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MATERNO-FETAL MEDICINE

Combination of biochemical markers in predicting pre-term delivery

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Abstract
Purpose The aim of this study was to evaluate the predictive performances of some biochemical markers in predicting pre-term delivery in asymptomatic women.
Methods We included 491 asymptomatic women at 24 weeks' gestation, who underwent the endocervical phosphorylated insulin-like growth factor binding protein (phIGFBP-1) test, cervico-vaginal interleukins 6 (IL-6) and 8 (IL-8), and serum C-reactive protein (CRP). A receiver-operating characteristics (ROC) curve was used to determine the most useful cut off point. A multivariate logistic regression model was used in order to analyze the combination of significant predictive variables for pre-term delivery following univariate analysis.
Results ROC curves indicated that 33 μg/l was the optimal cut off value for phIGFBP-1 test, 21.3 ng/l for IL-6, 324 ng/l for IL-8, and 8.42 mg/l for CRP in predicting pre-term delivery. The univariate logistic regression analyses revealed an odds ratio of 3.04 for phIGFBP-1 test, 4.82 for IL-6, and 3.08 for CRP. The multivariate analysis of phIGFBP-1 test, IL-6, and CRP showed that they were independent variables and therefore useful in combination for predicting pre-term delivery.
Conclusions The phIGFBP-1 test, the cervico-vaginal IL-6, and the serum CRP are independent variables that can be used together to predict pre-term delivery in asymptomatic women.

Keywords Biochemical markers · Phosphorylated insulin-like growth factor binding protein-1 test · Cervico-vaginal interleukins · Serum C-reactive protein · Pre-term delivery · Spontaneous pre-term delivery

Abbreviations

phIGFBP-1	Phosphorylated insulin-like growth factor binding protein
IL	Interleukin
CRP	C-reactive protein
PPV	Positive predictive value
NPV	Negative predictive value
NS	Not significant
OR	Odds ratio

The endocervical phosphorylated insulin-like growth factor binding protein test, the cervico-vaginal interleukins 6 and serum the serum C-reactive protein can be used together to predict pre-term delivery in asymptomatic women.

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Introduction

Pre-term deliveries occur before 37 weeks' gestation, and account for 5–7% of deliveries in Europe and 12–13% in

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the United States [1, 2]. About 5% of pre-term births occur before 28 weeks' gestation, 15% at 28–31 weeks, 20% at 32–33 weeks, and 60% at 34–36 weeks [1, 2]. There is a considerable interest in biochemical markers capable of differentiating patients at a truly high risk of pre-term delivery from those requiring no treatment. Assessing the probability of pre-term delivery is important, because clinical interventions are potentially risky and expensive [2]. Recent attempts to predict pre-term delivery have included the use of biochemical markers [3–20]. Considerable interest has been shown in developing biomarker assays for the prediction of pre-term birth, including one rapid test for determining the phosphorylated isoform of insulin-like growth factor binding protein-1 (phIGFBP-1) in endocervical secretions and the cervico-vaginal interleukins 6 (IL-6) and 8 (IL-8). The nonphosphorylated isoform of IGFBP-1 is secreted by the fetal and the adult liver, and is contained in amniotic fluid, fetal serum and maternal plasma (4.5), with a concentration in amniotic fluid 100–1,000 times higher than that in fetal serum (4.5). However, phIGFBP-1 is mainly secreted by maternal decidual cells, and may be an indicator of tissue damage of the chorio-decidual interface (4.5). In the early stages of labor, the fetal membrane begins to detach from the decidua and a small amount of phIGFBP-1 may be found in cervical secretions (4.5). Kekki et al. [10] have reported that a phIGFBP-1 concentration of at least 10 μg/l in a cervical swab sample indicates a tenfold greater risk of pre-term delivery.

