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Clinical application of the combined determination of neutrophil elastase, fetal fibronectin, and cervical length in predicting preterm birth of twin pregnancies.

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Abstract

In total, 115 pregnant women underwent detection of Neutrophil Elastase (NE) and Fetal Fibronectin (fFN), and measurement of Cervical Length (CL) 20-34 weeks into pregnancy. The clinical value of these values in predicting preterm birth was analysed retrospectively. In the 29 women who gave birth before 34 weeks, the CL value (21.3 ± 9.8 mm) and the fFN and NE rates (65.5% and 75.9%, respectively) were significantly different compared with women undergoing childbirth after 34 weeks (P<0.05). The combined detection rate of CL and fFN was 78.6%, CL and NE was 84.4%, and fFN and NE was 88.9%. Moreover, the combined detection rate of CL, NE and fFN was 100%. The predictive value of premature labor in twin pregnancy using multiple markers was more successful than that of a single marker; therefore, these multiple markers could be used to evaluate clinical intervention.

Keywords: Twin pregnancies, Preterm birth, Prediction, fFN, NE, Cervical length, Clinical intervention, Probiotics.

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Introduction

Preterm birth is a common complication in obstetrics [1]. Preterm birth can result in an increase in the mortality rate and incidence of follow-up complications, compared to full term infants [2]. In recent years, the prediction and prevention of preterm birth has become a topic of interest in the area of obstetrics [3,4]; however, few studies have focused on twin pregnancies. Additionally, with the development of science and the improvement of society, environmental problems have become a serious hazard to pregnant women and infants. Studies have indicated that air pollution, could increase the risk of preterm birth [5-8], especially polycyclic aromatic hydrocarbons [9-12]. Therefore, the prevention and treatment of premature birth is an important and urgent issue. At present, a method for clinically predicting preterm birth includes Cervical Length (CL) measurement [13,14] and fetal Fibronectin (fFN) detection [15]; however, little research has been reported on how Neutrophil Elastase (NE) relates to infection, resulting in preterm birth. The use of a single indicator to predict preterm birth is easily susceptible to subjective factors, resulting in low accuracy. Therefore, this study chose to evaluate fFN, NE, and CL as predictors; CL is an evaluation index of cervical function, fFN is used to assess the cervical tension, and local chorioamnionitis is evaluated with NE. This study investigated the value of the combined determination of CL, fFN, and NE for prediction of preterm birth in women with twin pregnancies.

Materials and Methods

Subjects

One hundred and fifteen women with twin pregnancies and a Creasy score of more than 12, at the First People's Hospital of Yunnan Province, were enrolled in this study from January 2012 to June 2015. They conformed to the following conditions; 1) 20 to 34 weeks of pregnancy [16,17], 2) pregnant with twins, 3) fetal membranes were intact without colporrhagia, 4) no vaginal surgery, use of anti-uterine contraction agents, or sexual intercourse within 24 h, and 5) no severe complications during pregnancy. Dynamic monitoring was used to repeatedly detect NE and fFN at different time points through the pregnancy, and CL was measured by ultrasonography. This study was conducted in accordance with the declaration of Helsinki [18]. This study was conducted with approval from the Ethics Committee of the First People's Hospital of Yunnan Province. Written informed consent was obtained from all participants.

Vaginal secretions collection

The women were placed in the lithotomy position, a bivalve speculum examination was performed, and vaginal secretions were collected via swabbing of the posterior fornix for 10 to 15 s.

Detection of fFN

fFN was detected using a solid phase immunosorbent assay kit (Hologic Company, USA). Per the manufacturer's instructions, the swab was placed in the buffer for 10 s. Results were shown as either a positive or negative line.

Detection of NE

An Enzyme Linked Immunosorbent Assay (ELISA) method was used to detect the levels of NE (Merck, Germany). In brief, the swab was placed in the buffer for 10 min and the results were read within 15 min. Results appeared as either a positive or negative line.

CL measurement

ALOKAα10 four dimensional diasonography (ALOKA, Japan) was used to measure CL through the vagina, after emptying the bladder. The conditions of the internal and external orifice of the uterus were also observed. The CL value was measured three times and a mean value calculated. A CL value less than 25 mm was defined as an abnormal value [14].

