Interleukin 6 and fetal fibronectin as a predictors of preterm delivery in symptomatic patients

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Abstract

Preterm delivery is the leading cause of neonatal mortality and morbidity. The rate of preterm births has been estimated to be about 15 million, which accounts for 11.1% of all live births worldwide. The purpose of this study was to evaluate the cervico-vaginal (CVF) cytokine IL-6 and fetal fibronectin (fFN) status as predictors of preterm delivery in patients with symptoms of preterm labor. Patients with symptoms suggestive of preterm labor were recruited from September 2013 to March 2014. Vaginal swabs were taken for fetal fibronectin test (fFN) and CVF IL-6. Antibiotics, steroids and tocolytics were administered, where appropriate. The outcome was measured by the occurrence of preterm delivery within 14 days from the day of hospital admission. Cut-off value of 1305 pg/mL for the concentration of IL-6 in the CVF was the best predictor of preterm delivery, with the sensitivity of 69.4% and specificity of 68.2%. Patients with positive fFN test had the OR of 6.429 (95%CI 1.991-20.758) to deliver prematurely. The multivariate analysis of combined fFN and CVF IL-6 tests resulted in risk of 86.7% to deliver prematurely, if both tests were positive. The combination of both tests performed better than the individual tests and decreased the false positive rate, which in turn reduced the chances for inappropriate patient treatment, bringing down the costs.

KEY WORDS: Preterm labor; fFN; IL-6; predictive value
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INTRODUCTION

Preterm delivery is defined as the delivery before completing 37 weeks of gestation. It is and a leading cause of neonatal mortality and morbidity worldwide. The rate of preterm births has been estimated to be about 15 million, which accounts for 11.1% of all live births worldwide [1]. Individual countries' incidence rates are highly dependent on the degree of development and range from 5% in the most developed European countries to 18% in several African countries [1]. More than 60% of all preterm births are registered in the underdeveloped regions of sub-Saharan Africa and South Asia, and at the same time these are the regions that account for 52% of all global live births [1].

In an effort to prevent serious neonatal mortality and morbidity, women diagnosed with threatened preterm labor are hospitalized and tocolytics and corticosteroids are administered to them. Most randomized studies on the use of tocolytics for treatment of threatened preterm labor demonstrate a significant delay in delivery of about 7 days, but no significant reduction in the incidence of preterm delivery and consequential neonatal mortality and morbidity [2,3]. Early detection and confirmation of preterm labor is difficult, since the initial symptoms are often mild, and the later symptoms manifest when the process is beyond intervention.

Preterm birth has been ranked among the top 10 causes of global burden of disease, making the reduction of the incidence of preterm labor to be the main goal of the global community [4].

In the past three decades there have been many efforts to develop the appropriate methods that will correctly predict preterm delivery. Obstetric history, clinical symptoms, epidemiological risk factors, maternal indicators, such as age and anthropometric parameters, pregnancy characteristics (e.g. bleeding), different physical examination parameters and biological markers were considered as potential predictive factors. Unfortunately, most of these methods are neither sensitive nor specific enough [5,6].

One of the most studied biochemical markers used to predict preterm labor is fetal fibronectin (fFN). The isolation of this glycoprotein in the cervical-vaginal fluid (CVF) indicates

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a choriodecidual disruption [7]. fFN is usually absent from the CVF starting at the 24th gestational week until near the delivery term. In a study by Goldenberg and al., involving women undergoing routine screening at 24-26 weeks of gestation, 3-4% of the tested population had CVF positive for fFN, and all of these patients had a significantly increased risk of preterm delivery [7-10].

Positive fFN tests at 22nd-24th gestational week predicted more than half of the spontaneous preterm deliveries before 28th week of gestation, with a calculated sensitivity of 63% [7]. Another study conducted on asymptomatic pregnant women revealed that a positive fFN test increased the relative risk of delivery before 34 weeks of gestation with a pooled likelihood ration (LR) of 4.0 (95% CI: 2.9-5.5) and a corresponding summed LR for a negative test of 0.78 (95% CI 0.72-0.84) [9]. The most important characteristic of the fFN test, when taking into account its clinical applicability and use, was its negative predictive value (NPV) [10].

