CASE STUDY

Cervicovaginal Fetal Fibronectin Levels in Preterm Labor: Just Association or Reliable Screening Biomarker

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ABSTRACT

Background: Preterm birth (PTB) is a chief reason for neonatal illness and death. Prediction of PTB can prevent complications. The cervico-vaginal fetal fibronectin (fFN) test is a good predictor for preterm labor (PTL) within 7 to 14 days from testing. The levels of fFN are high during the first 16 to 22 weeks of gestation in normal pregnancy before it drops to very low values and rises over again as the gestation reaches term. The current work aimed to assess the association and reliability of cervicovaginal fFN in predicting PTL.

Patient and method: The study was cross-sectional that included hundred pregnant (aging from 20–34 years), gestational age ranging (from 24–34 weeks), and presented with abdominal pain. All applicants' Detailed medical history regarding age, gravidity, and parity were taken from all applicants. Gestational age was calculated through general examination and abdominal and vaginal examination. During speculum vaginal examination, fFN samples were taken from the cervix and were examined for fFN using the quick check for assay. Statistical scrutiny was carried out by SPSS version 17. A $p \leq 0.05$ was measured as significant.

Result: The mean age was (27.53 ± 4.23), range (of 20–34). Positive fFN results were detected in only (22%) of patients. Around two-thirds of the women end their pregnancy by normal vaginal delivery. Less than half (45.5%) of the included women delivered within one week after presentation, around 1/3rd (31.8%) delivered after the second week, and only 22.2% delivered during the first day of presentation. There was a significant association between pregnancy outcome and fFN results. A majority (90.9%) of patients end with preterm labor during 2 weeks of follow-up were presented with positive fetal fibronectin. The majority (90.9%) of patients end with preterm labor during 2 weeks of follow-up were presented with positive fFN results. There were non-significant variations between means of gestational ages, parity, and gestational age/weeks between those with positive and negative fFN results.

Conclusion: Among pregnant women with uterine contraction before advanced cervical dilatation, the presence of cervicovaginal fFN is an associated and reliable screening test in predicting the risk of PTL.

Keywords: Cervicovaginal, Fetal fibronectin, Gestational age, Predictor, Preterm birth, Preterm labor.

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INTRODUCTION

Pregnant women at term are normally revealed a reduced risk for later preterm birth (PTB). However, a prior PTB is the main predictor of an upcoming one. The greater risk of recurrent PTB reflects insistent maternal or environmental causal factors.[1] The term "great obstetrical syndromes" is projected in order to highlight the likelihood of joint pathways joining gestational disorders with outcomes like abruptio placenta, pre-eclampsia, fetal undergrowth, and death.[2] Some scientists have believed that these conditions are manifestations of "ischemic placental disease" rather than distinct entities.[3]

Presently, the management of susceptible PTB is based on improving newborn products. Prescription of pre-natal corticosteroids (for pulmonary fetal maturation), magnesium sulfate neuroprotective agent (for cerebral palsy), and tocolytic agents (inhibit contractions of the uterus) can delay parturition up to one week, allowing time for further management.[4]

Estimations from vital health statistics data put forward that generally, 9 to 12% of human births ensue before 37 gestational weeks, causing about 14.8 million PTBs annually. Extreme PTBs form about 0.42% of all births.[5] The definition

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of PTBs by the World Health Organization is birth at <37 weeks or 259 days of gestation.[6] Alarmingly, the worldwide occurrence of PTB is believed to be rising.[4] Precise current statistical records in other states are limited. However, some revisions propose that frequencies of extreme PTB have been steady in high-income states during the previous decades.[7] Relative risks for PTBs were 2 to 4 times higher than term neonates. The PTBs are at higher risk of cerebral palsy, neonatal respiratory distress syndrome, cognitive defects, as well as neonatal sepsis and death.[1]

Most pregnant females susceptible to preterm labor (PTL) will labor at term without any interventions. Moreover, some females who have prophylactic management may still give birth prematurely. Hence, the prediction of which pregnant are at a higher risk of PTB is a real challenge for clinicians to introduce preventive measures, hospital admittance, choices of birthplace, and in-utero remedies for better neonatal outcomes.

