# Foetal Fibronectin (fFN) in Cervico Vaginal Lavage (CVL) as a Predictor Marker of Preterm Labour

Geetha Damodaran K.[1], Lalithambika Karunakaran[2], Sreedevi N.[3], Rajesh K.M.[4]

[1]Professor, Department of Biochemistry, Government Medical College, Thrissur, Kerala, India. [2]Professor, Department of Obstetrics and Gynaecology, Government T.D. Medical College, Alappuzha, Kerala, India. [3]Professor, Department of Biochemistry, Mount Zion Medical College, Ezhamkulam, Kerala, India. [4]Assistant Professor, Department of Biochemistry, Government Medical College, Ernakulam, Kerala, India.

**ABSTRACT**

**BACKGROUND**

Presently used clinical and ultrasound diagnostic criteria for predicting preterm labour (PTL) have limitations. Instead an objective diagnostic marker will be more appropriate.

**METHODS**

Pregnant women during 24 – 35 weeks of gestation who attended T.D. Medical College for antenatal check-up were included in this prospective observational study as per inclusion and exclusion criteria with informed consent. The cervicovaginal lavage fFN was assayed by ELISA and total protein (TP) by photometric Biuret method. To counter dilutional errors, fFN is expressed in ng / 0.1 gram of TP in CVL. The type of delivery of enrolled subjects was recorded as preterm (14/216) and term (202/216). MedCalc trial version 18.2 was used for statistical analysis.

**RESULTS**

Reference interval of fFn in CVL ranged from 6.2 to 69 ng / 0.1 g TP. The best diagnostic cut off value of CVL fFN during 24 – 35 weeks of gestation to predict PTL in asymptomatic pregnant women is 63 ng / 0.1 g TP (AUC: 0.891; P: <0.0001) with a sensitivity of 79 and specificity of 97%.

**CONCLUSIONS**

The CVL fFN assay is useful as a predictive marker of PTL. Its high negative predictive value will help in reducing unnecessary hospital admissions. Merit of this study is reduction in dilutional inaccuracies by expressing fFN as ng per 0.1 g of TP in CVL.

**KEYWORDS**

Cervico Vaginal Lavage (CVL), Clinical Decision Limit, Foetal Fibronectin (fFN), Reference Interval, Preterm Labour (PTL)

*Corresponding Author:*
*Dr. Rajesh K.M.,*
*Assistant Professor,*
*Department of Biochemistry,*
*Government Medical College,*
*HMT Colony, Kalamassery P.O.,*
*Ernakulam- 683503, Kerala, India.*
*E-mail: raj.bhat.18@gmail.com*

*DOI: 10.18410/jebmh/2020/353*

*How to Cite This Article:*
*Damodaran GK, Karunakaran L, Sreedevi N, et al. Foetal fibronectin (fFN) in cervico vaginal lavage (CVL) as a predictor marker of preterm labour. J Evid Based Med Healthc 2020; 7(33), 1694-1698. DOI: 10.18410/jebmh/2020/353*

*Submission 09-06-2020,*
*Peer Review 13-06-2020,*
*Acceptance 13-07-2020,*
*Published 17-08-2020.*

## BACKGROUND

Preterm Labour (PTL) refers to delivery that occurs before 37 weeks of gestation. It is the major cause of neonatal mortality and morbidity. As per sample registration system 2013 the neonatal mortality rate (NMR) in India was 28 per 1000 population.[1] Around 43.7% of causes of neonatal mortality are due to complications resulting from PTL.[1] Neonatal morbidity due to preterm labour is serious, like bronchopulmonary dysplasia, intra ventricular haemorrhage, retrolental fibroplasia, neuro developmental problems and cognitive difficulties.[2]

Approximately 70% preterm births have pregnancy associated and epidemiological risk factors but no cause traceable to 30% of cases.[3] However these risk factors are neither sensitive nor specific predictors of PTL. Identification of women at risk of preterm delivery is critical because administration of antenatal corticosteroids at least 48 hours prior to delivery of a premature infant significantly reduces neonatal morbidity and mortality.[4] Asymptomatic cases will go unnoticed.

