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Comparison of Vaginal and Plasma Fibronectin Concentrations for Prognosis of Preterm Delivery: A Cross-Sectional Study

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Abstract

Background & Objective: Prediction of preterm delivery can reduce a large number of its complications. The present study aimed to compare vaginal and plasma fibronectin concentrations in the diagnosis of preterm delivery.

Materials & Methods: Serum samples were obtained from 105 women at 24-36 weeks of gestation. However, only 40 women gave permission to collect vaginal samples. Fibronectin concentration was measured using the ELISA technique. Then, plasma and vaginal fibronectin levels were compared in term and preterm deliveries.

Results: The mean plasma fibronectin level was 6226.43±7174.97 ng/mL among the mothers with term infants and 7724.01±1143.82 ng/mL among those with preterm infants (p=0.667). The mean fetal fibronectin level was 156.61±126.42 ng/mL among the mothers with term infants and 127.71±43.14 ng/mL among those with preterm infants (p=0.241). The cut-off point of plasma fibronectin level was 1750 ng/mL with a sensitivity of 80.25% and specificity of 85.17%. Additionally, the cut-off point of vaginal fibronectin level was 158.98 ng/mL with a sensitivity of 94.62% and specificity of 22.08%.

Conclusion: Plasma fibronectin analysis had lower sensitivity and higher specificity compared to vaginal fibronectin analysis. This implies that plasma testing has lower false-positive cases and can identify a more significant number of true positive cases of preterm delivery.

Keywords: Premature Births, fibronectins, vaginal, plasma, Screening Tests

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Introduction

Pregnancy is a complicated phenomenon accompanied by challenges, which can cause concerns for mothers. One of the most common problems that can disrupt the normal duration of pregnancy, particularly in pregnancies that do not follow the natural process and are accompanied by problems, is preterm delivery that has been introduced as the main factor in the incidence of infant mortality and morbidity (1). Preterm delivery refers to delivery before 37th week of gestation or 259 days after the last menstrual period (LMP) (2, 3) which has been identified as the cause of 75-80% of prenatal mortalities and

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50% of neonatal neurological complications (4).

Preterm delivery is accompanied by a wide range of health problems and developmental disabilities, including respiratory issues, gastrointestinal complications, central nervous system disorders, and cognitive, motor, and behavioural disorders (5, 6).

Consequently, attempts should be made to predict and prevent preterm delivery. In this context, identifying high-risk women using reliable tests can provide the ground for faster interventional care services for the mother, fetus, and infant (6).

Considering the importance of preterm delivery whose prediction can prevent prenatal problems, various biochemical measurements have been carried out to promote maternal and fetal outcomes (7, 8). Vaginal Fibronectin is yet a more potent marker for the diagnosis of preterm delivery. Fibronectin is a glycoprotein produced by the chorionic and acts as a glue between the placenta and the decidua. It is normally secreted in the vagina and the cervix within 16-20 week of gestation, but it is rarely found in the vaginal secretions after the 21st week. It increases again in the vaginal secretions before delivery. Hence, the early presence of fibronectin in vaginal and cervical discharges can predict preterm delivery (9). In other words, fibronectin concentration follows a descending trend during pregnancy, but it may increase weeks or months before delivery under such conditions as preterm labour, preeclampsia, and intrauterine growth restriction (8). A large number of cells including liver cells, amnion cells and fibroblast cells produce fibronectin. This glycoprotein is present in the amniotic fluid and can enter the maternal plasma through placental circulation. Fibronectin plays a role in the process of intercellular adhesion during implantation and also in stabilizing the adhesion of the placenta to the decidua of the uterus. Therefore, the presence of this glycoprotein in vaginal secretions can be a sign of rupture of the fetal membranes and the risk of premature birth (2).

Vaginal fibronectin test is a standard method for predicting preterm delivery, which is carried out through the vaginal or blood plasma. Nonetheless, blood sampling is more accepted. Moreover, no studies have been conducted on comparing vaginal and plasma fibronectin levels to determine which test has a higher predictive value and can help predict preterm delivery sooner. Therefore, the present study aims to determine a cut-off point for fetal and plasma fibronectin levels that predict preterm delivery.

Materials and Methods

This study was conducted on 105 pregnant women who were referred to the gynecology clinic in Vali-e-Asr Hospital, Fasa, Iran, for routine prenatal care. The inclusion criteria were women aged between 18-35 years old, singleton pregnancy, and gestational age of 24-36 weeks, with no history of chronic hypertension, diabetes, renal problems, and inflammatory disorders such as lupus. The exclusion criterion was having a history of receiving tocolytic agents (terbutaline, ritodrine, magnesium sulphate, salbutamol, and isoxsuprine).