Fetal fibronectin (fFN) is a complex glycoprotein found in the uterine amniotic fluid. In cervicovaginal fluids, its existence indicates disrupted fetal membranes. Previously the quality assessment of fFN was applied at a 50 ng/mL threshold, which is nowadays replaced by quantitative fFN tests that allow a more precise predictor of PTB in asymptomatic and symptomatic females with PTL.[8,9] Levels of fFN glycoprotein found in plasma are 1/5th that found in amniotic fluid; it is absent in urine. Normally, this protein remains in the space between decidua and chorion and is found in minimum concentrations (<50 ng/mL) in cervicovaginal fluids after 22 weeks of gestation.[10] This study aimed to evaluate the diagnostic ability of cervicovaginal fFN levels to predict PTL.

PATIENTS AND METHODS

Study Setting and Patients

This was a cross-sectional prospective study completed in the Department of Obstetrics at Babylon Maternity and Children Teaching Hospital, Babylon, Iraq, during the period from (November 2019 to July 2020). The study included 210 pregnant women with a gestation age range of 24 to 34 weeks and an age range of 20 to 34 years, who presented with abdominal pain. All participants have referred women from the labor room of Babylon Maternity and Pediatric Hospital. All pregnant females who received care at this hospital were suitable for participation if they presented with intact membranes.

All applicants' detailed medical history regarding age, gravidity, and parity were taken from all applicants. The women's gestational age was considered from the first day of the latest cycle or 1st-trimester ultrasound. Detail review of current complaints like low backache, cramping, pelvic pressure, increase discharge, or coexistence of vaginal bleeding.

Those women who fulfilled the study criteria were subjected to general examination, and abdominal examination of fundal height, lie, presenting part, uterine tenderness (signifying chorioamnionitis or abruption), contractions, and auscultation of the fetal heart. If any abnormal vaginal discharge, aspiration of amniotic fluid, or blood, and visual assessment of cervical dilatation using speculum examination.

PTL was identified as uterine contractions detected by tocodynamometry, at a rate of 4/20 minutes or 8/1 hour or uterine activity to some extent correlated with variations in cervical effacement (minimum 50%) besides dilatation (minimum 2 cm).

Exclusion Criteria

Any pregnant with any of the following criteria were excluded from this study: History of PTL, cervical surgery, uterine overdistention (multiple pregnancy or polyhydramnios), genital tract infection, antepartum hemorrhage, rupture membrane, cervical cerclage, >3cm cervical dilatation, pre-eclampsia, placenta previa, and major fetal anomaly were excluded. Women are also excepted if they performed cervical handling (intercourse, cervicovaginal exam, or sonography) during the last 24 hours. Medically directed PTLs were not included too.

Laboratory investigations and imaging

Blood samples were obtained by venipuncture to assess complete blood count, FBS, renal function test, and liver function tests. A vaginal swab was done to exclude infection. Cervicovaginal testing of fFN was done by the cervical sampling of fFN obtained from the posterior fornix after cervical digital examination, using examination speculum and "Hologic QuikCheck^{TM}" fFN from Hologic, Inc. (CA-USA), which is a solid-phase immunogold assay.

A cervical digital examination was executed with sterilized hand gloves and oil to evaluate dilatation and effacement of the cervix. The standard care during hospitalization for all women with PTL. The managing gynecologist was unaware of fFN-tests results, so as not to influence the therapy.

Patients with negative fFN results whose symptoms subside are discharged home, while women with positive fFN results, and regular/painful contractions, are admitted for further follow-up.

Ethical consideration

The protocol of the study was ratified by the "Iraqi Board of Medical Specialization of Obstetrics and Gynecology" in consistence with Helsinki Declaration guidelines. After a full explanation of the authors to the candidates the importance of the study for her condition and future works, written consent had been obtained before being involved in the study.

Data investigation

The statistical examination was finalized via SPSS software (version-17). Categorical data were expressed as frequencies and percentages. Continuous data were expressed as Means ± SD. To compare the means between any two groups, the "Independent Student-test" was used. Fisher-exact tests and Pearson's chi-square were applied to find any association between categorical parameters. A *p-value* of ≤ 0.05 was considered significant.

RESULTS

Table 1 displays the distribution of patients based on study characteristics including age, parity, and fFN levels. The mean age was (27.53 ± 4.23), range (of 20–34). Nearly half of the women were unipara (48%). Positive fFN results were detected in only (22%) of patients. Around two-thirds of the women end their pregnancy by normal vaginal delivery. Less than half (45.5%) of the included women delivered within one week after presentation, around 1/3rd (31.8%) delivered after the second week, and only 22.2% delivered during the first day of presentation. The majority (78%) of patients continue normal pregnancy by the end of follow-up.

