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Difference in the D-dimer rise between women with singleton and multifetal pregnancies

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ABSTRACT

Introduction: The differences in the D-dimer rise between women with singleton and multifetal pregnancies have not been studied extensively.

Materials and Methods: D-Dimer levels were determined in 1089 blood specimens from 1089 women in various stages of pregnancy, including 977 and 112 women with singleton and multifetal pregnancies, respectively. None of the 1089 women developed hypertension or clinical venous thromboembolism during pregnancy or in the postpartum period.

Results: The D-dimer levels were significantly and positively correlated with gestational week at examination in women with singleton or multifetal pregnancies. The D-dimer levels (μg/ml, mean ± SD [number of specimens]) determined at the 1st trimester did not differ significantly (0.81 ± 0.82 [102] for singleton vs. 1.20 ± 0.77 [7] for multifetal), but those at the 2nd (1.61 ± 1.45 [216] vs. 2.62 ± 2.26 [59]) and 3rd (2.37 ± 2.22 [659] vs. 4.02 ± 2.14 [46]) trimesters were significantly higher in women with multifetal than singleton pregnancies. The 90th percentile value was 4.31 μg/ml for 1089 specimens. A significantly greater number of women exceeded 4.31 μg/ml during the 2nd (16.9% vs. 5.6%, $P = 0.0043$) and 3rd (34.8% vs. 10.6%, $P < 0.0001$) trimesters among those with multifetal than with singleton pregnancies.

Conclusions: The degree of D-dimer rise in pregnancy was greater in women with multifetal than with singleton pregnancies.

Keywords: D-dimer, twin, pregnancy, venous thromboembolism

Abbreviations used:

VTE: venous thromboembolism

GW: gestational week

INTRODUCTION

Pregnancy is a state characterized by hypercoagulability [1] and is associated with an increased risk of venous thromboembolism (VTE) [2]. Although D-dimer level is elevated physiologically during pregnancy [3 – 9] and the cut-off value for non-pregnant individuals is not useful for excluding pregnant women with VTE, D-dimer level is often determined in pregnancy.

Multifetal pregnancy is a risk factor for VTE in pregnancy [10 – 12]. As D-dimer levels are suggested to be elevated in patients who develop VTE later [13 – 15], a high baseline D-dimer level is a risk factor for subsequent VTE [13 – 15]. Although a limited number of studies [16 – 18] have suggested that D-dimer level is higher in multifetal than singleton pregnancies, it has not been determined how often women with multifetal pregnancies exhibit a high D-dimer level according to gestational stage. We conducted this retrospective study to address this issue.

MATERIALS AND METHODS

This study was conducted with the approval of the Ethics Committee of Hokkaido University Hospital, a tertiary teaching hospital managing mainly high-risk pregnant women. Laboratory tests, including a complete blood count, biochemistry, and parameters of coagulation-fibrinolysis such as the D-dimer level, are performed routinely in pregnant women visiting our clinic even for minor symptoms as well as women admitted to the hospital for management of various obstetric and incidental complications.

After exclusion of 93 and 3 women who developed pregnancy hypertension and venous thromboembolism, respectively, this study included 1089 of such women who underwent blood tests including D-dimer level, but did not develop VTE or hypertension during pregnancy or the postpartum period, and gave birth at Hokkaido University Hospital between April 2007 and May 2012. **Of the 96 women who were excluded from the present analysis, 7 of the 93 with hypertension and one of the three with venous thromboembolism were twin gestations.** These 1089 women accounted for 71.5% of 1523 women who gave birth at our hospital during the study period. Data regarding patient age, parity, and clinical outcomes were collected from the medial

