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Original Article

Prediction of spontaneous preterm delivery in asymptomatic twin pregnancies using cervical length and granulocyte elastase

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ABSTRACT

Objective: The purpose of this study was to evaluate sonographic cervical length (CL) and granulocyte elastase (GE) in cervical secretion as predictors of preterm delivery in asymptomatic twin pregnancies. **Materials and methods:** This study prospectively enrolled asymptomatic twin pregnancies with CL < 25 mm at 22–29 weeks of gestation. All women were hospitalized for preterm labor, and the cervical secretion was obtained for GE testing on admission. The results of CL measurement and GE testing were reviewed, and the relationship between each variables and preterm delivery prior to 34 weeks of gestation was assessed.

Results: Overall, we included 54 women with twin pregnancies, of which 12 (22.2%) had preterm deliveries prior to 34 weeks of gestation. A CL of <20 mm was significantly associated with preterm delivery with an odds ratio of 4.88 (95% confidence limit, 1.15–20.73). GE was not an independent predictive marker for preterm delivery. We also performed a subgroup analysis on the combination of CL and GE for predicting preterm delivery. Among the patients with GE(–), CL < 20 mm markedly increased the risk of preterm delivery with an odds ratio of 10.89 (95% confidence limit, 1.40–77.10). CL was not associated with preterm delivery among those with GE(+). Those with negative GE and shorter CL demonstrated the shortest duration of pregnancy after admission.

Conclusion: The combination of sonographic CL and GE of cervical secretion is useful to predict the risk of preterm delivery in asymptomatic twin pregnancies.

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Introduction

The number and rate of twin births has increased over the past decades in developed countries [1]. Prematurity is the major cause of neonatal morbidity and mortality in twin births. The rates of preterm birth <37 weeks and <32 weeks for twin gestations were 10.8 and 7.7 times the rates for singleton gestations, respectively [2]. Prediction of spontaneous preterm delivery in twin pregnancies is of major importance. The identification of the patients at higher risk for preterm birth in twin pregnancies would allow more effective management to prevent adverse perinatal outcomes including antenatal administration of steroid. Moreover, the ability to predict preterm births would be useful to avoid unnecessary interventions and hospitalization in patients with lower risk.

It has been proposed that a short cervical length (CL) measured by transvaginal ultrasound is useful to predict spontaneous preterm delivery in singleton pregnancies [3,4]. More recently, there have been reports suggesting a predictive role in twin pregnancies [5].

Granulocyte elastase (GE) is a serine protease released from leukocytes, which is capable of degrading extracellular matrix proteins. This enzyme has been isolated in human cervical mucus and believed to play an important role in depolymerizing collagen in ripening cervix [6]. It has been reported that polymorphonuclear leukocyte and GE are abundant in the vagina of patients with preterm labor compared with those of normal pregnant women [7]. A more recent study demonstrated that the increased level of GE in cervical secretions was an independent predictive marker for preterm delivery prior to 34 weeks of gestation in singleton pregnancies [8]. To our knowledge, there have been no studies on GE in cervical secretion as a predictive marker for preterm delivery in twin pregnancies.

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The objective of this study was to evaluate CL, GE, and the combination of both as predictors of spontaneous preterm delivery prior to 34 weeks in preterm labor of asymptomatic twin pregnancies.

Materials and methods

We conducted a prospective observational study of women with twin pregnancies who received prenatal care and delivered at the Kyorin University hospital, the tertiary perinatal center in Tokyo, Japan, between January 2009 and July 2015. We prospectively included women with asymptomatic twin pregnancies whose CL was less than 25 mm between 22 weeks and 29 weeks of gestation with intact fetal membrane as the study participants. All participants provided written informed consent for participation in the study, which was approved by the institutional review board. For risk assessment of preterm delivery, we routinely performed CL measurement by transvaginal ultrasound on all outpatients with twin pregnancies every 2 weeks from 20 weeks' gestation. We used the cutoff of 25 mm for indication of hospitalization, which is below the 10th percentile at 24 weeks of gestation in a population delivering at term [4]. Sonographic scan of the uterine cervix was performed with a 7.5-MHz transvaginal probe (SONOVISTA-C3000; Siemens Medical Systems, Tokyo, Japan) with the patient in the lithotomy position and having an empty bladder. The optimal image was defined according to the criteria reported by Iams et al [4]. CL was measured from the internal to the external cervical os. Each scan was performed by a trained obstetrician. A short CL was defined as a CL of less than 20 mm, as the cutoff of 20 mm has been validated in other studies [9,10].