The clinical diagnosis of PTL is done when uterine contraction frequency ≥ 6 per hour, cervical dilatation ≥ 3cm or cervical effacement is ≥80%. But these clinical findings often occur too late for intervention.[4] Ultrasound transvaginal cervical length cut off, of 25 mm at less than 34 weeks of gestation has a sensitivity of 92.86%. The major limitation of this investigation is poor patient acceptance because of its invasive nature and limited resources.[5]

The problem of subjectivity associated with clinical diagnosis of PTL can be eliminated by using an objective biochemical marker. Measurement of foetal fibronectin (fFN) in cervico vaginal lavage fluid (CVL) may serve the purpose. The fFN is an extracellular matrix protein which is distributed in the extracellular matrix located in the chorio decidual junction between maternal decidua and foetal membranes, in the uterus and placenta. It is normally present in cervico vaginal secretions during the first 22 weeks of pregnancy until the foetal membranes completely fuse to maternal decidua and also after 37 weeks' gestation, when fFN becomes heavily glycosylated, loses its adhesive properties. The disappearance of fFN in CVL from 20 – 37 weeks of gestation coincides with the fusion of the chorion and adjacent decidua capsularis remnant with decidua parietalis of the uterine wall.[6] This fusion creates a seal that prevents the further release of chorionic fFN. Break down of maternal decidua and foetal membranes cause release of fFN into cervicovaginal secretions. Hence CVL specimen is more ideal for biomarker determination to predict preterm labour.[7]

It is postulated that mechanical or inflammatory mediated damage to the membranes before preterm delivery might result in its release into the cervix and vagina. Appearance of fFN ≥50 µg/L in CVL of pregnant women between 24 to 35 weeks gestation, is associated with increased risk for PTL.[6] The biomarkers found in cervico vaginal fluid, being the proximal fluid shows more specificity and sensitivity than blood in diagnosing PTL.[7]

Effective management strategies are now available to reduce the complications associated with preterm births. But the accurate prediction of spontaneous preterm births is essential for the timely institution of treatment. Studies on fFN in CVL as an objective predictor marker of PTL in Indians is inevitable because of the high rate of preterm births in India and the consequent high neonatal mortality and morbidity. In this scenario, an objective marker will be more effective to assess the risk of PTL. If the fFN is found to have high negative predictive value that will help to reduce referring mothers with symptoms of preterm labour to tertiary or regional centres unnecessarily and to decrease the associated costs.

The determination of reference interval and medical decision limit of fFN in our setting will improve identification of pregnant women who need corticosteroid and tocolytic therapy. It will also help to provide reassurance to mothers and their families that premature birth of their baby is unlikely within next 14 days. The current study aims to evaluate fFN in CVL as a marker for predicting preterm labour.

## METHODS

This observational cohort study was conducted in Departments of Biochemistry and Obstetrics & Gynaecology of Govt. T. D. Medical College, Alappuzha, Kerala state, India, after getting clearance from institutional ethics committee. International Federation of Clinical Chemistry (IFCC) guideline was used for choosing sample size of 200 for reference interval determination.

To compensate dropout rate of 20%,

N= n/1-(z/100),

Where N = expected sample size; n= sample size; z = dropout rate (20%)

N = 200 /1-(20/100) = 250

Hence, additional 50 included to adjust for anticipated dropout rate of 20% and further eleven subjects included to adjust for outliers if any occur in the data. Hence a total of two hundred and sixty-one (n = 261) pregnant women[8] who had attended outpatient antenatal clinic between 24 – 35 weeks were selected by consecutive enrolment after getting informed written consent, conforming to inclusion and exclusion criteria.

### Inclusion Criteria

Inclusion criteria were singleton pregnancies, 24-35 weeks of gestation with or without symptoms but with intact membranes, and cervical dilatation <3 cm.

### Exclusion Criteria

The exclusion criteria were multiple pregnancy, pre-eclampsia, gestational age <24 weeks or >35 completed weeks, preterm rupture of membranes, cervix ≥3 cm

dilatation, active vaginal bleeding, vaginal examination or sexual intercourse in the past 24 hours and clinically detectable bacterial vaginosis.

The cervico - vaginal fluid (CVF) sample was collected during an unlubricated speculum examination before vaginal ultrasound or digital examination done as a part of antenatal care. Using a disposable sterile syringe, two mL of sterilized phosphate buffered saline (PBS pH = 7.4) was gently pushed deep into the vagina by the obstetrician. Using the same syringe, the CVF is sucked in and pushed out of the syringe three times to rinse the area. Then 1.0 - 1.5 mL of cervico vaginal lavage (CVL) was collected by the same syringe and transferred to polypropylene microcentrifuge tubes with locking snap cap and stored at - $70^0$C.