records (Table 1). As our hospital is a tertiary perinatal center, the numbers of women with multifetal pregnancies and/or preterm births were greater than those of the general population. The 1089 women were divided into two groups according to number of fetuses: 977 women with singleton pregnancies and 112 women with multifetal pregnancies consisting of 106 with twins, 5 with triplets, and 1 with quadruplets. Changes in D-dimer levels during pregnancy were analyzed in the two groups (singleton pregnancy vs. multifetal pregnancy). Pregnancy stage was grouped into three categories: first trimester (until the end of gestational week [GW] 13), second trimester (GW 14−27), and third trimester (GW 28−42). Each subject usually underwent blood tests including determination of D-dimer level several times during pregnancy. However, only the D-dimer value determined in the earliest trimester of pregnancy was used as the datum for each study subject. When D-dimer value was determined several times within a trimester, the highest value was used as the datum for the study subject. Thus, 1089 blood specimens obtained from 1089 women in various stages of pregnancy were analyzed in this study. Actual gestational week at determination of D-dimer levels for each trimester except for the third trimester did not differ between women with singleton and multifetal pregnancies (Table 1). The D-dimer level was determined significantly earlier in third trimester in women with multifetal than singleton pregnancies.

The D-dimer levels were measured using the latex agglutination assay (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan) in citrated blood samples after centrifugation. Blood levels of D-dimer ranging from 0.0 μg/mL to 0.99 μg/mL are considered normal at our institution. Intra- and interassay coefficients of variation were 4.4% and 3.3%, respectively, at our institution. Correlation of D-dimer levels determined by our assay system (y) with those by the Vidas DD new assay© (bioMérieux, Marcy l'Etoile, France) (x_1) [19−21] or Tinaquant assay© (Roche Diagnostics, Mannheim, Germany) (x_2) [21−23] were as follows: $y = 3.577\ x_1 - 1.71$, $r = 0.943$ or $y = 2.471\ x_2 - 0.59$, $r = 0.951$ for 302 blood samples with D-dimer levels of 0.0−40.0 μg/mL determined by our method; and $y = 0.999\ x_1 + 0.31$, $r = 0.819$ or $y = 1.446\ x_2 + 0.32$, $r = 0.882$ for 90 blood samples with D-dimer levels < 2.0 μg/mL determined by our method.

Data are presented as means ± standard deviation. Statistical analyses were performed

using the JMP10© statistical software package (SAS, Cary, NC). Differences between the means were tested by ANOVA and Tukey–Kramer HSD (honestly significant difference) test between each group and categorical variables were compared using the χ^2 test or Fisher's exact test. In all analyses, $P<0.05$ was taken to indicate statistical significance.

RESULTS

Changes in D-dimer levels during pregnancy

D-Dimer levels were significantly and positively correlated with GW at determination in either women with singleton or multifetal pregnancies, suggesting that D-dimer level increases with advancing gestation and its increase appeared to be marked in women with multifetal pregnancies compared to those with singleton pregnancies (Fig. 1). The 1st trimester D-dimer level in multifetal pregnancy (mean ± SD, μg/ml: 1.20 ± 0.77) was not significantly different from that in singleton pregnancy (0.81 ± 0.82), but increased to 2.62 ± 2.26 and 4.02 ± 2.14 during 2nd and 3rd trimesters, respectively (Fig. 2). Although D-dimer level in singleton pregnancy also increased with advancing gestation, the levels during 2nd and 3rd trimesters (1.61 ± 1.45 and 2.37 ± 2.22, respectively) were significantly lower than those in multifetal pregnancy.

Neither mean maternal age nor mean pre-pregnancy BMI differed significantly between women with singleton and multifetal pregnancies (Table 1). Neither maternal age (32.7 ± 5.6, 32.2 ± 5.2, 31.8 ± 5.3) nor pre-pregnancy BMI (21.8 ± 4.3, 22.0 ± 4.5, 21.5 ± 4.0) differed between women with singleton pregnancies whose D-dimer levels were examined during the first, second, and third trimesters, respectively. In women with multifetal pregnancies, maternal age (33.4 ± 3.6, 30.7 ± 5.1, 30.0 ± 4.3) did not differ significantly between women whose D-dimer levels were examined during the first, second, and third trimesters, respectively, while pre-pregnancy BMI was significantly greater in women whose D-dimer levels were examined during the first trimester than during the third trimester (*P*=0.0081) (24.2 ± 4.6, 21.6 ± 3.4, and 20.5 ± 2.2 for women with first, second, and third trimesters, respectively). Thus, D-dimer level appeared to be examined in earlier stage of pregnancy in women with a larger BMI and multifetal pregnancies.