Pregnancies with uterine anomalies, major fetal anomalies, intrauterine fetal death, twin–twin transfusion syndrome, cervical cerclage placement, or medically indicated delivery including preeclampsia prior to 34 weeks were excluded. We also excluded the symptomatic women with gross cervical bleeding, cervical change (effacement of at least 50% or dilatation of at least 2 cm), or painful and regular uterine contractions. The gestational age was based on the last menstrual period, a reliable menstrual history, and an ultrasound examination in the first trimester.

All women were hospitalized and underwent cervical secretion sampling for GE detection on admission. Prior to digital examinations, cervical secretion was taken with a sterile swab for GE assessment. The swab stick was rolled gently across the endocervix. The concentration of GE was measured using Inotech Elastase kit (Sanwakagaku Kenkyusho, Nagoya, Japan) based on latex immunoassay. The cutoff value was 1.6 µg/mL for GE, according to the manufacturer's instruction. According to the manufacturer's data, the minimal detectable dose was 0.1 µg/mL, and the intra- and interassay coefficients of variation were 3.4% and 6.5%, respectively.

All hospitalized women received the therapy for preterm labor including bed rest, intravenous administration of tocolytic agents (beta stimulator and/or magnesium sulfate), and vaginal administration of urinary trypsin inhibitor and metronidazole irrespective of the results of CL or GE until delivery or 34 weeks of gestation.

The relationship between each variables and preterm delivery prior to 34 weeks of gestation was assessed by Mann–Whitney *U* test or Fisher's exact test. Spontaneous labor and spontaneous preterm birth after the premature rupture of membranes were included as preterm delivery in the current study. An adjusted odds ratio was calculated by multivariate logistic regression, in which we controlled for maternal age, parity, chorionicity of the twins, and gestational age at testing.

Kaplan–Meier survival analysis was applied to evaluate the duration of pregnancy after admission according to each variables. The log rank test was used to compare differences in the duration of

pregnancy after admission between the groups. Data were examined using a statistics program (SPSS Statistics ver19; IBM, Tokyo, Japan). A *p* value < 0.05 was taken to indicate a significant test result.

Results

A total of 54 women with twin pregnancies were included during the study period. All were hospitalized at 22–29 weeks of gestation because the CL was below 25 mm. The demographic characteristics of participants are shown in Table 1. Among the 54 women, 12 (22.2%) had preterm deliveries prior to 34 weeks of gestation. There were no significant differences between those who delivered prior to and after 34 weeks with respect to maternal age, parity, chorionicity of the twins, or gestational age on admission.

The median (range) of CL on admission was 20 (6.2–24.0) mm, and 25 patients (46.3%) had a CL below 20 mm. Patients with a CL < 20 mm were significantly more likely to deliver at <34 weeks of gestation (*p* = 0.04), with an adjusted odds ratio of 4.88 [95% confidence interval (CI), 1.15–20.73] (Table 2).

Twenty-three patients (42.6%) showed a positive GE result on admission. As shown in Table 2, GE alone was not a significant predictive marker of spontaneous preterm delivery prior to 34 weeks (*p* = 0.53).

We then examined the combination of GE and CL testing as a risk factor for preterm delivery. Among the patients with positive GE, there was no significant difference in the rate of preterm delivery with respect to CL (*p* = 0.62, Table 3). In contrast, the patients with negative GE and CL < 20 mm had significantly higher rate of preterm delivery prior to 34 weeks, compared with those who had negative GE and CL ≥ 20 mm (*p* = 0.04), with an adjusted odds ratio of 10.89 (95% CI, 1.40–77.10) (Table 3).

The duration of pregnancy after admission according to each variables is shown in terms of survival curves in Figure 1. The

Table 1
Demographic data of twin pregnancies.

	Twin pregnancies included in the study
<i>N</i>	54
Maternal age (y)	35 (21–43)
Nulliparous women	39 (72.2)
Monochorionic twin	28 (51.8)
Gestational weeks on admission (wk)	27 (22–29)
Gestational weeks at delivery (wk)	35 (28–36)

Data are presented as median (range) or number (%).

