The Role of Inflammation in Pathogenesis of Preeclampsia: An Investigation of interleukin-6, interleukin 10, and the Ratio

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ABSTRACT
Introduction: Pre-eclampsia is a major cause of maternal mortality and morbidity, preterm birth, perinatal death, and intrauterine growth restriction in global maternity cases. Purpose: The study aims to investigate the role of pro-inflammatory and anti-inflammatory cytokines in the pathophysiology of preeclampsia. Method: In order to get cross sectional data, the study employed observation method. The serum IL-6 and IL-10 of the subjects with preeclampsia and normal pregnancies were measured. The systolic, diastolic blood pressures, and proteinuria of the subjects were also checked. The results were evaluated using the t-test, the Kruskal-Wallis test, and the Spearman correlation test (P<0.05) Results: Twenty subjects with severe preeclampsia, 15 subjects with mild preeclampsia, and 35 subjects with normal pregnancies were examined by taking blood sampling. The serum IL-6, IL-6:IL-10 ratio were significantly different between those groups, but IL-10 was not. The serum IL-6 and IL-6:IL-10 ratios were significantly correlated with the systolic, diastolic blood pressure, and proteinuria level. It indicates that pro-inflammatory cytokine (IL-6) has a role in the pathogenesis of preeclampsia. Conclusion: Pro-inflammatory cytokine (IL-6) has a role in pathogenesis in preeclampsia, but not anti-inflammatory cytokine (IL-10).

KEYWORDS Preeclampsia, interleukin-6, interleukin-10, inflammation, pathogenesis

Introduction
Preeclampsia is one of the causes of maternal and perinatal morbidity and mortality. Its symptoms are hypertension and proteinuria at 21 weeks of pregnancy or later.[1] The prevalence of preeclampsia is 5-10% of all pregnancies.[2] The pathogenesis of preeclampsia remains unclear. One proposed theory is that there is endothelial damage as the initial stage of preeclampsia.[3,4] The endothelial dysfunction is proceeded by an inflammatory response, which stimulates the secretion of various pro-inflammatory and anti-inflammatory cytokines. Some cytokines such as interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), IL-10 are considered as having the main role in eclampsia.[4] The deficiency of IL-10 and the increased TNF-α expression in placenta and decidua are found in preeclampsia compared to the normal pregnancy.[5] However, no difference in IL-10 level was found based on the severity of preeclampsia.[6,7]

The balance of pro-inflammatory and anti-inflammatory cytokines is important to maintain the homeostatic function of vital organs. Freeman et al.[8] found a significant difference in the IL-6:IL-10 ratio in preeclampsia compared to the normal pregnancy. A previous study concluded that some of the risk factors for preeclampsia among Asian women are the same as those of other ethnic groups, whereas some of the risk factors are different.[9] We tried to investigate the role of inflammation in preeclampsia by examining the IL-6, IL-10, and IL-6:IL-10 ratio in subjects with preeclampsia.
Subjects and Method

Subjects

The Riset was an observational, cross-sectional study. All of the subjects were examined at the departments of Obstetrics and Gynecology in hospitals in Makassar, Indonesia, from July to September 2012. A total of 35 subjects with preeclampsia and 35 normal control subjects were studied. The subjects included those in the third trimester of pregnancy (>28 weeks of gestation) with preeclampsia (mild and severe preeclampsia) and normal pregnancy. The exclusion criteria were those women in labour stage, smokers, having other diseases (such as Diabetes Mellitus, hypertension without proteinuria, autoimmune disease, cancer, infection, hypersensitivity, burn, and tissue graft.

Method

The procedures adhered to the tenets of the Declaration of Helsinki and were approved by the Ethics Committee of Hasanuddin University, Indonesia. Informed consent was obtained from the subjects after they were informed of the nature and possible complications of the examination procedures. Anamnesis and history taking, physical examinations, and laboratory examinations were performed in all subjects. The pregnancy age was determined using the Naegle formula of which the first day of last menstruation was known and the last three menstruation cycles were normal and regular. When the first day of last menstruation was not known, the age of pregnancy was determined based on the ultrasonography examination result. The blood pressure was measured using the mercury sphygmomanometer (presameter Nova Riester, Germany) and a stethoscope (3M Littmann Classic II, USA). The systolic pressure was determined as the first sound (Korotkoff I), and the diastolic pressure was determined when the brachial artery pulse disappeared (Korotkoff V). Blood pressure >140/90 mmHg accompanied with proteinuria (protein concentration 0.01 – 0.05%) is classified as mild preeclampsia, blood pressure >160/110 mmHg accompanied with proteinuria (protein concentration >0.05%) is classified as severe preeclampsia.[1]

Proteinuria examination was performed at Laboratory of Wahidin Sudirohusodo Hospital and other satellite hospitals in Makassar, while the serum IL-6 and IL-10 examination was done at Prodia Laboratory, Makassar.