Prevalence of women with a high D-dimer level (> 4.31 μg/ml)

The 90th percentile value was 4.31 μg/ml for all 1089 cases. In women with either singleton or multifetal pregnancies, the frequency of women above the 90th percentile D-dimer value increased significantly with advancing gestation (Table 2). The number of women above the 90th percentile D-dimer level during 2nd and 3rd trimesters was significantly larger in multifetal pregnancies than in singleton pregnancies.

DISCUSSION

This study confirmed that D-dimer level increases with advancing gestation, and the D-dimer rise in pregnancy is greater in women with multifetal than singleton pregnancies. Although these findings were not new, our study included the largest number of women with multifetal pregnancies, and therefore confirmed the latter suggestion.

To our knowledge, there have been three reports indicating that D-dimer rise in pregnancy is greater in women with multifetal than singleton pregnancies [16 – 18]. The first [16] included 22 non-pregnant women and 24 and 25 women with singleton and twin pregnancies, respectively; the results indicated that D-dimer levels determined around GW 30 were significantly higher in twin than singleton pregnancies and those of singleton pregnancies were significantly higher than those of non-pregnant women. The second [17] included 1106 and 25 women with singleton and twin pregnancies, respectively; the results indicated that D-dimer levels of twin pregnancies were not different from those of singleton pregnancies during the 1st trimester but were significantly higher during 3rd trimester compared with singleton pregnancies. The third [18] included 626 and 6 women with singleton and twin pregnancies, respectively, and indicated that women with twin pregnancies were likely to show a high (above 80th percentile value) D-dimer level compared to those with singleton pregnancies. Thus, the results of the present study were consistent with previous findings [16 – 18]. Our study added convincing data in a large cohort regarding the percentage of high D-dimer level (above 90th percentile value) among women with multifetal pregnancies; approximately one third of women with multifetal pregnancies showed D-dimer levels above 90th percentile during the 3rd trimester in this study.

The effect of gestational week on D-dimer level is well known; as also confirmed in this study, D-dimer level increases with advancing gestation [3 – 6, 8]. As pregnancy is associated with four- to sixfold increased risk of VTE [24,25], and as a further increased risk of VTE (approximately twofold compared with singleton pregnancies) is seen among women with multifetal pregnancies [10 – 12], the results of a series of studies [3 – 6,8, 16 – 18], including ours, may support three previous reports suggesting that elevated D-dimer levels are associated with an increased risk of subsequent VTE events [13 – 15]; the subjects consisted of a general population in one study [13] and unhealthy patients in the other two studies [14,15]. In a study of the general population [13], relative to the first quintile of the distribution of D-dimer, the age-adjusted odds ratios for future VTE for the second to fifth quintiles of D-dimer were 1.6, 2.3, 2.3, and 4.2, respectively (*P* for trend < 0.0001)[13]. Patients with an abnormal D-dimer level with qualitative D-dimer test one month after discontinuation of anticoagulation treatment had a significantly higher incidence of recurrent VTE compared to patients with normal D-dimer level during follow-up (15.0% vs. 6.2%, adjusted hazard ratio of 2.27 [95% confidence interval; 1.15 – 4.46])[14]. In another study [15], multivariate analysis showed that patients with elevated D-dimer levels (defined as > 0.5μg/ml) had a 3.2-fold increased risk of developing VTE (95% confidence interval, 1.5 – 6.5; $P = 0.002$) during the 90-day follow-up in comparison to subjects with normal levels among hospitalized patients more than 60 years old [15]. Although not yet verified, these results suggested that pregnant women with high D-dimer level may also have higher risk of developing VTE compared to pregnant women with a low D-dimer level. If this is the case, determination of D-dimer level in women with multifetal pregnancies would be helpful for identifying women at especially high risk of subsequent VTE.