On the day of analysis CVL specimens were thawed and centrifuged for 10 minutes at 3000 rpm. The supernatant was analysed for fFN and total protein. The fFN assayed by Enzyme Linked Immunosorbent Assay (ELISA) containing FDC-6 monoclonal antibody,[9] using ELISA Reader (Bio-Rad). Total protein content in the specimens was estimated by Biuret method in a clinical chemistry analyser. Since the specimen used for fFN assay is cervico vaginal lavage fluid, to counter the dilution errors, the value of fFN is expressed in ng per 0.1 gram of total protein present in the same lavage fluid.

All the enrolled patients were followed till delivery to record the type of delivery as preterm or term. The CVL fFN data of the pregnant women with term deliveries were used to determine reference interval. The whole data derived from term and preterm together was used to find out the optimum cut off value (clinical decision limit) of fFN in CVF in predicting preterm labour. The diagnostic accuracy of CVL fFN was found out using various metrics such as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, AUC (Area Under the Receiver Operating Characteristic (ROC) curve), and diagnostic odds ratio. Statistical analysis was done by MedCalc trial version 18.2.

## RESULTS

Two hundred and sixty-one asymptomatic pregnant women were recruited in this study as per inclusion and exclusion criteria. Out of these forty-five pregnant women lost follow up and the data of two hundred and sixteen pregnant women (n =216) were analysed. All were asymptomatic of PTL at the time of enrolment for the study. All were followed up till the delivery to know the type of delivery as preterm or term. Out of these, 14 deliveries were preterm and 202 term deliveries. General characteristics of pregnant women participated in the study are given in Table -1.

The fFN values expressed as ng/0.1 g TP in CVL of term delivery cases selected as the data for reference interval determination (n= 202). There were no far outliers as per Generalized ESD test (alpha level 0.05). Reference interval of fFN in CVL in pregnant women during 24 to 35 weeks of gestation by nonparametric percentile method 6.2 to 69 ng/0.1 g TP (Table 2 and Figure 1 & 2)

ROC curve analysis (Table 3, Fig 2) revealed diagnostic cut off value (clinical decision limit/optimum cut off value) of fFN in CVL as 63 ng /0.1 g TP with a sensitivity of 79% and specificity of 97% for anticipating preterm labour in a pregnant woman during the 24-35 weeks of gestation. The ability of this cut off value of fFN (63 ng /0.1 g TP in CVF) to predict preterm labour during the gestation period between 24-35 weeks is found to be good as indicated by area under the curve (AUC) of 0.891 (significance level P: <0.0001).

| Age | Term (n=202) | PTL (n=14) |
|---|---|---|
| **Age Distribution** | | |
| 41 - 45 yrs. | 1 | 0 |
| 36 - 40 | 4 | 3 |
| 31 - 35 | 29 | 2 |
| 26 - 30 | 64 | 5 |
| 21 - 25 | 89 | 3 |
| 18 - 20 | 15 | 1 |
| **Socio Economic Status** | | |
| APL | 94 | 3 |
| BPL | 108 | 11 |
| **Educational Status of Women Delivered at Term** | | |
| $8^{th}$ to $9^{th}$ std | 4 | 1 |
| $10^{th}$ | 40 | 3 |
| Plus 2 | 74 | 4 |
| Diploma | 15 | 0 |
| UG | 54 | 6 |
| PG | 5 | 0 |
| Professional Degree | 2 | 0 |
| **Job status** | | |
| Not working | 186 | 14 |
| Working | 16 | 0 |

***Table 1. Characteristics of Pregnant Women Who Participated in the Study***

PTL= Preterm labour; APL= Above poverty line; BPL= Below poverty line; UG= Undergraduate; PG= Postgraduate

