Cervicovaginal fetal fibronectin (FFN) for prediction of preterm delivery in symptomatic cases: a prospective study

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Summary

Objective: To assess the clinical value of cervicovaginal fetal fibronectin (FFN) in the prediction of preterm delivery (PTD) in women with signs and symptoms of preterm labor (PTL). *Method:* This investigation prospectively studied a cohort of a women with symptoms of PTL, between 24 and 37 weeks' gestation with < 3 cm of cervical dilatation and intact membranes. Cases were evaluated in terms of maternal demographic characteristics like age, body mass index, number of parities, previous PTL history, Bishop scores at admission, gestational age at delivery, mode of delivery, use of tocolytic or steroids, presence of histologic chorioamnionitis, neonatal outcomes and delivery before 34 weeks' gestation as well as within seven days of admission. *Results:* A total number of 68 cases were included in the study. There were no statistically significant differences between positive and negative FFN groups in terms of maternal characteristics, mode of delivery and adverse neonatal outcomes. However, FFN + cases had higher Bishop scores on admission (3.4 ± 1.2 vs 2.5 ± 0.3, p = 0.03) and lower gestational age at delivery (33.4 ± 3.1 weeks vs 36.8 ± 2.1 weeks, p = 0.002). Likelihood ratio (LR) for positive results was 1.83 (95% CI: 1.61-2.26) for predicting birth before 34 weeks' gestation, with a corresponding negative LR of 0.62 (95% CI: 0.3-1.2). LR for positive results was 4.34 (95% CI: 3.65-5.12) for predicting birth within seven days of testing, with a corresponding negative LR of 0.3 (95% CI: 0.2-0.5). *Conclusion:* Based on the results of cervicovaginal FFN, positive tests represent an increased likelihood of PTD among women with symptoms of threatened preterm labor.

Key words: Preterm labor; Fetal fibronectin; Preterm delivery; Prediction.

Introduction

Preterm labor complicates 8% to 12% of all deliveries and is responsible for 70% of perinatal mortality and morbidity [1]. The prediction and prevention of preterm birth have proven to be an obstetric challenge. The identification of women at risk of preterm delivery would allow the initiation of important interventions to delay delivery and to improve perinatal outcome, such as maternal transfer to a tertiary-care center, tocolysis and corticosteriod therapy [2]. Current evidence supports the screening of preterm delivery by maternal obstetric history, cervical ultrasonography, and several biomarkers in the serum and cervicovaginal secretions [3-5]. These above-mentioned markers have been extensively studied. Other markers like the presence of bacterial vaginosis, interleukin (IL)-6, ferritin, and granulocyte colony-stimulating factor levels have also been assessed, several of which have predictive values potentially useful for clinical practice [5, 6].

Fetal fibronectin (FFN) is a glycoprotein found in amniotic membranes, decidua, and cytotrophoblasts. The appearence of FFN in cervicovaginal secretions in the late second and early third trimester represent disruption of the chorio-decidual surface, leading to spontaneous preterm birth [7].

This prospective cohort study was conducted to assess the previously described association of FFN with preterm delivery (PTD) in women with symptoms suggestive of premature labor in whom no prior tocolytic treatment was initiated.

Materials and Methods

Approval for this study was obtained from the Institutional Ethical Board and all the authors conformed to the Declaration of Helsinki during the study period. This investigation prospectively studied a cohort of a 65 women with symptoms of PTL, between 24 and 37 weeks' gestation with < 3 cm cervical dilatation and intact membranes, from January 2004 to July 2006. Symptoms suggestive of preterm labor included regular uterine contractions, low back pain, minimal vaginal bleeding and increased vaginal discharge. Cases were excluded if they had cervical cerclage, massive vaginal bleeding, tocolysis at admission, or cervical manipulation such as vaginal douche, intercourse or digital examination within the previous 24 hours, preeclampsia, diabetes mellitus, hyperthyroidism or asthma. Symptomatic treatment included intravenous ritodrine hydrochloride or magnesium sulphate. Ritodrine hydrochloride was given as an intravenous infusion of 50-100 µg/mn in a 5% dextrose solution in water and increased by 50 µg/mn every 20 min until adequate tocolysis was achieved or up to a maximum dose of 350 µg/min. Magnesium sulphate was given as a bolus dose of 4 g in 100 ml saline solution, followed by a maintenence dose of 2 g/hour as an intravenous infusion. A total intramuscular dose of 24 mg betamethasone was given (12 mg) twice daily to enhance fetal lung maturation. Mode of delivery was dependent on obstetric indications.

