Review

Biochemical Markers as Predictor of Preterm Labor - and Their Clinical Relevance- the Current Status

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ABSTRACT

Preterm birth is associated with significant perinatal morbidity and mortality. Spontaneous preterm birth accounts for up to 75% of all preterm births. Several maternal and fetal characteristics have been associated with preterm birth. With a better understanding of the pathophysiology of preterm birth, various biochemical markers have been studied extensively to predict preterm birth efficiently to intervene appropriately and timely in the cases that would benefit from treatment. This paper provides a summary of the current literature on the use of biochemical markers in predicting spontaneous preterm birth in symptomatic and high-risk-asymptomatic women. For this review, we searched the Cochrane, Medline, PubMed databases and we have included studies from 2009 to date mentioning these important markers in predicting preterm labor. Evidence from the literature suggests cervicovaginal fetal fibronectin, interleukin-6, phosphorylated Insulin-like growth factor binding protein-1, placental alpha macroglobulin-1, and serumα-fetoprotein are promising biochemical markers in predicting spontaneous preterm birth.

Keywords: Fetal fibronectin, Interleukin 6, Phosphorylated Insulin-like growth factor binding protein-1, Placental alpha macroglobulin-1, Preterm birth, Preterm labor, Serum a-fetoprotein

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Introduction

Preterm delivery is defined as the delivery before completing 37 weeks of gestation. It is a leading cause of neonatal mortality and morbidity worldwide. As per WHO factsheet 2018, the rate of preterm births has been estimated to be about 15 million, accounting for 11.1% of all live births worldwide (1). Its incidence rate ranges from 5% in the most developed European countries to 18% in several African countries (2,3).

It is important for the clinician to identify the women at risk of having a preterm birth, as it has been documented that only 20% of the patients presenting with symptoms of preterm labor eventually have a preterm birth. This would avoid the problem of under-treatment as well as overtreatment.

A better understanding of the etiological factors and pathophysiology of preterm labor (4) has led to the development of numerous tools to identify women at greatest risk of preterm birth. But the quest for the most predictable and reliable biochemical marker continues.

Current screening tests for the prediction of spontaneous preterm birth (PTB) can be divided into three general categories: (i) risk factor assessment, (ii) cervical measurement, and (iii) biochemical markers.

In this article, we will elaborate on the clinically significant biochemical markers and their future prospects.

Biomarkers are substances, which are measured in a biological sample. For predicting PTB different types of markers are tested in various biological fluids such as cervicovaginal fluid (CVF), amniotic fluid, urine, blood serum, and saliva.

These body fluids provide rich sources of proteins and metabolites that vary in concentration in response to pregnancy and adverse pregnancy states (5). In the light of the development of genomic and proteomic technologies, screening of thousands of genes and gene products from small samples of body fluid has become possible (6).

We shall discuss samples that are easily collectible to those that need invasive intervention.

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1. Cervicovaginal fluid (C)

The CVF is a complex mixture of secretions derived from the vagina, endocervix, endometrial decidua, and amniochorion and it serves as an important diagnostic sample to monitor maternal and fetal health in pregnancy. Unlike the amniotic fluid, the CVF is readily accessible and its collection is minimally invasive and safe.

a) Fetal fibronectin (fFN)

This glycoprotein is produced by a variety of cell types, including fibroblasts and trophoblasts. It is thought to play a role in intercellular adhesion during implantation and in the maintenance of placental adhesion to uterine decidua. Generally, fFN is detectable from early gestation to the early second trimester. If found beyond 20 weeks' gestation, it suggests a disruption of the chorio-decidual interface and has been identified as a predictor of PTB (7). The release of fFN is thought to be due to mechanically mediated damage or inflammatory-mediated damage to the membrane or placenta before birth (8). Its presence in the CVF is a signal of spontaneous preterm birth within 7 days. Lockwood and co-workers (1991) reported that fFN detection in CVF prior to membrane rupture was a possible marker of PTB (9). Since then fFN has been used as a predictor of PTB and numerous studies have been published showing its positive correlation.

A systematic review in 2002 which included 64 articles with a total of 26,876 women on the predictive value of fFN for PTB identified 40 studies on symptomatic and 28 studies on asymptomatic women, it concluded that cervicovaginal fFN was the most accurate test in predicting spontaneous preterm birth within 7-10 days of testing. In practice, it is used as a negative marker. The absence of fFN suggests that the patient is at low risk of PTB (10).

