

Cervicovaginal Foetal Fibronectin in the Prediction of Preterm Labour in a Low-risk Population

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Abstract

The aim of this study was to evaluate the role of cervicovaginal fibronectin as a screening test in a low-risk population. Swabs were taken from the posterior fornix at 28 weeks gestation and foetal fibronectin levels were assayed using enzyme immunoassay. Eighteen patients (7.7%) delivered at less than 37 weeks of gestation, and 6 (2.4%) at less than 34 weeks of gestation. Five patients (2.1%) had a positive fibronectin test result. The sensitivity, specificity, positive predictive value and negative predictive value in the prediction of delivery less than 37 weeks of gestation were 16.7%, 99.1%, 60.0% and 93.4%, respectively. The sensitivity of foetal fibronectin in the prediction of delivery less than 34 weeks of gestation was higher (50%) compared with the prediction of delivery less than 37 weeks of gestation (16.7%). The low sensitivity of this test suggests a limited role for cervicovaginal fibronectin in the low-risk population. Further studies need to be carried out to assess whether multiple testing of cervicovaginal fibronectin at different gestational ages will further increase the sensitivity.

Ann Acad Med Singapore 1997; 26:776-80

Key words: Delivery, Prematurity, Obstetrics

Introduction

Preterm delivery (delivery less than 37 weeks gestation) is the major cause of perinatal mortality in the developed world and occurs in approximately 7% of all deliveries. Severe morbidity, especially respiratory distress syndrome, intraventricular haemorrhage, bronchopulmonary dysplasia, and necrotising enterocolitis, is far more common in preterm infants than in term infants, as is long-term impairment such as cerebral palsy, visual impairment or hearing loss.

The aims of good antenatal care include prevention of preterm labour, the prediction of pregnancies destined to end prematurely and effective tocolysis once preterm labour is diagnosed. The accurate identification of pregnant women who subsequently develop preterm labour allows the appropriate use of maternal steroid therapy, thereby resulting in reduced perinatal morbidity and mortality.¹ The prediction of premature delivery has previously been based on past obstetric history. Although there are numerous risk factors for preterm labour, these risk factors are generally low in sensitivity

(<50%) and in positive predictive value (17% to 34%).^{2,3} Furthermore, more than 50% of women who deliver prematurely have no known risk factors.⁴ A recent multicentre randomised controlled trial of preterm birth demonstrated that the prevention programme involving 2395 women with a risk factor for preterm labour had no beneficial effect on preterm birth rates.⁵ Frequent vaginal examinations to assess cervical status⁶ and ultrasound visualisations of cervical length and dilatation⁷ have also been suggested to be useful in the prediction of preterm labour. However, these methods of predicting preterm labour have yet to be proven to be clinically useful in large studies.

In view of the above limitations, there is a need for a test which accurately predicts preterm labour. The presence of foetal fibronectin in cervicovaginal secretions has been proposed as a specific indicator of preterm delivery.⁸ There is evidence that preterm labour may be preceded by alterations in the biologic activity of chorionic and adjacent decidual cells. Genital infections are associated with preterm labour.⁹ Chorionic and decidual

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cell prostaglandin production can be stimulated by bacterial endotoxins and inflammatory cytokines such as interleukin-1 and tumour necrosis factor.¹⁰ These and other cytokines promote the release of extracellular matrix-degrading collagenases by chorionic, decidual and cervical cells.¹¹ The collagenases result in preterm labour by promoting cervical change and disrupting the chorionic-decidual matrix interface. On the basis of this model, Lockwood et al⁸ hypothesised that preterm labour is preceded by progressive proteolytic disruption of the chorionic-decidual extracellular matrix interface, which results in the release of degraded matrix proteins into the cervical and vaginal secretions. Fibronectins are a family of widely distributed proteins located in the extracellular ground substance. A particular fibronectin has been identified in amniotic fluid, placental tissue and malignant cell lines. This can be detected by a specific monoclonal antibody FDC-6¹² and is called foetal fibronectin. Immunohistochemical studies suggest that foetal fibronectin is present in the extracellular matrix of the decidua basalis adjacent to the intervillous space.⁸

Numerous studies have suggested that foetal fibronectin is useful in predicting preterm labour in high-risk pregnancies^{8,13-15} and the success of induction of labour at term.¹⁶ Foetal fibronectin has also been useful in distinguishing those patients with threatened preterm labour who subsequently deliver preterm from those who do not.¹⁷⁻¹⁹ However, for a test to be clinically useful, its ability as a screening test to predict preterm labour in a low-risk unselected population should also be evaluated. A screening study was therefore designed to evaluate the usefulness of foetal fibronectin in cervicovaginal secretions in the prediction of preterm labour in a low-risk obstetric population at Kandang Kerbau Hospital, Singapore.