Statistical Analyses

The characteristics of those with preeclampsia and normal subjects were compared using the t-test. Significant differences in the serum IL-6, IL-10, the ratio of IL-6:IL-10 of the groups were determined by using the Kruskal-Wallis test. Spearman correlation test was performed to examine the relationship between the serum IL-6, IL-10, IL-6:IL-10 ratio and the systolic, diastolic blood pressure. Statistical analyses were performed using the SPSS version 16.0 software program (SPSS, Inc., Chicago, USA).

Results

In a total of riset, there were 70 subjects in this study: 35 pregnant women with preeclampsia and 35 women with normal pregnancy. Among those with preeclampsia, 15 of them were classified as mild preeclampsia and 20 of them were classified as severe preeclampsia. The characteristics of the subjects are shown in Table 1. There were no significant differences in age, pregnancy age, and parity of the two groups.

IL-6, IL-10, and The Ratio

The comparisons of IL-6, IL-10, and IL-6: IL-10 ratio between normal pregnancies, pregnancies with mild, and severe preeclampsia are displayed in Table 2. It was found that there were significant differences between severe preeclampsia and mild preeclampsia, normal pregnancies. However, there was no significant difference between normal pregnancies and mild preeclampsia. The serum IL-10 was not significantly different between those three groups. The IL-6: IL-10 ratio was significantly different between normal and mild preeclampsia, normal and severe preeclampsia, mild and severe preeclampsia.

Relationship between serum IL-6:IL-10 ratio, systolic blood pressure, diastolic blood pressure, proteinuria

Other crucial findings that there were significant relationships between serum IL-6 and systolic blood pressure, diastolic blood pressure, proteinuria (r=0.706, 0.583, 0.553, respectively, all P<0.05). (Table 3) The relationships between serum IL-6:IL-10 ratio and systolic blood pressure, diastolic blood pressure, proteinuria were also significant (r=0.608, 0.507, 0.534, respectively all P<0.05). Nevertheless serum IL-10 was not significantly correlated with systolic blood pressure, diastolic blood pressure and proteinuria.

Discussion

Some principal findings are: first the serum IL-6 level was higher in severe preeclampsia compared to mild preeclampsia and normal pregnancy. Previous studies also found a significant increase of IL-6 compared to normal pregnancy.[10,11] In preeclampsia aponecrosis replaces the normal apoptotic mechanism leading to placental damage.[12] This will cause the release of cytokines (IL-6, tumour necrosis factor-α (TNF-α)) to the maternal circulation which will damage the endothelium clinically manifests as preeclampsia.[3,4] Free radical oxygen also induces IL-6 synthesis. Podjarny et al.[13] investigated the cytokines (TNF-α, IL-1, IL-6) function as pro-inflammatory mediators during hypoxia and found that only IL-6 increased during preeclampsia.

Another crucial finding there were significant differences in IL-10 between subjects with preeclampsia and normal pregnancies while there was a decreased serum IL-10 level in mild preeclampsia, it was not significantly different. Some previous studies found a significant decrease of IL-10 in subjects with preeclampsia compared to normal pregnancies.[6,14] No differences were found between mild preeclampsia and severe preeclampsia.[6]

It was hypothesized that due to preeclampsia the placenta has inadequate growth leading to the decreased of IL-10 production.[14] On the other hand, other studies found a significant IL-10 increase compared to the normal pregnancies.[15,16] The increasing serum IL-10 in patients with preeclampsia was connected with the theory that preeclampsia is an immune rejection manifestation of which an increased serum IL-10 is a protective response to suppress the rejection process.[15] Those different results resulted in a hypothesis that there is a dynamic serum IL-10 change in preeclampsia.

The next essential findings are that there were higher serum IL-6:IL-10 ratio in those with preeclampsia compared to those with normal pregnancies. A previous study found a significantly higher difference of IL-6:IL-10 ratios in preeclampsia compared to normal pregnancies.[8] Taniguchi et al. [17] reported the
### Table 1 The characteristics of the subjects of the two groups.

| Variable            | Pregnancy                  | p-value |
|---------------------|----------------------------|---------|
|                     | Normal (n=35)              |         |
|                     | Preeclampsia (n=35)        |         |
| age (years)         | 29.29 (6.16)               |         |
|                     | 30.26 (6.46)               | p=0.522 |
| pregnancy age (weeks)| 35.06 (2.50)              |         |
|                     | 35.31 (2.15)               | p=0.646 |
| parity              | 2.20 (1.12)                |         |
|                     | 2.28 (1.17)                | p=0.752 |