Using the study of Zygmunt et al (10) and considering the error of the first type 0.5 and the test power of 80%, the sample size was determined to be 72 people. Due to the possibility of falling samples, 105 people were included in the study.

Gestational age was determined according to the LMP. In case of doubt about the LMP, the first-trimester sonography was taken into consideration. The demographic data (Mother's age, occupations, and education level and pre-pregnancy body mass index), husband's cigarette smoking status, family history of preterm delivery, contraception method, and utilization of assisted reproductive techniques and obstetric information (gravida, history of miscarriage, planned pregnancy and delivery type) were collected for each participant. Then, venous blood samples were collected, and the sera were separated and kept at -70°C. All the samples proceeded for the assessment of fibronectin level using 96-well ELISA kits

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Blood samples were taken from all 105 participants. However, only 40 women gave permission to collect their vaginal samples. Vaginal samples were obtained from the posterior fornix using a cotton swab. In doing so, after inserting a speculum, a cotton swab was put in the posterior fornix for 10 sec. The sample was then put in a tube containing normal saline and preserved at -70ºC. The criteria observed for taking vaginal samples included not having had sexual activities during the past 24-48 hours and not having vaginal bleeding at the time of sampling.

All participants were followed until delivery, and information about the infant's weight, type of delivery, and gestational age at delivery was collected and recorded.

Results

Demographic Characteristics

The mothers' mean age was 28.81±6.257. The results showed no significant differences between the women with term and preterm deliveries regarding mother's age (p=0.51).

The results also showed no significant differences between the two groups concerning occupation, education level, parity, history of miscarriage, husband's cigarette smoking status, family history of preterm delivery, contraception method, and utilization of assisted reproductive techniques (p=0.49) (Table 1).

Among the 105 participants, 28 (26.7%) had a preterm delivery, and 77 (73.3%) had term delivery. Out of the 40 women who gave vaginal samples, seven had preterm delivery, bringing to account that the mean gestational age at the time of sampling was 28.11±2.98 weeks (Table 1).

Table 1. Demographic characteristics of study participants in two groups

Variables	Preterm (n=28)	Term (n=77)	Total (n=105)	p-value
Mother's age (yr)	56.5±47.29	50.6±57.28	6.257±28.81	0.518*
Gestational age at sampling (wk)	2.68±27.54	2.33±28.16	2.98±28.1	0.870*
Gestational age at birth (wk)	1.10±35.12	2.13±39.25	2.03±38.24	0.012*
Mother's Weight before pregnancy (kg)	56.10±3/65	74.10±42.60	10.867±61.72	0.42*
Maternal BMI before pregnancy	46.3±24.26	88.3±18.24	3.87±24.73	0.015*
Neonatal Birth weight (g)	89.573±2425	6.369±61.3229	559.59±3015.05	<0.001*

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Delivery type	NVD C/S	13 (46.4%) 15 (53.6%)	44 (57.1%) 37 (42.9%)	0.33**
Mother's job	Housewife Employed	22 (78.6%) 6 (21.4%)	71 (92.2%) 6 (7.8%)	0.158**
Mother education	Illiterate Under the diploma Academic	2 (7.1%) 7 (15%) 19 (67.8%)	1 (1.3%) 27 (35.1%) 49 (63.7%)	0.372**
Gravida	1 2 ≤3	13 (28.6%) 8 (25%) 7 (6.4%)	27 (35.1%) 27 (35.1%) 23 (29.8%)	0.703**
Abortion history	Yes No	4 (14.3%) 24 (85.7%)	19 (24.7%) 58 (75.3%)	0.255**
Smoking by Husband	Yes No	6 (21.4%) 22 (78.6%)	14 (18.2%) 63 (81.8%)	0.708**
Preterm labor history	Yes No	23 (82.1%) 5 (17.9%)	69 (89.6%) 8 (10.4%)	0.304**
Use of fertility assisted methods	Yes No	2 (7.1%) 26 (92.9%)	3 (3.9%) 74 (96.1%)	0.49**
Disease in the current pregnancy	GDM PEC Infection Vaginal bleeding	4 (14.28%) 4 (14.28%) 7 (25%) 1 (3.57%)	1 (1.31%) 2 (2.63%) 19 (25%) 10 (13.15%)	0.009**