However, a recent meta-analysis published in 2016 which included 6 trials on 546 symptomatic singleton pregnancies reported that fFN testing in singleton gestations was neither associated with the prevention of PB nor improvement in perinatal outcomes. It is also expensive to test (11).

In an observational study on 9410 nulliparous women with a singleton pregnancy, the accuracy of combined serial cervical length measurements and fetal fibronectin for predicting PTB was studied and it was found to be low and the findings do not support the routine use of this test in asymptomatic primigravid patients (12).

Systemic umbrella review by Lucaroni et al in 2017, which included 542 articles including 14 systemic reviews suggested that cervical fetal fibronectin showed the highest strength of association with the occurrence of preterm birth delivery within 7 days- (OR 12, 95% CI 8-16) (13).

Cochrane meta-analysis 2019 review suggests that management based on knowledge of fFN results may reduce preterm birth before 37 weeks (14).

The Society for Maternal-Fetal Medicine (SMFM) supports fFN use in conjunction with transvaginal ultrasound (TVU), stating, "fFN seems to be most helpful for women with a 'borderline' TVU CL (cervical length) of 20 to 29 mm." (15). NICE guideline on preterm labor also recommends the fFN at a threshold of 50ng/ml to be a reliable predictor of preterm birth in borderline TVU CL or where Ultrasound reports are not available (16).

Recently, experts published an evidence-based standardized protocol for the diagnosis of PTL (17). The algorithm (Figure 1) recommends that fFN be used in women with a cervical length between 20 and 30 mm and cervical dilation <3 cm, which is similar to an algorithm published by Ness et al in 2007 (18).

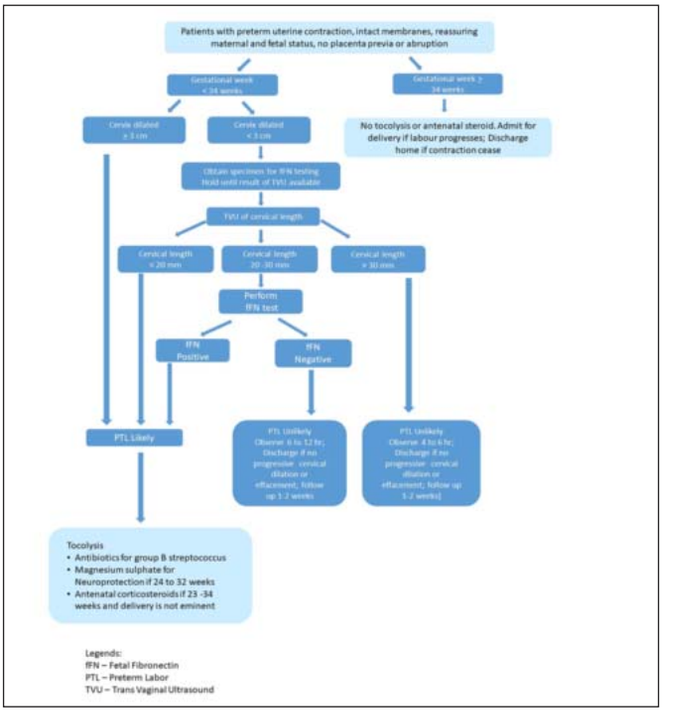

Figure 1: *Up to date algorithm for diagnosis and management of PTL (17)*

Thus, research until now indicates that fFN is a reliable biomarker and is suggestive of PTB within 7 days both in singleton and twin pregnancy. The fFN levels, along with the cervical length measurement, are used as a sensitive method of detection for PTB.

b) Phosphorylated Insulin-like growth factor binding protein-1 (phIGFBP-1)

It is secreted by decidual cells and leaks into cervical secretions when fetal membranes detach from decidua.

Actim® Partus is a type of qualitative immunochromatographic test, designed to detect phIGFBP-1 in cervical secretions during pregnancy.

A study by Lembet et al showed that cervical detection of phIGFBP-1 is a rapid and easily applicable test that highly anticipates preterm delivery in patients at risk (19). A few other

studies concluded that the phIGFBP-1 test with an OR of 10.08 may be better than the fFN test in predicting preterm delivery before 34 weeks (20,21)

When combined with cervical length, the Actim® Partus test (IGFBP-1) was suggested as an alternative for fFN to identify the women who are at risk of delivering in 7 days (22).

So, it can be concluded that the presence of cervical phIGFBP-1 is predictive of preterm delivery.

c) Placental alpha macroglobulin-1 (PAMG-1)

PAMG-1 is a protein released from the decidual cells into the amniotic cavity throughout pregnancy.