Another group of biochemical markers involved in the prediction of preterm delivery is inflammatory molecules, such as cytokines. Cytokines may be involved in the etiology of preterm birth through their influence on prostaglandin synthesis and secretion [11]. A number of studies have reported increased concentrations of certain cytokines, most notably interleukin 6 (IL-6) in the serum and amniotic fluid of patients with preterm labor [12-16]. Several studies have investigated IL-6 detection in the CVF and demonstrated that the presence of IL-6 in the CVF is associated with preterm delivery [17-19].

The purpose of this study was to evaluate the usefulness of measuring cervical-vaginal pro-inflammatory cytokine IL-6 and fFN as predictors of preterm delivery in patients with symptoms of preterm labor. We hypothesize that adding cervical vaginal IL-6 determinations as a second marker to fFN will improve the positive predictive value of fFN testing for preterm birth.

MATERIALS AND METHODS

Study population and specimens

Fifty-eight pregnant women were eligible to join the study. They were admitted to the Department of High Risk pregnancy at University Clinic for Gynecology and Obstetrics, Skopje, with symptoms of uterine activity indicative of preterm labor at 24.0 to 36.6 weeks of gestation. They were recruited in the period of 6 months (September 2013 to March 2014). Inclusion criteria were presence of symptoms or complaints suggestive of preterm labor, including uterine contractions, intermittent lower abdominal pain and pelvic pressure, intact amniotic membranes determined by speculum examination and cervical dilatation ≤3 cm. Women were not included if they had ruptured membranes, hemorrhage, active labor, a cervical cerclage in place and suspected chorioamnionitis, defined by fever, abdominal pain and leukocytosis.

With a prior consent, they were treated according to usual hospital protocol, with additional vaginal swabs taken for fFN and cervicovaginal IL-6. First, women were asked to empty their bladders and were placed in dorsal lithotomy position. After sterile speculum introduction, prior to ultrasound or digital examination, sample of cervical fluid was collected from the external os with a Dacron swabs. Two swabs were placed in the posterior vaginal fornix for 15 sec to achieve saturation. Following collection, each individual sample was placed into a sterile cryovial with extraction buffer. The swab for IL-6 was inserted into a tube containing phosphate-buffered saline (PBS), NaCl, 0.1 mg of aprotinin per ml, fetal bovine serum (FBS) and 0.001% sodium azide. Collection for fFN was with commercial specimen collection kit for fFN, containing 1ml fFN extraction buffer (Fetal Fibronectin Enzyme Immunoassay or Rapid fFN; Hologic Inc., Marlborough, USA).

Laboratory analyses

Sample for IL-6 was incubated for 1h at ambient temperature and then centrifuged at 16,000g in a Spin-x centrifuge filter unit for 15min. A second wash of the swab with another 500µl of extraction buffer was followed by immediate centrifugation in the Spin-X tube. After extraction, samples were stored at -40°C until measurements were performed.

IL-6 in both serum or swab extracts, were quantified on fully automated immunoassay analyzer by use of sequential, two-site, solid-phase, chemiluminiscent enzyme immunometric assays (Immulite 2000 HP, Diagnostic Products Corp). Analytic sensitivity for IL 6 test is 2pg/ml with highest level of 1000pg/ml.

fFN was measured by membrane immunoassay on a Rapid fFN Analyzer. The Rapid fFN Cassette is a lateral flow, solid-phase immune-chromatographic assay. Specimens with fFN concentrations > 0.05µg/mL are interpreted as positive.

Clinical investigations

After collection of the cervical sample, a trans-vaginal ultrasound measurement was performed using 6.5 MHz trans-vaginal probe, in accord with the Fetal Medicine Foundation Criteria [34]. The mean of three measurements was used. A digital examination of the cervix was then performed, and cervical status was documented according to the modified Bishop score.

Following the taking of the swabs, the attending clinician did a standard ultrasound exam to determine fetal biometry,

fetal position, cervical length, as well as a digital exam of the cervix. A 30 minute cardio-tocogram was performed in order to evaluate the well-being of the fetus and to grade contractions. Urine analysis was performed in all cases to exclude urinary tract infection. After the initial evaluation and admission of the patients, corticosteroids, beta-mimetic tocolytics and antibiotics were administered.

Outcome variable was occurrence of preterm delivery within 14 days from the day of hospital admission.

Statistical analysis

IBM SPSS Statistics 20 was used for analysis. Test for logistic regression (binary) and receiver operating characteristic curves (ROC) were used and p-values less than 0.05 were considered significant.