Materials and Method

A prospective observational screening study was carried out between April 1995 and September 1995. A total of 240 asymptomatic women at 28 weeks of gestation with no risk factors for preterm labour were recruited into the study from the out-patient clinics. The gestational age was assessed by both the patient's last menstrual age and first trimester crown-rump length dating. Risk factors for preterm labour were defined as a history of preterm labour in previous pregnancies, multiple pregnancies or a history of spurious labour during the antecedent pregnancy. Patients with active vaginal bleeding, placenta praevia, uncertain gestational age, hypertension, or suspected rupture of membranes were also excluded from the study.

After obtaining informed consent, a speculum examination was performed once at 28 weeks gestational age to obtain a sample for cervicovaginal fibronectin. At the

time of the speculum examination, premature rupture of membranes was excluded and samples were taken in the absence of bleeding in the lower genital tract. Foetal fibronectin sample was obtained by rolling a Dacron swab gently in the posterior fornix for 10 seconds. The swab was then extracted in 750 µml of assay buffer (Tris buffer containing bovine serum albumin as a stabiliser and protease inhibitors). The solution was then frozen immediately at -20°C. The samples were then stored until the time of assay.

The concentration of foetal fibronectin was measured by a solid-phase, enzyme-linked immunosorbent assay using the monoclonal antibody FDC-6 to specifically bind foetal fibronectin (Adeza Biochemical, USA). These assays were performed in batches. The absorbance of duplicate negative and positive controls and patient samples were determined at a wavelength of 550 nm with an automated microtitre plate reader. Patients samples with an absorbance greater than the positive control (50 ng/ml foetal fibronectin) were deemed positive for the presence of foetal fibronectin. The methodology described here is consistent with that reported previously.^{8,13} Results of the fibronectin levels were not revealed to the clinicians.

The outcome measures evaluated were delivery less than 37 weeks of gestation and delivery less than 34 weeks of gestation.

To decide on the number of patients (n) to be enrolled into the study, we performed a power calculation based on the assumption that the test was expected to have a specificity (Sp) of 80% and sensitivity (Se) of 65% to within five percentage points (0.05). Using the formulae $0.05 = 1.96 [Sp (1-Sp/n)]^{0.05}$ and $0.05 = 1.96 [(Se (1-Se)/n)]^{0.05}$ respectively, the number of subjects would have to be between 190 and 368. The sensitivity, specificity, positive predictive value and negative predictive value were calculated to evaluate the usefulness of cervicovaginal fibronectin as a screening test.

Results

From the 240 women recruited into the study, 6 were excluded because of subsequent development of hypertensive disease. Data from 234 women were therefore used in the final analysis. Of these, 150 were primigravidae and 84 were multigravidae.

Eighteen patients (7.7%) subsequently delivered at less than 37 weeks of gestation. Six patients (2.4%) delivered at less than 34 weeks of gestation. None of the patients delivered prematurely for introgenic reasons and none developed premature labour which was subsequently inhibited pharmacologically. The number of patients who had a positive fibronectin test result was 5 (2.1%). The sensitivity, specificity, positive predictive value and negative predictive value of foetal fibronectin

in the prediction of delivery at less than 37 weeks gestation are shown in Table I. While the sensitivity was low (16.7%), the positive predictive value was high (60%) with a high specificity (99.1%) and negative predictive value (93.4%). The sensitivity, specificity, positive predictive value and negative predictive value of foetal fibronectin in the prediction of delivery at less than 34 weeks of gestation are shown in Table II. The sensitivity of foetal fibronectin in the prediction of delivery at less than 34 weeks of gestation was higher (50%) compared with the prediction of delivery at less than 37 weeks of gestation (16.7%).