Table 2 shows the association between outcome and fetal fibronectin results. A significant association was observed between outcome and fFN results. A majority (90.9%) of patients end with preterm labor during 2 weeks of follow-up were presented with positive fetal fibronectin.

Table 3 displays the mean age difference (years) of pregnant women according to fFN. There were non-significant differences in the means of age between the study groups.

Table 4 displays the differences in the gestational age/weeks according to fFN results. There were non-significant differences in the means of gestational age between the two groups of the study.

Table 5 The fetal fibronectin was not significantly associated with parity.

Table 1: Distribution of women according to variables of the study

Parameters	*Descriptive data*	
Age/year #	27.53 ± 4.23	Range (20–34%)
Parity*		
Primigravida	27	27.0
Unipara	48	48.0
Multipara	25	25.0
Fetal Fibronectin	Positive 22%	Negative 78
Mode of delivery		
Normal vaginal delivery	14	63.6
Caesarian section	8	36.4
Period until delivery		
< 1 day	5	22.7
(1 day to <7 days)	10	45.5
(7 days to 14 days)	7	31.8
Pregnancy outcomes	Delivered during follow-up (22%)	Continue normal pregnancy (78%)

* Number %, # mean ±SD,

Table 2: Association between fetal fibronectin results and pregnancy outcome

Cervicovaginal fFN N (%)	*Pregnancy outcome*		*p-value*	*Odds*	*95% CI*
	Preterm labor	*Continue pregnancy*			
Positive	20 (90.9)	2 (2.6)	<0.001	380	50.36–286.17
Negative	2 (9.1)	76 (97.4)			

Table 3: The mean age differences of the women according to fetal fibronectin results

	Fetal fibronectin	*N*	*Mean ± SD*	*p-value*
Age/year	Positive	22	27.13 ± 4.62	0.6
	Negative	78	27.64 ± 4.05	

Table 4: The differences in the mean gestational age according to fetal fibronectin results

Variable	*Fetal fibronectin*	*N*	*Mean ± SD*	*p-value*
Gestational age (weeks)	Positive	22	30.77 ± 1.92	0.8
	Negative	78	30.89 ± 1.88	

DISCUSSION

This prospective cross-sectional study included 100 pregnant women at their 24 to 36th gestational week who presented to the hospital with abdominal cramps and manifestations suggesting PTL. The main finding of the current study is the highly significant association of fFN concentrations with the incidence of PTL. Pregnant women with higher levels of fFN have 360 times more chance to develop PTL. Consequently, cervicovaginal levels of fFN can be considered a useful predictor of the incidence of PTL among women in their 24 to 36th gestational weeks. Thus, obstetricians can apply the additional risk indicator provided by fFN measures to assure women who are otherwise low risk according to the cervicovaginal fFN values, even when other risk factors for PTB exist.

According to the "American College of Obstetricians and Gynecologists," PTL is defined as childbirth before the 37 week of pregnancy and is a recognized cause of most non-genetic perinatal morbidity and/or mortality.[11,12] Threatened PTL is manifested usually by backache, altered vaginal discharge, uterine contractions, vaginal spotting, and abdominal and pelvic cramps. Threatened PTL can be diagnosed by various modalities like uterine monitoring and digital vaginal examination, to assess cervical sizes. However, such techniques are of limited value, as < 3 cervical dilatation and uterine contraction usually arise and remain not inevitably diagnostic of impending PTL.[11,13]

In contrast, cervicovaginal fFN analysis in singleton conceptions with threatened PTL is not linked with preventing 73-PTB or improving perinatal outcome; nevertheless, is associated with additional costs reported by a study conducted in 2016 in the USA.[14]

C-reactive protein is a hepatic acute-phase protein.[15-19] A recent Chinese case-control study revealed that inflammatory biomarkers, including C-reactive protein, may have a critical

Table 5: Association between fetal fibronectin and the parity

Parity	*Fetal fibronectin*		*p-value*	*Odds*	*95% CI*
	Positive	*Negative*			
Primigravida	6 (27.3)	21 (26.9)	0.9	0.961	0.311 – 2.974
Unipara	11 (50.0)	37 (47.5)		1.143	0.301 – 4.345
Multipara	5 (22.7)	20 (25.6)			

role in the initial analysis or prognosis of PTL.[20] On the same channel, interleukins are inflammatory cytokines.[16,21] in addition to "tumor necrosis factor-alpha" as proinflammatory cytokines[22,23] and several other inflammatory biomarkers have been also evaluated to test PTL.[24] Nevertheless, more than a few plasma biomarkers have been assessed, but none have been extensively adopted for clinical practice.[23,25-27]

