Research Article

High Level of Tumor Necrosis Factor (TNF)-α is a Risk Factor for Preeclampsia

Peningkatan Kadar TNF-α merupakan Faktor Risiko Terjadinya Preeklampsia

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Abstract

Objective: To define that elevated TNF-α serum level was the risk factor of preeclampsia in pregnancy.

Method: This research is a case-control study. From 56 pregnant women, there are 28 women with preeclampsia and the other 28 women with normal pregnancy. Then the serum level of TNF-α was obtained at Prodia's clinical Laboratory Denpasar. Data's normality test was done with Kolmogorov-Smirnov, then an analysis of data was done with Independent Sample Test, predictive value a = 0.05. To define the role of TNF-α level in preeclampsia, Chi-Square test was chosen.

Result: From this research we found the average level of TNF-α in preeclampsia (6.64 ± 7.64 pg/ml) was higher than in normal pregnancy (2.42 ± 1.77 pg/ml). Analysis with t-independent test shows that the t-value was 2.85 and p-value was 0.006, which means that the average level of TNF-α between the two group was significantly different (with predictive value, p<0.05). Based on cut-off value 2.42 pg/ml, the relative risk for preeclampsia was six time (RO = 6.33, IK 95%=1.97-20.34, p = 0.001) in patient with TNF-α level greater than 2.42 pg/ml.

Conclusion: TNF-α level in preeclampsia was significantly different with TNF-α level in normal pregnancy and the elevated serum level of TNF-α in pregnancy could be one of the risk for preeclampsia.

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Keywords: normal pregnancy, preeclampsia, TNF-α

Abstrak

***Tujuan**: Untuk mengetahui apakah peningkatan kadar serum TNF-α merupakan factor risiko terjadinya preeklampsia pada kehamilan.*

***Metode**: Penelitian ini merupakan studi kasus-kontrol. Dari 56 orang ibu hamil, didapatkan 28 ibu hamil dengan preeklampsia dan 28 dengan kehamilan normal. Selanjutnya dilakukan pemeriksaan kadar serum TNF-α di laboratorium klinik Prodia Denpasar. Dari data yang terkumpul dilakukan pengujian normalitas data dengan Kolmogorov-Smirnov, kemudian dilakukan analisis data dengan independent sample test dengan tingkat kemaknaan a=0,05. Untuk mengetahui peranan kadar TNF-α terhadap preeklampsia dipakai uji Chi-Square.*

***Hasil**: Dari penelitian ini didapatkan kadar rerata TNF-α pada preeklampsia 6,64 ± 7,64 pg/ml lebih tinggi dari kehamilan normal dengan kadar rerata TNF-α 2,42 ± 1,77 pg/ml. Analisis kemaknaan dengan uji t-independent menunjukkan bahwa nilai t = 2,85 dan nilai p=0,006. Hal ini berarti bahwa rerata kadar TNF-α pada kedua kelompok berbeda secara bermakna (p<0,05). Berdasarkan nilai titik potong 2,42 pg/ml, didapatkan bahwa risiko relatif terjadinya preeklampsia adalah sebesar 6 kali (RO = 6,33; IK 95% = 1,97-20,34; p=0,001).*

***Kesimpulan**: Kadar TNF-α pada preeklampsia berbeda secara bermakna dibandingkan dengan kadar TNF-α kehamilan normal. Dan adanya peningkatan kadar serum TNF-α pada kehamilan dapat berisiko terjadinya preeklampsia.*

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***Kata kunci**: kehamilan normal, preeklampsia, TNF-α*

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INTRODUCTION

Pre-eclampsia is a specific gestational disorder, which occupies approximately 3-5% from whole number of pregnancy. The manifestation seen in the pregnant mother varies from mild hypertension, severe/critical hypertension, eclampsia to HELLP syndrome (hemolysis, elevated liver enzyme, low platelet count). Meanwhile, in the fetus, the manifestation of this disorder also varies from premature birth, retarded fetal growth, to fetal death.

