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Prediction of spontaneous preterm birth using fetal fibronectin in women with a low-lying placenta.

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List of Abbreviations

CVF - cervicovaginal fluid

ELISA - enzyme linked immunosorbent assay

fFN - foetal fibronectin

FNR - false negative rate

FPR - false positive rate

PROM - premature rupture of membranes

PTB - preterm birth

qfFN - quantitative fetal fibronectin

sPTB - spontaneous preterm birth

Abstract

Objective

To determine the effect of a low lying placenta on the concentration of quantitative fetal fibronectin (qfFN) in the cervicovaginal fluid (CVF), and predictive accuracy for spontaneous preterm birth in asymptomatic high-risk women (18⁺⁰-24⁺⁰ weeks gestation).

Methods

Median concentrations of qfFN were compared in women who had a low lying placenta, covering the cervical os (n = 61) to matched controls (n= 61) without a low lying placenta. Proportions of women with raised qfFN concentrations (>10ng/ml), and false positive and negative rates (FPR and FNR) for spontaneous preterm delivery were also compared.

Results

The median concentration of qfFN in women with low lying placenta was 5.0 ng/mL, compared with 6.0 ng/mL in controls. Proportion of women with raised levels (>10 ng/mL), positive levels (>50ng/mL) and very high levels (>200ng/mL) were similar in both groups (62.3% vs 59.0%, 16.3% vs 22.0% and 6.5% vs 4.9%, $p>0.05$ for all thresholds). The FPR and FNR rate for delivery before 34 and 37 weeks were also comparable (FPR 90.0% vs 85.7% and 80.0% vs 78.6%; FNR 5.8% vs 4.3% and 9.8% vs 8.5%)

Conclusions

CVF qfFN concentrations in asymptomatic high-risk women are not affected by the presence of a low-lying placenta.

Introduction

Preterm birth (PTB) is a major cause of neonatal morbidity and mortality worldwide; accurate identification of those women who are at greatest risk facilitates treatment, including targeted administration of steroids or in utero transfer, potentially improving fetal outcome, and reducing unnecessary treatment of those destined to deliver closer to term.

Fetal fibronectin (fFN) is a leading predictor of spontaneous PTB (sPTB)^{1,2}. fFN is an extracellular matrix protein, found at the interface of the fetal membranes and decidua. Although it is commonly found in the cervicovaginal fluid (CVF) before 18 weeks and later as labour approaches, it usually present in low concentrations between 18 and 37 weeks of gestation³. Higher concentrations of fFN detected in the CVF after 18 weeks may indicate disruption between this interface and is associated with subsequent sPTB. Initial studies demonstrated that detection (>50 ng/mL) confers a six-fold risk for sPTB before 35 weeks and 14-fold risk for birth before 28 weeks⁴.

fFN testing is recommended in clinical practice for the identification of true preterm labour in those with symptoms and as a screening tool for high-risk asymptomatic women^{1,5-7}. Used as a qualitative test (Rapid fFN Casette: TLiQ[®] system), a positive or negative result is generated at a threshold of 50 ng/mL. It has been demonstrated that quantification of actual fFN concentration (qfFN) improves prediction of sPTB, providing alternative thresholds at which to define high and low

risk in both symptomatic and asymptomatic high risk women^{8,9}. Quantification can now be performed with a bed-side test (Rapid fFN Cassette: 10Q System[®]).

In certain circumstances, including vaginal bleeding, recent sexual intercourse and low lying placenta, the concentration of fibronectin and predictive value of the test may be affected due to interference with the enzyme linked immunosorbent assay (ELISA). Additionally there is the theoretical potential that a low lying placenta may cause increased fFN secretion into cervico-vaginal fluid¹⁰⁻¹². However, the predictive value of fetal fibronectin in women with a low lying placenta or placenta praevia has never been formally assessed.

The aim of this study was to examine the median concentrations of fetal fibronectin, the proportion of women with a raised concentration of fibronectin (>10 ng/ml) and the incidence of false positive and false negative rates (with a positive result defined as >50ng/mL for prediction of spontaneous delivery prior to 34 and 37 weeks of gestation) in asymptomatic women between 18⁺⁰ and 24⁺⁰ who were deemed to be at high-risk of preterm birth and who had a low placenta (defined as covering the internal cervical os) compared with a matched group of high-risk women who do not have a low placenta.

