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<tbody>
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<td>Author(s)</td>
<td>Morikawa, Mamoru; Yamada, Takashi; Turuga, Noriko; Cho, Kazutoshi; Yamada, Hideto; Sakuragi, Noriaki; Minakami, Hisanori</td>
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Coagulation-fibrinolysis in twin pregnancies

[ORIGINAL ARTICLE]

Coagulation-fibrinolysis is more enhanced in twin than in singleton pregnancies

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Coagulation-fibrinolysis in twin pregnancies

ABSTRACT

Aims: To evaluate that the coagulation-fibrinolysis in late pregnancy in women with twin pregnancies is more pronounced than in women with singleton pregnancies.

Patients and Methods: The plasma levels of D-dimer, fibrinogen/fibrin degradation products (FDP), and fibrinogen, the platelet count, and the antithrombin activity were assessed from 3 weeks before delivery until postpartum day 7 in 48 women (24 singleton and 24 twin pregnancies) without preeclampsia who underwent cesarean sections. The women with singleton or twin pregnancies gave birth at 37.3 ± 1.2 weeks or 35.2 ± 1.4 weeks, respectively.

Results: Compared with the women who had singleton pregnancies, the prenatal D-dimer and FDP levels were consistently and significantly higher and a significantly larger number of women exhibited prenatal levels of D-dimer > 5.0 μg/mL and of FDP > 10.0 μg/mL among the women with twin pregnancies. A significantly larger number of women with twin pregnancies exhibited a prenatal level of fibrinogen < 420 mg/mL. The prenatal antithrombin activity in the plasma was significantly lower and a significantly larger number of women exhibited a prenatal level of antithrombin activity < 70% among the women with twin pregnancies.

Conclusions: Coagulation-fibrinolysis is more enhanced in women with twin gestation than in women with singleton gestation.

Key Words: antithrombin activity, D-dimer, coagulation, fibrinolysis, twin
Coagulation-fibrinolysis in twin pregnancies

Over the past two decades, the frequency of multiple births, especially twins, has increased dramatically in many industrialized countries. For instance, the rate of twin pregnancies in the United States increased by 42% between 1980 and 1997 [1]. Twin pregnancy differs from singleton pregnancy in many aspects. The length of gestation is much shorter, and the perinatal mortality rate is much higher for twin pregnancies than for singleton pregnancies [2]. In general, the degree of physiological change in the mother is also greater in women with twin pregnancies than in women with singleton pregnancies. For example, the normal expansion in maternal blood volume is greater in women with twin pregnancies [3]; the average increase in late pregnancy is about 40% to 50% with a single fetus, whereas it is about 50% to 60% with twins [3]. Better understanding of the physiology associated with twin pregnancy may be important for the management of women with twin pregnancies.

The hemostatic balance is physiologically displaced toward hypercoagulability in normal singleton pregnancies [4]. This hypercoagulability is more exaggerated in women with preeclampsia [5,6], in whom the risk of pulmonary thromboembolism is increased [7]. Because women with twin pregnancy are more likely to develop both preeclampsia [8] and pulmonary thromboembolism [7], compared with women with singleton pregnancies, hypercoagulability may further exaggerated in women with twin pregnancies. However, this issue has not been extensively studied.

We compared perinatal changes in coagulation-fibrinolysis parameters, like the D-dimer, FDP, and fibrinogen levels, the platelet counts, and the antithrombin activity levels, between women with singleton and those with twin pregnancies.
Coagulation-fibrinolysis in twin pregnancies

Patients and Methods

A total of 48 women (24 with singleton pregnancies and 24 with twin pregnancies) who gave birth by cesarean section at our hospital between 2001 and 2004 were studied. All 48 women gave their informed consent to participate in this study, and their D-dimer, FDP, and fibrinogen levels, platelet counts, antithrombin activity, and Ht were determined at 3 weeks, 2 weeks, one week, and one day before delivery, the day after the cesarean section, and on postpartum days 3 and 7. Twenty-four patients with singleton pregnancies underwent cesarean section for various reasons: previous cesarean in 10 patients, breech presentation in 3 patients, dystocia in 3 patients, uterine fibromata in 3 patients, fetal malformations in 3 patients, impaired lung function in one patient, and spinal cord injury in one patient. Thus, none of the patients in the singleton group had preeclampsia. Cesarean section is liberally used for women with twin pregnancies in our hospital. None of the women with twin pregnancies had preeclampsia, but two of them had gestational proteinuria.