During the initial physical examination, a speculum was introduced into the vagina before digital examination. The FFN specimen collection kit (*Quick*Check fFN, Adeza Biochemical Cooperation, Sunnyvale, CA) contains a dacron® swab (Dupont, Kinston, NC) an a buffer-filled collection tube. The dacron

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polyester swab was rolled against the posterior lip of the cervix. The collected specimen was placed in a buffer solution and sealed witin the collection tube. All samples were sent to the hospital laboratory and the FFN was processed by monoclonal antibody ELISA rapid assay (Adeza), with results avilable within 30 minutes. All the digital examinations were made by a single experienced physician.

Results were blinded to managing obstetricians during the study. Decisions on tocolytic and steroid use after specimen collection were made by managing physicians. Positive and ngative FFN cases were evaluated in terms of maternal demographic characteristics like age, body mass index (BMI), number of parities, previous PTL history, smoking status, number of pregnancies from assisted reproductive techniques (ART), Bishop scores at admission, gestational age at delivery, mode of delivery, use of tocolytics, antibiotics or steroids, presence of histologic chorioamnionitis, neonatal outcomes such as birthweight, Apgars scores, days in the neonatal intensive care unit (NICU), newborn sepsis, neonatal death, and delivery before 34 weeks'gestation as well as within seven days of admission. A positive test was defined as a fetal fibronectin concentration > 50 ng/ml. Histological chorioamnionitis was defined by the criteria of Salafia *et al*. [7].

Statistical analysis was performed using the SPSS 10.0 (SPSS10.0, Chicago, IL, USA) statistical package. Results are presented as the mean ± standard deviation. Patient demographic chatracteristics were analyzed by the Student's t-test, and the chi-square test was used for discrete variables. Univariate and multivariate logistic regression analyses were performed to evaluate the association of various confounding variables and FFN with the outcome of pregnancy. Kaplan-Meier curves were compared with the Wilcoxon log-rank test. Statistical significance was assumed at $p < 0.05$. The sample size was predetermined using a power analysis. We calculated 68 patients would be required to demonstrate a significant association between FFN and outcome with a positive predictive value (PPV) of at least 40% and a negative predictive value (NPV) of 70%. In order to deal with the uncertainty in estimation, we generated 95% confidence intervals (CI) for post-test probabilities arount the point estimate.

Results

In this cohort, the rate of preterm delivery before 37 and 34 weeks was 19.1% (13/68) and 8.8% (6/68), respectively. As shown in Table 1, there were no statistically significant differences between FFN positive and negative groups in terms of maternal characteristics, mode of delivery and adverse neonatal outcomes. However, positive FFN cases had higher Bishop scores on admission ($p = 0.04$) and longer duration of tocolysis ($p = 0.01$) but lower gestational age at delivery ($p = 0.002$), time from admission to delivery ($p = 0.003$ and birthweight ($p = 0.04$). As shown in Table 2, univariate analysis showed that the strongest predictors of PTD < 34 weeks' gestation was FFN positivity (RR: 55.2, 95%, CI: 9-335, $p < 0.001$) and the history of PTL (RR: 4.89, 95% CI: 1.21-19.76, $p = 0.02$). For deliveries within seven days of admission, FFN positivity (RR: 14.6, 95% CI: 4.3-49.9, $p < 0.0001$), Bishop score (RR: 1.3, 95% CI: 1.01-1.66, $p = 0.03$) and cervical dilatation on admission (RR: 1.63, 95% CI: 1.03-2.57, $p = 0.03$) were found to be statistically significant (Table 3).