Materials

This study is a prospective masked sub-analysis of a larger observational study (Evaluation of Quantitative fetal fibronectin in the Prediction of Preterm Birth – EQUIPP) which included data from 6 UK hospital sites collected between October 2010 through July 2014 of CVF qfFN concentration and obstetric outcomes in women deemed to be at high risk of preterm birth. Ethical approval was obtained from the South East London Research Ethics Committee. Written informed consent was obtained from all participants prior to involvement. Women were eligible to take part in the study if they were between 18⁺⁰ and 24⁺⁰ weeks of gestation, and considered high risk for pre-term birth; one or more of previous preterm birth (<37 weeks), previous premature rupture of membranes (PROM) <37 weeks, previous late miscarriage (16-23⁺⁶ weeks), previous invasive cervical surgery (e.g. large loop excision of the transformation zone, cone biopsy), uterine abnormality, or a cervical length less than 25 mm in the current pregnancy. Gestational ages were confirmed with standard early ultrasound scans. Participant baseline demographics, obstetric history and risk factors were entered onto an online secure study specific database (www.medscinet.net/ptbstudies)

Eligible women underwent CVF fetal fibronectin testing between 18⁺⁰ and 24⁺⁰ weeks of gestation. qfFN swabs were taken according to the manufacturer's instructions; during speculum examination, dacron swabs were placed in the posterior fornix and rotated for 10 seconds to become fully saturated. The swabs were placed in a buffer containing a specific monoclonal antibody (FDC-6) against the oncofetal domain of fFN^{13,14}. Aliquots were then analysed simultaneously with the

commercially available qualitative analyser, Rapid fFN Cassette: TLiQ[®], and quantitative analyser, Rapid fFN Cassette: 10Q System[®]. The qualitative fFN result was made available to the clinician (positive at a value >50 ng/mL), whereas the quantitative concentration remained masked (random code generation). Samples from women who reported prior sexual intercourse (within 24 hours), who had confirmed or suspected rupture of membranes, or who had frank bleeding visible on the swab were excluded from analysis due to known interference with fetal fibronectin measurement. If a transvaginal ultrasonographic cervical length was performed, this was done after the swab was taken.

For this sub-analysis, asymptomatic high-risk women between 18⁺⁰ and 24⁺⁰ weeks of gestation, who during ultrasound scanning, were found to have a placenta covering the internal os were included in the study as having a low lying placenta and considered 'cases'. Cases were matched with asymptomatic high-risk women from the same database without low-lying placenta according to the following: risk factor for preterm birth (previous sPTB, previous PROM, previous late miscarriage [16-23⁺⁶ weeks], invasive cervical surgery, uterine anomaly, or cervical length <25 mm in current pregnancy) and both gestational age at testing and delivery (+/- 7 days).

Statistical analysis was performed using Stata software (version 12.0; Stata-Corp LP, College Station, TX). Women undergoing iatrogenic delivery before 37 weeks and 34 weeks of gestation were excluded from the analysis.

Standard distributional checks were carried out. Quantitative fFN values were found to be highly asymmetric, with large standard deviations compared with the

means in both groups of women. Values were therefore logged using ln skew (0.0468) using censoring to accommodate for qfFN concentrations '0' and >500 ng/ml, and checks repeated. Geometric means were generated after transformation of log-normal distributions. The qfFN values were compared between groups using Student t tests on the logged values. Results are reported as ratios of geometric means. Linear regression was used to look for differences in the qfFN levels according to placental location. To allow for matching, robust standard errors were used.

The number and percentages of women who tested within each concentration category of qfFN (<10, 10-49, >50-199, >200 and >500 ng/mL) was analysed for cases and controls. These were compared using a chi-squared test.