The patient characteristics are shown in Table 1. The mean age and number of nulliparous women did not differ between the two groups. Three of the 24 women (12.5%) with singleton pregnancies gave birth before 37 weeks of gestation: one at 35 weeks because of breech presentation and premature membrane rupture, one at 36 weeks because of a huge uterine fibromata, and one at 36 weeks because of impaired lung function. Seventeen women (70.8%) with twin pregnancies gave birth before 37 weeks because of increased uterine activity.

The estimated blood loss, including amniotic fluid, during the cesarean section was significantly larger among the women with twin pregnancies than among the women with singleton pregnancies. Neither the body mass index [body weight /
Coagulation-fibrinolysis in twin pregnancies

(height)²] at the time of cesarean section nor the birth weight differed between the two groups. None of the infants in either group had an Apgar score of less than 8 at 5 minutes.

Plasma levels of D-dimer and fibrinogen/fibrin degradation products (FDP) were measured using the latex agglutination assay (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). Plasma fibrinogen level was measured using a thrombin clotting time method (Sysmex Co., Kobe, Japan). Antithrombin activity was measured using the chromogenic substrate assay (Daiichi Pure Chemicals Co., Tokyo, Japan). Platelet counts and Ht were determined using an electronic particle counting system (Beckman Coulter Int., Fullerton, CA, USA).

The data are presented as the means or medians. Paired Wilcoxon and Mann-Whitney U tests were used to analyze the results. A \( \chi^2 \) test was used to compare frequencies. \( P < 0.05 \) was considered statistically significant.

Results

Changes in the mean and median values are shown in Figure. 1 and Table 2, respectively. The plasma D-dimer levels gradually increased during the period before delivery and peaked on postpartum day 1 in both groups. The prenatal D-dimer level was consistently and significantly higher in women with twin pregnancies than in women with singleton pregnancies. Similar patterns in perinatal plasma FDP levels were also observed. The prenatal FDP level was also consistently and significantly higher in women with twin pregnancies than in women with singleton pregnancies. In contrast, the plasma fibrinogen level appeared to be lower in women twin than pregnancies than in women with singleton pregnancies, and a nadir value was observed on postpartum day 1 in both groups (Figure. 1). The median value of plasma fibrinogen
Coagulation-fibrinolysis in twin pregnancies

was significantly lower at one week before delivery and on postpartum day 3 in the twin group than in the singleton group (Table 2). The prenatal Ht value (two and one weeks prior to delivery) was significantly lower in women with twin pregnancies than in women with singleton pregnancies. The platelet count gradually decreased during the period before delivery and increased promptly after delivery in both groups. No significant difference in platelet count was observed between the two groups. Antithrombin activity gradually decreased during the period before delivery, and a nadir value was observed on postpartum day 1; the antithrombin activity had normalized by one week after delivery in both groups. The prenatal antithrombin activity level was significantly lower, except at 2 weeks before delivery, in women with twin pregnancies than in those with singleton pregnancies. Mean prenatal values of these parameters according to gestational age was shown in Figure. 2. Difference between the two groups was more pronounced.

Compared with women with singleton pregnancies, a significantly larger number of women with twin pregnancies exhibited high prenatal levels of D-dimer > 5.0 μ g/mL and FDP >10.0 μ g/mL (Table 3). Furthermore, a significantly larger number of women with twin pregnancies exhibited prenatal fibrinogen levels of < 420 mg/dL and antithrombin activity levels of < 70%.