As both advanced maternal age and obesity are risk factors for venous thromboembolism in pregnancy [24], these two factors may increase D-dimer level. However, neither mean maternal age nor mean pre-pregnancy BMI differed significantly between women with singleton and multifetal pregnancies. Among women with multifetal pregnancies, the pre-pregnancy BMI was rather greater in women whose D-dimer levels were determined in the first trimester than in the third trimester. Thus, these two factors may have not distorted our conclusion that

D-dimer level increases with advancing gestation, and the D-dimer rise in pregnancy is greater in women with multifetal than singleton pregnancies.

Although we determined the 90th percentile D-dimer levels using latex agglutination assay (Mitsubishi Kagaku Iatron Inc.), the numerical results differed between assay methods employed. As described in the Materials and Methods section, our method gave relatively high D-dimer values. In addition, the patterns of rising D-dimer levels during pregnancy differ depending on the assay methods used [16]; the Innovance D-Dimer assay® (Siemens Medical Solutions, Malvern, PA) increased significantly with the advancement of pregnancy, and is more sensitive than D-Dimer PLUS assay® (Dade Behring, Marburg, Germany) in the pregnant population [16]. Our data were comparable to those obtained by the Innovance D-Dimer assay, and our assay showed sensitivity similar to Innovance D-Dimer assay for the detection of D-dimer change during pregnancy (data not shown).

In conclusion, the present study confirmed that D-dimer increases with advancing gestation and the D-dimer rise in pregnancy is greater in women with multifetal than singleton pregnancies. Approximately one third of women with multifetal pregnancies exhibited above 90th percentile D-dimer value during the third trimester. Previous studies suggested that subjects with a high D-dimer level are at increased risk of subsequent development of VTE [13 – 15]. Epidemiology suggested that pregnant women, especially with multifetal pregnancies, are at increased risk of developing VTE [10 – 12]. These results together with those of the present study suggest that determination of D-dimer level in women with multifetal pregnancies would be helpful for identifying those at especially high risk of subsequent VTE.

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DISCLOSURE

The authors have no conflicts of interest to declare.

REFERENCES

1. Cerneca F, Ricci G, Simone R, Malisano M, Alberico S, and Guaschino S. Coagulation and fibrinolysis changes in normal pregnancy. Increased levels of procoagulants and reduced levels of inhibitors during pregnancy induce a hypercoagulable state, combined with a reactive fibrinolysis. Eur J Obstet Gynecol Reprod Biol 1997;73:31–6.
2. Ros Salonen H, Lichtenstein P, Bellocco R, Petersson G, and Cnattingius S. Increased risks of circulatory diseases in late pregnancy and puerperium. Epidemiology 2001;12:456–60.
3. Chabloz P, Reber G, Boehlen F, Hohlfeld P, and de Moerloose P. TAFI antigen and D-dimer levels during normal pregnancy and at delivery. Br J Haematol 2001;115:150–2.
4. Giavarina D, Mezzena G, Dorizzi RM, and Soffiati G. Reference interval of D-dimer in pregnant women. Clin Biochem 2001;34:331–3.
5. Morse M. Establishing a normal range for D-dimer levels through pregnancy to aid in the diagnosis of pulmonary embolism and deep vein thrombosis. J Thromb Haemost 2004;2:1202–4.
6. Szecsi PB, Jorgensen M, Klajnbard A, Andersen MR, Colov NP, and Stender S. Haemostatic reference intervals in pregnancy. Thromb Haemost 2010;103:718–27.
7. Chan W-S, Chunilal S, Lee A, Crowther M, Rodger M, and Ginsber JS. A red blood cell agglutination D-dimer test to exclude deep venous thrombosis in pregnancy. Ann Intern Med 2007;147:165–70.
8. Kovac M, Mikovic Z, Rakicevic L, Srzentic S, Mandic V, Djordjevic V, Radojkovic D, and Elezovic I. The use of D -dimer with new cutoff can be useful in diagnosis of venous thromboembolism in pregnancy. Eur J Obstet Gynecol Reprod Biol 2010;148:27–30.
9. Chan WS, Lee A, Spencer FA, Chunilal S, Crowther M, Wu W, Johnston M, Rodger M, and Ginsberg JS. D-dimer testing in pregnant patients: toward determining the next level in the diagnosis of deep vein thrombosis. J Thromb Haemost 2010;8:1004–11. Epub 2010 Jan 30.
10. Jacobsen AF and Sandset PM. Venous thromboembolism associated with pregnancy and hormonal therapy. Best Prac & Res Clin Haematol 2012; 25: 319–332.