Chi- Square test
* Independent t-test, ** Chi-Square
NVD: normal vaginal delivery.
C/S: caesarian section
GDM: gestational diabetes mellitus.
PEC: preeclampsia
BMI: Body Mass Index

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Factors Affecting Preterm Birth

In this study, logistic regression analysis was used to explore the effective factors in preterm delivery. Firstly, the variables were entered into the univariate model. In this model, pre-pregnancy body mass index (BMI), family history of preterm delivery, planned pregnancy, and suffering from disorders such as hypertension and gestational diabetes mellitus during the pregnancy period were significant. Accordingly, an increase in BMI was accompanied by a 15% increase in the odds of preterm delivery. Additionally, the odds of preterm delivery were 2.57 folds higher among the individuals who had a family history of this disorder compared to those who did not. Besides, the odds of preterm delivery were 84% lower among the individuals with planned pregnancies in comparison with those with unplanned pregnancies. Finally, the odds of preterm delivery were respectively 7.33 and 14.66 folds higher among the individuals who had a history of hypertension and gestational diabetes mellitus in comparison with those who did not. After adjustment, the results revealed that BMI and pregnancy intention were associated with preterm delivery. Accordingly, an increase in BMI caused an 18% increase in the odds of preterm delivery [CI (1.04-1.35), p=0.009]. In addition, the odds of preterm delivery were 87.7 lower among the individuals with planned pregnancy compared to those with unplanned pregnancy [CI (0.02-0.59), p=0.009] (Table 2).

Table 2. Crude and adjusted Odds Ratio (OR) estimates of different variables in the preterm based on logistic regression

Variables	Crude			Adjusted		
	OR	95% CI	P-value*	OR	95% CI	p-value
Mother's age	1.042	.96 - 1.12	0.282	-		
Pre-pregnancyBMI	1.15	1.02-1.29	0.020	1.188	1.04-1.35	0.009
Gravidia	0 .766					
Family history						
No	Reference					
Yes	2.548	1.05-6.17	0 .038			
ART						
NO	Reference	-				
YES	0.527	0.08-3.33	0.496			

Pregnancy disease				-		
No	Reference					
Yes	0.56	0.23-1.34	0.197			
Abortion	1.96	0.60-6.38	0 .261	-		
planned pregnancy						
No	Reference					
Yes	0.160	0.03-0.72	0 .018	0.123	0 .02-0.59	0.009
Pregnancy disease				-		
No	Reference					
Hypertension	7.33	1.19-44.96	0.031			
GDM	14.66	1.49-143.7	0.021			
Vaginitis	2.61	0.70-9.73	0.151			
UTI	0.611					
PROM	0.00	-	1			
Trauma	0.40	0.04-3.54	0.416			
Vaginal bleeding	0					

ART: Artificial Reproductive Technology
GMD: Gestational Diabetes Mellitus
Uti: Urinary tract Infection
PROM: Premature Rupture of fetal Membranes
BMI: Body Mass Index
OR: odds ratio, CI: confidence interval.

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Plasma and Vaginal Fibronectin Comparison

The mean plasma fibronectin level was 6226.43±7174.97 ng/mL among the mothers with term infants and 7724.01±1143.82 ng/mL among those with preterm infants. Although the mean plasma fibronectin level was higher in preterm delivery, the difference was not statistically significant (p=0.667). The mean vaginal fibronectin level was 156.61±126.42 ng/mL among the mothers with term infants and 127.71±43.14 ng/mL among those with preterm infants, but the difference was not statistically significant (p=0.241) (Table 3).

Table 3. Comparison of plasma concentration with a vaginal concentration of fibronectin (n=40)

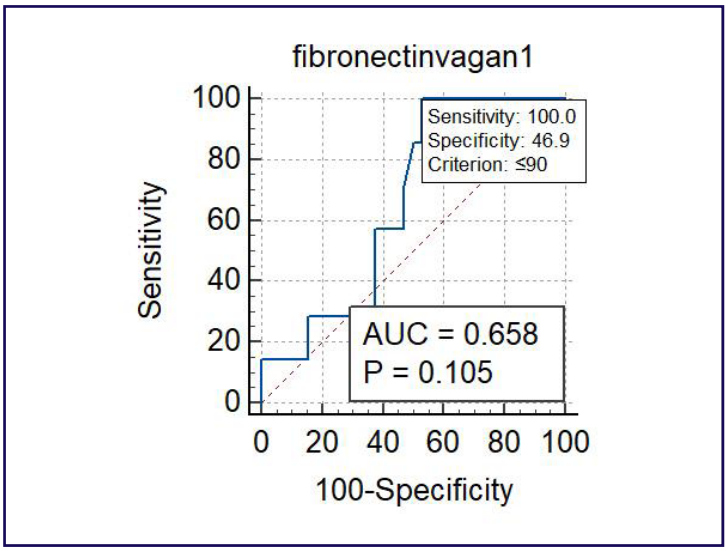
Variable	Term	Preterm	p-value
vaginal fibronectin	156.61±126.42	127.71±43.14	0.199
Plasma fibronectin	6226.43±7174.97	6726.43 ± 7174.91	0.668

