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RESEARCH ARTICLE

DIFFERENCES OF *FIBRONECTIN* LEVELS IN FIRST TRIMESTER NORMAL PREGNANCY AND MISCARRIAGE

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Abstract

Introduction: Miscarriage is a reflection of the low quality of health in pregnant women. The incidence of miscarriage in the world accounts for 23 million (15%) of the 130 million births per year and up to 80% of miscarriages occur in the first trimester of pregnancy. Miscarriage will affect a woman's social, physical, and psychological. The complexity of the negative impact of miscarriage makes it necessary to pay special attention. Biomarker examination is needed to more accurately identify pregnancies at risk of miscarriage before the appearance of clinical symptoms. The use of fetal fibronectin (fFN) levels have been used as a marker of unexpected labor and as evidence of premature rupture of the fetal membranes. Normally fFN can be detected in cervical and vaginal secretions at <20 weeks gestation. The presence of fFN at >22 weeks gestation indicates disruption of the uteroplacental surface. Therefore, if screening for fibronectin levels using the Enzyme-Linked Immunosorbent Assay (ELISA) test can be carried out in the first trimester of pregnancy, there is a high possibility that miscarriage can be prevented.

Method: The type of research is analytical observational research with a cross-sectional research design. The stored samples were examined for fibronectin levels using the ELISA test, which included 21 blood serum samples from normal pregnancy patients in the first trimester and 21 miscarriage samples.

Results: The results of the ELISA test produced an average first-trimester normal pregnancy fibronectin level of 118.8 ± 18.4 ng/mL while the miscarriage fibronectin level was 208.2 ± 152.0 ng/mL. Data analysis using the Mann-Whitney test obtained a p-value = 0.138, which means there was no significant difference in fibronectin levels between normal pregnancy in the first trimester and miscarriage.

Conclusion: This study concludes that fibronectin levels are not specific biomarkers in detecting miscarriage in the first trimester of pregnancy.

Keywords: Miscarriage, Fibronectin, Normal pregnancy, First trimester

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INTRODUCTION

Miscarriage is the end of a pregnancy before the fetus can live outside the womb (nonviable) with the criteria of gestational age of <20 weeks or a fetus weighing <500 grams. Miscarriage can occur without intervention (spontaneous) or through deliberate action (provocate).[1] Cases of miscarriage are a reflection of the low quality of health of pregnant women. The incidence of miscarriage in the world accounts for 23 million (15%) of the 130 million births per year.[2] In Indonesia, miscarriage accounts for 10-15% of 6 million pregnancies or around 600,000-900,000 annually.[3] Up to 80% of miscarriages occur in the first trimester of pregnancy and decrease after 12 weeks of gestation.[4] Several factors play a role in miscarriage, that are maternal, neonatal, and external factors. Neonatal factors are considered to be the main cause of the increase in cases of spontaneous miscarriage and 50-60% of fetuses have chromosomal abnormalities.[5]

Risk factors for miscarriage are old age, body mass index, ethnicity, smoking, alcohol, air pollution, pesticides, stress, and previous history of miscarriage.[3] Miscarriage will affect a woman's social, physical, and psychological state. Clinical manifestations of miscarriage include abdominal pain, bleeding, infection, or hemorrhagic shock. Women who experience a miscarriage will experience sadness, trauma, feel blamed and depressed due to the loss they feel. The complexity of the negative impact of miscarriage makes it necessary to pay special attention to the treatment and prevention of miscarriage.[6]

Miscarriage can be managed by taking drugs (mifepristone and misoprostol) or surgically. The provision of these drugs and medical devices should be a priority in all countries, including low and middle-income countries. These actions are taken to reduce further bleeding and gynecological infections due to retained conception. Complications sometimes occur as a result of miscarriage management, namely surgery resulting in infection, massive bleeding, and uterine perforation. On the other hand, the use of misoprostol has side effects such as nausea, vomiting, and vaginal bleeding. Another problem in developing countries with miscarriage is the unavailability of drugs and tools to support the treatment of miscarriage.[3]

Routine prenatal check-ups in every symptomatic or asymptomatic pregnant woman are needed for early detection of miscarriage risk. A common examination is transvaginal ultrasonography (TVUS) as the gold standard to determine the condition of the womb and fetal viability through gestational sac diameter (GSD) to fetal node ratio, yolk sac diameter, and embryonic heart rate and velocity.[4,7] he use of TVUS is often combined with pregnancy hormones, such as progesterone or human (HCG) to aid in the diagnosis and early management of pregnancy. Incorrect use and interpretation of such examinations can create significant damage.[7] Thus, additional biomarker examination is needed to more accurately identify pregnancies at risk of miscarriage before the appearance of clinical symptoms.