Delivery before 37 weeks gestation with a qfFN concentration greater than 50ng/mL was defined as a true-positive result; likewise a false positive result was defined as cervicovaginal qfFN concentrations $\geq$ 50ng/mL at testing and delivery after 37 weeks of gestation. The false-positive and false negative rates in women with a term delivery were compared between the cases and controls. Analysis was repeated for prediction of delivery before 34 weeks of gestation.

Results

A total of 62 participants with singleton pregnancies between and 18⁺⁰ and 24⁺⁰ weeks' gestation who had a low-lying placenta at the time of testing were identified. One woman was excluded from the analysis because of iatrogenic preterm delivery, leaving a total of 61 women fulfilling criteria for analysis. Demographic, obstetric and background characteristics for study participants are described in Table 1.

[Insert Table 1 here]

The mean gestational age for both groups at testing was 21⁺³ (range 18⁺⁰ to 24⁺⁶) and the mean gestational age at delivery was 38⁺³ (range 19⁺³ to 41⁺⁶). The sPTB rate was 6.6% (4/61) (95% Confidence Interval [CI]: 1.8-15.9) at less than 34 weeks' gestation and 11.5% (7/61) (CI: 4.7-22.2) at less than 37 weeks' gestation.

The median concentration of qfFN in the women who had low lying placenta was 5.0 ng/mL (quartiles 3.0, 18.0), compared with 6.0 ng/mL (quartiles 3.0, 36.0) in the control group. The mean concentration of qfFN in women who had a low-lying placenta was 41.9 ng/mL compared with 47.0 ng/mL in the control group. The mean concentration of qfFN in the two groups did not differ significantly (mean difference 0.82 [CI: 0.43-1.53], ratio of geometric means cases vs controls; 7.67 and 9.31 ng/mL p= 0.548). The distribution of qfFN concentrations for low lying placenta and controls are summarized in Figure 1.

[Insert Figure 1 here: Figure 1: Dot Plot of Fetal Fibronectin Results in Low Lying Placenta and Controls Illustrating a Similar Distribution of qfFN Results in Both Groups]

The number and proportion of women with qfFN concentration (ng/mL) in each category (<10, 10-49, 50-199, 200-499, >500 ng/ml) is summarized in table 2. 38/61 (62.3%) of the cases and 36/61 (59.0%) of the controls had fFN concentrations <10ng/mL (chi square 0.65, $p>0.05$). 10/61 (16.3%) and 14/61 (22.9%) of the cases had positive results by our predefined criteria, (concentrations >50ng/mL, chi square 1.37, $p>0.05$).

[Insert Table 2 here]

For prediction of delivery before 34 weeks of gestation, there was no statistical difference in the false positive rate or false negative rate in women with a low placenta compared with controls; false positive rate in the low lying placenta group was 9/10 (90.0%) vs. 12/14 (85.7%) in the control group (difference 5.3%; $p = 0.47$). The false-negative rate was 3/51 (5.8%) vs 2/47 (4.3%) in the controls (difference 1.6%; $p= 0.47$). The false positive rate for delivery before 37 weeks in the low lying placenta group was 8/10 (80.0%) vs 11/14 (78.6%) in the control group (difference 2.4%; $P = 0.45$). The false negative rate was 5/51 (9.8%) in the low lying placenta group and 4/47 (8.5%) in the control group (difference 6.3%; $P = 0.58$).

Discussion

This is the first study to report the effect of low-lying placenta on CVF qfFN concentrations in the pregnant population. The results demonstrate that there is no difference in CVF qfFN concentration between women with a low lying placenta, and those whose placenta does not partially or wholly obscure the cervical os. Contrary to common belief, the presence of a low-lying placenta does not preclude the use of qfFN as a predictive test for preterm birth in this population.

Consistent with other studies, the qfFN concentrations in both the low placenta and control groups were low^{8,9}. Approximately 60% of swab results were <10ng/mL, compared to 5-6% of women with results >200 ng/mL. Only one of the cases and two of the controls had results >500ng/mL. There was no statistical or clinically relevant difference in proportions of case and controls falling into each of the qfFN concentration categories.