TABLE I: CERVICOVAGINAL FIBRONECTIN IN THE PREDICTION OF DELIVERY LESS THAN 37 WEEKS OF GESTATION

	Delivery less than 37 weeks	
	Yes	No
Positive fibronectin (>50 ng/ml)	3	2
Negative fibronectin (<50 ng/ml)	15	214

Sensitivity 16.7%; specificity 99.1%; positive predictive value 60.0%; negative predictive value 93.4%

TABLE II: CERVICOVAGINAL FIBRONECTIN IN THE PREDICTION OF DELIVERY LESS THAN 34 WEEKS OF GESTATION

	Delivery less than 34 weeks	
	Yes	No
Positive fibronectin (>50 ng/ml)	3	2
Negative fibronectin (<50 ng/ml)	3	226

Sensitivity 50.0%; specificity 99.1%; positive predictive value 60.0%; negative predictive value 98.7%

Discussion

The antenatal identification of pregnant women who are at increased risk of preterm labour is an important aspect of obstetric care. Of all the methods which have previously been described to predict preterm labour and delivery, none have been shown to be clinically useful in low-risk pregnancies.²⁰ Recent studies have suggested that the presence of foetal fibronectin in vaginal secretion predicts imminent preterm delivery in high-risk pregnancies and in pregnancies with evidence of preterm labour.^{15,17-19} There has only been one previous study assessing the role of foetal fibronectin in the low-risk population.²¹ The results of the present study, the largest to date, suggest a role for foetal fibronectin screening for preterm delivery within a low-risk obstetric population.

The obstetric population we studied was at low risk of preterm labour and delivery. We specifically excluded patients with an antecedent history of spurious labour.

Patients with vaginal bleeding were excluded as the monoclonal antibody FDC-6 also binds to maternal plasma fibronectin.^{22,23} Fibronectin is also elevated in patients who later develop pre-eclampsia and its release into the circulation is closely related to the pathophysiology of vascular endothelial injury.²⁴ The study therefore excluded results from patients who developed hypertensive disease during pregnancy. In our study, we elected to evaluate the usefulness of a single test of cervicovaginal fibronectin level at 28 weeks of gestation in the prediction of subsequent preterm delivery. This is in contrast to some previous studies where patients were sampled up to 6 times during the second and third trimesters of their pregnancies.^{15,21} We felt that for a screening test to be clinically meaningful and acceptable in otherwise asymptomatic women, testing for cervicovaginal levels of fibronectin should be confined to one gestational age, thereby minimising the inconvenience to the patient and reducing the number of vaginal examinations. Sampling was performed from the posterior vaginal fornix. A cut-off value of 50 ng/ml, consistent with previous studies,^{15,17-19,21} was used in our study to define raised levels of cervicovaginal fibronectin.

Previous studies evaluating the use of foetal fibronectin in high-risk pregnancies have reported high sensitivities and positive predictive values in the prediction of preterm delivery. Leeson et al¹⁵ evaluated the role of foetal fibronectin in 43 women at high risk of preterm delivery. High vaginal swabs were taken fortnightly from 24 to 34 weeks of gestation. The sensitivity, specificity and positive predictive value in the prediction of delivery before 37 weeks were 70%, 93% and 50%, respectively. In a study of 36 patients with threatened preterm labour, the presence of foetal fibronectin was found to be predictive of delivery prior to 34 weeks gestational age.¹⁷ All patients with a positive fibronectin test delivered before 34 weeks (sensitivity of 100%) with a positive predictive value of 46%. In that study, 25 patients had a negative fibronectin test and none delivered before 34 weeks (negative predictive value of 100%). In another study of 37 patients with preterm labour, cervical foetal fibronectin test identified 89% of patients who delivered before 34 weeks gestation with a positive predictive value of 79%.¹⁸ Iams et al¹⁹ reported a sensitivity of 44% and positive predictive value of 60% in the prediction of delivery less than 37 of weeks gestation in a group of 192 women with preterm labour.

The sensitivity was increased to 93% and the positive predictive value reduced to 29% in the prediction of delivery within 7 days. In a study based on a high-risk inner city population in New York, foetal fibronectin predicted 68% of preterm deliveries with a specificity and positive predictive value of 80% and 30%, respectively.¹⁴ In a small study of 28 women with false labour,¹³ 14 had positive fibronectin and all delivered preterm

(positive predictive value of 100%). In a study of 87 women at increased risk for preterm delivery, the presence of cervicovaginal fibronectin identified 93% of women who delivered before 37 weeks gestation with a positive predictive value of 46%.²⁵ It should be noted that all the above studies were carried out in pregnancies at high risk of preterm delivery and the results may not necessarily be replicated in a low-risk population.