PartoSure is one such qualitative lateral flow, an immunochromatographic test designed to detect PAMG-1 in vaginal secretions. It was compared with fFN and cervical length measurement and it was reported to be more accurate in predicting PTB within 7 days with 80% sensitivity and 95% specificity and had the greatest utility in patients when the cervical length was 15-35 mm (23).

A meta-analysis of 15 studies published in 2019 revealed a pooled sensitivity of 66.2% (95% CI: 59.1, 72.7) and specificity of 96.1% (95% CI: 95.1, 97.0) for prediction of delivery within 7 days of testing for PAMG (24).

In a recent meta-analysis by Melchor et al (2018) which compared PAMG-1, fFN, and phIGFBP-1 in symptomatic women, PAMG-1 was reported to have the highest positive predictive value of 76.3% as compared to 34.1% for fFN and 35.2% for phIGFBP-1 for value and the negative predictive value was 96.6% (25).

d) Interleukins

Various members of the IL family have been studied by many researchers and found to be involved in PTD.

IL-6 and IL-8 levels in the cervicovaginal fluid were associated with PTB within 7 days especially when combined with cervical length. Charles et al studied CVFIL6 concentration to predict PTB and found it to be associated but with low sensitivity (26). Thereafter, a lot of studies were carried out and they showed a positive correlation between CVF IL6 and IL8 with PTB both in symptomatic and high-risk asymptomatic women (27,28).

In a meta-analysis (29), an increased risk of PTB in asymptomatic women was associated with elevated levels of cervical IL-6 (OR=3.1, 95% CI: 2.0, 4.7).

e) Matrix metalloproteinase (MMP) 2, 8, 9

MMP family is involved in tissue remodeling and collagen breakdown. MMP 8, which is also called the neutrophil collagenase, is the product of the endocervical neutrophil, that is produced due to the inflammation in the cervix or its surroundings. MMP-8 is one of the proteolytic enzymes that degrade collagen in the cervix and helps in cervical ripening (30).

Becher et al studied the levels of MMP-2, MMP-8, and MMP-9 in cervical mucus plug (CMP) in 15 women with PTL and compared that with 15 women with TL. They found that the concentration of MMP-8 was double and MMP 9 was fourfold higher in the PTL group (31).

These are a few of the significant biochemical markers which can be studied on cervicovaginal fluid. Comparing the sensitivity and specificity of the above by different studies is difficult because of the different study populations, different inclusion criteria, and outcomes.

Table I compares the sensitivity and specificity of CVF fFN, phIBP-I, and PAMG-I by different studies

2. Maternal Serum

Table I: *Sensitivity and Specificity of cervicovaginal fluid fetal fibronectin, Phosphorylated Insulin-like growth factor binding protein-1, and Placental alpha macroglobulin-1*

Fetal fibronectin	**Delivery**	**Sensitivity (%), specificity (%)**
Boots et al, 2014 (65)	Delivery within 48 h	Sensitivity: 62%, specificity: 81%
	Delivery within 7 days	Sensitivity: 79%, specificity: 79%
Sanchez-Ramos et al, 2009 (66)	Delivery within 7 days	Sensitivity: 76%, specificity: 82%
Cooper, 2011 (67)	Delivery within 7 days	Sensitivity: 33%, specificity: 95%
Tripathi et al, 2016 (21)	Delivery within 7 days	Sensitivity: 19.4%, specificity: 99.4%
Brujin et al, 2016 (23)	Delivery within 7 days	Sensitivity: 58%, specificity: 96%
Melchor et al, 2018 (25)	Delivery within 7 days	Sensitivity: 58%, specificity: 84%
phIGFBP-I	**Delivery**	**Sensitivity (%), specificity (%)**
Tripathi et al, 2014 (39)	Delivery within 7 days	Sensitivity: 81.1%, specificity: 97%
Cooper et al, 2011 (67)	Delivery within 7 days	Sensitivity: 39%, specificity: 76%
Conde-Agudelo et al, 2015 (68)	Within 7 days	Sensitivity: 67%, specificity: 77 %
	Within 14 days	Sensitivity: 66%, specificity: 79%
Melchor et al, 2018 (25)	Delivery within 7 days	Sensitivity: 93%, specificity: 76%
PMAG-I	**Delivery**	**Sensitivity (%), specificity (%)**
Brujin et al, 2016 (23)	Delivery within 7 days	Sensitivity: 75%, specificity: 91%
Melchor et al, 2018 (25)	Delivery within 7 days	Sensitivity: 76%, specificity: 97%

phIGFBP-I: Phosphorylated Insulin-like growth factor binding protein-1, PAMG-1: Placental alpha macroglobulin-1

a) C-reactive protein (CRP)

