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Profile Tumor Necrosis Factor Alpha and Procalcitonin in Preeclampsia and Preeclampsia with Sepsis

Bambang Rahardjo*, Tatit Nurseta, Aqua Rossalinda Sinaga

Department of Obstetric and Gynecology, Faculty of Medicine, University of Brawijaya, dr. Saiful Anwar Hospital, Malang, Indonesia

Abstract

BACKGROUND: Preeclampsia is hypertension in pregnancy which are characterized by high blood pressure, proteinuria that occur after 20 weeks of GA. Preeclampsia remains a massive cause of maternal morbidity and mortality that 50.000 death annually. The cause of preeclampsia is still unclear but there is a possibility caused by immunological processes in micro placenta environment during the early age of pregnancy. It is suggested that cytokines such as tumor necrosis factor (TNF-α) has an important role in the pathogenesis of preeclampsia. Preeclampsia is an extreme feature of the systemic inflammatory response during pregnancy. Systemic inflammation in preeclampsia can cause organ damage and induce sepsis. The pathophysiology is initiated by a high level of pro-inflammatory cytokine that released by peripheral blood mononuclear cell (PBMC). Beside pro-inflammatory cytokine, the marker of sepsis can be shown by procalcitonin (PCT) that produced by PBMC which is activated by TNF-α.

AIM: The objective of the study is to evaluate profile maternal plasma levels of TNF-α and PCT and analyze their correlation in normotensive pregnant woman, preeclamptic and preeclampsia with sepsis.

METHODS: An observational cross-sectional study. The sample were normotensive, preeclamptic, and preeclamptic with sepsis (n = 18) in Bangil Hospital, Pasuruan. The level of TNF-α and PCT was measured by ELISA. The statistical analysis with SPSS 18.0 with $p < 0.05$.

RESULTS: This study showed level of TNF-α and PCT in preeclamptic with sepsis was significantly higher than control ($p < 0.05$) and not a significant difference in preeclampsia ($p > 0.05$). The level of TNF-α and PCT in preeclampsia compared with control was not a significant difference ($p > 0.05$). This study showed there was no correlation between TNF-α and PCT in patients with preeclampsia with sepsis.

CONCLUSION: The plasma level of TNF-α and PCT was statistically different between the control group, preeclampsia and preeclampsia with sepsis. There was no significant difference of TNF-α and PCT plasma level in preeclampsia with sepsis than preeclampsia group. There was no significant correlation between preeclampsia in woman and preeclampsia with sepsis in maternal plasma TNF-α and PCT levels.

Introduction

Preeclampsia is one type of gestational hypertension which is characterized by new onset hypertension with systolic blood pressure>140 mmHg or diastolic blood pressure>90 mmHg, measured on two occasions at least 4 h apart and proteinuria (>100 mg/dL with urine analysis or >300 mg in 24-h urine collection) occurring at gestational age after 20 weeks of pregnancy or new onset of hypertension, in the absence of proteinuria with new onset one of this criteria: thrombocytopaenia (<100.000/μL), renal insufficiency (creatinine serum >1.1 mg/dL or doubling of the creatinine serum concentration in the absence of other renal disease, high concentration of liver function to twice normal level, cerebral, or visual problems [1].

The morbidity and mortality of preeclampsia affect 5–7% of all pregnant women but it is responsible for over 70.000 maternal deaths and 500.000 fetal deaths worldwide per annually. Preeclampsia is one of the leading causes of maternal death, severe maternal morbidity, maternal intensive care admission, cesarean section, and prematurity [2], [3].

The pathogenesis of preeclampsia remains largely unknown. However, many researchers support the two-step theory [4]. In stage 1 of early-onset preeclampsia, impaired extravillous trophoblast invasion into maternal spiral arteries causes poor vascular modeling and induces placental and endothelial damage. In stage 2, these damaged tissues release antiangiogenic factors such as the soluble form of the vascular endothelial growth factor receptor (sFlt-1) and soluble endoglin, a coreceptor for transforming growth factor-β1 and -β3, which induces maternal intravascular systemic inflammatory responses and endothelial dysfunction [5].

System inflammation plays an important role in the pathophysiology of preeclampsia. There is increasing evidence suggesting that both innate and adaptive

immune processes are involved in the pathogenesis of preeclampsia. It is believed that there is a shift to Th-1 type from Th-2 type of immunity. Predominance of Th1 immunity can produce INF-γ and tumor necrosis factor (TNF)-α and it can not only related to poor placentation but also to the exaggerated inflammatory response and endothelial dysfunction in circulation. Immunologic response in preeclampsia starts when the peripheral blood mononuclear cell (PBMC) (monocyte) activity is occured. This activity causes increasing of pro-inflammatory cytokines (TNF-α and IL-6) and is known to be involved in many pathological processes. This activity causes an increase in the synthesis of pro-inflammatory and anti-inflammatory mediators [1], [6].