RESULTS

The main demographic characteristics of the individuals studied are summarized in Table 1. The average maternal age was 30.12 years. The average gestational age was 31.55 weeks at recruitment. The average height was 164.34 cm. The average weight was 74.05 kg. The average BMI was 27.54. From the total of 58 patients enrolled in the study, 9 (15.5%) had a history of previous preterm delivery. We also evaluated the number of previous spontaneous abortions, parity and smoking habits of the patients with threatened preterm labor.

Twenty-six patients (44.8%) were delivered within 7 days from admission, while 3610 more patients (62.0717.24%) were delivered within 14 days from admission., bringing the total number of patients delivered within 14 days to 36 (62.07%).

In the group of the patients that were delivered within 14 days of admission, we found significantly higher concentrations of IL-6 in the CVF (p=0.0011). The average measured concentration of IL-6 was 3139.8±2646.2 pg/ml in the group of patients that delivered within 14 days, while the average concentration in the group that exceeded the period of 14 days was 1755.7±3165.7 pg/ml (Table 2).

TABLE 1. Demographic characteristics of the individuals examine (n=58).

Variable	Mean±SD (range)
Maternal age (years)	30.12±4.82 (20-40)
Gestation age at examination	31.55±3.95 (22-36)
BMI	27.54±4.93 (18.7-43.8)
	N (%)
Parity	
Nulliparous	13 (22.41)
Multiparous	45 (77.59)
Previous preterm delivery	10 (17.24)
Smoker	11 (18.96)

Using the data, we calculated a receiver operating characteristic (ROC) curve in order to determine the cut-off value for the concentration of IL-6 in the CVF that accurately predicts preterm delivery (Figure 1).

The best cut-off value for the concentration of IL-6 in the CVF that correctly predicts preterm delivery in our study was 1305 pg/mL, which gave the test a sensitivity of 69.4%, specificity of 68.2%, a positive likelihood ratio (LR+) of 2.18 and a negative likelihood ratio (LR-) of 0.45 (Figure 1 and Table 3). The calculated rate of preterm delivery was 78.13% in patients with a CVF concentration of IL-6 higher than 1305 pg/mL, and 42.31% in the patients with concentrations lower than the established cut-off.

The difference in the CVF concentration of IL-6 classified above or below the determined cut-off of 1305 pg/mL, between the two groups of patients was statistically significant (p=0.005).

The univariate logistic regression analysis for the CVF concentrations of IL-6 as a predictor of preterm delivery revealed

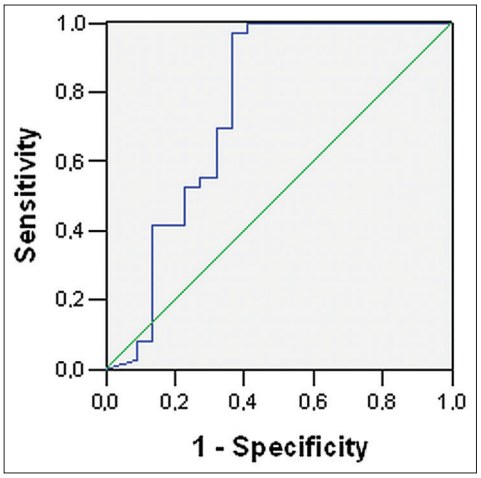

FIGURE 1. ROC curve for the performance of IL-6 in the CVF as a predictor of preterm delivery

TABLE 2. Concentration of IL-6 in the CVF of the individuals studied

Variable	Outcome within 14 days of admission		p value
	Undelivered (n=22)	Delivered (n=36)	
IL-6 in CVF (pg/mL, mean±SD, range)	1755.7±3165.7 (19.9-10001.0)	3139.8±2646.2 (190.9-10001.0)	p=0.0011

TABLE 3. Diagnostic performance of IL-6 in the CVF as a predictor of preterm delivery

Concentration of IL-6 in CVF cut-off=1305 pg/mL	Outcome within 14 days of admission		Total
	Undelivered (n=22)	Delivered (n=36)	
Below	15 (57.69%)	11 (42.31%)	26
Above	7 (21.88%)	25 (78.13%)	32
Total	22	36	58

Sensitivity=69.4%; Specificity=68.2%; PPV=78.1%; NPV=57.69%; LR+=2.18; LR-=0.45; Area under the curve (AUC) =0.759; 95% CI=0.607-0.91; Chi-square=7.82; df=1; P=0.005;

that patients with the concentrations of IL-6 higher than 1305pg/mL had an odds ratio (OR) of 3.87 (95% CI 1.23-11.282).