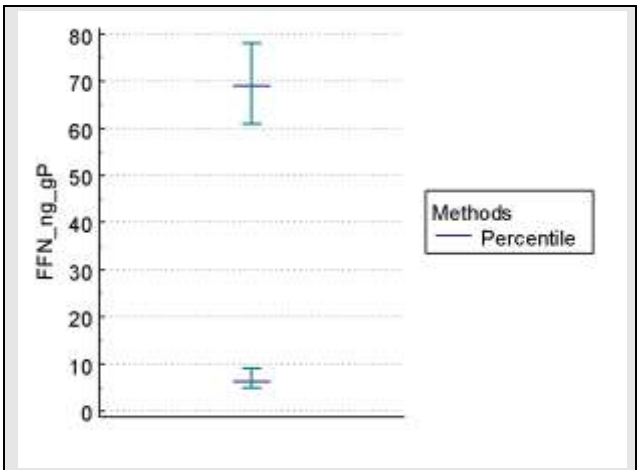


***Figure 1. Reference Interval by Non-Parametric Percentile Method***

| Non-Parametric Percentile Method | |
|---|---|
| **Limits** | **fFN in CVL (ng /0.1 g Total Protein)** |
| Lower limit | 6.2 |
| Upper limit | 65.6 |

***Table 2. Reference Interval of fFN in CVL***

fFN= foetal Fibronectin; CVL= Cervico-vaginal Lavage fluid

The diagnostic accuracy of cut off value of 63 ng/0.1 g of CVL fFN was revealed in terms of sensitivity - 78.6%, specificity: 96.5%, positive predictive value (PPV): 71.6, negative predictive value (NPV): 97.6, positive likelihood ratio: 22.7, negative likelihood ratio: 0.22, Youden's index J = 0.751 (Table 3) and Odds ratio: 102.14 (P < 0.0001).

| Variable: fFN ng*/0.1g TP in CVF | |
|---|---|
| Classification variable based on type of delivery: PTL and Term | |
| Clinical decision limit (associated criterion) | 63 |
| 95% Confidence interval | >61 to >78 |
| Area under ROC curve (AUC) | 0.891 |
| Sensitivity | 78.6 |
| Specificity | 96.5 |
| Youden index J | 0.751 |
| PPV** | 71.6% |
| NPV*** | 97.6% |
| Positive Likelihood ratio (LR +) | 22.7% |
| Negative Likelihood ratio (LR -) | 0.221% |
| Odds ratio | 102 (95% CI: 23.2 – 449.9) |
| Significance level | (p < 0.0001) |

***Table 3. Metrics of Diagnostic Accuracy of CVF fFN***

ng=Nanogram; PPV=Positive predictive value; NPV=Negative predictive value; CVF=Cervico-vaginal fluid; ROC=Receiver Operator curve; CI=Confidence Interval; TP=Total protein

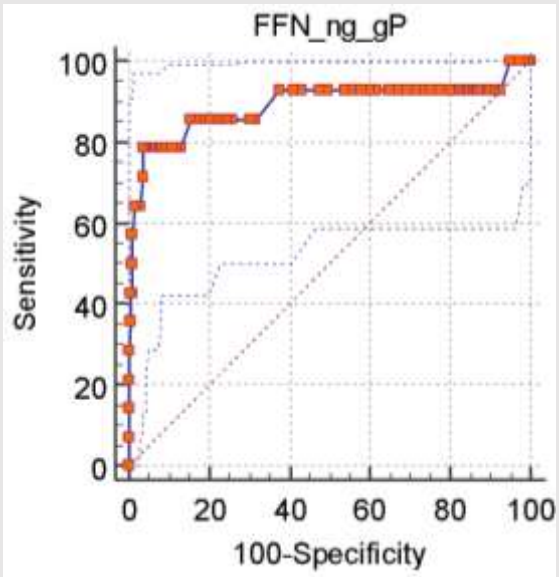


***Figure 2. ROC of CVL fFN for Predicting PTL in Pregnant Women during 24 – 35 Weeks of Gestation***

## DISCUSSION

Preterm labour and prematurity are the most important causes of neonatal and infant morbidity and mortality. Preterm labour often occurs unexpectedly in women with no obvious risks. In this context an objective marker as a screening test of PTL having a high sensitivity and specificity will be useful for early detection and timely intervention to reduce the incidence of PTL. The test for this purpose should be reproducible, accurate, cost effective and widely available.

In the case of CVL foetal fibronectin assay, the CVL sample can be collected by simple outpatient clinical procedure as a part of regular ante natal check-up. The CVL fFN determination needs only an ELISA reader to estimate fFN and a clinical chemistry analyser to determine protein content in a clinical chemistry laboratory. Hence laboratories even in the rural areas can offer this test without additional sophisticated equipment. The diagnostic accuracy of CVL fFN was found to be good based on the various metrics of diagnostic accuracy of a biomarker.

