Research Report

IL-10 Serum Concentration was Observed Higher in Threatened Preterm Labor

Kadar serum IL-10 lebih tinggi pada kasus persalinan preterm

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Abstract

Objective: To determine the serum concentration of Th-1 and Th-2 cytokine in threatened preterm labor compared to that in normal pregnancy.

Method: The design was analytical cross-sectional, comparing the serum levels of TNF-α, IL-2, IL-10, TNF-α to IL-10 ratio and IL-2 to IL-10 ratio between 29 subjects with threatened preterm labor and 29 normal pregnant women. The cytokine concentration was measured with ELISA. T test and Mann-Whitney U test was used for statistical analysis.

Results: The mean concentration of TNF-α and IL-2 in both groups did not reveal any difference (p = 0.188 and p = 0.493). Median of IL-10 serum concentration in the threatened preterm labor (PTL) group was observed higher than that observed in the normal pregnancy group (p = 0.001). Compared to normal pregnancy group, the TNF-α to IL-10 ratio in the PTL group was observed lower (p = 0.009). Both groups did not show any difference in the IL-2 to IL-10 ratio (p = 0.057).

Conclusion: The IL-10 serum concentration was increased in threatened preterm labor. There was no difference observed in the Th-1 to Th-2 cytokines serum ratio in threatened preterm labor as compared to normal pregnancy.

[Indones J Obstet Gynecol 2010; 34-4: 191-4]

Keywords: IL-10, preterm labor, Th-1 and Th-2 cytokines

Abstrak

***Tujuan**: Untuk menilai kadar sitokin Th-1 dan Th-2 dalam serum perempuan hamil dengan ancaman persalinan preterm dan dibandingkan dengan kadar tersebut pada perempuan hamil normal.*

***Metode**: Kajian potong lintang, yang membandingkan kadar TNF-α, IL-2, IL-10, nisbah TNF-α dengan IL-10 dan nisbah IL-2 dengan IL-10, pada 29 subjek penelitian perempuan hamil dengan ancaman persalinan preterm dan pada 29 perempuan hamil normal. Kadar sitokin diukur dengan teknik ELISA. Uji statistik dilakukan dengan menggunakan uji T dan uji Mann-Whitney.*

***Hasil**: Tidak dijumpai perbedaan yang bermakna dari rerata TNF-α dan IL-2 dari kedua grup (p = 0,188 dan p = 0,493). Konsentrasi median dari IL-10 serum perempuan hamil dengan ancaman preterm dijumpai lebih tinggi dibandingkan dengan kadar tersebut pada kehamilan normal. (p = 0,001). Dibandingkan dengan kehamilan normal, maka kehamilan dengan ancaman persalinan preterm menunjukkan nisbah TNF-α dengan IL-10 yang lebih rendah. Tidak terdapat perbedaan pada nisbah IL-2 dan IL-10 antara kedua grup.*

***Kesimpulan**: Kehamilan dengan ancaman persalinan preterm menunjukkan kadar IL-10 yang lebih tinggi dibandingkan dengan kehamilan normal. Tidak terdapat perbedaan nisbah sitokin Th-1 dan Th-2 pada kedua group.*

[Maj Obstet Ginekol Indones 2010; 34-4: 191-4]

***Kata kunci**: IL-10, persalinan preterm, sitokin Th-1 dan Th-2*

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INTRODUCTION

Prematurity is one leading cause in perinatal morbidity and mortality.[1] Although some theories and assumptions had ever been reported for the mechanism of preterm labor, however, the definite pathophysiology of preterm labor remains uncovered well until now. Over few decades, the incidence of preterm labor seems never to be reduced significantly.[1-4] In Indonesia the incidence of preterm labor is around 10 - 20%.[4]

Intrauterine infection is considered as the most frequent cause of preterm labor, however, it is only proven in 50% cases which shows increasing levels of pro and anti-inflammatory cytokines.[5] Even in the absence of infection, there are increasing productions of pro and anti-inflammatory cytokines in maternal circulation.[6]