Table 2
Risk of spontaneous preterm delivery in twin pregnancies based on cervical length and granulocyte elastase alone.

	<i>N</i>	Rate of preterm birth <34 wk (%)
Cervical length (CL)		
<20 mm	25	36.0
≥20 mm	29	10.3
<i>p</i> value		0.04
OR (95% CI)		4.88 (1.15–20.73)
PPV (%)		17.3
NPV (%)		74.2
Granulocyte elastase (GE)		
GE(+)	23	17.3
GE(–)	31	25.8
<i>p</i> value		0.53
OR (95% CI)		0.61 (0.17–2.22)
PPV (%)		36.0
NPV (%)		89.7

CI = confidence interval; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value.

Table 3

Risk of spontaneous preterm delivery based on the combination of cervical length and granulocyte elastase.

	Rate of preterm birth <34 wk (%)	
	GE(+) (N = 23)	GE(−) (N = 31)
Cervical length (CL)		
<20 mm	22.2	43.8
≥20 mm	14.3	6.7
p value	0.62	0.03
OR (95% CI)	1.71 (0.24–12.27)	10.89 (1.40–77.10)
PPV (%)	22.2	43.8
NPV (%)	85.7	93.3

CI = confidence interval; GE = granulocyte elastase; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value.

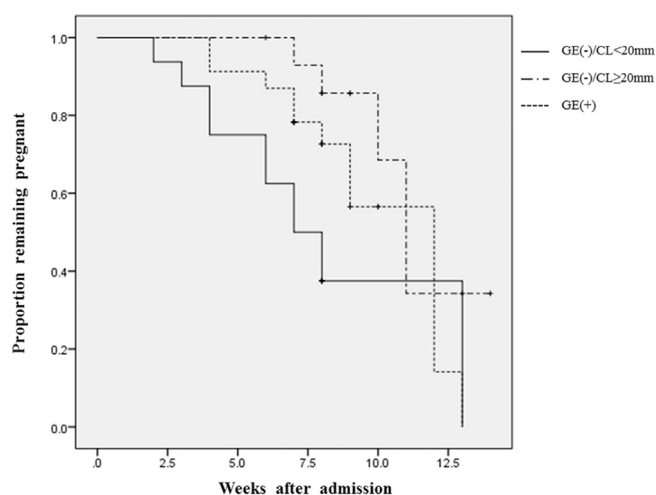


Figure 1. Fraction of twin pregnancies undelivered at each week after admission grouped by the results of cervical length (CL) and granulocyte elastase (GE) testing.

patients with negative GE and CL < 20 mm had the shortest duration of pregnancy with a mean value of 8.3 (95% CI, 6.3–10.3) weeks, whereas those with negative GE and CL ≥ 20 mm had a mean value of 11.4 (9.9–12.9) weeks. The mean duration of patients with positive GE was 10.0 (8.7–11.4) weeks, and the difference in pregnancy duration among the three groups was statistically significant ($p = 0.03$).

Discussion

In this study, GE was not an independent predictor of spontaneous preterm delivery prior to 34 weeks in preterm labor of twin pregnancies. Compared with a previous study [8], which predominantly included women with singleton pregnancies as participants, the result of our study showed a comparable positive predictive value (17.3% vs. 22.2%). However, the negative predictive value of GE was found to be considerably lower in our study (74.2% vs. 92.2%), which we believe was one of the reasons why GE was not a significant predictor.

A short CL was significantly associated with spontaneous preterm delivery prior to 34 weeks, which was consistent with the results of a systematic review [5]. The positive predictive value of 36.0% with a cutoff value of 20 mm was as good as that in other reported studies [9,10]. Our study proved that transvaginal ultrasound measurement of CL prior to 30 weeks was a good test to predict preterm delivery prior to 34 weeks in twin pregnancies.

We did not find any significant difference in the rate of preterm delivery with respect to CL among the patients with positive GE. In

contrast, the patients with the combination of negative GE and shorter CL showed the highest risk of spontaneous preterm delivery (Table 3). The same group of patients also showed a significantly shorter duration of pregnancy after admission as described in Figure 1.