There is an increasing evidence that inflammation of the upper genital tract may play a role in the pathogenesis of pre-term delivery [14]. New markers of infection and inflammation, such as cervico-vaginal IL-6 or IL-8, can facilitate an early diagnosis [14]. Throughout gestation, IL-6 is a multifunctional cytokine and women with high values of cervico-vaginal IL-6 between 24 and 32 weeks' gestation were at high risk of pre-term delivery [14–17]. IL-8 is produced at the levels of the chorionic-decidual interface in response to inflammatory cytokines such as IL-1 and TNF-α. The cervico-vaginal IL-8 played a role in cervical ripening and disruption of the chorion-decidual interface that occurred in pre-term delivery [18, 19].

Recent studies have identified the serum C-reactive protein (CRP) as an acute-phase reactant associated with chorioamnionitis, and perhaps also as a risk indicator for pre-term delivery [20]. CRP begin a sensitive marker of tissue damage and inflammation, can be a potential marker and play a role in eliciting the inflammatory response characteristic of pre-term delivery [20]. CRP is an easily detectable serological marker, and is primarily produced in the hepatocytes in response to cytokines, such as IL-1 and IL-6, released from the inflammatory site [20].

In English literature, we have not found any studies that compare the value of endocervical phIGFBP-1 with cervico-vaginal IL-6 and IL-8, and blood serum CRP in the prediction of pre-term delivery. The aim of this study was to analyze if a combination of biochemical markers is useful in predicting pre-term delivery in asymptomatic patients at 24 weeks' gestation.

Materials and methods

This prospective study involved a series of 491 Caucasian asymptomatic women who received prenatal care between January 2006 and June 2007 in our departments of obstetrics. All the patients signed an informed consent form approved by the local Health Sciences in Human Subjects Committee. The inclusion criteria were: a singleton pregnancy at 24 weeks' gestational and intact membranes. The exclusion criteria were: uterine contractions, vaginal bleeding, placenta previa, multiple gestations, fetal abnormalities and uterine anomalies. Gestational age was based on menstrual data, confirmed by an early first trimester ultrasound scan; the other recorded data included demographic information, pre-term labor management and delivery outcomes.

Immediately upon admission, a rapid cervical sample for phIGFBP-1 determination (Actim Partus Test, Medix Biochemica, Kauniainen, Finland) was taken by means of a polyester-tipped swab during speculum examination of the cervix, and extracted with specimen-extraction solution. The lower end of the swab was inserted into the external cervical orifice and left in that place for about 10 s, after which it was placed in a test tube containing 0.5 ml of sample buffer solution for 15 s. The polyester swab absorbs approximately 150 μl fluid when saturated, and the average dilution of cervical sample in the buffer will be approximately 1:5. The specimens were frozen and stored at −20°C until phIGFBP-1 concentration was measured. The concentration of phIGFBP-1 was measured by immunoenzymometric assay with monoclonal antibody 6303 as the detecting antibody. The detection limit of the assay was 0.3 μg/l. All sample were measured in duplicates.

We performed a cervico-vaginal IL-6 and IL-8 that were monitored by placing the sterile gauze swabs into women's vaginas for a period of at least 20 s in order to saturate with the secretions from the cervix and vagina. Cervico-vaginal IL-6 and IL-8 were measured with chemiluminescent immunometric assay (DPC, Los Angeles, USA), in which inter-assay and intra-assay coefficients of variation were 10%. A blood sample was drawn for CRP analysis. CRP was measured with a highly sensitive method by immunonephelometric assay. The polystyrene particles sensitized with monoclonal anti-CRP Ig are agglutinated while

mixing with samples containing the CRP to be measured. The intensity of the light dispersed by the nephelometer is determined by the CRP content of the sample.

The results of the various clinical markers were not available to care providers of these women and could not be considered to modify their clinical management.