Cytokines have a contribution in maintaining the continuation of successful pregnancy.[7,8] IL-2, IFN-γ and TNF-α belong to pro-inflammatory cytokine Th-1 group, that is reported harmful to pregnancy, while on the other hand Th-2 cytokines including IL-4, IL-5, IL-6, IL-10 and IL-13, are believed to be conducive to pregnancy.[8] Pregnancy is a Th-2 phenomenon. Studies postulated that the balance concentration between Th-1 and Th-2 cytokines would be more valueable in maintaining the continuation of pregnancy instead of their exact concentration.[8] But others investigator found no changes in ratio levels of Th-1/Th-2 neither in preterm labor nor in recurrent abortion.[9] Th-1 had been proven to be important in murine pregnancy.[10] Another study demonstrate that IL-10 which was believed to be important in pregnancy maintenance, actually has a paradoxical effect toward promoting labor initiation.[11]

Only 30% patients diagnosed clinically as having threatened preterm labor, gave birth within 24 - 48 hours. It is important to combine clinical manifestation with biophysical and biochemical sign in order to make optimal prediction.[1,12]

The objective of this study is to compare the concentration of serum Th-1 and Th-2 cytokine in threatened preterm labor to normal pregnancy. We expect this study might explain the correlation between some cytokines and incidence of preterm labor.

MATERIAL AND METHOD

Subjects

The study design was analytical cross-sectional, comparing the serum level of TNF-α, IL-2, IL-10, TNF-α/IL-10 ratio, IL-2/IL-10 ratio between threatened preterm labor group (PTL) and reference group (normal pregnancy). Each group consisted of 29 pregnant women, age 19 to 40 years old, who came to obstetric emergency unit and obstetric polyclinic in Dr. Cipto Mangunkusumo Hospital and Fatmawati Hospital. Threatened preterm labor was defined as pregnancy ≥ 28 weeks and ≤ 36 weeks with painful contraction 4 times in 20' or 8 times in 60' and either one of cervical dilatation ≥ 2 cm, cervical effacement ≥ 50% or progressive cervical changes. All of the subjects were having singleton pregnancy, normal fetus, no hydramnios, placenta praevia nor abruptio placentae. They did not suffer from preeclampsia, malignancy or cardiovascular diseases and were non smokers. This study was approved by medical ethical committee, fa-culty of medicine, university of Indonesia. Informed consent was obtained before blood samples were collected.

Serum Preparation

A 5 cc of maternal venous blood was collected in SST tube with clot activator. After centrifugation, the serum was removed into polypropilene tube and stored in -80°C until the time of cytokine assay.

Determination of Cytokine Concentration

Serum TNF-α, IL-2 and IL-10 concentration was assayed with ELISA kit from R & D system, Minneapolis. Microplates covered with specific monoclonal antibody for TNF-α, IL-2 and IL-10 were used. Serum was pipetted into the wells on the microplates. TNF-α, IL-2 and IL-10 would bind to the antibody. The wells were washed to remove unbound substances. For TNF-α and IL-2, specific polyclonal antibody was added, while specific monoclonal antibody was added for IL-10. After the wells were washed again, substrate solution was added. The colour intensity was measured using microplate reader (BioRad) with optical density between 450 nm and 540 nm.

Statistical Analysis

The Mann-Whitney U test was used for non-parametric comparisons of median IL-10 concentrations because the concentrations did not follow a normal distribution. The concentration of TNF-α, IL-2, ratios of Th-1 to Th-2 cytokines were compared using t test.

RESULT

This study was held between January 2006 and November 2006. TNF-α was chosen to represent Th-1 cytokine due to a study by Sato et al, that demonstrate TNF-α as the principal regulator in IL-10, IL-1ß and PGE_2 production in LPS stimulated inflammation.[2] IL-2 which also belong to proinflammatory cytokine group, was also increase in preterm labor especially in intrauterine infection.[13] IL-10 was chosen to represent Th-2 cytokine in this study, because some studies revealed that IL-10 inhibits TNF-α action and synthesis.[2]

There were no significant differences between maternal age. The gestational age were almost similar in the two groups. 10% of the subject in PTL group had history of previous preterm labor, but not significantly different with the control group. The demographic data of the subject are outlined in Table 1.