11. Lussana F, Coppens M, Cattaneo M, and Middeldorp S. Pregnancy-related venous thromboembolism: risk and the effect of thromboprophylaxis. Thromb Res 2012; 129:673–80.
12. Henriksson P, Westerlund E, Wallén H, Brandt L, Hovatta O, and Ekbom A. Incidence of pulmonary and venous thromboembolism in pregnancies after in vitro fertilization: cross sectional study. BMJ 2013;346:e8632 doi: 10.1136/bmj.e8632 (Published 15 January 2013)
13. Cushman M, Folsom AR, Wang L, Aleksic N, Rosamond WD, Tracy RP, and Heckbert SR. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood 2003;101:1243–8.
14. Palareti G, Cosmi B, Legnani C, Tosetto A, Brusi C, Iorio A, Pengo V, Ghirarduzzi A, Pattacini C, Testa S, Lensing AW, and Tripodi A. D-dimer testing to determine the duration of anticoagulation therapy. N Engl J Med 2006;335:1780–9.
15. Fan J, Li X, Cheng Y, Yao C, and Zhong N; Investigators Group. Measurement of D-dimer as aid in risk evaluation of VTE in elderly patients hospitalized for acute illness: a prospective, multicenter study in China. Clin Invest Med 2011;34:E96–104.
16. Bar J, Blickstein D, Hod M, Bar-Hava1, Ben-Rafael Z, Rahmany-Babai J, and Lahav J. Increased D-dimer levels in twin gestation. Thromb Res 2000;98:485–9.
17. Nishii A, Noda Y, Nemoto R, Ushiro K, Ohno T, Mochizuki Y, Yoshihara H, Taguchi A, Uchino N, and Ohkawara S. Evaluation of D-dimer during pregnancy. J Obstet Gynaecol Res 2009;35:689–93.
18. Khalafallah AA, Morse M, Al-Barzan AM, Adams M, Dennis A, Bates G, Robertson I, Seaton D, and Brain T. D-Dimer levels at different stages of pregnancy in Australian women: A single centre study using two different immunoturbidimetric assays. Thromb Res 2012, doi:10.1016/j.thromres.2012.05.022
19. Epiney M, Boehlen F, Boulvain M, Reber G, Antonelli E, Morales M, Irion O, and De Moerloose P. D-dimer levels during delivery and the postpartum. J Thromb Haemost 2005;3:268–71.
20. Dargaud Y, Hierso S, Rugeri L, Battie C, Gaucherand P, Négrier C, and Trzeciak MC. Endogenous thrombin potential, prothrombin fragment 1+2 and D-dimer during pregnancy. Thromb Haemost 2010;103:469–71.

