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Full Length Article

Platelet reactivity in twin pregnancies

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ABSTRACT

Background: Gestational thrombocytopenia is more likely to occur in twin than singleton pregnancies. However, it is unclear whether platelets are more reactive in twin than singleton pregnancies.
Methods: Changes in spontaneous platelet aggregation and concomitant fall in platelet count were examined over 90 min after blood sampling in 171 and 52 citrated whole blood (CWB) samples from 59 and 17 women with singleton and twin pregnancies, respectively. Soluble P-selectin (sP-selectin) levels in the plasma were also determined.
Results: CWB 60 min after blood sampling during 2nd trimester exhibited significantly larger numbers of platelet aggregates (1297 ± 1600 vs. 497 ± 432/μl, $P = 0.040$) concomitant with significantly greater net decrease in platelet count (152 ± 55 vs. $115 \pm 45 \times 10^9$/μl, $P = 0.036$) in twin than singleton pregnancies, respectively. This was followed by significantly lower 3rd trimester platelet count (181 ± 43 vs. $229 \pm 62 \times 10^9$/l, $P = 0.009$) with significantly greater mean platelet volume (8.0 ± 1.2 vs. 7.1 ± 1.1 fl, $P = 0.021$) in twin than singleton pregnancies, respectively. The 3rd trimester sP-selectin per platelet was significantly higher in twin than singleton pregnancies.
Conclusions: Platelets were more reactive in the 2nd trimester of twin than singleton pregnancies. This enhanced platelet reactivity may explain the decreased platelet count in the 3rd trimester of twin pregnancy.

1. Introduction

Pregnancy is associated with a hypercoagulable state and increased risk of venous thromboembolism [1,2]. As women with essential thrombocythemia have increased likelihood of experiencing adverse pregnancy outcomes, such as abortion and fetal demise [3,4], the degree of platelet reactivity may be associated with pregnancy outcome. Theoretically, the circulating platelet count may decrease in the presence of disproportionately enhanced platelet reactivity for the platelet production in the bone marrow. Indeed, some pregnant women exhibit a gradual decline in platelet count during pregnancy (gestational thrombocytopenia) [5–7] and women with gestational thrombocytopenia are prone to the syndrome of hemolysis, elevated liver enzymes, and low platelet counts (HELLP syndrome) [6,8,9]. Gestational thrombocytopenia and HELLP syndrome occur significantly more often in women with twin than singleton pregnancies [5–7,10].

In addition, the coagulation–fibrinolysis system is activated to a greater extent in twin than in singleton pregnancies. D-dimer levels are consistently higher [11] and blood fibrinogen level in the late stage of pregnancy is lower in twin than in singleton pregnancies, suggesting exaggerated fibrinogen consumption in twin pregnancy [12]. All of these observations suggest that platelet reactivity is enhanced to a greater extent in twin than in singleton pregnancies.

Traditional platelet function tests have limited clinical application as they require blood sample processing, are time consuming, labor intensive, and require skilled laboratory staff to perform and interpret the assay. However, when citrated whole blood (CWB) is stirred or mixed in some other way, spontaneous platelet aggregation and concomitant decrease in single platelet count occur as a result of platelet activation [13–16]. A hematology analyzer (CELL-DYN Sapphire Hematology System®; Abbott Diagnostics, Abbott Park, IL) with a newly developed software package can specifically count the numbers of platelet aggregates (PA) and single platelets simultaneously in CWB [17,18]. The number of PA detected by this analyzer is suggested to reflect the degree of platelet reactivity [18].

Upon activation of platelets or endothelial cells, P-selectin is quickly expressed on the surface membrane [19]. Circulating degranulated platelets rapidly shed surface P-selectin [20], producing the circulating plasma protein soluble P-selectin (sP-selectin) [21]. Although P-selectin is present in both platelets and endothelial cells, several authors

Abbreviations: CWB, citrated whole blood; HELLP syndrome, syndrome of hemolysis, elevated liver enzymes, and low platelet counts; IPF, immature platelet fraction; MPV, mean platelet volume; PA, platelet aggregate.