Table 1. Maternal demographic characteristic.

Data	Reference group n = 29	PTL group n = 29	p value
Maternal age	29.90 ± 4.28 years	27.66 ± 4.45 years	0.056
Gestational age	32.00 ± 1.94 weeks	32.55 ± 1.88 weeks	0.945
History of preterm labor	0 (0%)	3 (10%)	0.503

The evaluated Th-1 cytokines were TNF-α and IL-2. The mean concentration of TNF-α in PTL group were higher than the reference group, but did not reach significancy with p = 0.188. The mean IL-2 concentration in PTL group were lower than in the reference group, but also were not significantly difference, with p = 0.439.

Since, the data of IL-10 concentration did not show a normal distribution, therefore Mann-Whitney U test was used in this study. The median concentration of IL-10 serum in PTL group were significantly higher compared to the reference group, with p = 0.001. The difference between mean and median concentration of the cytokines are outlined in Table II and III.

Table 2. Mean concentration of serum TNF-α and IL-2 in threatened preterm labor compared to normal pregnancy.

Serum concentration (pg/ml)	PTL and Reference	Mean (pg/ml)	Deviation standard	95% CI	p value
TNF-α	PTL	10.32	2.73	9.28 - 11.36	0.188
	Reference	9.45	2.20	8.60 - 10.29	
IL-2	PTL	7.98	5.53	5.87 - 10.08	0.439
	Reference	9.17	6.12	6.81 - 11.49	

Table 3. Median concentration of serum Th-2 (IL-10) in threatened preterm labor compared to normal pregnancy.

	IL-10 serum concentration (pg/ml)				
	Median	**Min**	**Max**	**95% CI**	**p value**
PTL	35.56	20.95	234.09	33.73 - 70.84	0.001
Reference	27.08	15.05	39.29	24.94 - 29.29	

In this study, we also compared the ratio of TNF-α/IL-10 and IL-2/IL-10. TNF-α/IL-10 ratio in PTL group were significantly lower than in the reference group, with p = 0.009. While IL-2/IL-10 ratio in the two groups were not different significantly with p = 0.057. The comparison of the ratios are outlined in Table IV.

Table 4. Comparison of Th-1/Th-2 ratios in threatened preterm labor and normal pregnancy.

Ratio	**PTL and Reference**	**Mean**	**Deviatio standard**	**95% CI**	**p value**
TNF-α/IL-10	PTL	0.28	0.13	0.23 - 0.33	0.009
	Reference	0.36	0.11	0.32 - 0.40	
IL-2/IL-10	PTL	0.23	0.22	0.15 - 0.31	0.057
	Reference	0.35	0.25	0.26 - 0.45	

Nineteen from 29 (65.5%) subjects in PTL group gave birth in 24 hours after diagnosed. The data in this study were the cytokine level after clinical signs of preterm labor had occured, while the inflammation process should have been begun before. But in this study we could not reveal the time needed from the beginning of inflammation process to the clinical manifestation.

DISCUSSION

Influenced by different cytokines and immunological interactions in the feto-maternal interface, pregnancy can be viewed as a condition with a bias or deviation in immunological status, in which maternal immune response differ quantitatively and qualitatively during pregnancy.[14]

Studies demonstrate that immunological mechanism plays important role in the maintenance of pregnancy. In pregnancy, there is a bias toward Th-2 domination, known as Th-2 paradigm.[8,15,16] Th-1 cytokine concentration was found to be higher in patients with history of recurrent abortion compared to normal patients.[7,15-18] Meanwhile, others found that Th-1 cytokines that is dangerous for pregnancy in the second and third trimester, were needed in early pregnancy and during labor.[14,19]

