roles in the pathogenesis of preeclampsia is interleukin-

Copyright © 2018 Authors. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (<http://creativecommons.org/licenses/by-nc-sa/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are properly cited.

6 (IL-6) (Lockwood et al., 2008). Interleukin-6 may significantly increase endothelial permeability by changing the endothelial cells shape and altering intracellular actin fibers (Didion, 2017). Interleukin-6 can also reduce prostacyclin synthesis by inhibiting the cyclooxygenase enzyme. This can increase the thromboxane A2 to prostacyclin ratio, an abnormality that commonly occurs in preeclampsia (Lewis et al., 2010; Lubrano et al., 2008). IL-6 also stimulates placenta-derived growth factor (PlGF), which also induces systemic vascular dysfunction as a central underlying pathogenesis of preeclampsia (Goulopoulou and Davidge, 2015). Oxygen free radicals which were abundant during preeclampsia can induce synthesis of IL-6 by endothelial cells. Oxygen free radicals are implicated in the pathogenesis of preeclampsia, because it can cause endothelial injury, which later leads to reduction in nitric oxide synthesis and prostaglandin balance (Aouache et al., 2018).

Concentrations of serum IL-6 were significantly higher ( $p < 0.001$ ) in preeclamptic women (median 7,9 pg/mL) compared to normal pregnant patients (0, 6 pg/mL) (Kalinderis et al., 2011). The increasing levels of serum IL-6 in preeclamptic women also has been demonstrated by several studies (Molvarec et al., 2011; Xie et al., 2011) Another study also found that serum IL-6 levels were significantly higher in preeclamptic women (median [minimum-maximum] 15,5 [12.0-32.0] pg/mL) compared to normotensive pregnant women (7,0 [5.0-9.0] pg/mL) and normotensive non-pregnant women (6,0 [5.0-8.0] pg/mL) ( $p < 0,001$ ) (Szarka et al., 2010).

However, study to evaluate serum IL-6 level in pregnant women with HELLP (hemolysis, elevated liver enzyme, and low platelet) syndrome is not well documented yet. This study aimed to evaluate serum IL-6 levels between women with HELLP syndrome compared to normotensive pregnant.

## METHODS

The research design was observational cross-sectional study. Forty six samples were recruited consecutively using inclusion and exclusion criteria

from emergency department and inpatient wards at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. Samples were assigned into two groups, HELLP group and normotensive group, 23 subjects of each. Inclusion criteria were (a) HELLP syndrome or normotensive pregnant women with gestational age  $\geq 32$  weeks, (b) subjects voluntarily confirmed to participate in the study and having been informed of the full details of study protocols and the implications that might be happen during study. Exclusion criteria were (a) maternal systemic disorders, (b) premature rupture of the membrane, (c) in labor state. The study protocols have been approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada – Dr. Sardjito General Hospital.

## Interleukin-6 Measurement

Serum IL-6 were collected from venous blood sample, stored in  $-80^{\circ}\text{C}$  refrigerator, and measured by enzyme-linked immunoassay (ELISA) method (Quantikine ELISA, Human IL-6 Immunoassay D6050, R&D Systems, Inc, USA)

## Management and treatment for the patients

Management and treatment for the patients or subjects were according to standard operating procedure which available in Dr. Sardjito General Hospital and did not affected by the study protocols. Independent-samples t-test were used to compare serum IL-6 values between women with HELLP syndrome and normotensive pregnancy. A statistical measurement conducted using SPSS IBM Statistics 19® and considered significant when  $p < 0,05$ .