Table 1. — *Maternal and neonatal characteristics of cases with cervicovaginal positive and negative FFN (ns: not significant).*

	FFN test		
	(+) (n = 36)	(–) (n = 32)	p value
Age (years)	28.5 ± 3.5	28.3 ± 2.3	ns
BMI (kg/m^2)	25.8 ± 1.2	26.7 ± 1.2	ns
Gravidity (n)	2.1 ± 1.2	2.2 ± 1.4	ns
Parity (n)	0.69 ± 0.7	0.69 ± 0.2	ns
Abortion (n)	1.7 ± 0.6	1.5 ± 0.5	ns
Gestational age on admission (weeks)	31.1± 2.5	30.6 ± 2.3	ns
History of abortion (n)	10	12	ns
Tocolytic use (n)	34	29	ns
Cesarean delivery (n)	14	15	ns
Vaginal delivery (n)	21	18	ns
Histological chorioamnionitis (n)	11	5	ns
Bishop score on admission	3.4 ± 2.1	2.5 ± 2.0	0.04
Cervical dilatation on admission (cm)	1.8 ± 1.2	1.4 ± 1.0	ns
Cervical effacement on admission (%)	32.2±10.5	29.0 ± 10.3	ns
Duration of tocolysis (days)	7.1 ± 1.3	5.9 ± 2.8	0.01
Gsetational age at delivery (weeks)	33.4 ± 3.1	36.8 ± 2.1	0.002
Time from admission to delivery (days)	10.3 ±4.5	23.2 ± 9.1	0.003
Apgar score (1 min)	6.5 ± 2.1	6.9 ± 2.2	ns
Apgar score (5 min)	8.4 ± 1.5	8.6 ± 1.7	ns
Birthweight (g)	2514 ± 716	2796 ±784	0.04
Days in NICU	4.96 ± 5.9	4.51 ± 6.1	ns
Newborn sepsis (n)	2	3	ns
Neonatal death (n)	3	1	ns

NICU: neonatal intensive care unit.

Table 2. — *Univarite analysis of several confounding factors to determine deliveries < 34 weeks' gestation.*

	PTD < 34 weeks' gestation		
	Relative risk	95% CI	p value
Age	0.97	0.86-1.11	0.735
Body mass index	1.07	0.93-1.24	0.304
Multiple pregnancy	1.70	0.38-7.55	0.483
History of PTL	4.89	1.21-19.76	0.026
pH IGFBP-1 +	55.2	9-335	< 0.001
Bishop score	1.16	0.87-1.55	0.295
Cervical dilatation	1.31	0.76-2.55	0.325
Cervical effacement	1.02	0.98-1.03	0.235
Corticosteriod use	1.03	0.9-1.9	0.341
Histological chorioamnionitis	1.38	0.32-5.86	0.655

Table 3. — *Univariate analysis of counfounding factors to determine the deliveries within 7 days of admission.*

	Delivery within 7 days of admission		
	Relative risk	95% CI	p value
Age	0.92	0.83-1.02	0.115
Body mass index	0.91	0.81-1.03	0.157
Multiple pregnancy	0.60	0.16-2.22	0.444
History of PTL	3.23	0.86-12.09	0.081
FFN (+)	14.6	4.3-49.9	< 0.001
Bishop score	1.30	1.01-1.66	0.036
Cervical dilatation	1.63	1.03-2.57	0.034
Cervical effacement	1.02	0.99-1.05	0.062
Tocolysis	0.24	0.02-2.44	0.229
Corticosteroid use	0.61	0.20-1.80	0.374
Histological chorioamnionitis	0.82	0.24-2.75	0.753

As depicted in Table 4, for deliveries < 34 weeks' gestation, the FFN test had a sensitivity, specificity, PPV, NPV, positive LR and negative LR of 57.1%, 69.2%, 66.7%, 70%, 1.8 and 0.6, respectively. For deliveries within seven days of admission, the corresponding figures were: 68.6%, 84.4%, 52.8%, 82.4%, 4.3 and 0.3, respectively. As shown in Figure 1, Kaplan-Meier survival analyses showed that a higher percentage of women with positive FFN delivered within 14 days of sampling, compared to those with negative FFN (Mantel-Cox, log-rank analysis, χ^2 value: 12.1, $p < 0.001$).