Statistical analysis

Student's *t* test was used to compare the average values of the continuous variables, with the values being expressed as the mean ± SD. Pearson's chi-square test or Fisher's exact test was used to analyze the categorical variables, whose values are expressed as percentages. A receiver-operating characteristics (ROC) curve was constructed and used to determine the most useful cut off point (with highest overall accuracy) for biochemical markers in predicting preterm delivery. Univariate logistic regression analysis was performed to assess the capability of the test to predict pre-term delivery. A multivariate logistic regression model was then developed using the forward stepwise method and 95% confidence levels in order to analyze the significant predictive variables in combination.

Results

The study involved 491 asymptomatic women. The pre-term birth rate was 8.9%. The average maternal age of the patients delivering pre-term was 32.02 ± 5.78 years, and that of the patients delivering at term was 30.45 ± 5.57 years [not significant (NS)]; their average body mass index was 22.79 ± 2.99 and 24.24 ± 4.22, respectively (p = NS). The 52.1% were nulliparous and 47.8% multiparous. The mean gestational age at delivery was of 33.75 ± 3.02 years in the patients delivering pre-term and 38.99 ± 1.27 years in the patients delivering at term ($p < 0.0001$). The ROC curve showed that the best cut off value in terms of predicting pre-term delivery was 33 µg/l for phIGFBP-1 test, 21.3 ng/l for IL-6, 324 ng/l for IL-8, and 8.42 mg/l for CRP (Figs. 1, 2, 3). The univariate logistic regressions of phIGFBP-1, IL-6, and CRP were statistically significant in predicting pre-term delivery with an odds ratio (OR) of 3.04 for phIGFBP-1 test ($p = 0.001$), an OR of 4.82 for IL-6 ($p < 0.0001$), and an OR of 3.08 for CRP ($p = 0.005$) (Table 1). The ability of the IL-8 to predict delivery was not statistically significant at Fisher's exact test (Table 1). The sensitivity, specificity, positive predicting value (PPV) and negative predicting value (NPV) of these biochemical markers are shown in Table 2. Multivariate analysis showed that the combination of phIGFBP-1 test (OR 3.48), IL-6 (OR 3.91) and CRP (OR 3.19) was statistically significant (Table 1). The association of phIGFBP-1, IL-6 and CRP is to predict pre-term delivery with a specificity of 99%, a PPV of 83% and, a NPV of 90% (Table 2).

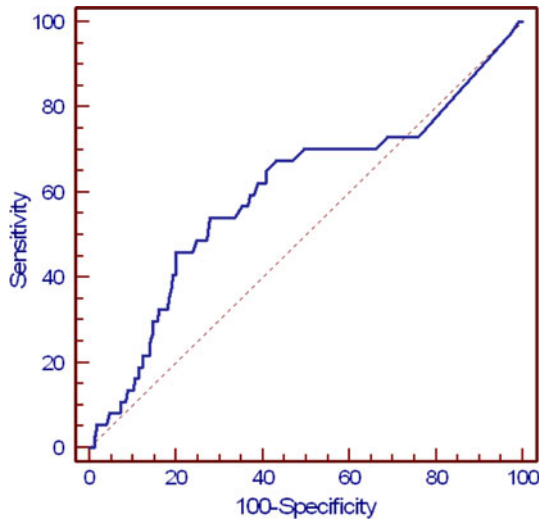

Fig. 1 Receiver-operating curves for the performance of the phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1) test. Cut off value 33 µg/l