Studies of placental immunohistochemistry have exposed that normally fFN is kept within the "extracellular matrix" of the junctional area of the fetal and maternal tissues inside the uterus.[11] Normally, fFN is raised in cervicovaginal fluids during the first trimester, then is reduced at 22–35 weeks, although physiologically, this is indefinite. Still, it might replicate a typical growth of the placenta and the extravillous trophoblasts.

There is growing support for the claim that fFN is a useful predictor of PTL, which seems to validate the outcomes of the current study. Identification of fFN in cervicovaginal discharges in symptomatic and asymptomatic women with PTL.[8,9] Early gestational fFN tests (less than 22 weeks) do not provide a strong predictive ability among twin pregnancies, unlike singleton, and must not be depended on until additional research can approve or contradict this.[28] The qualitative assay of fFN in the current study was not associated with age, parity, or gestational ages/week.

Because the majority of negative fFN tests with a good *95% CI* (50.36–286.17) in the current study, consistent with preceding studies,[29] the use of fFN values provides a first-hand, exclusive prospect to reassure the most pregnant who are hypothetically at potentially high risk, possibly decreasing substantial expenses.

CONCLUSION

Among pregnant women with uterine contraction before advanced cervical dilatation, the presence of cervicovaginal fFN is an associated and reliable screening tool in predicting the risk of PTL.

LIMITATIONS

We have encountered several limitations in this study. Firstly, experimental limitations of the fFN-test, as the test can basically be applied for the qualitative recognition of cervicovaginal fFN. The assay outcomes had better be evaluated in combination with further clinical findings and lab tests for precise management of the women.

REFERENCES

1. Kvalvik LG, Wilcox AJ, Skjærven R, Østbye T, Harmon QE. Term complications and subsequent risk of preterm birth: registry based study. BMJ. 2020;369:m1007.
2. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. American journal of obstetrics and gynecology. 2011;204(3):193-201.
3. Roberts JM. Pathophysiology of ischemic placental disease. Seminars in perinatology. 2014;38(3):139-45.
4. Hezelgrave NL, Shennan AH. Quantitative fetal fibronectin to predict spontaneous preterm birth: a review. Women's health (London, England). 2016;12(1):121-8.
5. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. The Lancet Global health. 2019;7(1):e37-e46.
6. Morgan AS, Mendonça M, Thiele N, David AL. Management and outcomes of extreme preterm birth. BMJ. 2022;376:e055924.
7. Ancel PY, Goffinet F, Kuhn P, Langer B, Matis J, Hernandorena X, et al. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. JAMA pediatrics. 2015;169(3):230-8.
8. Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, et al. Quantitative Fetal Fibronectin to Predict Preterm Birth in Asymptomatic Women at High Risk. 2015;125(5):1168-76.
9. Kuhrt K, Smout E, Hezelgrave N, Seed PT, Carter J, Shennan AH. Development and validation of a tool incorporating cervical length and quantitative fetal fibronectin to predict spontaneous preterm birth in asymptomatic high-risk women. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016;47(1):104-9.
10. Berghella V, Hayes E, Visintine J, Baxter JK. Fetal fibronectin testing for reducing the risk of preterm birth. Cochrane Database Syst Rev. 2008;2008(4):CD006843-CD.
11. Lockwood CJ, Senyei AE, Dische MR, Casal D, Shah KD, Thung SN, et al. Fetal Fibronectin in Cervical and Vaginal Secretions as a Predictor of Preterm Delivery. New England Journal of Medicine. 1991;325(10):669-74.
12. Morrison JC. Preterm birth: a puzzle worth solving. Obstetrics and gynecology. 1990;76(1 Suppl):5s-12s.
13. Iams JD, Casal D, McGregor JA, Goodwin TM, Kreaden US, Lowensohn R, et al. Fetal fibronectin improves the accuracy of diagnosis of preterm labor. American journal of obstetrics and gynecology. 1995;173(1):141-5.
14. Berghella V, Saccone G. Fetal fibronectin testing for prevention of preterm birth in singleton pregnancies with threatened preterm labor: a systematic review and metaanalysis of randomized controlled trials. American journal of obstetrics and gynecology. 2016;215(4):431-8.
15. Al-Agam A OA, Alzughaibi M, Makki H, Alhaideri AF. The Association of Depressive Symptoms With Plasma C-Reactive Protein in Patients With Major Depressive Disorder Under Treatment. Iranian Rehabilitation Journal. 2021;19(4):425-32.
16. Al-Hindy HA-AM, AJHA-A, MJM, SJH, SHO. The Utility of Serum IL-1β and CRP Together with Fractional Exhaled Nitric Oxide in the Diagnosis of Asthma in Adults. NeuroQuantology. 2021;19(8):119-24.
17. Amer Fadhil Alhaideri ANMA-A, Hayder Abdul-Amir M. Al-Hindy, Mazin J. Mousa, Hawraa Kadhum, Saja Hatem. Inflammatory Associations of Peripheral Oxytocin, C-Reactive Protein Levels with Depression Among Adult Age Group with Major Depressive Disorder. Clinical Schizophrenia & Related Psychoses. 2022.
18. Qasim J. HS, Ghada H., Hayder AA. High-Sensitivity C-reactive protein Assessment in Bronchial Asthma: Impact of Exhaled Nitric Oxide and Body Mass Index. Systematic Reviews in Pharmacy. 2020;11(3):705-11.
19. Raghdan Z. AKS, Dleikh F., Al-Hindy H. . Is There any Association Between Highly Sensitive C-Reactive Protein and

