



RESEARCH ARTICLE

Differences in Serum IL-6 Levels Between First-Trimester Normal Pregnancy and Spontaneous Abortion

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Abstract

Objectives: In spontaneous abortion cases without previously known predisposing factors, an increase proinflammatory cytokines, especially interleukin 6 (IL-6), found in local and systemic specimens compared to normal 1st -trimester pregnancies. Therefore, IL-6 is thought to have a role in implantation failure in early pregnancy resulting in spontaneous abortion. However, research on serum IL-6 levels in spontaneous abortion patients is still limited in Indonesia. This study aims to determine the difference in serum IL-6 levels in normal pregnancy 1 st - trimester and spontaneous abortion in hospitals in Padang City;

Method: This study is an analytical study with a cross-sectional design using secondary data of 21 blood serum samples from each research group at the Biomedical Laboratory of FK Unand. Samples were collected using a consecutive sampling technique. A serum examination was performed with the ELISA technique. Bivariate data analysis using Mann-Whitney statistical test based on data type and distribution;

Result: The results showed that the mean value of serum IL-6 levels was 280,56 ng/L in the normal pregnancy group in the 1st -trimester and the median value of the spontaneous abortion group was 279,78 ng/L. There was no significant difference in serum IL-6 levels in normal pregnancy in the 1st - trimester and spontaneous abortion ($p = 0,980$);

Conclusion: This study concluded that there was no difference in serum IL-6 levels between patients with normal pregnancy in the 1st -trimester and spontaneous abortion in hospitals in Padang City.

Keywords: Spontaneous abortion; first trimester; serum interleukin 6; cytokines; ELISA

INTRODUCTION

In every normal pregnancy, an innate immune cell response will occur during implantation on the fetomaternal surface, resulting in an inflammatory process.¹ The presence of leukocyte cells is considered to be an immune response to paternal antigens expressed during infiltration of trophoblast cells into the maternal endometrial decidua. This innate immune cell response is necessary for successful implantation and pregnancy, but an increase in the number of infiltrating leukocyte cells can cause pregnancy complications, especially in the early trimester.²⁻⁵

Failure of fetal growth in the first-trimester phase can lead to abortion. In habitual abortion patients, there was a decrease in mRNA expression for IL-6, but an increase in uterine synthesis and secretion of IL-6 was found during the menstrual cycle, especially in the mid to late secretory phase of the endometrial cycle. This study used endometrial epithelial and stromal cell specimens using immunohistochemical techniques and RNAase protection assay. Uterine secretion can also be used using the ELISA technique.⁶ At the stage of endometrial destruction and reconstruction, angiogenesis occurs and IL-6 is expressed temporarily during this process. The next process is the formation of folliculogenesis and decidua early post implantation if fertilization occurs, but IL-6 production is physiologically inhibited by estrogen and/or progesterone. In addition, it was found that the effect of stopping zygote mitosis until the morula phase, blastocyst implantation, until failure of embryo development or abortion on the incidence of increased IL-6 indicates that IL-6 has an inhibitory effect on the preimplantation process of the embryo.⁷

According to world epidemiological studies, spontaneous abortion is the most common complication in pregnancy specifically in developing countries. The incidence of abortion reaches 15-20% of all pregnancies with 80% of abortions occurring at gestational age before 13 weeks (first-trimester).^{8,9} In 2008, there were 21,600,000 unsafe abortions worldwide and 21,200,000 in developing countries according to WHO.⁸ In Indonesia, the incidence of spontaneous abortion is 500,000-750,000, and artificial abortion is 750,000-1,500,000 annually.^{10,11} If we take into account unknown pregnancies, such as very early pregnancy and a few days late menstruation, the number of spontaneous abortions is 2,5 times higher than the data presented.

Risk factors such as maternal age, trisomy chromosomal abnormalities, alcohol, smoking, and methylxanthine consumption are directly related to the incidence of spontaneous abortion.^{9,12} In early pregnancy, the most common cause of abortion is chromosomal abnormalities.⁹ In addition, cytokines are also thought to have a role in the occurrence of spontaneous abortion in pregnant women. This is proven by the findings of significant IL-6 expression during endometrial growth, embryo implantation, trophoblast growth and development, and also angiogenesis of the formation of chorionic villi. In this case, it is suspected that the increase in IL-6 expression is directly proportional to the risk of spontaneous abortion based on previous research in abortive murrines there was an increase in local IL-6 levels from placental and decidual specimens by flowsitometry and immunohistochemical methods compared to normal pregnant murrines.¹³ However, there was no significant difference in

systemic IL-6 levels from blood specimens using ELISA methods.¹³

IL-6 expression is found since preimplantation in the endometrial cycle during the phases of menses, proliferation, and secretion as well as during the angiogenesis process during folliculogenesis and endometrial decidual formation early after implantation.^{2,14} Uterine stromal tissue and endothelial cells are also found to excrete IL-6, but production by these cells inhibit by estrogen and or progesterone. Trophoblast cells also produce IL-6 which stimulates the secretion of human chorionic gonadotropin (HCG) through a different pathway involving gonadotropin-releasing hormone (GnRH). As HCG functions to stimulate the corpus luteum to continue producing progesterone during the first 6-8 weeks of pregnancy, it also plays a role in uterine tissue in maintaining a large number of blood vessels. Placenta derived IL-6 may play a major role in the initiation of the angiogenic process.⁷ The capacity of HCG to support cytotrophoblast development has also been shown, so IL-6 is mentioned as a trophoblast growth factor because of its ability to stimulate HCG release.