- Sensitivity - 78.6%: 79/100 of pregnant women with CVL fFN values more than 63 ng / 0.1 g TP during 25 to 34 weeks will go for PTL but 21/100 will not develop PTL.
- Specificity - 96.5%: 97/100 of pregnant women with CVL fFN values less than 63 ng / 0.1 g TP during 25 to 34 weeks will not go for PTL but 3/100 will develop PTL.
- Receiver operating characteristic curve (ROC) analysis revealed optimum medical decision limit of fFN in CVL for predicting preterm labour when sample is taken between 25 to 34 weeks, as 63 ng / 0.1 g TP. This study showed that discriminatory ability of CVL fFN in predicting PTL in an asymptomatic pregnant woman between 24 to 35 weeks of gestation is fair as the AUC is 0.891. (figure 2)
- Youden index J - 0.75: It indicates, good performance of the test, with associated cut off of CVL fFN > 63 ng / 0.1 g TP but there are chances of false positive and false negative results. For a perfect test, Youden index J equals 1.
- Diagnostic Odds Ratio - 102 (P < 0.0001) Higher odds ratio suggests better test performance.
- Positive Predictive Value (PPV) – 71.6): The probability of, pregnant women with CVL fFN more than 63 ng/0.1g TP, to go for PTL is ~ 72%.
- Negative Predictive Value (NPV) - 97.6: The probability of pregnant women, with CVL fFN less than 63 ng / 0.1 g TP, not going for preterm labours ~ 98%. The high negative predictive value will help to reduce admission of mothers with symptoms of preterm labour to tertiary or regional centres and also to decrease the associated costs.
- Positive Likelihood Ratio (LR +): 26.452: Away from 1, indicates the chances of pregnant women with CVL fFN > 63 ng / 0.1 g TP going for PTL is high.
- Negative Likelihood Ratio (LR -): 0.22: In this study LR (–) is less than one which indicates chances of pregnant women with CVL fFN < 63 ng / 0.1 g TP going for PTL is very less.

The other studies on fFN as a predictive marker of PTL in asymptomatic women used the unit, ng of fFN/mL of CVL. In these studies, CVL were collected by swab and phosphate buffered saline where dilution errors are not eliminated. But in our study, cervico vaginal lavage (CVL) fluid is used and to adjust the dilution variability and the concentration of fFN is corrected by protein concentration in the same CVL. The medical decision limit of CVL fFN, thus obtained is 63 ng / 0.1 gTP whereas in most of the studies it is 50 ng / mL.[10]

According to the multicentre study (n= 2929) by Goldenberg et al in asymptomatic women between 22-30 weeks of gestation, a positive fFN test early in gestation (24-26 weeks) was more sensitive than late in gestation (28-30 weeks) in predicting PTL.[11] In this study fFN values ≥50 ng/mL were considered positive.

The meta-analysis by Leitich et al of asymptomatic women reported a sensitivity of 22% and specificity of 97% of fFN to predict spontaneous PTB within seven days. Goepfert et al. found out the optimal cut off point of CVF fFN as 45-60 ng/mL at 24-30 weeks' gestation to predict PTL before 35 weeks.[12]

## CONCLUSIONS

Clinical decision limit (cut off value) of CVL fFN to predict preterm labour, in pregnant women during 24 – 35 weeks of gestation is 63 ng / 0.1 g TP. The diagnostic accuracy of this clinical decision limit of CVL fFN was found to be good based on the various metrics of diagnostic accuracy. Hence CVL fFN can be used as a reliable biomarker for predicting PTL in pregnant women. The high negative predictive value will confer confidence to clinician to reduce admission of mothers with CVL fFN less than 63 ng / 0.1 g TP for PTL, One notable merit of this study over other studies of fFN as a predictive marker of PTL is that the reduction of dilution inaccuracies by expressing CVL fFN as ng per 0.1 g of TP.

The study was conducted after obtaining approval from Institutional Ethics Committee, TDMC, Alappuzha, as per the order no: B3/1573/2010/TDMCA dtd 22/4/2013

Concept, design, analysis, interpretation of data, drafting & revising manuscript, ensuring accuracy, integrity of all aspects of research- Principal investigator and all the other co-authors have actively contributed.

Financial or Other Competing Interests: The source of funding was from State Board for Medical Research (SBMR), Kerala, India.

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