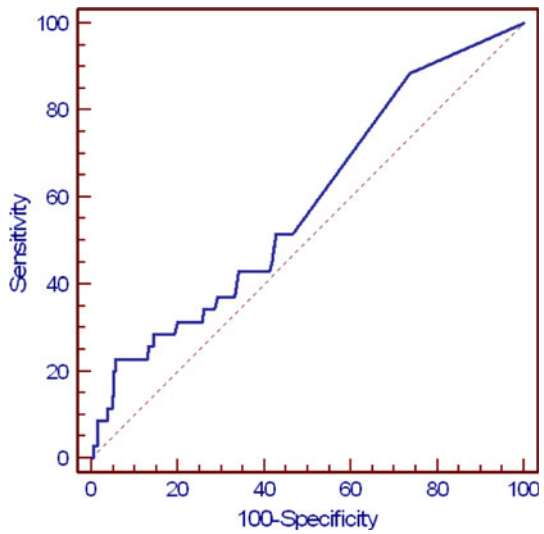

Fig. 2 Receiver-operating curves for the performance of the cervico-vaginal interleukins 6 (IL-6). Cut off value 21.3 ng/l

Conclusion

Recent attempts to predict pre-term delivery have included the use of biochemical markers. The introduction of new cervico-vaginal test based on the search of phIGFBP-1, considering the decidual and not the amniochorial synthesis, may improve the accuracy to predict pre-term delivery [4–13]. In this study, also cervico-vaginal cytokines and

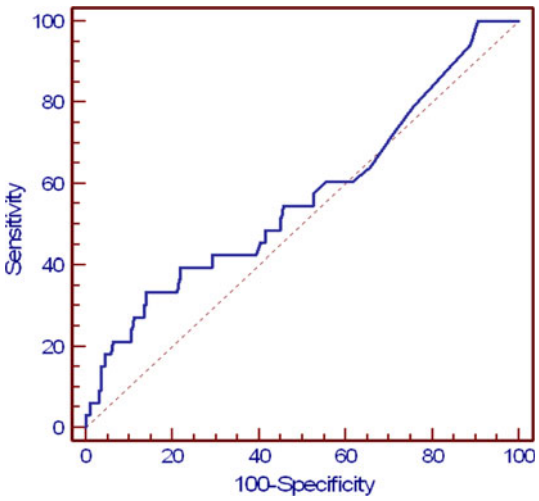

Fig. 3 Receiver-operating curves for the performance of serum C-reactive protein (CRP). Cut off value 8.42 mg/l

Table 1 Univariate and multivariate analysis of the ability of the phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1) test, cervico-vaginal interleukins 6 (IL-6) and 8 (IL-8), and serum C-reactive protein (CRP) in predicting preterm delivery

	Univariate analysis			Multivariate analysis		
	OR	(95% CI)	*p*	OR	(95% CI)	*p*
phIGFBP-1	3.04	(1.52–6.07)	0.001	3.48	(1.46–8.25)	0.004
IL-6	4.82	(1.94–12.02)	<0.0001	3.91	(1.31–11.67)	0.01
CRP	3.08	(1.38–6.87)	0.005	3.19	(1.25–8.13)	0.01
IL-8	2.49	(0.92–6.77)	NS			

NS Not significant

serum CRP were considered as useful markers to avoid pre-term delivery [14–20]. We assessed phIGFBP-1 test in endocervical secretions, IL-6 and IL-8 in cervico-vaginal secretions, and serum CRP in a large number of asymptomatic patients. We analyzed the optimal cut off values of these markers and we found a cut off of 33 μg/l for phIGFBP-1 test, 21.3 ng/l for IL-6, 324 ng/l for IL-8, and 8.42 mg/l for CRP. Our data showed that phIGFBP-1 test has a high NPV of 93% with a specificity of 72%. Therefore, we analyzed the cytokine values and the univariate analysis revealed that IL-6 was significantly associated with pre-term delivery. The phIGFBP-1 test, IL-6, and CRP were independently and significantly associated with the prediction of pre-term delivery. The multivariate analysis showed that the combination of phIGFBP-1 test, IL-6, and CRP was significantly associated to prediction of pre-term delivery with a greater specificity, a NPV of 90% and a PPV of 83%, than either method alone. Thus indicating that these three markers can be used together to predict pre-term delivery. In our study, IL-8 could not be considered as a useful marker to avoid pre-term delivery in a population of asymptomatic women, without contractions and with intact membranes.