Our study population demographics and sPTB rates are comparable to previously published preterm prediction studies⁷. Moreover there was a varied ethnic origin and thus the test can be considered transferable to a broad population. 5 women, 4 cases and 1 control, underwent cerclage (history or ultrasound indicated) for prevention of preterm birth (progesterone was not routinely prescribed in the UK at the time of study data collection). Given the relative rarity of a low placenta at this gestation, the small sample size precluded analysis of the predictive power of qfFN for meaningful clinical outcomes. However the fact that the median levels of fetal fibronectin, the proportion with raised levels of fibronectin and the incidence

of false positive and negative rates in women with a low placenta is comparable to those without low lying placenta is reassuring. This indicates that low lying placenta does not interfere with the ELISA nor cause increased fFN secretion into cervico-vaginal fluid.

The prevalence of placenta praevia at term (placenta which has implanted in the lower uterine segment) is 5.2 per 1000 and is thought to be on the rise secondary to the increased incidence of caesarean sections in the past three decades¹³. Numerous risk factors have been associated including previous uterine surgery and advanced maternal age, multiparity, smoking, cocaine abuse, history of induced abortions and multiple pregnancies – risk factors which also confer risk for sPTB^{14,15}. It is associated with poor maternal and neonatal outcomes¹⁶.

At earlier gestations, a placenta partially or totally covering the os is more common, and this becomes less common with increasing gestational age, thought to be thought to be due to placental migration¹⁹. Whilst the incidence of placenta praevia at weeks 18-24 of gestation ranges from 0.97-5.3%, there is a 90% resolution rate by the third trimester²⁰⁻²².

As many of the women screened with fetal fibronectin (either due to a previous history of preterm birth, or other obstetric risk factor) are well into their second trimester, it is likely that a significant proportion will have pregnancies complicated by low lying placentas. These are all women who may benefit from qfFN testing. Our findings that a low lying placenta has no effect on the levels of qfFN nor incidence of false positive or negative results, is reassuring. Moreover, the qfFN

test was used successfully in 62 women with low lying placenta without any adverse events, including bleeding. Therefore it is reasonable to offer this test to women between 18 and 24 weeks of gestation regardless of their placental location, as long as the other caveats to qfFN testing are fulfilled.

Declaration of Interest

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References

Berghella V, Hayes E, Visintine J, Baxter JK. Fetal fibronectin testing for reducing the risk of preterm birth. *Cochrane Database Syst Rev* 2008(4): CD006843.

Deshpande SN, van Asselt AD, Tomini F, Armstrong N, Allen A, Noake C, Khan K, Severens JL, Kleijnen J, Westwood ME. Rapid fetal fibronectin testing to predict preterm birth in women with symptoms of premature labour: A systematic review and cost analysis. *Health Technol Assess* 2013;17(40): 1-138.

Iams JD. Prediction and early detection of preterm labor. *Obstet Gynecol* 2003;101(2): 402-412.

Goldenberg RL, Mercer BM, Meis PJ, Copper RL, Das A, McNellis D. The preterm prediction study: Fetal fibronectin testing and spontaneous preterm birth. *Nichd maternal fetal medicine units network. Obstet Gynecol* 1996;87(5 Pt 1): 643-648.

RCOG. Tocolysis for women in preterm labour. In: RCOG editor. Tocolysis for women in preterm labour. Green-top guideline 1B; 2011.

Fetal fibronectin or actim partus testing - query bank

Honest H, Bachmann LM, Gupta JK, Kleijnen J, Khan KS. Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: Systematic review. *BMJ* 2002;325(7359): 301.

Abbott DS, Radford SK, Seed PT, Tribe RM, Shennan AH. Evaluation of a quantitative fetal fibronectin test for spontaneous preterm birth in symptomatic women. *Am J Obstet Gynecol* 2013;208(2): 122.e121-126.

Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, Girling JC, Chandiramani M, Stock SJ, Carter J et al. Quantitative fetal fibronectin to

predict preterm birth in asymptomatic women at high risk. *Obstet Gynecol* 2015;125(5): 1168-1176.

McLaren JS, Hezelgrave NL, Ayubi H, Seed PT, Shennan AH. Prediction of spontaneous preterm birth using quantitative fetal fibronectin after recent sexual intercourse. *Am J Obstet Gynecol* 2014.