Fetal fibronectin (fFN) is a glycoprotein produced by the chorion and has a role in the implantation and attachment of the placenta to the uterus. Normally fFN can be detected in cervical and vaginal secretions at <20 weeks gestation. The presence of fFN at >22 weeks gestation indicates disruption of the uteroplacental surface.[8] FFN levels have been used as a marker of unexpected labor and used as evidence of premature rupture of the fetal membranes.[9]

Based on this background, the author is interested in knowing the differences in fibronectin levels in the blood of first-trimester normal pregnancy and miscarriage patients using the Enzyme-Linked Immunosorbent Assay (ELISA). It is hoped that the results of this research can develop knowledge related to the diagnosis of non-communicable diseases so that it can reduce the mortality rate of pregnant women in Indonesia.

METHOD

This type of research is analytic observational with cross-sectional research design. The research sample used stored blood serum from women with normal pregnancy in the first

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trimester and miscarriage, each of which amounted to 21 samples. The research was conducted at the Biomedical Laboratory of the Faculty of Medicine, Andalas University, which started from December 2022 to November 2023. This research has been submitted for ethical clearance and passed ethical review through the Research Ethics Commission of the Faculty of Medicine, Andalas University with a research ethics code number 336/UN.16.2/KEP-FK/2023.

The research procedure begins with taking blood serum stored in the Biomedical Laboratory of the Faculty of Medicine, Andalas University, and then detecting fibronectin levels using an ELISA test, namely sandwich ELISA. Samples were compared using standard serum contained in the human fibronectin ELISA kit (E2002Hu). The Standard serum concentrations used were 0, 25, 50, 100, 200, and 400 ng/mL. The results were read using an ELISA reader in the form of fibronectin levels. The value of fibronectin levels >50 ng/mL indicates an increase in fibronectin levels above normal limits. The data obtained were presented in graphs and tables analyzed using the Statistical Product and Service Solution (SPSS) 15.0 program with the Mann-Whitney test to determine the difference in fibronectin levels between normal pregnancy in the first trimester and miscarriage.

RESULT

A total of 42 blood serums consisting of 21 serums of normal pregnancy patients in the first trimester and 21 serums of miscarriage patients were examined for fibronectin levels using the sandwich ELISA test. Samples were compared using 6 standard serums with various concentrations of 0, 25, 50, 100, 200, and 400 ng/mL obtained from the Bioassay Technology Laboratory. Furthermore, the absorbance value (OD value) and fibronectin levels of each well (standard serum and sample serum) were immediately read after the stop solution was applied using an ELISA reader. The results of sample fibronectin levels are listed in Table 1.

Table 1. ELISA Test Results

Sample	Fibronectin level (ng/mL)	
	Miscarriage	**Normal pregnancy**
01	110.5	154.5
02	123.7	110.5
03	466.0	131.3
04	134.4	106.7
05	387.3	115.5
06	383.6	146.4
07	82.8	127.5
08	118.0	121.8
09	336.4	107.3
10	519.5	94.1
11	108.0	91.6
12	108.6	118.0
13	115.5	114.3
14	512.5	114.3
15	145.1	121.2
16	150.8	85.3
17	104.8	143.8
18	92.9	139.4
19	130.0	120.6
20	116.8	132.5
21	125.6	98.5
Max	519.5	154.5
Min	82.8	85.3
Mean	208.2 ± 152.0	118.8 ± 18.4

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In this study, fibronectin in the blood serum of pregnancy and miscarriage was detected at levels >50 ng/mL. The average normal pregnancy fibronectin level was 118.8 ± 18.4 ng/mL. The lowest normal pregnancy fibronectin level was 85.3 ng/mL and the highest was 154.5 ng/mL. Meanwhile, the average fibronectin level of miscarriage was 208.2 ± 152.0 ng/mL. The lowest abortus fibronectin level was 82.8 ng/mL and the highest was 519.5 ng/mL.

The examination also found that the fibronectin levels of miscarriage samples A03, A11, and A15 produced extreme values, that are 466.0, 519.5, and 512.5 ng/mL. In addition, as many as 12 of the 21 miscarriage samples had increased fibronectin levels above the average value of normal pregnancy fibronectin levels in trimester 1 (118.8 ± 18.4 ng/mL).

Table 2. Mann-Whitney Test Result

Variable	Sample				p-value
	Normal pregnancy		Miscarriage		
	f	Median (ng/mL)	f	Median (ng/mL)	
Fibronectin levels	21	118,0	21	125,6	0,138

The results obtained were analyzed bivariate using the Mann-Whitney test to determine the difference in fibronectin levels between the two samples. The Mann-Whitney test results showed that the median fibronectin of normal pregnancy in trimester 1 was 118.0 ng/mL while the median pregnancy miscarriage was 125.6 mg/mL. The significance test obtained a p-value of 0.138 which indicates there is no difference in fibronectin levels between normal pregnancy and miscarriage (table 2).