Clinical intervention

Pregnant women with the threat of preterm birth received vaginal micro-ecological treatment: placement of lactobacillus capsules (Inner Mongolia Shuang Qi Pharmaceutical, China) into the vagina. Frequency and strength of uterine contraction was monitored, while a uterine contraction inhibitor and glucocorticoids were used to treat the symptoms. Furthermore, changes in NE, fFN and CL were monitored dynamically.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software. Statistical tests used for analysis were the chi-square test and Student's t-test. $P<0.05$ denoted statistical significance.

Results

General conditions

Assessment of 115 women with twin pregnancies is summarized in Table 1. The positive rate of fFN and NE was 34.8% and 45.2%, respectively, in the women with twin pregnancies during the first visit. The mean CL was 22.6 ± 7.7 mm, which was less than the predicted critical value of 25 mm. The CL of 86 (74.8%) pregnant women was less than 25 mm, of which 28 cases had positive fFN (24.3%), 32 cases had positive NE (27.8%), and 27 cases had both positive fFN and NE (23.5%). Among the 115 twin pregnancies, 29 cases gave birth before 34 weeks, and the other 86 after 34 weeks.

Table 1. *General feature of twin pregnancies (115 cases).*

Feature	No.	Percentage (%)	Mean ± SD
Age			31.7 ± 4.9
20-35 years	95	82.6	
≥ 35years	20	17.4	
Frequency of pregnancy			
First pregnancy	54	47	
Multiple pregnancy	61	53	
Frequency of reproduction			
Primipara	96	83.5	
Multipara	19	16.5	
Natural abortive history (≥ 1)	35	34.8	
Assisted reproduction	42	36.5	
Primary fFN (+)	40	34.8	
Primary NE (+)	52	45.2	
CL ≤ 25 mm	86	74.8	
CL ≤ 25 mm			
fFN (+)	28	24.3	
NE (+)	32	27.8	
Primary both fFN and NE (+)	27	23.5	

Pregnant week of first detection (wk)			27.5 ± 4.6
Pregnant week of having child (wk)			34.9 ± 2.3
Birth week	Total 115		
Before 34 weeks	29	25.2	
After 34 weeks	86	74.8	
Primary cervical length measurement (mm)			22.6 ± 7.7

Relationships between preterm birth before 34 weeks and predictors and predictive value

Among the 115 study subjects, 29 cases occurred premature birth before 34 GW, with the incidence rate as 25.2%. As shown in Table 2, for those preterm labor before 34 GW, the positive rate of fFN, NE and CL value in preterm labor before 34 weeks group were significantly different from those birth after 34 weeks group ($p<0.05$). Table 3 showed that the prediction value of fFN, NE and CL for those birth before 34 weeks were 65.5%, 75.9% and 72.1% respectively.

Table 2. *Relationship between first detection results of three predictors and pregnant weeks.*

Predictor	Result	No.	Pregnant weeks ≥ 34 weeks: 86 cases		Pregnant weeks<34 weeks: 29 cases		P value
			No.	%	No.	%	
fFN	+	40	21	52.5	19	47.5	<0.05
	-	75	65	86.7	10	13.3	
NE	+	52	30	57.7	22	42.3	<0.05
	-	63	56	88.9	7	11.1	
CL	≤ 25 mm	86	68	79.1	18	20.9	<0.05
	>25 mm	29	18	62.1	11	37.9	

Table 3. *Prediction value of three predictors for preterm labor before 34 weeks (29 cases).*

Predictor	Predict standard	Positive no.	Predict value (%)
fFN	+	19	65.5
NE	+	22	75.8
CL	≤ 25 mm	18	62.1

Predictive value of combining NE, fFN, and CL

When combining the values of NE and fFN, the predicted rate of preterm birth was far higher (88.9%) than preterm births predicted using a single indicator (Table 4).

Table 4. *Relationship between preterm birth and two predictors (NE and fFN).*

Pregnant weeks	E+, F+	E-F+/E+F-/E-F-	Total
<34 weeks	24 (88.9%)	5 (5.7%)	29
≥ 34 weeks	3 (11.1%)	83 (94.3%)	86
No.	27	88	115

E=NE, F=fFN, P=0.001.