CRP is a sensitive marker of systemic inflammation and is primarily synthesized in hepatocytes in response to infection and tissue injury. It is also raised in other conditions like obesity, cigarette smoking, hormone use, metabolic syndrome, and cardiovascular disease (32). Infection and inflammation are one of the pathways leading to preterm birth, therefore CRP has been evaluated in the literature as a potential marker for preterm birth. Studies by Hvilsom et al (33) and Lohssonthorn et al (34) suggested that elevated maternal serum CRP levels (≥85th percentile) in early pregnancy are positively associated with PTB risk.

A Systemic review by Lucaroni et al showed that maternal C-reactive protein had an OR of 2 (95% CI 1-2) (13).

b) Maternal serum alpha-fetoprotein

Waller and associates observed a strong gradient of increasing risk of preterm birth with increasing levels of serum AFP. Among women with high levels of serum AFP, 24.3% had PTB (35). Allen et al also showed that with PTB (36). In an umbrella systemic review, high AFP was associated with an OR of 4 and 3 for early and late PTB (13).

c) Alkaline phosphatase (ALP)

Patients with elevated placental ALP levels are at an increased risk for PTB (37,38). A significant correlation between PTB and serum ALP levels at 24-28 weeks was observed but its values were affected in conditions such as hemolysis and liver disorders, etc. (39). It can thus be concluded that ALP is an important but not a reliable marker. Further studies are needed to demonstrate its role in predicting PTB.

d) Pregnancy-associated plasma protein A (PAPP-A)

Pregnancy-associated plasma protein A is measured in the maternal serum as a part of the first-trimester screening. A study by Grisaru-Granovsky et al suggested that a decrease in PAPP-A levels (≤30,000 mU/l) was noted in women with PTB (40). Other studies also suggest that PAPP-A is associated with PTL and its measurement can further help in predicting PTD (41,42).

e) Serum Ferritin

Ferritin is an intracellular storage protein that holds iron in an insoluble form. It also increases the number of acute reactions such as inflammation. A study conducted by Singh et al observed that serum ferritin showed an increase in its level in patients who had PTB despite the prevalence of anemia during pregnancy (43). Erdinc et al also published similar results of serum ferritin levels being associated with PTB (44). A study by Tayebeh et al which excluded cases of anemia showed similar results with serum ferritin >37.5 ng/ml with a sensitivity of 78.7 % and specificity of 68.7% could indicate preterm delivery (45).

f) Other serum markers

A review article by J Shah et al in 2016 mentioned that serum placental protein 13, corticotropin-releasing hormone, prolactin, caeruloplasmin, IL2, IL6, IL 8, and TNF α have been studied as a predictor of PTB but have shown unpredicted role and hence are not recommended as a predictor of PTB (46).

3. Saliva

a) Salivary estriol

A study by McGregor et al showed that saliva estriol exceeding a 2.3 ng/ml level was associated with the occurrence of preterm labor (71% sensitivity, 77% specificity) (47). A study by Heine et al concluded an increased risk of spontaneous preterm labor and delivery, in the low-risk population (RR 4.0), and the high-risk population (RR 3.4) (48). Shogra et al observed that the negative predictive value of salivary estriol was much higher than its positive predictive value (82% versus 18.3%) (49).

It is US FDA approved for prediction of preterm birth in singleton pregnancy.

b) Salivary Progesterone

A small study by Lachelin et al in the UK has indicated that salivary progesterone concentrations are significantly lower from 24 weeks of pregnancy in women, who had a spontaneous PTB (< 34 weeks' gestation) (50). Subsequently, further studies have confirmed that a single cut-off value for salivary progesterone of 2575 pg/mL produced a sensitivity of 83%, specificity 86%, positive predictive value 60%, and negative predictive value 95%, for PTB at <34 weeks (51).

4. Amniotic fluid

Amniocentesis is unlikely to become a routine practice to collect a sample to predict PTB as the procedure itself can precipitate preterm labor. Several Biomarkers have been studied in amniotic fluid, and in recent times genome and proteome of AF have been extensively investigated too.