21. de Moerloose P, Palareti G, Aguilar C, Legnani C, Reber G, and Peetz D. A multicenter evaluation of a new quantitative highly sensitive D-dimer assay for exclusion of venous thromboembolism. Thromb Haemost 2008;100:505–12.
22. Janssen MC, Heebels AE, de Metz M, Verbruggen H, Wollersheim H, Janssen S, Schuurmans MM, and Nováková IR. Reliability of five rapid D-dimer assays compared to ELISA in the exclusion of deep venous thrombosis. Thromb Haemost 1997;77:262–6.
23. Agterof MJ, van Bladel ER, Schutgens REG, Snijder RJ, Tromp EA, Prins MH, and Biesma DH. Risk stratification of patients with pulmonary embolism based on pulse rate and D-dimer concentration. Thromb Haemost 2009;102:683–7.
24. Royal College of Obstetricians and Gynaecologists. Reducing the Risk of Thrombosis and Embolism During Pregnancy and the Puerperium. Green-top Guideline No. 37a, November 2009.
25. Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos A-M, Vandvik PO. VTE, thrombophilia, antithrombotic therapy, and pregnancy. Chest 2012;141:e691S–e736S.

330 **FIGURE LEGENDS**

331 **Fig. 1: Distribution of D-dimer levels according to gestational week**

332

333 **Fig. 2: Changes in D-dimer levels during pregnancy according to number of**
334 **fetuses**

335 Dashed and solid lines indicate multifetal pregnancy and singleton pregnancy,
336 respectively. Vertical bar indicates 1 SD.

337 *, $P < 0.001$ between singleton and multifetal pregnancies; ‡ and †: comparison within
338 a group, and ‡, $P < 0.01$ vs. 1st- and 2nd-trimester levels for multifetal pregnancy and vs.
339 1st- trimester level for singleton pregnancy; and †, $P < 0.0001$ vs. 1st- and 2nd-trimester
340 levels.

341

Table 1: Demographic characteristics of study subjects

	Singleton	Multifetal*	*P*-value
Number of women	977	112*	
Maternal age (year)	32.0 ± 5.3	30.6 ± 4.7	0.0074
Primiparity	552 (56.5%)	62 (55.3%)	0.8174
Pre-pregnancy BMI (kg/m^2)	21.6 ± 4.1	21.3 ± 3.2	0.4142
Gestational week at delivery	37.1 ± 4.5	33.9 ± 5.5	< 0.0001
< 37 weeks	226 (23.1%)	75 (67.0%)	<0.0001
Gestational week at determination	30.5 ± 9.5	27.4 ± 7.1	0.0010
Fist trimester	9.6 ± 2.9 [102]	11.8 ± 2.4 [7]	0.0576
Second trimester	23.0 ± 4.1 [216]	23.8 ± 3.7 [59]	0.1396
Third trimester	36.2 ± 2.9 [659]	34.4 ± 1.7 [46]	< 0.0001

Date are presented as means ± SD.

*, including 106, 5, and 1 women with twin, triplet, and quadruplet pregnancies, respectively.