As shown by our group in 2002, the cervico-vaginal IL-6 concentrations, due to local inflammatory response, were significantly higher in women who delivered pre-term than those who delivered at term [4]. The deciduas is a major producer of cytokines and likely played a role in the inflammatory response to infection, demonstrated by the elevated concentrations of IL-6 in patients who delivered pre-term [4]. According to our previous study, these data demonstrated that IL-8 could not be correlated with pre-term delivery [4]. Instead, Kurkinen-Raty et al. [9], in a small group of symptomatic patients between 22 and 32 weeks' gestation, showed that both cervical IL-6 and IL-8 concentrations were significantly higher in cervical secretions of women who incurred in pre-term delivery. They reported that IL-6 is an independent risk factor of pre-term delivery with a sensitivity of 73% and a specificity of 61% [9]. In our opinion, cervical IL-8 played a role in cervical ripening and disruption of the chorion-decidual interface that occurred in symptomatic patients with cervical modifications [9], but IL-8 was not increased in asymptomatic patients without contractions and intact membranes [9]. Kurkinen-Raty et al. [9] found that the association of IL-6, IL-8 and ultrasonographically measured cervical index is the best predictor of pre-term delivery. They have shown that also a phIGFBP-1 > 6.4 μg/l increased the risk of pre-term birth and if the concentration was elevated (>21 μg/l), there was a significantly increased incidence of puerperal infections [9]. They concluded that elevated concentration of cervical

Table 2 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of positive test for endocervical phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1), cervico-vaginal interleukins 6 (IL-6), and serum C-reactive protein (CRP) in predicting pre-term delivery (%)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
phIGFBP-1	54.1	72.1	17.4	93.5
IL-6	22.9	94.2	28.6	92.3
CRP	33.3	86.1	21.6	91.8
phIGFBP-1 + IL-6 + CRP	17.9	99.5	83.3	90.1

phIGFBP-1 seemed to be a marker of puerperal and neonatal infections. We thought that the patients number and the percentage of pre-term deliveries were not great enough to show a statistically significant correlation with pre-term delivery.

In this study, we assessed the serum CRP and we found an optimal cut off value of 8.4 mg/l. Concluding, the serum CRP was significantly associated in prediction of pre-term delivery. In 2002, Hvilsom et al. [20] showed an association between the CRP level in maternal serum early in the second trimester with pre-term delivery in 84 cases of preterm deliveries and in 400 controls. They found a statistically significant difference in CRP levels with a cut off value ranging from 5.6 (75th percentile) to 16.4 mg/l (95th percentile [20]. A high CRP level at the beginning of pregnancy is associated with a nearly twofold increased risk of pre-term delivery [20]. As cytokines stimulate CRP production, one could speculate that CRP levels are increased by an infectious process [20].

In conclusion, the combination of phIGFBP-1 test, IL-6, and CRP was significantly associated to prediction of preterm delivery. In asymptomatic patients, the identification of biomarkers associated with pre-term delivery provided not only an insight into the pathophysiological conditions of these pregnancy complications, but also a tool that can be applied to identify the women who were at high risk for pre-term delivery, to allow for targeted interventions. However, various biological markers may have the potential to better define the population in which this intervention will be more effective.

The phIGFBP-1 test, IL-6, and serum CRP are noninvasive tests that could be together applicable in clinics with a high specificity, NPV, and PPV. These biochemical markers, by evaluating women at earlier weeks' gestation, can be a potential marker for pre-term delivery and they may differentiate the truly high risk patient requiring treatment, in order to reduce the prematurity and the neonatal morbidity and mortality, although further comparative studies using the same population and study design are needed to confirm our results. Only when the use of a marker and subsequent treatment have been shown to result in a significant reduction in pre-term delivery should any single or multiple marker test for spontaneous pre-term birth, be introduced as a part of routine prenatal care.

Conflict of interest None.

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