Data presented as mean±SD.
independent t-test

The plasma and vaginal fibronectin concentrations in the diagnosis of preterm delivery have been presented in ROC curves. Accordingly, the cut-off point of plasma fibronectin level was ≥4000 ng/mL with a sensitivity of 60.7% and specificity of 52.6%.

Additionally, the cut-off point of vaginal fibronectin level was ≤90 ng/mL with a sensitivity of 100% and specificity of 46.9%. Thus, plasma fibronectin testing had lower sensitivity and higher specificity compared to vaginal fibronectin testing.

This implies that plasma testing had lower false-positive cases and could identify a more significant number of true positive cases of preterm delivery (Figure 1).



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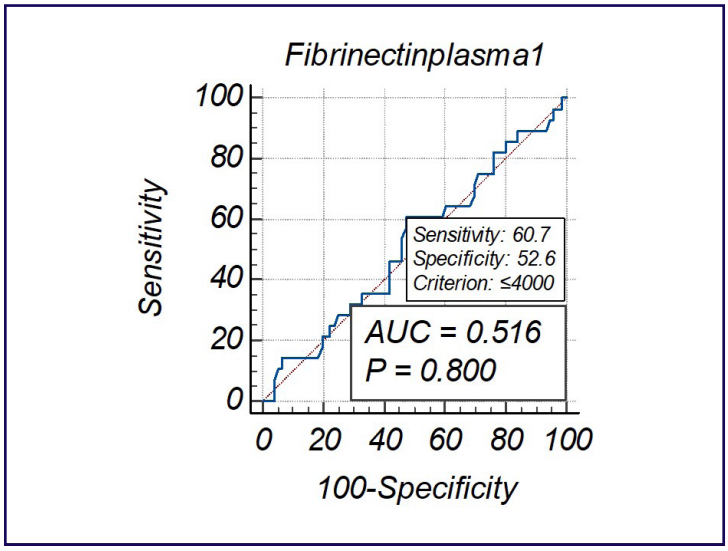

Figure 1. Rock curve of vaginal and plasma fibronectin concentrations

Furthermore, the Kappa coefficient was 40% for vaginal fibronectin testing and 45.19% for plasma fibronectin testing. Thus, the Kappa coefficient was higher for plasma testing compared to vaginal testing. Although the Kappa coefficient was lower than 0.4 for both types of testing, the rate of getting a positive result by chance was lower for plasma testing compared to vaginal testing (Table 4).

Table 4. Comparison of diagnosis of preterm delivery based on tests performed with the actual condition of the newborn

		Baby age at delivery		p-value	Agreement	Kappa
		Preterm	Term			
vaginal fibronectin	Preterm	28 (100)	57 (75)	0.002*	40%	-0.015
	Term	0	19 (25)			
Plasma fibronectin	Preterm	19 (67.9)	54 (70.01)	0.823**	45.19%	0.152
	Term	9 (32.1)	23 (29.9)			

Data presented as Number and percentage
*Fisher exact test
**Chi-square test

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Discussion

The study results indicated that plasma fibronectin testing had lower sensitivity and higher specificity compared to vaginal fibronectin testing. This implies that plasma testing had lower false-positive cases and could identify a larger number of true positive cases of preterm delivery. A study compared three groups of pregnant women (with the symptoms and risk factors of preterm delivery, with the symptoms and without the risk factors of preterm delivery, and healthy pregnant women) regarding plasma fibronectin levels. The results indicated significantly higher plasma fibronectin levels among women with preterm delivery compared to those with term delivery (8). Another study also conducted a 10-month study in obstetrics and genecology hospital affiliated with Gibbon University in Germany in 1996 to investigate plasma fibronectin concentration as a predictive marker of preterm delivery. In that study, 80 pregnant women with the symptoms of preterm delivery were enrolled into the case group, and 64 healthy pregnant women were allocated to the control group. All pregnant women were at 22-36 weeks of gestation. Plasma fibronectin concentration was measured in both groups, and the mean values were determined. The results revealed a significant difference between the twogroups concerning the mean plasma fibronectin level. In the control group also, the mean plasma fibronectin level was higher among the women with preterm labour compared to their peers. Hence, it was concluded that ascending plasma fibronectin concentrations could be influential in the prediction of preterm delivery (10).