In the US, the number of incident of pre-eclampsia is approximately 5% of all pregnancy, and about 0.5-2% from them will develop into eclampsia, which is the second most common cause of death in pregnancy after thromboembolic disease. In Indonesia, the incident varies from 2.1-8.5%. In Sanglah Hospital Denpasar, from the study conducted by Ardhana (1997), the incident of preeclampsia is 1.8%. Meanwhile, Oka and Surya (2002-2003) reported that the incident is as much as 5.83% from 7552 number of labor within the duration of the study. By comparing these two studies, we can con-

clude that the incident of preeclampsia is increasing during the previous 3-4 years.[1]

Based on the Working Group of The National High Blood Pressure Education Programme (NHBPEP), the diagnosis of preeclampsia is established when there is an increment in blood pressure by ≥140/90 mmHg after the 20th week of gestational age, followed by proteinuria ≥300mg/24 hours or from dipstick test result of ≥1+.[2-5]

The early cause of preeclampsia is still unknown; the latest development tries to explain about an underlying molecular mechanism and most importantly the abnormal development, placental hypoxia, and endothelial dysfunction. Cytokine is a messenger protein secreted by inflammatory cell and immune cell, so as for growth factor, oncogene, chemokine, and another dissolved factor which influencing the differentiation of growth and viability of the cell. Cytokine is divided into 6 groups: interleukin, colony-stimulating factor, interferon, tumor necrosis factor, growth factor, and chemokine. Some type of cytokine is proven to be increased in preeclampsia and it probably can be used as a marker of preeclampsia.[4,6-7]

The inflammatory cytokines, such as IL-6 and TNF–α, are reported to be increased in preeclampsia. But the importance of those cytokine in mediating the cardiovascular and renal dysfunction, as the response towards placental ischemia during pregnancy is not fully established. From the study using pregnant mice, the important role of TNF–α and IL-6 in mediating hypertension and decreased renal hemodynamic can be observed during the reduction of uterine perfusion pressure.[8]

The purpose of this study is to describe the role of TNF–α in preeclampsia, since several studies reported contradictory result, where the correlation between the increase in serum level of TNF–α and the incident of preeclampsia is not significant.[10]

METHOD

The study design was case-control study non-paired. The population of the case and control group is pregnant mother with preeclampsia and mother with normal pregnancy, respectively, who came to Emergency Department and Polyclinic of Obstetrics and Gynecology of Sanglah Hospital Denpasar during the period of July 2010-2011. The inclusion criteria was pregnant woman who came to Emergency Department and Polyclinic of Obstetrics and Gynecology of Sanglah Hospital Denpasar with gestational age more than 20 weeks and willing to be included in the study. The exclusion criteria were pregnant woman with diabetes mellitus, renal disorder, cardiovascular disorder, chronic hypertension, premature rupture of the membrane, clinical sign of infection, twin pregnancy, and intrauterine fetal death. The patients who match all the criteria for the study sample was then further informed about the study detail and the consent was obtained.

The method for the serum level investigation of TNF–α was using the reagent set Quantikine HS Human TNF–α Immunoassay product R&D system Minneapolis with the standard range 0.5-32 pg/ml detection limit 0.106 pg/ml.[11] The data was collected and analysed using computer programme Statistical Product and Service Solution (SPSS) for Windows version 16.0.

RESULT

During the study, 56 pregnant patients were included as the sample of this research after fulfilling the inclusion criteria and devoid of exclusion criteria. They were further divided into case and control group, each consists of 28 mothers. The characteristic data of the subject from respective group are presented in Table 1.