Sepsis is a major problem with the mortality rate of septic shock currently 25%. The morbidity and mortality of sepsis remain high. Sepsis is characterized by increased vascular permeability, unbalanced inflammation, and immune modulation which form a complex, crosslinked networks of cells, mediators, and signaling pathways [7]. In patients with septic shock, monocyte and macrophage is the main source of cytokines. Stimulation of lipopolysaccharides (LPS)-containing-endotoxin and the increase of pro-inflammatory cytokines activate the monocytes and macrophages to activate pro-inflammatory (TNF-α, IL-1, IL-6) which will then cause a more severe inflammatory reaction. Understanding the cytokine profile in patients with sepsis may be very useful in the diagnosis of disease severity and prediction of mortality and better patient management [8].

Current evidence suggests that a dysregulated, i.e., systemic and extensive, immune response causes sepssi- induced multiple organ failure. This not only implies the initial inflammatory reaction but also delays sepsis-association depression of the immune system. Endothelial dysfunction and sepis induced coagulopathy represent additional major pathomechanisms [9]. Systemic inflammatory response syndrome (SIRS) is a persistent inflammatory response that is not caused by infection. This condition can trigger by non-infection state (trauma, heat stroke, hypovolemic shock, immunologic response such as SLE) [10]. In Septic condition, the infection or necrotic tissue can cause a systemic inflammatory response by activating TLR-4 and binding with CD14 and MD2 for mediated LPS recognition; this complex further interacts and activates endothelial and smooth muscle cells. The mechanism in which the Gram-positive bacteria activates monocyte remains unclear, however, the CD14-dependent mechanism may play a role. Stimulation of lipopolysaccharide-containing endotoxin to monocyte and macrophage will induce the expression of pro-inflammatory cytokines (TNF-α, IL-1, IL-6) which then causes inflammatory reaction [9], [11].

The clinical operational definition of organ dysfunction is the increase of sequential organ failure (related to sepsis) shown by a sequential organ failure assessment (SOFA) score of 2 points or more, which is associated with in-hospital mortality >10%. Among critically ill patients with suspected sepsis, the predictive validity of the SOFA score for in-hospital mortality was superior to that of the SIRS criteria (area under the receiver operating characteristic curve 0.74 vs. 0.64). Patients who fulfill SOFA score have a predicted mortality of ≥10%. According to Levi *et al*., there is a quick SOFA score that are more applicable in clinical condition. It consists of (1) Temperature more than 38°C or <36°C; (2) Tachycardia (HR >90 bpm); (3) Tachypnea (RR >20 times per minute) or PaCO2 <32 mmHg; and (4) Leukocyte >12.000/μL or <4.000/μL or neutrophil >10% [12], [13].

This systemic inflammatory response that found in patients with preeclampsia can cause impairment of organ function which can induce sepsis [14]. In systemic inflammation response, TNF-α and IL-1 induce the PBMC to produce procalcitonin (PCT), where the increase of pro-inflammatory cytokines is followed by the increase of PCT release. Activated endothelial is probably associated to the increase of PCT level. PCT is a biomarker which is usually elevated significantly in patients with sepsis caused by bacterial infection [8].

This aimed of this study was to evaluate maternal plasma levels of TNF- α and PCT and to investigate their correlation in woman with preeclampsia and preeclampsia with sepsis.

Materials and Methods

Research design

This study was a cross-sectional observational study. There was no treatment given to subject, the characteristics of the subjects were then observed and analyzed. The data were explained as descriptive data. The analysis of variance (ANOVA) was used to test the dependence of cytokine level on several parameters. A $p < 0.05$ was considered statistically significant.

Sample and population

This study was design since period of May 2021 at Bangil Hospital, Pasuruan and the study was approved by the local Ethical Hospital Committee for scientific studies at University of Brawijaya. Three group of patients were studied: 18 normotensive pregnant woman as control, 18 preeclampsia and 18 preeclampsia that accompanied with sepsis. The diagnosis of preeeclampsia was established according to the International Society for the Study of Hypertension in Pregnancy [15]: Systolic blood pressure of more than 140 mmHg, diastolic blood pressure more than 90 mmHg on two different occasions after 20 weeks

of pregnancy and accompanied with maternal and neonatal complications proteinuria >2 g in a 24-h urine collection, blurred vision, epigastric or upper quadrant abdominal pain, vomiting or subnormal fetal growth). Blood pressure was measured with the blood pressure cuff placed on the left arm at heart level in sitting position. The pregnancies were >28 weeks of gestation which got Informed consent for this study.