Matrix metalloproteinase 8

Several biomarkers have been proposed to identify intra-amniotic inflammation, the accumulated data suggest that the determination of amniotic fluid matrix metalloproteinase-8 (MMP-8), or neutrophil collagenase, is a powerful predictor of spontaneous preterm delivery. In asymptomatic mid-trimester women undergoing amniocentesis, a rapid bedside test of matrix metalloproteinase-8 (MMP-8) was reported to predict nearly half of spontaneous preterm births. MMP-8 bedside test had a sensitivity of 42.2%, and a specificity of 100% in the prediction of spontaneous preterm delivery (<30 weeks) following a mid-trimester genetic amniocentesis (52).

Vascular endothelium growth factor (VEGF)

It is a critical mediator of inflammation that can be linked to PTB. A study by Hong et al showed that amniotic fluid

VEGF levels in the preterm group (32.24±4.87 pg/mL) were significantly higher than those in the control group, thus favoring VEGF as a predictor of PTB (53). A study by S E Lee also has a similar finding (54). But a study by Yilmaz et al observed no correlation between VEGF values and PTB (55). Thus, the correlation of VEGF with PTB is uncertain.

Glucose, Caeruloplasmin

Glucose as a biomarker for detecting PTB is uncertain and not efficient (46). No association of caeruloplasmin with PTB was observed (56).

Future prospects

a) Proteomic Technologies

Cervicovaginal fluid proteome of symptomatic women who spontaneously delivered preterm within 7 days was compared with gestation-matched women who delivered at term. Four biomarkers, Thioredoxin (TXN), interleukin 1 receptor antagonist (IL1RN), Vitamin D binding protein (GC, group-specific component), and albumin (ALB) were identified and further investigated and all four were significantly altered. GC displayed 77.8% sensitivity and 98.1% specificity while ALB displayed 83.3% sensitivity and 73.3% specificity (57).

In asymptomatic high-risk women, TXN and IL1RN concentrations in the CVF were found to be significantly reduced up to 90 days prior to spontaneous preterm labor. TXN showed a high positive predictive value and negative predictive value of 75.0% and 96.4%, respectively. IL1RN also showed comparable positive and negative predictive values of 72.7% and 95.7%, respectively (58).

Vitamin D binding protein (GC, group-specific component) was significantly increased by up to 7-fold, 14 days before the onset of labor, and had a positive predictive value of 82.8% at 3 days and 78.8% at 7 days before labor onset (59).

Molecular techniques

Production of 25 proteins in maternal serum at 16-17 weeks of gestation was analyzed and proteomic imbalance in 25 proteins as antioxidant enzymes, chaperons, cytoskeleton proteins, cell adhesion molecules, and proteins involved in angiogenesis, proteolysis, transcription, inflammation, binding, and transportation of various ligands was detected. This means that changes that promote PTB start as early as second trimester. For predicting PB within 2 weeks Annexin A2 was reported to have a sensitivity of 81.25%, a specificity of 88.89%, and a positive predicting value (PPV) of 92.86% (60).

c) Epigenetics

A growing body of literature shows emerging evidence for the role of gene-gene interactions and gene-environment interactions and the role of epigenetics in understanding PTB (61-64). Elaborating further on this is beyond the scope of this article.

Conclusion

Four distinct mechanisms for the pathogenesis of preterm labor have been described and include premature activation of the fetal hypothalamic-pituitary axis, mechanical stretch, inflammation/matrix remodeling, and placental abruption. PTB has multifactorial etiology and therefore it is unlikely that a single marker can be of desired predictive efficacy. CRH is suggestive of premature activation of the fetal hypothalamic-pituitary axis. Fetal fibronectin, phIGBP-I, and PAMG-1 are released because of mechanical stretch. Interleukins, MMPs, CRP, caeruloplasmin, and ferritin suggest the inflammatory pathway activation.

Therefore, multiple biomarker modeling is gaining increased attention. Studies have shown that fFN + cervical length has higher sensitivity and specificity to predict PTB.

Currently in the review of literature CVF fFN (>50 ng/ml) is most effective and useful in predicting PTB both in symptomatic and high-risk asymptomatic women. The sample collection is safe and easy, without any added risk to the mother and fetus. The test kit is easily available and results are rapidly available. At present, it is justifiable to use fFN as a screening tool to select the cases likely to have PTB and provide a timely intervention with positive results. Placental alpha macroglobulin-1 (PAMG-1) is also showing promising results. In high-risk asymptomatic women CVF fFN, IL6, serum AFP, PAPP-A, and salivary progesterone seems to be showing promising potential as a predictor of PTB. Genomic, proteomic, and metabolomic approaches are also being investigated with increased vigor and will soon enable the discovery of novel biomarkers as per the pathophysiology of PTB.

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