Table 1. Age Average, Gestational Age, Parity, and Level of TNF–α from Case Group and Control Group

Variable	Pregnant		p
	Preeclampsia (Case) (n=28)	Normal (Control) (n=28)	
Age (yr)	29.04±7.66	26.36±5.45	0.137
Gestational Age (wk)	35.96±4.11	37.21±3.85	0.245
Parity	0.68±0.95	0.64±0.78	0.878
Level of TNF–α	6.64±7.64	2.42±1.77	0.006

Table 1 above showed that the average maternal age of the case group and control group is 29.04±7.66 years and 26.36±5.45 years, respectively. The average gestational age of the case group and control group is 35.96±4.11 and 37.21±3.85 weeks, respectively. The average parity of the case group and control group is 0.68±0.95

Table 2. The Risk of Incident of Preeclampsia on TNF–α

		Preeclampsia	Normal Pregnancy	OR	CI 95%	p
TNF-α	≥ 2.42	19	7	6.33	1.97–20.34	0.001
	< 2.42	9	21			

and 0.64±0.78, respectively. The average level of TNF–α of the case group and control group is 6.64 ±7.64 and 2.42±1.77. The analysis with indepedentt-test on the maternal age, gestational age, and parity, showed the p value of >0.05. This means that there were no differences in those three variables between the case and control group. Meanwhile, the level of TNF–α of both groups were significantly different with p = 0.006.

To identify the risk of incident of preeclampsia on the high level of TNF–α, Chi-Square test was used. The result is presented in Table 2.

Table 2 shows that the odd ratio of high level of TNF–α (≥ 2.42 pq/ml) is 6.33 (OR = 6.33, CI 95% = 1.97-20.34, p=0.001), meaning that subject with high level of serum TNF–α is 6 times more likely to suffer preeclampsia compared to the subject with low level of serum TNF–α.

DISCUSSION

Preeclampsia is a specific pregnancy disorder that complicates approximately 3-5% of pregnancy. The incident of preeclampsia in Indonesia varies from 2.1%-8.5%, from that number, 4.91% causing death. The early cause of preeclampsia is still unknown, the latest development explaining the molecular mechanism underlying the manifestation, most importantly placental hypoxia and endothelial dysfunction. The factor that present before the endothelial dysfunction such as increase of cytokine (IL-6 and TNF–α) on the preeclampsia patient which stimulates the activation of neutrophil and endothelial dysfunction, lipid peroxydase resulting from the breakdown of unsaturated lipid chain is increased in preeclampsia, sincytiotrophoblast microvillus membrane affecting the activity of neutrophil and luminal endothelial cell growth that further causing endothelial dysfunction.[12-13]

The over secretion of TNF–α will destroy the endothelial cell, causing blood vessel occlusion, reducing regional blood flow, and increasing the endothelial permeability. One of the possible mechanisms of preeclampsia is placental factor that induce the monocyte and neutrophil to produce TNF–α that causing endothelial disorder. Regarding these reasons, the increase of TNF–α serum level is assumed to be part of the pathogenesis of preeclampsia. In normal pregnancy, TNF–α can modify the growth and invasion of trophoblast on the maternal spiral arteries. Apart from them, this might contribute to abnormal formation of placenta, oxidative stress and endothelial disorder.[10]

In this research, based on the case distribution in both groups, the average maternal age for case group and control group is 29.04±7.66 years and 26.36±5.45 years, respectively. The average gestational age in case group and control group is 35.96±4.11 weeks and 37.21±3.85 weeks, respectively. The average parity in case group and control group is 0.68±0.95 and 0.64±0.78, respectively. The average TNF–α level in case group and control group is 6.64±7.64 and 2.42±1.77, respectively. The analysis of significance with t-independent test on the variable of maternal age, gestational age, and parity showed that the value of p>0.05. This means that those three variables did not differ between case group and control group. Meanwhile, on the variable of TNF–α level showed the value of p = 0.006. It means that the average TNF–α level in both groups vary significantly (p<0.05).