IL-6 levels can be identified through local (vaginal cervical fluid or amniotic fluid) or systemic (blood) specimens through supporting examinations. IL-6 can be a cytokine indicator for the diagnosis of intraamnion infection. In normal pregnancy in the 2nd or 3rd trimester, IL-6 levels in amniotic fluid are detected but in low levels, namely 50-1500 pg/ml. Whereas in preterm labor with intraamnion infection, there is a significant increase in IL-6 levels to 5 µg/ ml, with 1 µg = 1 million pg. Riddhi Thaker, et al 2021 conducted research on 126 subjects, namely 63 spontaneous abortus women and 63 were at least primiparous women without spontaneous abortus as controls to see differences in IL-6 and IL-10 levels of the two subjects. Mean IL-6 levels were higher among women with spontaneous abortion, recurrent spontaneous abortion, and controls.¹⁵ Spearman correlation showed that IL-6 was positively correlated with the number of spontaneous abortion events.¹⁶ However, in Indonesia, there are still limited data showing significant IL-6 levels in the systemic circulation during 1st trimester of pregnancy. Increased levels of IL-6 in the blood during pregnancy lead to the presence of the fetus, as well as due to the effects of pregnancy hormones.

Based on previous studies, the researchers hypothesized that the difference in cytokine concentrations, especially IL-6, in normal pregnant women and women with 1st-trimester abortion without a clear etiology may be related to the mechanism causing abortion and may be indicative of the involvement of the immune system mechanism.

METHODS

This type of research is an analytical observational study with a cross-sectional design that first-trimester spontaneous abortion patients as the study group and normal pregnancy women in the first-trimester as a control group. The location of the research study was carried out by Biomedic Laboratory Faculty of Medicine Andalas University in December 2022 - November 2023, which is to start conducting initial surveys, literature searches, data collection, and analysis, as well as the final thesis report.

The samples of this study used blood serum stored in the Biomedical Laboratory of the Faculty of Medicine, Andalas University which had been taken from research on Pengaruh Kadar Vitamin D dan Tumor Necrosis Factor Alpha terhadap Kehamilan Normal Trimester 1 dan Abortus Spontan with ethical number 717/UN.16.2/KEP-FK/2022 which met the inclusion and exclusion criteria until a minimum sample size of 40 is reached.¹⁷ The independent variable of the study was serum interleukin 6 levels and the dependent variable of the study was normal pregnancy in the first trimester and spontaneous abortion. Each group requires 20 samples minimum to obtain important clinical results and meaningful statistical test. Researcher succeeded in collecting 21 samples in each group for this research. The data collected were subjected to statistical analysis with a computer program. Bivariate analysis began with normality analysis using the Shapiro-Wilk test to see the relationship between normal pregnancy and spontaneous abortion with interleukin 6. Based on the normality test using Saphiro-Wilk method, it was found that the serum levels of interleukin 6 in the first trimester normal pregnancy group were normally distributed ($p = 0,523$), but in the spontaneous abortion group, the data showed that is were not normally distributed ($p = 0,000$). Therefore, bivariate analysis was continued with the Mann-Whitney test to determine differences in serum interleukin 6 levels between the two groups. This research was conducted after obtaining ethical clearance from the research ethics committee of the Faculty of Medicine, Andalas University with ethical number 475/UN.16.2/KEP-FK/2023.

RESULT AND DISCUSSION

Table 1 shows the difference in optical density (OD) values in the standard serum which is influenced by the number of antibodies that bind to the serum. The fewer antibodies that bind to the serum, the fewer conjugates that bind, resulting in less visible color changes. The color density will be proportional to the OD value.¹⁸

Table1. Standard Absorbance Value

Standard	Concentration(ng/L)	Value OD
1	0	0,047
2	20	0,050
3	40	0,054
4	80	0,072
5	160	0,112
6	320	0,180

ELISA examination on each groups using the Human Interleukin 6 ELISA kit with Cat.No E0090Hu and there was an extreme increase in serum interleukin 6 levels in three serum samples of the spontaneous abortion group with sample codes A03, A10, and A14, which were 1088.293 ng/L, 1183.275 ng/L, and 1035.010 ng/L, respectively. This situation can be influenced by the process of taking, handling, and storing samples. The difference in distance from sample collection to the centrifugation process could have an effect because centrifugation is thought

to be another factor in the activation of immune cells in the sample. Serum samples stored at 37°C for at least 8 hours showed an increase in serum interleukin 6 levels by more than nine times. Even in previous studies, serum interleukin 6 levels can increase hundreds of times after prolonged storage at room temperature (20-25°C) or in an incubator.¹⁹ In addition, Table 2 shows the mean serum interleukin 6 levels in the normal pregnancy group in the first trimester and spontaneous abortion.