Table 4 presents the distribution of the patients that delivered within or exceeded the period of 14 days since admission in regards to the results of the fFN test. Of the 36 patients that delivered within 14 days of admission, 27 patients (75%) had a positive fFN test, while 15 patients (68.18%) of the 22 patients that surpassed the period of 14 days from admission had a negative fFN test. The Chi-square test confirmed observed the observed difference (p=0.0011).

The fFN test was a significant predictor of preterm delivery. The patients with a positive fFN test had an OR of 6.429 (95%CI 1.991-20.758) to deliver prematurely. The diagnostic performances of the fFN test in our study were as follows: Sensitivity=75%; Specificity=68.2%; PPV=79%; NPV=62.5%; LR+=2.46; LR-=0.37; Area under the curve (AUC) =0.716; 95%CI = 0.575-0.856 (Figure 2).

The multivariate analysis of the combination of the fFN test and the concentration of IL-6 in the CVF showed that patients with a positive fFN test and a concentration of IL-6 in the CVF above 1305pg/mL had a likelihood of 86.7% to deliver prematurely. This combination of tests had a sensitivity of 97.2% and a specificity of 63.6% in the prediction of preterm delivery (Figure 3)

The calculated AUC was 0.759 with 95% CI of 0.61-0.908, which indicates that the combination of the two tests was a good predictor that correctly divided the patients who would deliver prematurely from patients who will remain pregnant after 14 days of admission.

TABLE 4. Detection of fFN in the CVF of the individuals studied

Variable	Outcome within 14 days of admission		p-value
	Undelivered (n=22)	Delivered (n=36)	
Fetal fibronectin n (%)			p=0.0011
Positive	7 (31.82%)	27 (75.0%)	
Negative	15 (68.18%)	9 (25.0%)	

DISCUSSION

Despite developments in obstetric care, preterm delivery remains a major cause of neonatal morbidity and mortality. The recommended treatment of women with acute risk of preterm delivery includes tocolytics, steroids and in utero transfer to a center with neonatal intensive care [21]. This involves unnecessary treatment and complex management in a number of symptomatic women who eventually will not deliver preterm. Therefore, there is a need for assessment tools to reliably differentiate between cases of high risk of early delivery and those with low risk, in which the treatment can be avoided.

In our study, we recruited patients with symptoms of preterm labor, focusing on the most clinically significant group of patients i.e. the patients that will give birth prematurely within 14 days of admission. We measured the concentration of IL-6 in the CVF and did a fFN test in all enrolled patients.

Out of the 58 patients recruited, 36 (62.07%) were delivered within 14 days of admission. That means that the remaining 22 patients (47.93%) were needlessly admitted and treated with tocolytics and corticosteroids.

Our data suggested that IL-6 at a cut-off of 1305pg/mL correctly identifies the patients that will deliver within 14 days from admission, with a sensitivity of 69.4%, specificity of 68.2%, a positive likelihood ratio (LR+) of 2.18 and a negative likelihood ratio (LR-) of 0.45. The calculated rate of preterm delivery was 78.13% in patients with a concentration of IL-6 in the CVF higher than 1305 pg/mL, and 42.31% in the patients with concentrations lower than the cut-off. This cut-off value for the concentration of IL-6 in the CVF yielded a PPV of 78.1% and a NPV of 57.69.

Previous studies demonstrate that patients with preterm labor and clinical chorioamnionitis have increased concentrations of IL-6 in the amniotic fluid and umbilical cord blood serum [22-27].

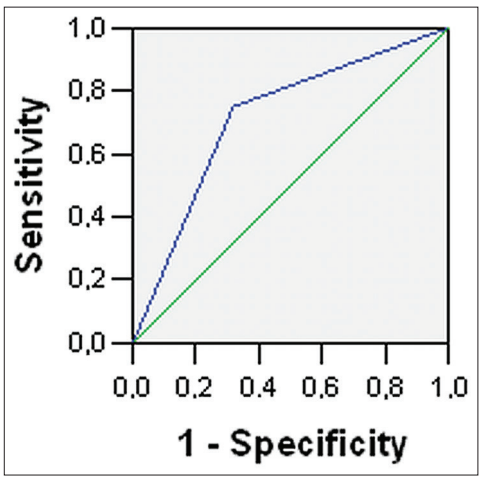

FIGURE 2. ROC curve for the performance of the fFN test in the prediction of preterm delivery