In a study to evaluate fetal fibronectin test as a predictor of labour onset showed that vaginal fibronectin measurement in pregnant women after 36 weeks alone has not been a predictor of labor so that it can assist the specialist and pregnant women in deciding when to visit maternity centres (11). The results of a study performed to evaluate the accuracy of predicting serial transvaginal cervical length and fetal vaginal fibronectin for preterm delivery in nulliparous women, showed little accuracy for predicting preterm delivery. This study does not recommend the routine use of these tests in these women (12).

Another study in 2020 reported that plasma fibronectin testing after the 30th week of gestation could be effective in the prediction of preterm delivery, thereby providing the mothers as well as the medical team to carry out care interventions at due time and have access to intensive care services for infants (13). Furthermore, a study demonstrated that in comparison with the overall assessment of fibronectin concentration at a particular age, longitudinal measurement of plasma fibronectin concentration at 26, 30, and 34 weeks of gestation was a better predictor of the incidence of preeclampsia (14). Consistently, Juha Rasanen indicated that plasma fibronectin testing could be effective in the prediction of preeclampsia (15). On the contrary, a systematic review in 2018 showed the unsatisfactory results of using plasma fibronectin testing in the identification of pregnant women who required interventions. Additionally, physicians' information about vaginal fibronectin concentration was not influential in the reduction of the rate of preterm delivery and had no benefits for either the mother or the fetus (16). Vaginal fibronectin testing could not improve the prognosis of preterm delivery. In symptomatic women, however, this test could help identify at-risk cases before 34 and 37 wks of gestation (17).

Based on what was mentioned above, plasma fibronectin testing has been used as a screening test for preeclampsia considering the two studies. 14 and 15 and that blood sampling is easier than vaginal sampling during pregnancy, and it is time to change the type of sampling for the prediction of preterm delivery.

Considering the cut-off points computed for diagnosis of preterm delivery, infants' actual status as the gold standard, and Kappa coefficient <0.4 in both plasma and vaginal samples, this criterion was very weak and, as a result, vaginal and plasma fibronectin levels at this

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gestational age could not predict preterm delivery properly. Nonetheless, this test might be useful at higher gestational ages. Moradi et al. also disclosed that sampling after the 30th week of gestation could have a better predictive value (13).

Since no studies have been conducted on the comparison of vaginal and plasma fibronectin testing, the findings of the present study cannot be compared to those of other investigations. Future studies in this field can help achieve more definite results.

Conclusions

Vaginal fibronectin testing has no preventive role and is usually useful in the diagnosis of preterm delivery when the delivery process has begun. Nevertheless, plasma fibronectin testing can have a better prognostic value compared to vaginal fibronectin testing. Since plasma fibronectin testing is more easily performed and more accepted by mothers, it is recommended to be used as a screening test for the prediction of preterm delivery after 30th week of gestation.

Limitations

It is true that the best time for collecting vaginal sample to predict preterm labour is after 30th week of gestational age, but unfortunately pregnant women in our study refused to give a vaginal sample after 30th week. This is one of the limitations of the present study due to culturalconditions. Due to the fact that pregnant women did not allow us to do vaginal sampling for the second time, inevitably only one sampling was performed. Since the number of preterm births was small, it would take much longer to study if we just wanted to enter spontaneous preterm births.While there were no diseases at the time of sampling, unfortunately this is one of the limitations of our study.

Statistical Analysis

All data were analysed using SPSS statistical software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.”). Descriptive statistics such as mean, standard deviation (SD), and percentage were used. The chi-square independent and paired t-tests and logistic regression analysis were also employed. In addition, the receiver operating characteristic Receiver Operating Characteristic (ROC) curve was used to assess the cut-off points. The significance level was set at $p< 0.05$.

Ethical Considerations

Women were informed about the study and those who were interested in joining provided written informed consents. This article was extracted from a research proposal (93104) approved by Fasa University of Medical Sciences.

Conflict of Interests

Corresponding Author (Zahra Moradi) has received research grants from Company Fasa University of Medical Sciences. The authors declare that they have no conflict of interest.

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