## RESULTS

### Baseline characteristics of study population

Baseline characteristics of subjects which participated in this study are illustrated in Table 1. There were significant differences in the average gestational age at diagnosis between HELLP syndrome women and normotensive pregnant women  $38,0 \pm 1.7$  weeks vs.  $39,7 \pm 1,3$  weeks respectively ( $p < 0,001$ ). There were also

**Table 1. Baseline comparisons for HELLP syndrome group to normotensive group.**

Variable	HELLP syndrome (n = 23) Mean±SD	Normotensive (n = 23) Mean±SD	T test	p
Age (years)	32.08 ± 7.1	29.2 ± 5.3	1.61	0.11
Gestational age (weeks)	38.0 ± 1.7	39.7 ± 1.3	-3.95	<0.001
Body Mass Index	25.5 ± 3.5	24.8 ± 5.3	0.55	0.60
Leukocyte ( $10^5/\text{mm}^3$ )	13.4 ± 5.8	9.9 ± 2.1	2.80	0.01

Ganap, et al.

**Table 2.** Mean serum IL-6 levels difference between HELLP syndrome group and Normotensive group

Variable	HELLP Synd-G	Normotensive-G
	Mean±SD	
IL-6	0.11 ± 0.08	0.15 ± 0.2

significant differences in white blood cell (WBC) count between HELLP syndrome group and normotensive group  $13,4 \pm 5,8$  vs.  $9,9 \pm 2,1$  ( $p=0,01$ ). This data means that baseline characteristic of women in this study were not homogenous.

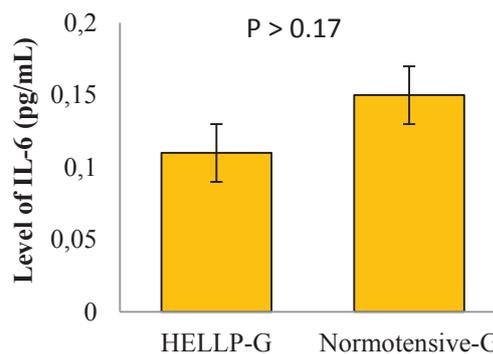
**The levels of IL-6 in serum were not significantly increased in women with preeclampsia**

There was no significant difference between pro-inflammatory circulating IL-6 in women with preeclampsia compared to healthy pregnant women. Independent-samples T-test was conducted to analysis the mean difference of serum IL-6 levels between each group. Mean serum IL-6 levels was not significantly different in HELLP syndrome group ( $0,11 \pm 0,08$  pg/mL) compared to that of normotensive pregnant patients ( $0,15 \pm 0,20$ ),  $p$ -value= $0,17$  as shown in Table 2 and Figure 1.

**DISCUSSION**

Preeclampsia has been thought to be a state of inappropriate increased inflammatory response to increased secretion of inflammatory cytokines (Aouache et al., 2018). Several literatures and guidelines defined preeclampsia as maternal hypertension ( $>140/90$  mmHg systolic/diastolic blood pressure) and proteinuria ( $>300\text{mg}/24$  h) (ACOG, 2013; Butalia et al., 2018; Williams et al., 2018). In severe preeclampsia-spectrum of disease, it might occurs several comorbidities such as hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) in maternal (Abildgaard and Heimdal, 2013).

Although what triggers, etiology, and exact mechanisms underlying preeclampsia are still incompletely understood, the roles of cytokines including pro-inflammatory cytokines appear to be significant key players in maternal systemic inflammatory response. Significant body of evidences have reported the increased levels of circulating serum



**Figure 1.** Independent T test on IL-6 Concentration between Two groups.

IL-6 levels in women with preeclampsia regardless its severity (Kalinderis et al., 2011; Ozler et al., 2012; Xiao et al., 2012).

A recent review highlighted the potential roles of IL-6 in the pathophysiology of preeclampsia (LaMarca et al., 2011). It was thought that IL-6 together with other pro-inflammatory cytokines including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) stimulated T-helper 1 and B-lymphocyte activation, that further leads to the increased secretion of endothelin-1, oxidative stress, and sFlt-1, which finally contributing to the development of hypertension in preeclampsia (LaMarca et al., 2011). IL-6 can be secreted by several types of white blood cells (WBC) such as monocyte, lymphocyte, macrophage, granulocyte, and mast cells. Serum IL-6 levels increased linearly with total WBC count (Suzuki et al., 2009).