DISCUSSION

Clinical diagnosis of pregnancy is the discovery of fetal heart movement through ultrasound at 6 weeks postmenstrual or 4 weeks after conception by recognizing fetal heart movement. As many as 10-15% of pregnancies at weeks 6 to 12 will experience pregnancy failure. Accurate pregnancy screening is very important, especially in the first trimester of pregnancy which is prone to pathological conditions, such as miscarriage. [10] Miscarriage can be confirmed through ≥7 repeated ultrasound examinations with the criteria of gestational sac diameter ≥16 mm with an empty gestational sac or visualization of the embryo with crown-rump length ≥5 mm and no heartbeat.[11]

Current technological developments have provided a variety of more sophisticated examinations to investigate the risk of miscarriage in pregnant women. A biomarker examination was developed to obtain more accurate results. In this study, the examination of fibronectin levels in the patient's blood serum through the Enzyme-Linked Immunosorbent Assay (ELISA) test was carried out to detect the risk of miscarriage, especially in the first trimester of pregnancy.

Fibronectin is a multidomain glycoprotein (440 kDa) that can be found in most vertebrate tissues and organs.[12] Fetal fibronectin is a biological fluid produced by amniocytes and cytotrophoblasts during pregnancy. Fetal fibronectin functions as an adhesive between pregnancy and the uterus. The concentration of FFN can be detected in blood as much as 1/5 of the amount in amniotic fluid and cannot be found in urine. Under normal conditions, FFN will remain between the chorion and decidua at very low levels. FFN levels are found in high amounts in amniotic fluid (100 µg/mL), especially in the second trimester of pregnancy, and will decrease again at full term

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(30 µg/mL).[13]

In this study, fibronectin in the blood serum of pregnancy and miscarriage was detected at levels >50 ng/mL. The average normal pregnancy fibronectin level was 118.8 ± 18.4 ng/mL. Meanwhile, the average fibronectin level of miscarriage was 208.2 ± 152.0 ng/mL. In addition, as many as 12 of the 21 miscarriage samples had increased fibronectin levels above the average value of fibronectin levels in normal pregnancy first trimester (table 1).

Altered expression, degradation, and increased levels of fibronectin are associated with pathological states.[12] If abnormal levels of FFN are detected, it may indicate amniotic sac membrane disruption.[13] Normally, FFN levels will persist in cervicovaginal secretions in pregnant women until 22 weeks of gestation. Furthermore, it will decrease as the gestational age increases (<50 ng/mL).[14] The detection of fibronectin levels before 20 weeks of gestation is thought to be due to the absence of complete fusion between the fetal membranes and the decidua. In addition, cervical or vaginal irregularities after this time are believed to be caused by the formation of a seal that prevents further release of fetal fibronectin.[12]

Until now, there are still no studies that explain the exact pathogenesis of FFN release with miscarriage. However, a study found a very high increase in fibronectin levels in patients with early spontaneous miscarriage which may be caused by immune mediator dysfunction, namely an increase in the number of lymphocytes in the decidual intersitial cells and the presence of decidual abnormalities and cytokine release by the decidua. This causes proteolytic destruction of the chorionic surface of the decidua, as well as the release of extracellular matrix proteins, one of which is fetal fibronectin from the surface into the cervix and vagina.[15] In this study, fibronectin levels of miscarriage samples A03, A11, and A15 produced extreme values, which are 466.0, 519.5, and 512.5 ng/mL (table 2).

Therefore, increased fibronectin levels cannot be used as a specific biomarker to detect the risk of miscarriage in first-trimester pregnancy due to the absence of a definite cut-off value for fibronectin levels that increase the risk of miscarriage. This finding is in line with a study that examined fibronectin levels in plasma, this study found that there was no difference in fibronectin levels in the plasma of women with normal pregnancy in the first trimester to the third trimester with women who were not pregnant.[16]

The results in the study obtained were reinforced by the results of bivariate analysis using the Mann-Whitney test listed in Table 2 obtained a p-value of 0.138, meaning that there was no significant difference in fibronectin levels between normal pregnancy and pregnancy miscarriage.

CONCLUSION

The average fibronectin level of normal pregnancy in the first trimester was 118.8 ± 18.4 ng/mL with the lowest level of 85.3 ng/mL and the highest of 154.5 ng/mL. Meanwhile, the mean fibronectin level of miscarriage was 208.2 ± 152.0 ng/mL with the lowest level of 82.8 ng/mL and the highest of 154.5 ng/mL with the lowest level of 82.8 ng/mL and the highest of 519.5 ng/mL.

Both samples were subjected to bivariate analysis and it was found that there was no statistically significant difference in fibronectin levels so it could not be used as a specific biomarker in detecting the risk of miscarriage in first-trimester pregnancy.

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