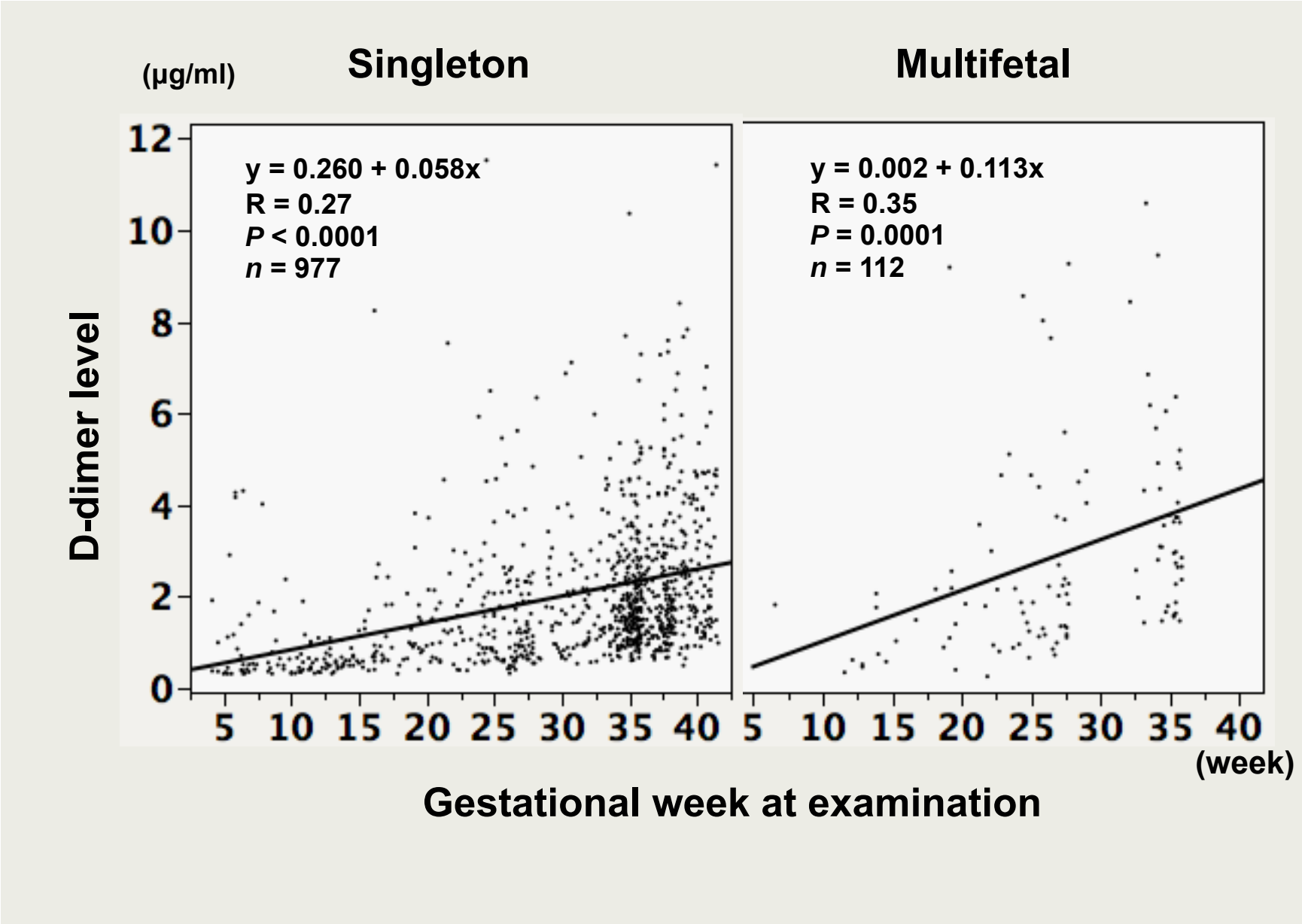
Numbers of women are indicated in square brackets.