Procedure blood samples

Venous blood samples (3 mL) from the patients were collected into EDTA vacutainers. The tubes then centrifuged at 1600 g for 15 min at a temperature 4°C. The supernatant plasma was separated and stored at −17°C.

The level of TNF-α was assessed using Human TNF-α enzyme-linked immunosorbent assay (ELISA) (Legend Max^{TM}). The minimum of detection of TNF-α was determined to be 3.5 pg/mL. Human PCT ELISA (Elabscience No: E-EL-H1492) method. The minimum detection of PCT was determined to be 0.01 ng/mL. All of these sampels were analyzed at Biomedical Laboratory Faculty of Medicine, University of Brawijaya.

Statistical analysis

Statistical analysis was performed by using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) statistical software. The normally distributed variable was analyzed with Shapiro-Wilk test and abnormally distributed variables were analyzed with Mann–Whitney U-test. Homogenity variable were analyzed with Levene test. The ANOVA was used to test the dependence of TNF-α and PCT level on several parameters. All p-value $\alpha \leq 0.05$ were considered statistically significant. Numerical data are presented as mean ± standard deviation (mean ± SD)

Results

Sample characteristics of the three groups are presented in Table 1.

Table 1: Sample characteristics

Item	Groups			p-value
	Control (n = 18)	PE (n = 18)	PE+sepsis (n = 18)	
Age (mean ± SD)	28 ± 6.71	26.44 ± 3.96	27.11 ± 7.09	0.745
GA (weeks), n (%)				
34–36	0	0	5 (9.3)	0.000
36–37	0	0	5 (9.3)	
37–38	7 (13)	7 (13)	6 (11.1)	
38–39	1 (1.9)	1 (1.9)	2 (3.7)	
39–40	10 (18.5)	10 (18.5)	0	
Parity, mean ± SD	1.89 ± 0.96	1.83 ± 0.79	1.44 ± 0.62	0.203
Systolic, mean ± SD	109.44 ± 8.73	164.44 ± 5.11	157.78 ± 9.43	0.000
Dyastolic, mean ± SD	73.33 ± 4.85	107.22 ± 8.26	95 ± 8.57	0.000

GA: Gestational age, PE: Preeclampsia, SD: Standard deviation.

There were no differences between the groups regarding maternal age (p = 0.745 (p > 0.05). Based on

gestational age (37–38 weeks GA) and blood pressure, there were significant differences (p = 0.000 (p < 0.05). Comparison of TNF-α in the three groups using ANOVA test was described in the Table 2.

Table 2: Mean tumor necrosis factor-alpha plasma level with analysis of variance

Group	Mean ± SD	p-value
Control	53.00 ± 26.22[a]	0.008
Preeclampsia	68.52 ± 34.42[a,b]	
Preeclampsia+sepsis	89.07 ± 42.20[b]	

Mean ± SD: When it contain with different subject show that was significant difference (p < 0.05) if it contain with same subject show that was no significant difference (p > 0.05). SD: Standard deviation.

One way ANOVA test showed that there was a significant difference in plasma level of TNF-α the p = 0.008 (p < 0.05) between each groups. Based on the comparison of TNF-α plasma level, mean plasma level of TNF-α in preeclampsia with sepsis group (89.07 ± 42.20) was significantly higher than the normal group (53.00 ± 26.22) and preeclampsia (68.52 ± 34.42).

The result of multiple comparison and least significant difference tests are described in Table 3. It showed that there was significant difference of mean value between control and preeclampsia + sepsis (p < 0.05). However, there was no significant difference between control and preeclampsia (p > 0.05). An increase, but not statistically significant was observed in plasma TNF-α level of the preeclampsia and preeclampsia + sepsis (p > 0.05).

Table 3: Comparison test of tumor necrosis factor-alpha with least significant difference 5%

Comparison	Difference mean	p-value
Control		
Preeclampsia	−0.253	0.132
Preeclampsia + sepsis	−0.540	0.002
Preeclampsia		
Preeclampsia + sepsis	−0.287	0.088

As shown in Table 4, Kruskal–Wallis test showed a significant difference of PCT level among those groups. The table showed that mean of PCT level was significant difference with p < 0.05 in preeclampsia + sepsis (1377.63 ± 277.04) versus normal (1123.97 ± 344.44) and preeclampsia (1148.69 ± 377.88).