Dental-Status in Ischemic Heart Diseases? A Comparative Stud. Biochemical and Cellular Archives. 2020;20(2):6069-75.
20. Huang S, Tian J, Liu C, Long Y, Cao D, Wei L, et al. Elevated C-reactive protein and complement C3 levels are associated with preterm birth: a nested case–control study in Chinese women. BMC Pregnancy and Childbirth. 2020;20(1):131.
21. Amjed H. Abbas MAR, Hayder Abdul-Amir Al-Hindy, Mazin J. Mousa and Hadeel Abd Ameir Al-Shalah. The Role of Serum IL-1β in Combination with Fractional Exhaled Nitric Oxide in the Diagnosis of Adult Bronchial Asthma. NeuroQuantology. 2021;19(9):13-9.
22. Hayder Abdul-Amir Alhindy and Mazin J. Mousa Enas K. Alkhazraji AAA, Mahir Abdulkhadhum Alzughaibi. Positive correlation of circulating tumor necrosis factor-alpha (TNFα) with spirometric pulmonary tests in adult asthmatic patients. Biochem Cell Arch. 2021;21:2605-10.
23. Wallenstein MB, Jelliffe-Pawlowski LL, Yang W, Carmichael SL, Stevenson DK, Ryckman KK, et al. Inflammatory biomarkers and spontaneous preterm birth among obese women. J Matern Fetal Neona. 2016;29(20):3317-22.
24. Bulletin: CP. Fetal Fibronectin, Inflammatory Biomarkers, and Salivary Estriol Testing for Preterm Labor. Aetna. 2015;http://qawww.aetna.com/cpb/medical/data/100_199/0166_draft.html:1-13.
25. Maymon R, Bahari C, Moroz C. Placental isoferritin measured by a specific monoclonal antibody as a predictive marker for preterm contraction outcome. Obstetrics and gynecology. 1989;74(4):597-9.
26. Wasmoen TL, Coulam CB, Leiferman KM, Gleich GJ. Increases of plasma eosinophil major basic protein levels late in pregnancy predict onset of labor. Proceedings of the National Academy of Sciences of the United States of America. 1987;84(9):3029-32.
27. McGregor JA, Jackson GM, Lachelin GC, Goodwin TM, Artal R, Hastings C, et al. Salivary estriol as risk assessment for preterm labor: a prospective trial. American journal of obstetrics and gynecology. 1995;173(4):1337-42.
28. Kuhrt K, Hezelgrave-Elliott N, Stock SJ, Tribe R, Seed PT, Shennan AH. Quantitative fetal fibronectin for prediction of preterm birth in asymptomatic twin pregnancy. Acta Obstet Gynecol Scand. 2020;99(9):1191-7.
29. Peaceman AM, Andrews WW, Thorp JM, Cliver SP, Lukes A, Iams JD, et al. Fetal fibronectin as a predictor of preterm birth in patients with symptoms: a multicenter trial. American journal of obstetrics and gynecology. 1997;177(1):13-8.