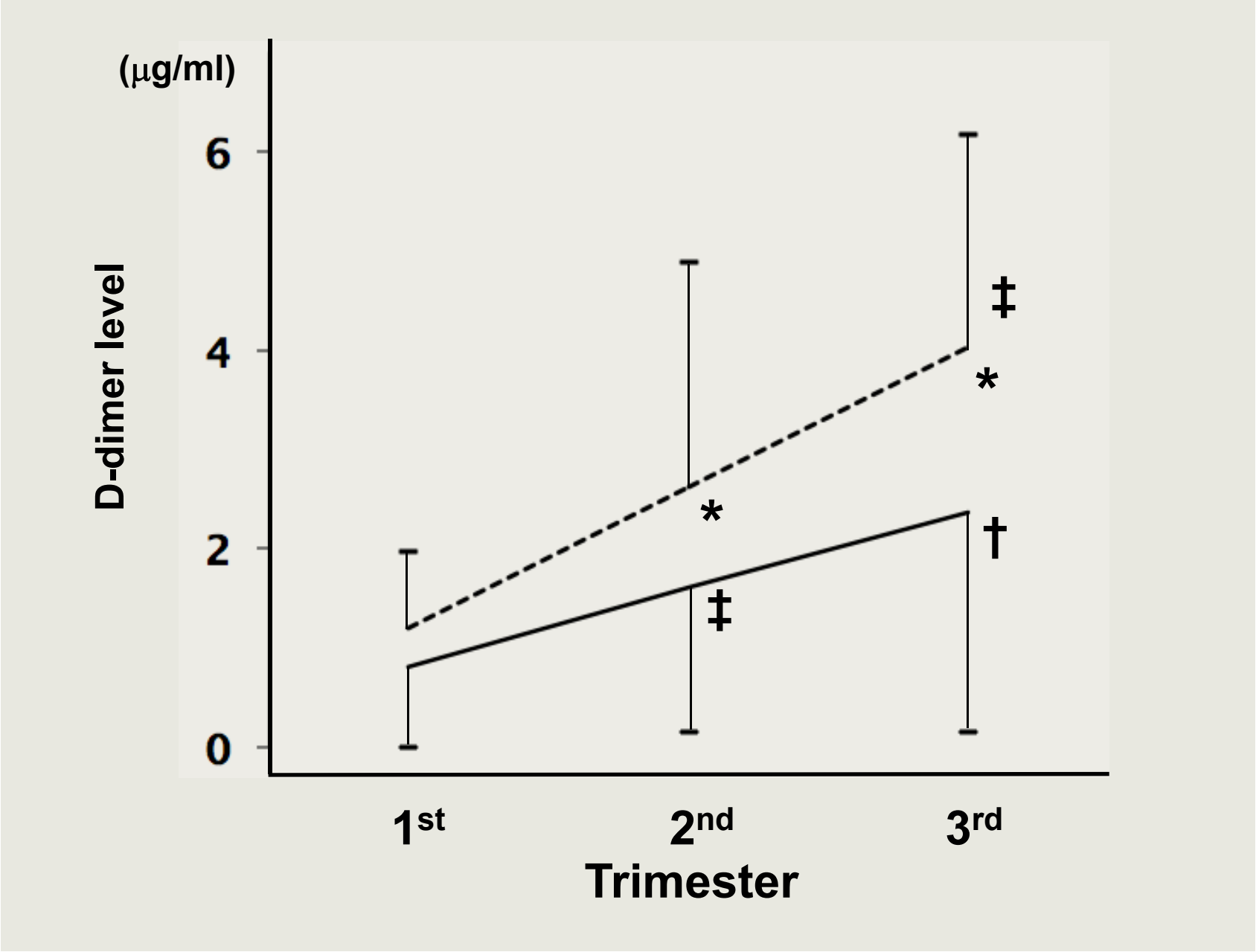
Table 2: Prevalence of women with a high D-dimer (> 4.31 μg/ml)

	Singleton	Multifetal	*P*-value
First trimester	1/102 (1.0%)*	0/7 (0.0%)	0.7924
Second trimester	12/216 (5.6%)*	10/59 (16.9%)	0.0043
Third trimester	70/659 (10.6%)*	16/46 (34.8%)†	< 0.0001
Overall	83/977 (8.5%)	26/112 (23.2%)	< 0.0001

*, $P < 0.05$ vs. frequency of any trimester; †, $P < 0.05$ vs. frequency of second trimester.

P-value was determined with χ^2 test or Fisher's exact test


(μg/ml)
Singleton
Multifetal
y = 0.260 + 0.058x
R = 0.27
P < 0.0001
n = 977
y = 0.002 + 0.113x
R = 0.35
P = 0.0001
n = 112
D-dimer level
0
2
4
6
8
10
12
5 10 15 20 25 30 35 40
5 10 15 20 25 30 35 40
(week)
Gestational week at examination


(μg/ml)
D-dimer level
6
4
2
0
‡
*
*
†
‡
1st
2nd
3rd
Trimester