Table 4. — *Sensitivity, specificity, PPV, NPV, positive and negative LR's of cervicovaginal FFN for predicting deliveries < 7 days, and < 14 days of admission as well as < 34 weeks' gestation.*

Parameters	< 7 days	< 14 days	< 34 weeks' gestation
Sensitivity (%)	68.6	82.9	57.1
Specificity (%)	84.4	62.5	69.2
PPV (%)	52.8	60.7	66.7
NPV (%)	82.4	86.9	70.0
LR +	4.3 (2.1-9.8)	2.2 (1.4-3.1)	1.83 (1.61-2.26)
LR –	0.3 (0.2-0.5)	0.2 (0.1-0.5)	0.62 (0.3-1.2)

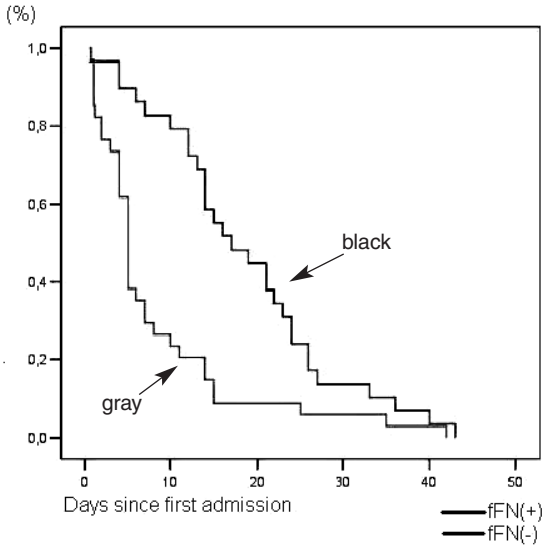

Figure 1. — Percentage of cases which remained undelivered (y-axis) since the first day of admission to the clinic (x-axis) in women with positive (light gray) and negative (black) cervicovaginal FFN.

Discussion

The data from this study demonstrated that in women with symptoms suggestive of preterm labor, positive cervicovaginal FFN was a strong predictor of preterm delivery but not the adverse neonatal outcomes. The presence of FFN was associated with increased risk of delivery within seven days of admission and < 34 weeks' gestation.

Since the first study by Lockwood *et al*. [8] showing the association of positive FFN and preterm delivery, several studies have concluded that in women with symptoms suggestive of preterm delivery, the negative predictive value of the test was over 90% [9, 10].

As shown in Table 4, the high NPV of cervicovaginal FFN for the prediction of deliveries within seven days of admission may indicate less intervention and avoid unnecessary medical procedures in women with threatened preterm labor. Swamy *et al*. [11] also concluded that the NPV of FFN was found to be 98% in 46 subjects with positive FFN. They also found that time to delivery and gestational age at delivery were lower in women with positive tests, conforming with our results. In contrast to the above-mentioned study, the present study showed no significant differences among women with positive or negative FFN in term of the frequency of therapeutic interventions. In a recent study by Eroglu *et al*. [12] that compared the predicting value of different cervicovaginal biomarkers with cerical length in 51 women between 24 to 35 weeks' gestation, NPV of fetal FFN was found to be 91.9%. In the present study, although the number of admissions and length of hospital stay were not mentioned, several strudies did find a significant difference in admissions to the antepartum service and length of stay in the antepartum ward with a negative FFN compared to positive FFN, emphasizing the reduction of unnecessary interventions and hospital stay, thus leading to a substantial cost savings [13, 14]. Similar to our results, in a recent study by Skoll *et al*. [15], of 149 women with symptoms suggestive of preterm labor tested, a negative FFN result was associated with a 97.4% likelihood of delivering more than seven days after testing and with a 91.4% chance of delivering after 34 weeks.

Both acute placental inflammation and positive mid-gestational cervico-vaginal fetal fibronectin assays have been independently correlated with preterm delivery [16]. However, in the present study, women with positive assays were no more likely to have histological evidence of acute inflammation noted at birth than women with negative FFN results. The same result was also observed in the study by Akers *et al*. [17]. Similar to our results, Rizzo *et al*. [18] found that a positive fetal fibronectin > 50 ng/ml was not associated with the presence of histological chorioamnionitis in women with intact membranes and signs sugestive of preterm labor. Hence, FFN is not a sensitive marker in identifying women at risk for the presence of histological chorioamnionitis.

The present study was an intent-to-treat study since the authors were blinded to the FFN results. However, the incidence of preterm birth was found to be higher in symptomatic women with negative FFN [19]. In contrast, blind sampling from the vagina for FFN was found to yield a sensitivity of 52% and specificity of 94.5% with a NPV of 99.1% in pregnant women at high risk for preterm delivery but without any symptoms [20].

Finally, based on the results of our study, the FFN test appears to provide useful information in the preterm delivery risk assessment in women with symptoms suggestive of preterm labor.

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