In this study, authors tried to measure circulating levels of IL-6 in pregnant women with HELLP syndrome and normotensive pregnant women as controls. According to our findings, in the baseline characteristics comparison between both groups showed that there was not homogenously distributed. It was probably due to two important limitations of this study. First, in our sample recruitment we used consecutive sampling method rather than purposive sampling (matched-pairs sampling). Second, since our location of study was in the tertiary hospital which was the highest referral hospital in Yogyakarta Special Province, it made researchers difficult to conduct matched-pairs sampling particularly to find normotensive pregnant women without any compelling co morbidities.

In this study, our data showed that there were no significant differences of circulating serum IL-6 levels in women with HELLP syndrome compared to healthy normotensive pregnant women. The result of our study did not support several previous studies which measured circulating serum IL-6 levels during preeclampsia (Kalinderis et al., 2011; Ozler et al., 2012; Xiao et al., 2012) including a meta-analysis conducted

by Xie et al., (2011). In particular, for example, a study conducted by Xiao and colleagues (2012) showed that there were significantly higher levels of pro-inflammatory cytokines IL-6 levels in women with preeclampsia and the highest result was in the group of severe preeclampsia. The similar result also has been supported by Kalinderis et al., 2011. However, both studies only mentioned severe preeclampsia and did not mention HELLP syndrome in particular.

According to our best knowledge, the only study which measured IL-6 in HELLP syndrome was conducted by Ozler et al., (2012). Ozler and colleagues did not found any significant differences of mean circulating serum IL-6 levels among mild, severe preeclampsia, and HELLP syndrome in compared to normal healthy pregnant women group.

## CONCLUSION

There was no significant difference in concentration of IL-6 levels in HELLP syndrome women compared to normal pregnant women. It is might be caused by the baseline characteristics of samples in this study which were not homogeneously distributed.

## CONFLICT OF INTEREST

There is no conflict of interest

## ACKNOWLEDGMENT

We were grateful to Aditya Doni Pradana, MD for helping preparing the research manuscript and data analysis.