Hellemans and coworkers²¹ reported data on the usefulness of foetal fibronectin as a screening test in a low-risk population. In a study of 133 women, speculum examinations were performed at fortnightly intervals from 26 to 36 weeks of gestation. The sensitivity and positive predictive values in the prediction of preterm contractions were 47% and 29%, respectively. The sensitivity and positive predictive values in the prediction of preterm delivery were 60% and 25%, respectively. The sensitivity reported in our study (16.7%) was much lower than that reported by Hellemans et al.²¹ The positive predictive value in our study (60%) was higher than that reported by Hellemans et al.²¹ While the prevalence of preterm delivery did not differ between the two studies (7.7% in the present study compared with 7.5% in the study of Hellemans et al.²¹), part of the difference in the results may be explained by the larger number of patients in our study ($n = 234$) and the considerably lower proportion of patients who were test positive in our study (2.1% compared with 18.0% in the study of Hellemans et al.²¹). It should be noted that maternal blood was detected in the vaginal specimens of 8 patients in the study of Hellemans et al.²¹ Of these 8 women, 7 had positive fibronectin test results but did not deliver preterm, possibly contributing to the large numbers of false positives and lower positive predictive value in that study. It is also noteworthy that whilst the sensitivity in the prediction of delivery less than 37 weeks of gestation in our study was low (16.7%), the sensitivity in the prediction of delivery less than 34 weeks of gestation was higher (50%). This is in agreement with previous workers who have reported higher sensitivities when an earlier gestation at delivery was used as the outcome criterion.^{15,19} Furthermore, morbidity data have shown that serious morbidity associated with preterm deliveries is greater in deliveries less than 34 weeks of gestation compared with deliveries after 34 weeks but before 37 weeks of gestation.³

Our study is the largest to date to evaluate the role of cervicovaginal fibronectin as a screening test for preterm delivery in a low-risk population. The high positive predictive value in our study suggests that a patient with a positive test result is likely to deliver preterm. Conversely, the high negative predictive value and specificity suggest that a negative result is likely to be reassuring to the clinician. However, the low sensitivity in our study suggests a limited role for the use of foetal fibronectin in

predicting preterm deliveries in the population as a whole. Before its widespread introduction as a screening test in the low-risk population, further studies need to be carried out to assess whether multiple testing of cervicovaginal fibronectin at different gestational ages will further increase the sensitivity of this test.

Acknowledgement

This study was supported by a research grant from the Chapter of Obstetricians and Gynaecologists, Academy of Medicine, Singapore.