Table 4: Mean procalcitonin level using Kruskal–Wallis and|Ri-Rj|5%

Group	Mean ± SD	p-value
Normal	1123.97 ± 344.44[a]	0.001
Preeclampsia	1148.69 ± 377.88[a,b]	
Preeclampsia + sepsis	1377.63 ± 277.04[b]	

Mean ± SD: When it contain with different subject show that was significant difference (p < 0.05) if it contain with same subject show that was no significant difference (p > 0.05). SD: Standard deviation.

Based on the result of |Ri-Rj| 5% test shown in Table 4, the normal patients group had the lowest mean PCT plasma level. However, the difference was not significant compared with the preeclampsia group. Compared to the preeclampsia + sepsis group, the mean 0.246 level of PCT in the preeclampsia + sepsis group was significantly higher than the normal group.

The correlation between of TNF-α and PCT level is described in Figure 1. It showed a scatter plot of association between TNF-α and PCT level. The scatter plot showed a linear pattern. Rank Spearman
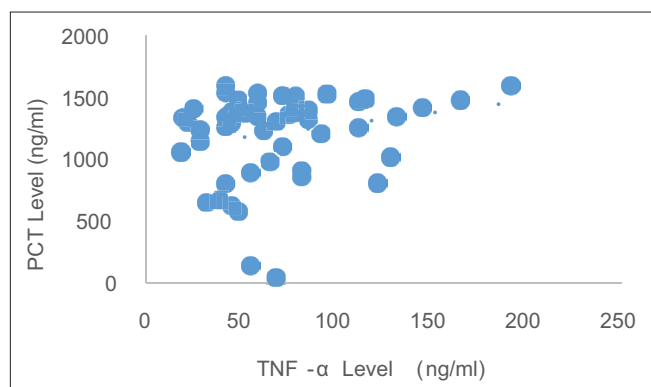
Figure 1: Scatter plot correlation between TNF-α and PCT level (r = 0.246)

correlation test showed a correlation coefficient of 0.246 with p = 0.073. The p > 0.05 (0.073) showed that there was no significant relationship between TNF-α and PCT plasma level.

Discussion

In this study, the plasma level of TNF-α and PCT were measured by ELISA method. There was an increase in TNF-α levels in plasma preeclampsia compared to the normal group but not significantly different. Several investigators have reported that serum concentration of TNF-α were significantly higher in the first and second trimester who subsequently developed preeclampsia compared to those in control group. Preeclampsia can occurred because of endothelial dysfunction which is caused by increasing level in pro-inflammatory cytokines, one of them is TNF-α. Although in normal pregnancy can stimulate a systemic inflammatory response, this is not enough to cause preeclampsia. Increased TNF-α levels in pregnancy with preeclampsia can caused by target cells (endothelial cells) have been damaged so that they cannot produce TNF-α but this still has to be proven by greater research. Type 1 cytokines including TNF- α are produce in preeclampsia induced inflammation. An abnormal stimulus from the placenta that experiences oxidative stress must be exceeded the inflammatory threshold [16].

The level of TNF-α in the preeclampsia with sepsis increased significantly compared to the normal and preeclampsia. There is increasing in possible relationship between endothelial dysfunction and infection, inflammation and preeclampsia. Infection may be a major risk factor for preeclampsia and it may cause increased cytokine levels sufficient to change vascular endothelial function and prime susceptible individuals for the future development of preeclampsia. According to Roudsari *et al*. there was an effect of genetic and environmental factors in preeclampsia. Some study

suggested that infection and inflammatory processes are related to preeclampsia. The role of inflammation and infection in the pathogenesis of preeclampsia is significant in developing countries, where the high incidence of chronic subclinical infection may contribute to the high incidence of preeclampsia [16].

As well as raised levels of inflammatory cytokines that constitute virtually a circular definition of inflammation, Douglas *et al*. have noted that all of these diseases are accompanied by dysregulation of iron metabolism, hypercoagulability and hypo fibrinolysis, blood microparticles, changes in the morphology of fibrin fibers and the role of a dormant blood and/or tissue microbiome, coupled in part with the shedding of highly inflammagenic bacterial components such as Gram-negative LPS and their Gram- positive cell wall equivalents such as lipoteichoic acids. There is strong evidence about a role for dormant microbes in the state of preeclampsia which subsequently leads to a state of sepsis. In this study, it was found that there were dormant bacteria and microbial tissue in the blood which would produce bacterial inflammatory components such as gram negative LPS and gram-positive lipoteichoic acid. Dormant bacteria are bacteria that is not viable and cannot be cultured. Bacteria in this situation have the possibility to reproduce in the right environmental conditions. One example is *Legionella pneumophila*, *Helicobacter pylori*, and *Coxiella burnetii* [17].