Table2. Mean Interleukin 6 Levels

Kadar Serum Interleukin 6	N	Mean $\pm$ SD	Min	Max
Normal pregnancy in the 1 st trimester	21	280,56 $\pm$ 30,60	219,55	335,38
Spontaneous abortion	21	440,63 $\pm$ 326,99	194,07	1183,28

The results of the Saphiro Wilk normality test on serum interleukin 6 levels in the normal pregnancy group in the first trimester showed a $p > 0.05$ value ($p = 0.523$), which means the data were normally distributed. However, in the spontaneous abortion group, the p -value is < 0.05 ($p = 0.000$) which indicates that the data is not normally distributed. After transforming the data scale with the mathematical functions Ln and sqrt, the p -value is < 0.05 , which means that the data is still not normally distributed. Because one of the data groups was not normally distributed, the bivariate analysis was continued with the Mann-Whitney test to determine the difference in serum interleukin 6 levels in normal pregnancy in the first trimester with spontaneous abortion.

Table3. Mann-Whitney Test

Variabel	Group				P value
	Normal pregnancy		Abortus		
	N	Mean	N	Median (min-max)	
Interleukin 6	21	280,56	21	279,78 (194,07 – 1183,28)	0,980

This study is not in line with other studies in 2018, where serum interleukin 6 levels were found to be higher in the recurrent spontaneous abortion group (habitual abortion) than in the normal pregnancy control group without a previous history of spontaneous abortion, and there were significant differences.²⁰ Interleukin 6 is a premature and sensitive indicator of inflammation, and in all stages of reproduction plays an important role in determining the success of pregnancy, especially in early pregnancy. Interleukin 6 levels were found to be higher in the spontaneous abortion group with seropositive anti-Toxoplasma antibodies than in the normal pregnancy control group in the same study, suggesting that the increase in inflammatory cells and the role of interleukin 6 in preventing infection with Toxoplasma contribute to the

transformation of the acute state into chronic inflammation. This increase in interleukin 6 levels is considered an early marker of infection in pregnancy before the modification of Th-2 cells into Th-1 cytokines that further induce inflammatory and cytotoxic reactions and are responsible for some abortions.²⁰

There is no fixed limit regarding serum interleukin 6 levels said to be elevated in pregnancy. In a 2020 study that found a significant difference in higher serum interleukin 6 levels in spontaneous abortion compared to controls, the mean level was 121.34 pg/ml or equivalent to 121.34 ng/L.¹⁵ This value is far below the average of the spontaneous abortion group and normal first-trimester pregnancy in this study. Research in 2019 in Bukit Tinggi related to serum interleukin 6 levels in preterm labor and normal pregnancy showed that the average level in preterm labor was significantly higher at 90.22 ng/L.²¹ This value is also below the average of the spontaneous abortion group and normal pregnancy in this study.

Patients who experience spontaneous abortion may show changes in systemic cytokine levels, compared to women whose pregnancies are uneventful, although results are highly variable. Increased concentrations of IL-6 in plasma and serum, as well as greater expression in peripheral blood mononuclear cell (PBMC) cultures, were found in spontaneous abortion and habitual abortion patients compared to the control group. Unchanged or decreased levels of interleukin 6 in the serum of spontaneous abortion and habitual abortion patients were also demonstrated.²² These inconsistent findings may be explained, at least in part, by methodological differences between studies (inclusion criteria of participants, gestational age, inclusion of spontaneous abortion and habitual abortion patients in the study, control group assigned, and others).

In summary, it can be concluded that circulating interleukin 6 levels fluctuate during pregnancy. This may reflect ongoing immune modulation throughout pregnancy, consistent with the goal of pregnancy maintenance. Identifying typical patterns in the trajectory of immune parameters during pregnancy should allow recognition of relevant deviations and prediction of adverse perinatal outcomes. However, the comparison of absolute cytokine levels between studies is often inconclusive in practice. The large number of inconsistent data may reflect methodological inconsistencies between studies and differences between assays (i.e., detection of different amounts of free and or bound cytokines, use of plasma or serum, differences in population size-statistical power, population characteristics), and other factors that can not be corrected. Conflicting results may also be related to several factors that influence cytokine levels, such as age, ethnicity, genetic polymorphisms and epigenetic marks, fetoplacental sex, prepregnancy BMI, HbA1c, diet, smoking, gut microbiota, and others.²³

CONCLUSION

In this study, there was no difference in serum interleukin 6 levels between normal pregnancy in the first trimester and spontaneous abortion in hospitals in Padang City. Differences in results between these studies can be caused by several factors, such as the large variety of samples used, methodological differences used, differences between tests carried out,

and a number of other factors that have not been excluded that influence cytokine levels between individuals. It is recommended that further research assess other parameters of Th-1 and Th-2 cytokines such as IL-2, IFN- γ , and TNF- β and their relationship which from the last study was found to be strongly related to the success of a pregnancy in the first trimester through its mechanism during the process of implantation and placentation and prevent the incidence of spontaneous abortion. The type of specimen used can also be used local samples that are minimally invasive for patients such as cervical or vaginal secretions.

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