**Discussion**

The progressive increase in prenatal D-dimer levels during late gestation and the marked elevation soon after delivery seen in women with singleton pregnancies in this study were consistent with the results of earlier studies in singleton pregnancies
Coagulation-fibrinolysis in twin pregnancies

[9-14]. Our longitudinal study demonstrated that the prenatal plasma levels of D-dimer and FDP were consistently higher in women with twin pregnancies than in women with singleton pregnancies, although the deliveries took place approximately 2 weeks earlier in the twin group than in the singleton group. Because the plasma D-dimer level increases as gestation advances [9-13], as confirmed in this study, the difference in the number of gestational weeks at the time of delivery between the two groups further supports our conclusion which was also demonstrated in the comparison of variables plotted against gestational weeks, although there were several missing data in this analysis (Figure 2). To our best knowledge, only one other report has dealt with this issue: the authors compared the plasma D-dimer levels in women with singleton and twin pregnancies at 30 weeks of and found a significantly higher plasma D-dimer level among the women with twin pregnancies [15].

Because D-dimer results from the digestion of cross-linked fibrin by plasmin, its plasma level provides information on the degree of fibrin formation and secondary fibrinolysis activated by the enhanced fibrin formation, thereby partially reflecting the coagulation activity. In contrast, because FDP includes the split products of both fibrinogen and fibrin, an enhanced level of FDP does not necessarily reflect the degree of fibrin formation [16]. However, the elevations in both the D-dimer and the FDP levels indicate both enhanced coagulation activity and enhanced fibrinolytic activity. Thus, our results suggest that the enhanced coagulation-fibrinolysis activity that normally occurs during late gestation was more exaggerated in women with twin pregnancies than in women with singleton pregnancies.

The mean plasma fibrinogen level appeared to be lower in women with twin pregnancies than in women with singleton pregnancies in this study, although the
difference did not reach a significant level possibly because of the relatively small number of study subjects. The lower level of fibrinogen in the twin group may be partly explained by the larger degree of hemodilution in the twin group than in the singleton group [3], as suggested by the changes in the Ht value. However, based on the changes in the D-dimer and FDP levels, the lower plasma fibrinogen level may partially reflect hyperconsumption of fibrinogen in women with twin pregnancies, compared with women with singleton pregnancies. This postulation is also supported by the fact that the nadir value of fibrinogen was seen on postpartum day 1, the day on which the D-dimer and FDP levels peaked in both the singleton and twin groups.

Thrombin generation, as monitored by the level of thrombin-antithrombin complex, is enhanced during the late stage of normal pregnancies [17, 18]. Antithrombin is the most important inhibitor of coagulation and is used to inactivate activated coagulation factors like Xa and thrombin. Therefore, antithrombin activity is reduced in the presence of enhanced thrombin generation in patients with disseminated intravascular coagulation [19]. An enhancement in thrombin generation and a reduction in antithrombin activity has also been reported in women with preeclampsia [18,20]. Prenatal antithrombin activity gradually decreased and was significantly lower in the twin group than in the singleton group in this study, consistent with an earlier report by Tsunoda et al. [21] in which they demonstrated that an antithrombin activity of 111% at 28.6 weeks of gestation decreased to 91% at 35.2 weeks of gestation in women with twin pregnancies, while the decrease was much smaller (from 111% to 106%) in women with singleton pregnancies. These results may also strengthen the concept that coagulation is exaggerated in women with twin pregnancies, compared with women with singleton pregnancies. The larger degree of decrease in antithrombin activity may
have reflected more pronounced thrombin generation in the twin group. Because thrombin converts fibrinogen into fibrin, plasma level of fibrinogen may be decreased in such a condition, as suggested in this study.

In conclusion, during the advanced stage of gestation, the plasma levels of D-dimer and FDP were higher and the plasma level of antithrombin activity was lower in women with twin pregnancies than in women with singleton pregnancies. All our results indicated that the enhanced coagulation-fibrinolysis activity that normally occurs during late gestation was more exaggerated in women twin pregnancies than in women with singleton pregnancies. This may partially explain why women with multifetal pregnancies are at a 3- to 4-fold increased risk of pulmonary embolism during the third trimester and peripartum compared with women with singleton pregnancies [7].