Increased TNF-α concentrations in preeclampsia with sepsis also associated with high changes in TNF-α and sepsis polymorphisms. In pathological conditions, TNF-α can remove damaged cells so that the condition of hemostasis can still be maintained. TNF-α originates from the TNF-α gene encoding. DNA damage can interfere with TNF-α function and give rise to conditions of inflammatory response and lead to cancer. In preeclampsia, the TNF-α gene was found in the major histocompatibility complex region on chromosome 6 p21.3. These genes creating several polymorphisms include microsatellite and single nucleotide polymorphisms (SNPs) [18].

Beside of that, it has been reported that phytohemagglutinin-stimulated IFN- γ production in PBMC in preeclampsia women is significantly higher compared to normotensive pregnant women. Elevated IFN-γ levels in pregnancy can be potentially harmful to the fetus. It is known that IFN- γ inhibits the outgrowth of trophoblast cells *in vitro* and synergistically stimulates the programmed death of primary villous trophoblast cells. Point mutations and SNPs in the regulatory regions of cytokine genes may affect cytokine transcription and influence its production [19].

In this study, there were significant differences between the levels of PCT in the preeclampsia group who had sepsis compared with preeclampsia. According to Linscheid *et al*., PCT is a precursor of calcitonin hormone found in C cells of the thyroidal gland and in extrathyroidal neurohormone cells. At the beginning of

sepsis, there will be an increase in the concentration of PCT. Under normal conditions without infection; PCT is only found in C cells in the thyroid gland. However, in the event of a bacterial infection, there will be an increase in the concentration of PCT produced by cells outside the thyroid C cells. This happens because if there is no infection, the transcription of extrathyroidal CALC-I genes will be suppressed. When bacterial infections occur, ion CALC-I genes from CT-RNA messenger will be expressed from various extrathyroidal neuroendocrine cells (parenchymal tissue) in the body so that all PCT molecules will spread systemically [20].

According Meisner, that during inflammation, PCT will also be produced by extrathyroidal cells. PCT concentrations in healthy subjects are <0.5 μg/L and will increase to 100 μg/L during acute bacterial, parasites, and/or fungal infections accompanied by systemic manifestations, even if there is no thyroid gland. One characteristic of PCT is that it is specific to bacterial endotoxins so that the concentration of PCT will not increase if a viral infection occurs. This means that PCT can distinguish whether the patient is infected with bacteria or viruses [21].

In a study conducted by Schuetz *et al.*, PCT levels found high in the circulation occurred in conditions of bacterial infection. PCT levels caused by viral infections and systemic inflammatory disorders tend to be low (<0.5 ng/mL). According to Schuetz, the mechanism for PCT formation caused by a systemic inflammatory reaction due to the presence of pro-inflammatory cytokines such as TNF-α, IL-6, IL-2, and IL-1 is still unclear. Further research is needed which can explain how PCT can be produced from various pro-inflammatory cytokines [22].

Polymorphism was also found in sepsis conditions, according to a study conducted by Yanjie *et al*. This study founded that there were multiple gene polymorphisms that occur in sepsis. This gene polymorphism is different in each country and race. According to Fu *et al.*, there a correlation between genetic factors and gene polymorphism in sepsis. For example, there is a TNF-α gene variant allele of rs 1800629 polymorphism that plays a role in the incidence of sepsis in 223 Han tribal populations in China, but the pathogenesis of sepsis still unclear [23].

In this study, there was no significant relation between TNF-α and PCT. Yanjie study showed that TNF is a kind of cytokine, it could regulate the immunologic function and mediated the inflammatory process. TNF includes two types (TNF-α and TNF-β) in accordance with resources. TNF-α is produced by multiple cells, and mainly in activated mononuclear macrophage. TNF-α is a cell signaling cytokine involving in inflammation. There are differences between TNF-α gene in preeclampsia or sepsis condition. TNF-α gene rs1800629 polymorphism correlated with sepsis susceptibility and TNF-α (G-308) are associated with preeclampsia [18], [23]. On the other hand, probably

because blood sampling did not take the microbial status of the subject. There is possibility that bacterial microbial status is in dormant or non-viable stage so that PCT levels did not significantly increase. Moreover, maternal and fetal genes interacting with each other interfere on the preeclampsia and outcome.

Based on these considerations, further studies are undoubtedly needed to clarify the association of genes polymorphisms and maternal cytokines production. In this sense, reproducing our findings in other populations will help defining the influence of genes polymorphisms and cytokine production in preeclampsia pathophysiology.

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