Predictive value of combining CL and NE, or CL and fFN

CL ≤ 25 mm was denoted as abnormal, and 28 cases were positive fFN, 22 of which occurred in the preterm birth (78.6%) and 32 cases were positive NE, 27 of which occurred in the preterm birth (84.4%). Combination of NE, fFN and CL: 24 cases whose NE and fFN were positive suffered from preterm birth, whose CL was obvious shorter than the predictive critical value 25 mm, and the average CL was 20.1 ± 6.7 mm. The detectable rate of combination of three predictors was 100% (Table 5).

Table 5. *Conditions of predicting preterm birth through combination of CL and NE or CL and fFN.*

Pregnant weeks	C+, F+	C-F+/C+F-/C-F-	C+, N+	C-N+/C+N-/C-N-	C+, F+, N+	C, F, N are not positive for all three
<34 weeks	22 (78.6%)	7 (8.0%)	27 (84.4%)	2 (2.4%)	19 (82.6%)	6 (6.5%)

≥ 34 weeks	6 (21.4%)	80 (92.0%)	7 (15.6%)	81 (97.6%)	4 (17.3%)	86 (93.4%)
No.	28	87	32	83	23	92

E=NE, F=fFN, C=CL, P=0.001.

Changes in fFN, CL and NE

fFN was continuously monitored during drug administration. Sixty-seven women always presented negative, one of who underwent preterm birth (1.5%), and 39 women always presented positive or went from negative to positive, 23 of who underwent preterm birth (59.0%). After clinical intervention, nine fFN positive cases turned fFN negative, one of whom underwent preterm birth (11.1%). The continuous positive rate of fFN was higher in preterm birth than in full term birth (P<0.05, Table 6).

The CL was dynamically monitored (Table 7). After clinical treatment, the CL decreased in both groups. In cases with a decreased CL, 54.8% underwent preterm birth, which significantly differed to cases where no change/increasing CL measurements were observed (6.8%).

Table 6. *fFN change after clinical intervention.*

Pregnancy weeks	F(+) → F(+)	F(+) → F(-)	F(-) → F(-)
≤ 34 weeks	23 (59.0%)	1 (11.1%)	1 (1.5%)
>34 weeks	16 (41.0%)	8 (88.9%)	66 (98.5%)
Total No.	39	9	67

F=fFN, P<0.05.

Table 7. *CL change after clinical intervention.*

Pregnant weeks	CL before intervention (mm)	Mean CL after intervention (mm)	P value	Shortened CL No.	CL with no change or increase No.
≤ 34 weeks	21.3 ± 9.8	14.7 ± 9.0	<0.05	23 (54.8%)	5 (6.8%)
>34 weeks	23.9 ± 7.9	21.2 ± 7.6	<0.05	19 (45.2%)	68 (93.2%)
Total No.				42	73
P value	>0.05 (t-test)	<0.05 (t-test)		<0.05 (χ^2 test)	

C=CL.

Of the NE positive cases, 66.7% underwent preterm birth. In the cases where NE went from positive to negative, 13.7% resulted in preterm birth. Furthermore, 17.3% of NE negative cases experienced preterm birth. Therefore, NE was significantly different between the two groups (Table 8).

Table 8. *NE change after clinical intervention.*

Pregnant weeks	NE (+) → NE (+)	NE (+) → NE (-)	NE (-) → NE (-)
≤ 34 weeks	8 (66.7%)	7 (13.7%)	9 (17.3%)
>34 weeks	4 (33.3%)	44 (86.3%)	43 (82.7%)
No.	12	51	52

E=NE, F=fFN, p<0.01.

After treatment, 22 of the 27 cases with both primary positive NE and fFN had no obvious improvement and underwent preterm birth. The remaining five women had a change in the NE and fFN values (which became negative), 60% of whom did not undergo preterm birth. This indicated that the treatment was effective (Table 9).