We assumed that these characteristic results of our study may reflect the pathophysiological variety of preterm birth in twin pregnancies. The intrauterine chorioamnionitis and the increased uterine pressure, such as multiple pregnancies and polyhydramnios, are two major causes of spontaneous preterm births [11,12]. GE in vaginal secretion or amniotic fluid has been reported to be associated with intrauterine infection in many studies [13–15], which implies that the GE positive group in our study could represent the population with active cervicitis, possibly at risk of chorioamnionitis and preterm delivery. By contrast, the group with negative GE, especially patients with shortened cervix, could be representative of those with increased uterine pressure, not complicated with active cervicitis. In twin pregnancies, which are subjected to relatively larger uterine content, the pathophysiology of cervical shortening due to uterine pressure might account for a larger portion of preterm deliveries, compared with singleton pregnancies at the same gestations. Recent studies and experiments showed that increased uterine wall stress induces inflammatory mediators in the amniotic fluid and myometrium that could lead to cervix shortening and preterm labor [16–18]. The highest risk of preterm delivery in patients with negative GE and shorter CL in our study supports this idea. In addition, we believe this idea could also explain the lower negative predictive value of GE in the current study than the previous study of singletons. Our study showed that the combination of GE testing and CL measurement is useful to understand the pathophysiology of preterm labor of twin pregnancies and also to predict the risk of spontaneous preterm delivery. The population with positive GE represents the cervicitis–chorioamnionitis type, whereas the patients with shortened cervix with negative GE represent the increased uterine pressure type. To our knowledge, this is the first report to reveal the usefulness of sonographic CL and GE of cervical secretions as predictive markers for spontaneous preterm delivery in twin pregnancies.

The positive predictive value of negative GE and shorter CL was 43.8%. The value of the current study was comparable with fetal fibronectin testing, which has been reported as one of the useful predictive biochemical markers for preterm delivery in twin pregnancies [10,19].

The lower rate of preterm delivery in the cervicitis–chorioamnionitis type compared with the increased uterine pressure type in our study might be attributed to therapeutic interventions such as vaginal administration of urinary trypsin inhibitor. Kanayama et al [20] have reported that intravaginal treatment with urinary trypsin inhibitor is effective for lengthening pregnancy in preterm labor.

The lowest preterm delivery rate (6.7%) in patients with negative GE and longer CL suggests that the combination of GE and CL also provides high negative predictive power, and it could enable us to avoid unnecessary interventions and hospitalization.

The limitations of this study include the small sample size and the fact that the study was conducted at a single institution. Although we acknowledge that the ultimate goal of prenatal surveillance is to improve the neonatal outcomes, the sample size of the study was not large enough to evaluate them. Future studies should be conducted using a larger number of cases to validate the utility of GE as a predictive marker for spontaneous preterm birth and neonatal prognosis of twin pregnancy. Another limitation is that therapeutic intervention is relatively unique in Japan, including the long-term use of tocolytic agents. Although some of the therapies described in the Materials and methods section have

not been proven to be beneficial in larger trials, they have been traditionally and widely accepted in Japan.

Conversely, the strength of our study is a uniform therapeutic intervention for preterm labor of twin pregnancy. All study participants received the therapy based on the same therapeutic protocol as described in Materials and methods, which could be an advantage when appropriately evaluating the predictive performance of CL and GE.

The therapeutic interventions to prevent preterm birth in twin pregnancies, such as vaginal progesterone and prophylactic cervical cerclage placement, have not been supported by prospective randomized trials [21,22]. However, as we discussed, the preterm deliveries in twin pregnancies may have a wider variety of pathogenesis than singleton, and the heterogeneity of the pathophysiological backgrounds of the patients might have made it difficult to prove the effectiveness of the interventions in previous trials. Some of the interventions that have been previously shown as ineffective might be actually effective with selected participants based on the pathogenesis. Our study showed that the potential abilities of CL measurement and GE testing, which reflect the pathogenesis of preterm delivery in asymptomatic twin pregnancies, cervicitis, chorioamnionitis, or increased uterine pressure, may help future studies to evaluate preterm birth prevention strategies appropriately.

In conclusion, the combination of sonographic CL measurement and GE testing of cervical secretions is useful to predict the risk of spontaneous preterm delivery prior to 34 weeks of gestation in asymptomatic twin pregnancies.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.tjog.2016.07.014>.

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