In this study, we did not found significant differences in TNF-α and IL-2 concentration. A study by Vassiliadis et al showed that cytokine Th-1 and Th-2 profile was stable during every stages of pregnancy and parturition.[20] IL-10 which was considered to be protective to pregnancy, still constant in the first two trimesters and reach maximal production during parturition. While in abortion, Th-1 cytokine concentration increased, but no significant changes in cytokine Th-2 concentration.[20]

We found no bias toward cytokine Th-1 in threatened preterm labor subjects. This result differs from some other studies.[7,15-18] The limitation of those studies was that the samples had recurrent abortions in which their immune system might have been primed with difference mechanism from normal pregnancy.[21] We also have to consider the weakness in measuring TNF-α concentration with ELISA, because bioactivity measurement will be more accurate than measuring bioactivity with ELISA.[14]

IL-10 serum concentration in the PTL group was significantly higher than in the reference group. Higher IL-10 concentration in subjects with threatened preterm labor might be caused by: 1) increasing IL-10 level was a response to increasing concentration of Th-1 cytokine other than TNF-α and IL-2; 2) high concentration of IL-10 as an antiinflammatory cytokine, in turn might accelerate parturition (paradoxical effect); 3) IL-10 itself initiates parturition.

A study by Mitchell concluded that amniotic response to IL-10 demonstrate a self-defence mechanism, in which acceleration of parturition was a respons to inflammation that had reached fetal side (amnion).[11]

Eventhough the presence of IL-10 can be detected, but in human it is not always express the Th-2 profile because IL-10 can also be produced by Th-0, 1, 2 and non-T cells, although the inhibition effect is still dominant.[22]

It would be better if in this study, we can prove whether there was any infection in chorioamnion or amniotic fluid. But it was not possible to obtain samples from chorioamnion and amniotic fluid since the action will be invasive and the examination was only performed at one time, that was when we diagnosed the patient had threatened preterm labor.

Many literatures concluded that Th-1/Th-2 cytokine ratio was more important than each concentration in maintaining pregnancy.[8] This study demonstrate that TNF-α/IL-10 ratio in PTL group was significantly lower than in reference group, while IL-2/IL-10 ratio in both groups showed no significant difference. The lower TNF-α/IL-10 ratio in PTL group was the result of marked increase in IL-10 concentration.

Some published literatures stated that Th-1/Th-2 paradigm in human was challenged.[7,21,23] Vince at al concluded that local blockade of Th-1 response at the level of IL-2 might be more effective in maintaining maternal toleration to intrauterine fetal allograft than any bias toward a Th-2.[23] Sacks et al reported that in pregnancy, there was monocytes activation that would lead to IL-12 production, while TNF-α production was not change eventhough the monocytes had been stimulated by endotoxin.[24]

Chaouat et al also proposed that Th-1/Th-2 concept implied in a steady state balance that was not fit with immunology profile during implantation nor during the preparation of parturition.[21]

In our study, the cytokine concentration was measured in serum. Although we found some studies that also measured cytokine level in serum or peripheral blood cultures, but the inflammation process that resulted in preterm labor acts locally. The systemic cytokine level might not always represent the cytokine level in amniotic fluid, decidua nor myometrium.[25]

CONCLUSION

We found no significant increase in serum proinflammatory cytokine TNF-α and IL-2, while the antiinflammatory cytokine IL-10 was significantly higher in patient with threatened preterm labor. Th-1/Th-2 ratio did not always increase in threatened preterm labor compared to normal pregnancy. It would be much better if in this study we also measure other cytokines that belong to Th-1 and Th-2 group, and also perform histopathological examination in order to correlate chorioamnionitis to cytokine Th-1 and Th-2 concentration.

ACKNOWLEDGEMENT

The authors thank Dr. Enud J. Suryana, ObGyn and Prof. Ali Baziad, ObGyn for providing the kits and laboratory facilities. We also thank Mondastri K, MS, DSc for assistance in statistical analysis.

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