## REFERENCES

- Abildgaard, U., Heimdal, K., 2013. Pathogenesis of the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP): A review. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 166, 117–123. <https://doi.org/10.1016/j.ejogrb.2012.09.026>
- Ballin A, Osdachi A, Klivitsky A, Dalal I, Lishner M. 2006. "Age-Related Leukocyte and Cytokine Patterns in Community-Acquired Bronchopneumonia". *IMAJ*, vol 8.
- The American College of Obstetricians and Gynecologists (ACOG)., 2013. *Hypertension in Pregnancy*. United States: Washington DC. ISBN: 978-1-934984-28-4
- Aouache, R., Biquard, L., Vaiman, D., Miralles, F., 2018. Oxidative stress in preeclampsia and placental diseases. *Int. J. Mol. Sci.* 19. <https://doi.org/10.3390/ijms19051496>
- Butalia, S., Audibert, F., Côté, A.M., Firoz, T., Logan, A.G., Magee, L.A., Mundle, W., Rey, E., Rabi, D.M., Daskalopoulou, S.S., Nerenberg, K.A., 2018. Hypertension Canada's 2018 Guidelines for the Management of Hypertension in Pregnancy. *Can. J. Cardiol.* 34, 526–531. <https://doi.org/10.1016/j.cjca.2018.02.021>
- Didion, S.P., 2017. Cellular and oxidative mechanisms associated with interleukin-6 signaling in the vasculature. *Int. J. Mol. Sci.* 18. <https://doi.org/10.3390/ijms18122563>
- Goulopoulou, S., Davidge, S.T., 2015. Molecular mechanisms of maternal vascular dysfunction in preeclampsia. *Trends Mol. Med.* 21, 88–97. <https://doi.org/10.1016/j.molmed.2014.11.009>
- Greer, I.A., Lyall, F., Perera, T., Boswell, F., Macara, L.M., 1994. Increased concentrations of cytokines interleukin-6 and interleukin-1 receptor antagonist in plasma of women with preeclampsia: a mechanism for endothelial dysfunction? *Obstet. Gynecol.* 84, 937–940.
- Jauniaux E, Gulbis B, Schandene L, Collette J, Hustin J. 1996. "Distribution of Interleukin-6 in Maternal and Embryonic Tissues During the First Trimester". *Molecular Human Reproduction* vol.2 no.4 pp. 239-243
- Kalinderis, M., Papanikolaou, A., Kalinderi, K., Ioannidou, E., Giannoulis, C., Karagiannis, V., Tarlatzis, B.C., 2011. Elevated Serum Levels of Interleukin-6, Interleukin-1 $\beta$  and Human Chorionic Gonadotropin in Pre-eclampsia. *Am. J. Reprod. Immunol.* 66, 468–475. <https://doi.org/10.1111/j.1600-0897.2011.01019.x>
- LaMarca, B., Brewer, J., Wallace, K., 2011. IL-6-induced pathophysiology during pre-eclampsia: Potential therapeutic role for magnesium sulfate? *Int. J. Interf. Cytokine Mediat. Res.* 3, 59–64. <https://doi.org/10.2147/IJICMR.S16320>
- Lewis, D.F., Canzoneri, B.J., Gu, Y., Zhao, S., Wang, Y., 2010. Maternal Levels of Prostacyclin, Thromboxane, ICAM, and VCAM in Normal and Preeclamptic Pregnancies. *Am. J. Reprod. Immunol.* 64, 376–383. <https://doi.org/10.1111/j.1600-0897.2010.00861.x>
- Lockwood, C.J., Yen, C.F., Basar, M., Kayisli, U.A., Martel, M., Buhimschi, I., Buhimschi, C., Huang, S.J., Krikun, G., Schatz, F., 2008. Preeclampsia-related inflammatory cytokines regulate interleukin-6 expression in human decidual cells. *Am. J. Pathol.* 172, 1571–1579. <https://doi.org/10.2353/ajpath.2008.070629>

*Ganap, et al.*

- Lubrano, V., Baldi, S., Ferrannini, E., L'Abbate, A., Natali, A., 2008. Role of thromboxane A2 receptor on the effects of oxidized LDL on microvascular endothelium nitric oxide, endothelin-1, and IL-6 production. *Microcirculation* 15, 543–553.
- Ozler, A., Turgut, A., Sak, M.E., Evsen, M.S., Soydinc, H.E., Evliyaoglu, O., Gul, T., 2012. Serum levels of neopterin, tumor necrosis factor-alpha and Interleukin-6 in preeclampsia: relationship with disease severity. *Eur. Rev. Med. Pharmacol. Sci.* 16, 1707–1712.
- Szarka, A., Rigó, J., Lázár, L., Beko, G., Molvarec, A., 2010. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol.* 11, 1–9. <https://doi.org/10.1186/1471-2172-11-59>
- Williams, B., Mancia, G., Spiering, W., Rosei, E.A., Azizi, M., Burnier, M., Clement, D.L., Coca, A., de Simone, G., Dominiczak, A., Kahan, T., Mahfoud, F., Redon, J., 2018. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur. Heart J.* 39, 3021–3104. <https://doi.org/10.1097/HJH>.
- Xiao, J.P., Yin, Y.X., Gao, Y.F., Lau, S., Shen, F., Zhao, M., Chen, Q., 2012. The increased maternal serum levels of IL-6 are associated with the severity and onset of preeclampsia. *Cytokine* 60, 856–860. <https://doi.org/10.1016/j.cyto.2012.07.039>
- Xie, C., Yao, M.Z., Liu, J.B., Xiong, L.K., 2011. A meta-analysis of tumor necrosis factor-alpha, interleukin-6, and interleukin-10 in preeclampsia. *Cytokine* 56, 550–559. <https://doi.org/10.1016/j.cyto.2011.09.021>