Coagulation-fibrinolysis in twin pregnancies

References


Coagulation-fibrinolysis in twin pregnancies


Coagulation-fibrinolysis in twin pregnancies

Figure Legends

Figure 1. Perinatal changes in the plasma levels of D-dimer, FDP (fibrinogen/fibrin degradation products), and fibrinogen, and in the hematocrit value, platelet count, and antithrombin activity of peripheral blood from 24 women with singleton (○) and 24 women with twin (●) pregnancies.

Data are presented as the mean.

*, p<0.05 and **, p<0.01 for singleton pregnancies vs. twin pregnancies.

Figure 2. Prenatal values of the plasma levels of D-dimer, FDP (fibrinogen/fibrin degradation products), and fibrinogen, and in the hematocrit value, platelet count, and antithrombin activity of peripheral blood.

Number of missing data was 2, 2, 2, and 2 for women with singleton pregnancies (○) at 33 weeks, 34 weeks, 35 weeks, and 36 weeks, respectively. Corresponding number of missing data for women with twin (●) pregnancies was 1, 4, 7, and 12, respectively.

Data are presented as the mean.

*, p<0.05 and **, p<0.01 for singleton pregnancies vs. twin pregnancies.
Coagulation-fibrinolysis in twin pregnancies

Table Legends

Table 1. Patient characteristics of 24 women with singleton pregnancies and 24 women with twin pregnancies

Data are presented as the mean ± SD and the range.

BMI, body mass index [(body weight, kg / (height, m)²)]

Table 2. Median and range of hemostasis parameters in 24 women with singleton pregnancies and 24 women with twin pregnancies

FDP, fibrinogen/fibrin degradation products

Data are presented as the median and range.

Table 3. Positive rate (%) of hemostasis parameters in 24 women with singleton pregnancies and 24 women with twin pregnancies

FDP, fibrinogen/fibrin degradation products

*, p<0.05 and **, p<0.01 for singleton pregnancies vs. twin pregnancies.
### Table 1. Patient characteristics of 24 women with singleton pregnancies and 24 women with twin pregnancies

<table>
<thead>
<tr>
<th></th>
<th>Twin pregnancy (n=24)</th>
<th>Singleton pregnancy (n=24)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.1±5.2 [18-41]</td>
<td>31.1±4.6 [24-41]</td>
<td>NS</td>
</tr>
<tr>
<td>Nullipara (%)</td>
<td>70.8</td>
<td>54.2</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational week at delivery (weeks)</td>
<td>35.2±1.4 [32-37]</td>
<td>37.3±1.2 [35-41]</td>
<td>NS</td>
</tr>
<tr>
<td>Preterm delivery (%)</td>
<td>70.8</td>
<td>12.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood loss at delivery (g)</td>
<td>1354±554 [280-2400]</td>
<td>869±510 [155-1790]</td>
<td>0.006</td>
</tr>
<tr>
<td>BMI at delivery</td>
<td>24.6±4.0 [19.9-37.5]</td>
<td>26.2±4.7 [19.2-39.2]</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2144±392 [1325-2930]</td>
<td>2771±499 [1925-4125]</td>
<td>NS</td>
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<tr>
<td>Apgar score &lt; 8 (5 min) (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>NS</td>
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Data are presented as the mean ± SD and the ranges, BMI: body mass index [body weight, kg / (height, m)^2]
Table 2. Median and range of hemostasis parameters in 24 women with singleton pregnancies and 24 women with twin pregnancies