Table 9. *FFN, NE were (+) cases of clinical changes after treatment.*

Pregnant weeks	F (+) N (+) → F (+) N (+)	F (+) N (+) → F (-) N (+)/F (+) N (-)	F (+) N (+) → F (+) N (+)
≤ 34 weeks	22 (100.0%)	2 (40.0%)	22 (100.0%)
>34 weeks	0 (0%)	3 (60.0%)	0 (0%)
No.	22	5	22

E=NE, F=fFN, p<0.01.

After treatment, 20 of the 28 cases with both primary positive CL and fFN had no obvious improvement and underwent preterm birth. The remaining eight cases had a change in the CL and fFN, which went negative, 75% of whom did not experience preterm birth, indicating that the treatment was effective (Table 9).

After treatment, 24 of the 32 cases with both primary positive CL and NE had no obvious improvement and underwent preterm birth. The remaining eight cases had a change in the

CL and NE, which went negative, 63.5% of whom did not experience preterm birth, indicating the treatment was effective (Table 10).

Table 10. *Changes of cases with CL (+) plus fFN/NE (+) after treatment.*

Indicator		Pregnant weeks ≥ 34 weeks	Pregnant weeks<34 weeks	Total no.
C (+)F (+)	C (+) F (+) → C (+) F (+)	20 (100.0%)	0	20
	C (+) F (+) → C (-) F (+)/C (+) F (-)	2 (25.0%)	6 (75.0%)	8
C (+)N (+)	C (+) N (+) → C (+) N (+)	24 (100%)	0	24
	C (+) N (+) → C (-) N (+)/C (+) N (-)	3 (37.5%)	5 (63.5%)	8

Discussion

In recent years, along with the development of assisted reproductive technology, the incidence of twin pregnancies has gradually increased. However, the occurrence of preterm birth is much higher in twin pregnancies, and brings in heavy burden for society [19,20]. Therefore, it has become increasingly important to acquire effective prediction indicators of preterm birth to enable treatment in time. Currently, fFN and CL are used as indicators of preterm birth. However, a single indicator can have limitations for predicting preterm birth as they only examine one risk factor. Some studies have demonstrated that combination with other indicators could increase the reliability of predicting premature birth [21,22]. In addition, increasing numbers of studies have indicated that preterm birth is related to infection [23,24], especially subclinical infection in second and third-trimester pregnancies [25]. NE is an important inflammatory indicator of infection [24,25]. In our study, we chose three indicators for predicting preterm birth: CL to evaluate cervical function, fFN to assess cervical tension, and NE to test for local chorioamnionitis. We aimed to explore the significance of combined application of these indicators for predicting preterm labor in women with twin pregnancies.

Alterations in CL is an important marker of pregnancy [26,27], and some experts have considered that detecting changes in CL could predict premature birth [28]. This study found that the mean value of CL was significantly shorter in preterm labor women than in women with full term pregnancies. If CL were the only predictor, the false positive rate would be increased. Therefore, dynamic monitoring was used to accurately evaluate the cervical function [29]. Fox et al. stated that if the CL decreased by more than 20% over two measurements, preterm birth can be predicted in twin pregnancies [28]. Furthermore, this study found that a significant decrease in CL resulted in a 54.8% incidence rate of preterm birth. In contrast, the incidence of preterm birth was significantly reduced when the CL increased or stayed the same length.

fFN is secreted from trophoblast cells [30]. When fFN is increased during pregnancy, it implies that the placental villus adhered to the cervical decidua has been destroyed. fFN is also a predictor for preterm birth. Some studies have reported that fFN had a high negative predictive rate for the preterm birth [31-34], but a low positive predictive rate was its defect. In this study, the positive rate of fFN was increased in the preterm birth group, compared to full term. These results demonstrate that fFN has a positive predictive value of preterm labor in twin pregnancies. Pregnant women with at risk of premature labor underwent dynamic fFN monitoring and we found that the incidence rate of preterm birth was significantly higher in the positive fFN group.