REFERENCES

- 1 Crowley I', Chalmers I, Keirse M J N C. The effects of corticosteroid administration before preterm delivery: an overview of evidence from controlled trials. *Br J Obstet Gynaecol* 1990; 97:11-25.
- 2 Carr-Hill R, Hall M. The repetition of spontaneous preterm labour. *Br J Obstet Gynaecol* 1985; 92:921-8.
- 3 Keirse M J N C. New perspectives for the effective treatment of preterm labour. *Am J Obstet Gynecol* 1995; 173:618-28.
- 4 Creasey R K, Merkatz I R. Prevention of preterm birth: clinical opinion. *Obstet Gynecol* 1990; 76:25-45.
- 5 Collaborative Group on Preterm Birth Prevention. Multicentre randomized controlled trial of a preterm birth prevention program. *Am J Obstet Gynecol* 1993; 169:352-66.
- 6 Buekens I', Alexander S, Boutsen M, Blondeel B, Kaminski M, Reid M, et al. Randomised controlled trial of routine cervical examinations in pregnancy. *Lancet* 1994; 344:841-4.
- 7 Gomez R, Galasso M, Romero R, Mazor M, Sorokin Y, Bauman R, et al. Ultrasonographic examination of the uterine cervix is better than cervical digital examination as a predictor of the likelihood of premature delivery in patients with preterm labor and intact membranes. *Am J Obstet Gynecol* 1994; 171:956-64.
- 8 Lockwood C J, Senyei A E, Dische M R, Casal D, Shah K, Jones L, et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. *N Engl J Med* 1991; 125:669-74.
- 9 Romero R, Avila C, Brekus CA, Marotti R. The role of systematic and intrauterine infection in preterm parturition. *Ann NY Acad Sci* 1991; 622:355-75.
- 10 Molner M, Romero R, Hertelendy F. Interleukin-1 and tumor necrosis factor stimulate arachidonic acid release and phospholipid metabolism in human myometrial cells. *Am J Obstet Gynecol* 1993; 169:825-9.
- 11 Kelly R W, Leask R, Calder A A. Chorionic production of interleukin-8 and mechanism of parturition. *Lancet* 1992; 339:776-7.
- 12 Matsuura H, Hakamori S. The oncofetal domain of fibronectin defined by monoclonal antibody FDC-6: its presence in fibronectins from fetal and tumour tissue and its absence in those from normal adult tissues and plasma. *Proc Natl Acad Sci USA* 1985; 82:6517-21.
- 13 Morrison J C, Albert J R, McLaughlin B N, Whitworth N S, Roberts W E, Martin R W. Oncofetal fibronectin in patients with false labor as a predictor of preterm delivery. *Am J Obstet Gynecol* 1993; 168:538-42.
- 14 Lockwood C J, Wein R, Lapinski R, Casal D, Berkowitzg, Alvarezm, et al. The presence of cervical and vaginal fetal fibronectin predicts preterm delivery in an inner-city obstetric population. *Am J Obstet Gynecol* 1993; 169:798-804.
- 15 Leeson S C, Maresh M J A, Martindale E A, Mahmood T, Muotone A, Hawkes A, et al. Detection of fetal fibronectin as a predictor of preterm delivery in high risk asymptomatic pregnancies. *Br J Obstet Gynaecol* 1996; 13:48-53.
- 16 Blanch G, Olah KS J, Walkinshaw S. The presence of fetal fibronectin in the cervicovaginal secretions of women at term-Its role in the assessment of

- women before labor induction and in the investigation of the physiologic mechanisms of labor. *Am J Obstet Gynecol* 1996; 174:262-6.
17. Parker J, Bell R, Brennecke S. Fetal fibronectin in the cervicovaginal fluid of women with threatened preterm labour as a predictor of delivery before 34 weeks gestation. *Aust NZ J Obstet Gynaecol* 1995; 35:257-61.
 18. Burrus DR, Ernewst JM, Veille JC. Fetal fibronectin, interleukin-6, and C-reactive protein are useful in establishing prognostic subcategories of idiopathic preterm labor. *Am J Obstet Gynecol* 1995; 173:1258-62.
 19. Iams JD, Casal D, McGregor JA, Goodwin TM, Kreaden US, Lowensohn R, et al. Fetal fibronectin improves the accuracy of diagnosis of preterm labour. *Am J Obstet Gynecol* 1995; 173:141-5.
 20. McLean M, Walters WA W, Smith R. Prediction and early diagnosis of preterm labor: a critical review. *Obstet Gynecol Surv* 1993; 48:209-25.
 21. Hellemans P, Gerris J, Verdonk P. Fetal fibronectin detection for prediction of preterm birth in low risk women. *Br J Obstet Gynaecol* 1995; 102:207-12.
 22. Sadovsky Y, Friedman SA. Fetal fibronectin and preterm labor. *N Engl J Med* 1992; 216:709.
 23. Feinberg FR, Wang C. Monoclonal antibody FDC-6 exhibits binding to human plasma fibronectin: A caveat for cervicovaginal oncofetal fibronectin testing? *Am J Obstet Gynecol* 1994; 171:1302-8.
 24. Taylor RN, Crombleholme WR, Friedman SA, Jones LA, Casal DC, Roberts JM. High plasma cellular fibronectin levels correlate with biochemical and clinical features of pre-eclampsia but cannot be attributed to hypertension alone. *Am J Obstet Gynecol* 1991; 165:895-901.
 25. Inglis SR, Jeremias J, Kuno K, Lescale K, Peeper Q, Chervenak FA, et al. Detection of tumour necrosis factor-alpha, interleukin-6 and fetal fibronectin in the lower genital tract during pregnancy: relation to outcome. *Am J Obstet Gynecol* 1994; 171:5-10.
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