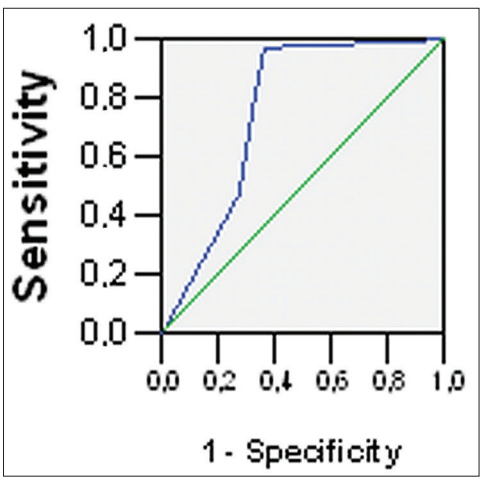

FIGURE 3. ROC curve for the diagnostic performance of the combination of fFN and IL-6 prediction of preterm delivery

A study of Grenacheand al. [20] revealed IL-6, IL-2R and tumor necrosis factor α (TNFα) in the CVF as predictors of the preterm labor. The authors concluded that IL-6 was the only cytokine that was significantly associated with preterm delivery (n=165). The study used an IL-6 cut-off value of 250ng/L, and the clinical sensitivity, specificity, PPV and NPV were nearly identical to those of the fFN test. The study also provided preliminary evidence that testing for IL—6 in the CVF could be used as a less expensive test to determine the likelihood of delivery within 14 days in patients with symptoms of preterm labor, given into account that the IL-6 assay costs in US 7$ per test, while the fFN assays cost around 100$ per test.

A recent large study conducted by Woodworth et al. [28] focused on the diagnostic accuracy of IL-6 detected in the CVF as a predictor of preterm delivery. The authors analyzed 660 CVF samples for IL-6 and concluded that the IL-6 test with a cut-off of 250pg/mL had a sensitivity of 35%, specificity 87%, PPV 19%, NPV 98% and a LR+ of 4.83 and LR- of 0.41. These results over perform the test in our analysis. Our regression analysis gave a significantly higher cut-off value for the concentration of IL-6, as opposed to the now almost universally accepted value of 250pg/mL, first determined by Lockwood *et al.* [17].This may be due to the fact that we calculated the likelihood of delivery within 14 days as opposed to 7 days, the small sample size and the fact that the study recruited a high risk group of patients that already had symptoms of preterm labor, a high proportion of which (over 60%) were delivered within 14 days.

A multitude of published studies undoubtedly demonstrated the clinical relevance of fFN testing for the assessment of patients at risk of preterm delivery. One of the more relevant such studies was published by Peaceman and al.[29]; it was a double blind study that evaluated the use of fFN in patients with a risk of preterm labor. The study enrolled 763 patients and used a cut-off of 50ng/mL. The calculated NPV for delivery within 14 days of admission was 99.2%. For patients that tested positive for fFN, the authors calculated the risk of delivery at 38.8%, although the PPV was only 13.4%. Most authors agree that the greatest value for fFN testing in symptomatic preterm patients is its high NPV with the potential to reduce unnecessary intervention.

Our data gave similar results. We found out that the fetal fibronectin test is a significant predictor of preterm delivery in our population with a sensitivity of 75%, specificity of 68.2%, PPV of 79% and NPV of 62.5%. Once again, the differences could be a product of the small sample size or the study design i.e. recruiting only high-risk symptomatic patients. We calculated that patients with a positive fetal fibronectin test have an OR of 6.429 (95%CI 1.991-20.758) to deliver prematurely.

The multivariate analysis of the combination of the fFN test and the concentration of IL-6 in the CVF showed that patients with a positive fFN test and a concentration of IL-6 in the CVF above 1305pg/mL had a likelihood of 86.7% to deliver prematurely. This combination of tests had a sensitivity of 97.2% and a specificity of 63.6% in the prediction of preterm delivery and performed better than each individual test in our population.

CONCLUSION

Our study confirms the usefulness of determining fFN and IL-6 in the CVF as biochemical markers for identifying patients with high risk of preterm delivery. The combination of both tests performed better than each individual test and decreased the false positive rate, which may reduce inappropriate patient treatment and bring down the costs. Further investigation on a larger sample that correctly reflects the general obstetrics population is necessary.

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