Studies have found that infection is an important cause of preterm birth. When the neck of uterus becomes infected, inflammatory factors stimulate neutrophilic granulocytes to release NE [35], which results in rupture of cervical elastic fibers and enhanced decomposition of amniotic collagen, leading to preterm birth. It has been reported that the NE concentration in the amniotic fluid was significantly increased in pregnant women with premature rupture of fetal membranes or threatened premature labor, suggesting that NE is a predictor of preterm birth [36]. In this study, the NE positivity rate was 45.2% in twin pregnancies, which implied that infection was an important cause of preterm birth. Clinical dynamic monitoring found that the incidence rate of preterm birth in the continually positive NE group was significantly different compared with the negative, and positive to negative NE group. Therefore, it suggested that NE was a good predictor of preterm labor to evaluate the clinical effect of prevention.

The study aimed to assess whether the combination of three indicators could accurately predict preterm birth in twin pregnancies. Retrospective analysis was used to calculate the predictive preterm birth rate of a single indicator: fFN, CL, and NE was 65.5%, 62.1% and 75.9%, respectively. Use of a single indicator had limited predictive value for preterm birth. A combination of indicators was used to acquire more accurate results; the preterm birth rate was 78.6%, 84.4%, and 88.9% through application of CL combined with fFN, NE combined with CL, and NE combined with fFN, respectively, which significantly increased the detectable rate of preterm birth. Moreover, NE combined with fFN and CL had 100% detectable rate of preterm birth, which implied that combination of multiple indicators was far more accurate than a single indicator.

Preterm birth is caused by many factors, and combined application of multiple indicators will improve the predictive value of preterm birth [28,34]. Previous studies have confirmed

that inflammation is the main cause of preterm birth, where inflammatory factors stimulate the neutrophilic granulocytes to release NE during genital tract infection. Therefore, NE is an inflammatory marker. fFN comprises the extracellular matrix component of the uteri chorion, and is located between the uteri chorion and the deciduas. Separation of the chorion and deciduas can result in the threat of premature labor [33]. Moreover, a change in CL is an important marker of pregnancy, and ultrasound examination of the uterine neck is used to predict preterm birth [26]. Indeed, the morphology of the uterine neck and certain cytokines related to premature birth from cervical secretions were altered before preterm birth. Based on this principle, we measured the CL combined with fFN and NE, to improve the predictive rate of preterm birth through comprehensive evaluation from morphological and bio-chemical indicators.

Preterm birth can be prevented by probiotics; the amount of lactobacillus, estrogen, and progesterone, and vaginal pH value play an important role in the ecological balance of the vagina. During pregnancy, estrogen and progesterone hormone levels are sharply altered and the amount of lactobacillus is reduced, which can result in vaginal dysbacteriosis. Inhibition of immunity whilst pregnant can lead to vaginal infections, which could result in adverse pregnancy outcomes, such as premature rupture of fetal membranes and preterm birth. Previous reports [37,38] stated that exogenous probiotics could be used to treat vulvovaginal candidiasis and thus recover the vaginal micro-ecological balance. All cases from this study received this treatment, as well as symptomatic treatment. After treatment, positive fFN in nine pregnant women had turned negative, one of which occurred in the preterm birth. Positive NE in 51 cases had turned negative, and seven of which occurred in preterm birth. These results indicate that effective intervention could turn an indicator negative and reduce the rate preterm birth. It is to be noted that some women with any two positive indicators of NE, fFN, and CL still underwent preterm birth after treatment at the first visit. Meanwhile, some cases where one or multiple indictors turned negative did not result in the preterm birth. These results suggested that any two positive indicators increased the predictive value of preterm birth; however, clinical treatment may reduce the incidence. This study used vaginal micro-ecological treatment to achieve a clinical effect, which is worth studying in further detail.

In conclusion, early detection and intervention is the key to preventing preterm birth and improving the related adverse outcomes [39]. The indicators fFN, CL, and NE were valuable to predict preterm birth in twin pregnancies; however, combination of these predictors was an extremely effective predictive method of preterm birth. Furthermore, air pollution is a serious problem, especially polycyclic aromatic hydrocarbons. Studies have shown that exposure to airborne polycyclic aromatic hydrocarbons during pregnancy can lead to an increased risk of preterm birth. Thus, environmental problems need to be given more attention with respect to preterm birth research in the future.

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Conflict of Interest

All authors have no conflict of interest regarding this paper.

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