### Table 2 Correlation of IL-6, IL-10 and ratio IL-6: IL-10 serum level with Blood Pressure and proteinuria.

| Variable          | Systolic (mmHg) | Diastolic (mmHg) | Degree of proteinuria |
|-------------------|-----------------|-----------------|----------------------|
|                   | Coefficient corelation | p-value | Coefficient corelation | p-value | Coefficient corelation | p-value |
| IL-6              | r = 0.706       | p = 0.000       |                       |         |                       |
| IL-10             | r = 0.054       | p = 0.329       |                       |         |                       |
| Ratio IL-6:IL-10  | r = 0.608       | p = 0.000       |                       |         |                       |
| IL-6              | r = 0.583       | p = 0.000       |                       |         |                       |
| IL-10             | r = 0.61      | p = 0.308       |                       |         |                       |
| Ratio IL-6:IL-10  | r = 0.507       | p = 0.000       |                       |         |                       |
| IL-6              | r = 0.553       | p = 0.000       |                       |         |                       |
| IL-10             | r = - 0.098      | p = 0.210       |                       |         |                       |
| Ratio IL-6:IL-10  | r = 0.534       | p = 0.000       |                       |         |                       |

### Table 3 Comparison of IL-6, IL-10 and ratio IL-6: IL-10 serum level.

| Variable          | Pregnancy                  | P-value |
|-------------------|----------------------------|---------|
|                   | Normal (n=35)              |         |
|                   | Mild preeclampsia (n=15)   |         |
|                   | Severe preeclampsia (n=20) |         |
| IL-6              | 2.98 (1.53) a              |         |
|                   | 3.78 (2.08) a              |         |
|                   | 8.40 (4.86) b              | p=0.000 |
| IL-10             | 3.81 (1.69) a              |         |
|                   | 3.15 (2.02) a              |         |
|                   | 3.96 (2.92) a              | p=0.160 |
| Ratio IL-6:IL-10  | 0.91 (0.61) a              |         |
|                   | 1.42 (0.88) b              |         |
|                   | 2.92 (2.37) c              | p=0.000 |

Kruskal Wallis test
correlation between the IL-6:IL-10 ratio and the systemic inflammatory response syndrome. It was associated with the role of those cytokines as a pro-inflammatory cytokine (IL-6) and anti-inflammatory cytokine (IL-10). The higher the pro-inflammatory and anti-inflammatory ratio the more severe the disease.[17]

Another important findings from this research: the results showed that the serum IL-6 and IL-6:IL-10 ratio have positive relationships with systolic and diastolic blood pressure. The result is similar to the previous result found by Mihu et al.[10] They concluded that the serum IL-6 has a positive correlation with systolic and diastolic blood pressure. Research in animals showed that IL-6 may cause increased arterial blood pressure, decreased endothelium vascular relaxation, and increased vascular contraction of blood vessels in an animal that gets IL-6 infusion in the long term.[18]

IL-6 as a pro-inflammatory cytokine influence the vascular function. IL-6 was found as one of a potential mediator which increases the vascular resistance that causes hypertension in preeclampsia.[2,18]

There was no significant relationship between serum IL-10 and blood pressure in our study. IL-10, a key immunosuppressive cytokine is increased early in pregnancy and remains elevated until the onset of labour.[19] The injection of preeclampsia serum in pregnant IL-10 knock-out mice causes hypertension and proteinuria.[20] Thus, the study in human needs more subjects than those in our study. This is one of the limitations of our study that really necessary to make another research with a bigger population.

The next interesting finding from this research that there is a positive correlation between IL-6 and proteinuria as well as IL-6:IL-10 ratio and proteinuria. However, we did not find any correlation between IL-10 and proteinuria. IL-6 was found to correlate with proteinuria[10]. IL-6 increases response to the chronic uterus perfusion by increasing blood pressure decreasing kidney perfusion and glomerulus filtration. IL-6 causes increased endothelial substance rate and decrease vasodilatation caused by acetylcholine.[21]

Conclusion
In conclusion we examined the relationships of IL-6, IL-10, and preeclampsia with blood pressure and proteinuria as indicators. The increased IL-6 level and IL-6:IL-10 ratio correlate with preeclampsia and the severity of preeclampsia while we could not find any relationship between IL-10 and preeclampsia and the severity of preeclampsia. Thus, the inflammation process plays its role in the pathogenesis of preeclampsia.

Conflict of interest
The authors have no conflicts to disclose. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Financial support and sponsorship
Nil.

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