Hologic. Rapid ffn for the tliq system. In: INC H editor. Rapid ffn for the tliq system; Marlborough, MA; 2009.

Hologic. The hologic rapid ffn 10q system. In: INC H editor. The hologic rapid ffn 10q system; Marlborough, MA; 2010.

Cresswell JA, Ronsmans C, Calvert C, Filippi V. Prevalence of placenta praevia by world region: A systematic review and meta-analysis. *Trop Med Int Health* 2013;18(6): 712-724.

Faiz AS, Ananth CV. Etiology and risk factors for placenta previa: An overview and meta-analysis of observational studies. *J Matern Fetal Neonatal Med* 2003;13(3): 175-190.

D'Antonio F, Bhide A. Ultrasound in placental disorders. *Best Pract Res Clin Obstet Gynaecol* 2014;28(3): 429-442.

Becker RH, Vonk R, Mende BC, Ragosch V, Entezami M. The relevance of placental location at 20-23 gestational weeks for prediction of placenta previa at delivery: Evaluation of 8650 cases. *Ultrasound Obstet Gynecol* 2001;17(6): 496-501.

Heller HT, Mullen KM, Gordon RW, Reiss RE, Benson CB. Outcomes of pregnancies with a low-lying placenta diagnosed on second-trimester sonography. *J Ultrasound Med* 2014;33(4): 691-696.

Lauria MR, Smith RS, Treadwell MC, Comstock CH, Kirk JS, Lee W, Bottoms SF. The use of second-trimester transvaginal sonography to predict placenta previa. *Ultrasound Obstet Gynecol* 1996;8(5): 337-340.

King DL. Placental migration demonstrated by ultrasonography. A hypothesis of dynamic placentation. *Radiology* 1973;109(1): 167-170.

Rizos N, Doran TA, Miskin M, Benzie RJ, Ford JA. Natural history of placenta previa ascertained by diagnostic ultrasound. *Am J Obstet Gynecol* 1979;133(3): 287-291.

Taipale P, Hiilesmaa V, Ylöstalo P. Diagnosis of placenta previa by transvaginal sonographic screening at 12-16 weeks in a nonselected population. *Obstet Gynecol* 1997;89(3): 364-367.

Taipale P, Hiilesmaa V, Ylöstalo P. Transvaginal ultrasonography at 18-23 weeks in predicting placenta previa at delivery. *Ultrasound Obstet Gynecol* 1998;12(6): 422-425.

Normal

Placenta position

Low

Tables

Table 1: Demographic and Obstetric Characteristics

Characteristic	Low Lying Placenta n = 61 Number (%)	Control n = 61 Number (%)
Maternal age	34 ± 4	32 ± 5
Gestation age at testing	21 ^{3/7} ± 2 ^{2/7}	21 ^{3/7} ± 2 ^{1/7}
Gestational age at delivery	38 ^{3/7} ± 3 ^{2/7}	38 ^{4/7} ± 4 ^{2/7}
Ethnicity		
White	37 (61)	28 (46)
Black	17 (28)	24 (39)
Asian	3 (5)	0 (0)
Other	4 (7)	9 (15)
Obstetric Risk Factor		
Previous spontaneous preterm birth	16 (26)	16 (26)
Previous PPRM	11 (18)	11 (18)
Previous late miscarriage	12 (20)	12 (20)

Previous cervical surgery	27 (45)	27 (45)
Multiple pregnancy	2 (3)	2 (3)
Cervical length <25 mm	6 (10)	6 (10)
Smoking		
Current	4 (7)	5 (8)
Ex-smoker	5 (8)	15 (25)
Never	52 (85)	41 (67)
Other Risk Factors		
Past or present history of domestic violence	3 (5)	1 (2)

Table 2: Proportion of Women in Each Quantification of fFN

fFN Results (ng/mL)	Low Lying Placenta n = 61 Number (%)	Control n = 61 Number (%)
<10	38 (62)	36 (59)
10 - 49	13 (21)	11 (18)
50 - 199	6 (10)	11 (18)
200 - 499	3 (5)	1 (2)
>500	1 (2)	2 (3)