<table>
<thead>
<tr>
<th>Days from delivery</th>
<th>-3weeks</th>
<th>-2weeks</th>
<th>-1week</th>
<th>-1day</th>
<th>+1day</th>
<th>+3days</th>
<th>+7days</th>
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<td><strong>2.5</strong></td>
<td><strong>3.2</strong></td>
<td><strong>3.1</strong></td>
<td><strong>4.5</strong></td>
<td>3.2</td>
<td>4.8</td>
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<td>Singleton</td>
<td>0.7-4.9</td>
<td>0.9-6.3</td>
<td>0.8-6.4</td>
<td>1.2-7.4</td>
<td>1.8-36.3</td>
<td>1.4-9.7</td>
<td>1.8-27.4</td>
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<tr>
<td>Twin</td>
<td>3.8</td>
<td>3.7</td>
<td>4.5</td>
<td>5.1</td>
<td>8.4</td>
<td>3.5</td>
<td>5.7</td>
</tr>
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<td>FDP (μg/mL)</td>
<td><strong>4.4</strong></td>
<td><strong>5.0</strong></td>
<td><strong>5.0</strong></td>
<td><strong>5.6</strong></td>
<td><strong>8.2</strong></td>
<td>5.9</td>
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<td>Fibrinogen (mg/dL)</td>
<td>449</td>
<td>437</td>
<td>445 **</td>
<td>440</td>
<td>422</td>
<td>485 *</td>
<td>403</td>
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<td>436</td>
<td>413</td>
<td>409</td>
<td>364</td>
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<td>373</td>
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<td>234</td>
<td>236</td>
<td>213</td>
<td>216</td>
<td>231</td>
<td>325</td>
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<td>132-463</td>
<td>125-473</td>
<td>120-466</td>
<td>108-408</td>
<td>161-383</td>
<td>200-491</td>
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<td>Twin</td>
<td>219</td>
<td>202</td>
<td>201</td>
<td>186</td>
<td>190</td>
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<td>351</td>
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<td>90.0</td>
<td><strong>87.0</strong></td>
<td><strong>85.0</strong></td>
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<td>99.0</td>
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<td>54-110</td>
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<tr>
<td>Twin</td>
<td>89.0</td>
<td>84.0</td>
<td>83.0</td>
<td>80.0</td>
<td>70.0</td>
<td>86.0</td>
<td>102.0</td>
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| Data are presented as the median and range, FDP:fibrinogen/fibrin degradation products
* p<0.05 and ** p<0.01 for singleton pregnancies vs. twin pregnancies.
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<thead>
<tr>
<th>Days from delivery</th>
<th>-3weeks</th>
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<th>-1week</th>
<th>-1day</th>
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<th>+3days</th>
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<td>&gt; 5.0 μg/mL</td>
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<td>0.0**</td>
<td>4.2*</td>
<td>16.7*</td>
<td>20.8*</td>
<td>45.8*</td>
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<td>29.2</td>
<td>45.8</td>
<td>50.0</td>
<td>83.3</td>
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<td>&gt; 10.0 μg/mL</td>
<td>Singleton</td>
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<td>4.2</td>
<td>4.2*</td>
<td>4.2*</td>
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<td>58.3</td>
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<td>&lt; 150x10⁹/L</td>
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<td>&lt; 70%</td>
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FDP: fibrinogen/fibrin degradation products, *, p<0.05 and **, p<0.01 for singleton pregnancies vs. twin pregnancies.
Figure 1 Morikawa et al.

D-dimer (µg/mL)

Hematocrit (%)

FDP (µg/mL)

Platelet count (x10⁹/L)

Fibrinogen (mg/dL)

Antithrombin activity (%)

Weeks from delivery
Figure 2 Morikawa et al.

- **D-dimer (µg/mL)**
  - Values: 10, 5, 0, 33, 34, 35, 36
  - Gestational weeks: 33, 34, 35, 36

- **FDP (µg/mL)**
  - Values: 10, 5, 0, 33, 34, 35, 36
  - Gestational weeks: 33, 34, 35, 36

- **Fibrinogen (mg/dL)**
  - Values: 500, 400, 300, 100
  - Values: 450, 350
  - Gestational weeks: 33, 34, 35, 36

- **Hematocrit (%)**
  - Values: 35, 30, 25, 20
  - Gestational weeks: 33, 34, 35, 36

- **Platelet count (x10⁹/L)**
  - Values: 250, 200, 150, 100
  - Values: 90, 70
  - Gestational weeks: 33, 34, 35, 36

- **Antithrombin activity (%)**
  - Values: 33, 34, 35, 36
  - Values: 80, 70
  - Gestational weeks: 33, 34, 35, 36