In the study conducted by Mihu, et al in 2008, they reported that the TNF–α serum level increased significantly in preeclampsia (14.15 pg/ml) compared to normal pregnancy (5.71 pg/ml), with p<0.001.[9] In 2003, a study conducted by Muzammil, et al in normal pregnancy, the TNF–α serum level is 9.3±0.56 pg/ml, meanwhile in preeclampsia is 67.66±61.83 pg/ml (p<0.001).[14]

Another research conducted by Afshari et al investigated another cytokine, i.e. IL-6. It is reported that the highest IL-6 serum level was found in preeclampsia compared to normal pregnancy, where the IL-6 serum level increased significantly in preeclampsia 5.8 (4.85) pg/ml compared to normal pregnancy, 3.01 (2.45) pg/ml (p=0.02).[15]

Based on the analysis result using ROC curve, the cut off point of TNF–α serum level between case group (preeclampsia) and control group (normal pregnancy) is 2.42 with sensitivity and specificity value of 75% and 68%, respectively. To identify the risk for incident of preeclampsia in the group with high level of TNF–α, Chi-Square test was chosen. It was found that the odd ratio of high TNF–α level (≥ 2.42 pg/ml) was 6.33 (OR = 6.33, CI 95% = 1.97-20.34, p=0.001). From these data, we concluded that the risk for preeclampsia in pregnancy with high level of TNF–α is 6 times higher. Considering those reasons, it is concluded that the increase in TNF–α serum level in pregnancy may put the mother at risk of preeclampsia. In this study, even though the risk of preeclampsia was found to be 6 times higher in TNF–α serum level of ≥ 2.42 pg/ml, the exact gestational age when the TNF–α level starts to increase and when the preeclampsia starts to develop in the condition of increased TNF–α serum level could not be identified.

In a study conducted by Williams, et al in 1998, it is reported that the odd ratio of TNF–α level (sTNFp55) in eclampsia is 5.00 (OR = 5.00, CI 95% = 1.20-20.92) and the odd ratio of sTNFp55 in preeclampsia is 2.37 (OR = 2.37, CI 95% = 1.11-5.06.[16]

In the study conducted by Sanchez, et al in 2000, it is reported that sTNFp55 serum level is 32.4% higher in the case of preeclampsia (920.1±30.4 pg/ml) compared to normal pregnancy (694.8±15.0 pg/ml, t-test analysis p<0.001), with the odds ratio in preeclampsia is 10.3 (OR = 10.3, CI 95% = 4.1-25.9).[17]

The difference in study design, gestational age at the time of sampling, characteristic of subjects, number of study population, and examination method used by other researcher may give different result from this study. We use reagent set Quantikine HS Human TNF–α Immunoassay product R&D system Minneapolis with standard range of 0.5 - 32 pg/ml. detection limit 0,106 pg/ml as the method of TNF–α serum level investigation.[11] In a study conducted by Mihu, et.al. in 2008, the method used in investigation of TNF–α serum level is the immunometric sandwich EIA, human TNF–α EIA kit 589201, Cayman Chemical Company, USA, with detection limit of 1.5 pg/ml.[9] Therefore, this may be one of the factors causing the gap in the result of study conducted by another researcher and this study.

In this study, the huge increase in TNF–α serum level (≥ 2.42 pq/ml) in a pregnancy can increase the risk of preeclampsia by 6 times. Understanding the risk factor of preeclampsia is very useful in healthcare system for pregnant women to observe the risk group in the early phase of pregnancy. It should be used as a preeclampsia predictor and one of the supporting diagnostic tools and as a predictor for prognosis of preeclampsia. In the subsequent study, a more variable and greater number of samples and longer duration of study are needed to get more accurate and complete result regarding the TNF–α as predictor of incident of preeclampsia.

CONCLUSION

TNF–α serum level differs in preeclampsia compared to normal pregnancy and there is statistically significant difference between the two groups (p<0.005), where the average TNF–α level in preeclampsia is higher than in normal pregnancy. The huge increase in TNF–α serum level (≥ 2.42 pq/ml) in pregnancy can increase the risk of incident of preeclampsia by 6 times.

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