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have concluded that platelets are the major source of sP-selectin in plasma [22,23].

The present study was performed to determine whether the degree of spontaneous platelet aggregation in the CWB differs between women with singleton and twin pregnancies and association between spontaneous platelet aggregation and plasma sP-selectin levels.

2. Material and methods

This study was performed in accordance with the provisions of the Declaration of Helsinki and was conducted after receiving approval from the Institutional Review Board of Hokkaido University Hospital. Written informed consent was obtained from all participants prior to the study.

2.1. Participants

A total of 76 healthy women consisting of 59 and 17 women with singleton and twin pregnancies, respectively, participated in this study (Table 1). None of the 76 participants showed positivity for anti-HIV-1/2 antibody, and all 76 women had unremarkable medical histories, were normotensive during pregnancy and postpartum, and experienced uneventful current pregnancies. Therefore, all participants were considered healthy and not to have pathological hypercoagulability. Participants provided blood samples several times at various stages of pregnancy, including the 1st, 2nd, and 3rd trimesters, and postpartum days 2–6 (designated as P1) and 24–39 (P2) (Table 1). Among the 59 women with singleton pregnancies vs. 17 women with twin pregnancies, only one blood sample was available for 13 (22%) vs. 1 (5.9%), two blood samples were available for 11 (19%) vs. 1 (5.9%), three blood samples were available for 14 (24%) vs. 11 (65%), four blood samples were available for 11 (19%) vs. 4 (24%), and five blood samples were available for 10 (17%) vs. 0 (0.0%), respectively.

2.2. Blood sample collection

Blood sampling was not performed at any particular time of the day, and without any particular time in relation to meal intake. A total of 9–10 ml of venous blood was drawn from the antecubital vein using a tourniquet and a 23-gauge needle connected to three successive vacuum tubes in the following order: 5–6 ml of blood in the first tube not used in this study, 2 ml of blood in the second tube containing 4.5 mg of EDTA, and 1.8 ml of blood in the third tube containing 0.2 ml of 3.2% sodium citrate solution. Two tubes containing whole blood anticoagulated with EDTA and citrate, designated as EDTA blood and CWB, respectively, were used in this study.

Table 1
Demographic characteristics between two groups.

	Singleton	Twin
No of women	59	17
Age (year)	34.2 ± 5.2	31.5 ± 6.1
Nulliparous women	33 (56%)	13 (76%)
GW at delivery	38.6 ± 1.3	36.1 ± 3.3*
Placenta weight (kg)†	0.6 ± 0.2	1.0 ± 0.2*
Infant weight (kg)†	3.0 ± 0.4	4.3 ± 1.0*
Total no. of blood samples	171	52
No. of blood samples/person	2.9 ± 1.4	3.1 ± 0.7
GW at blood sampling		
First trimester	11.6 ± 1.0 [49]	11.9 ± 1.3 [4]
Second trimester	25.4 ± 0.9 [42]	24.7 ± 2.6 [10]
Third trimester	36.2 ± 0.5 [35]	35.1 ± 0.8* [14]
PPD 2–6 (P1)	3.1 ± 0.8 [21]	3.8 ± 1.1 [12]
PPD 24–39 (P2)	29.8 ± 3.9 [24]	30.9 ± 3.3 [12]

The numbers of women that provided blood samples are indicated in square brackets.
* $P < 0.05$ vs. women with singleton pregnancies.
† Sum for twins; GW, gestational week; PPD, postpartum day.

2.3. Measurement of platelet count, number of platelet aggregates (PA), and sP-selectin

Two tubes containing EDTA blood and CWB were applied to the CELL-DYN Sapphire Hematology System® (Abbott Diagnostics) 30 min after blood sampling at room temperature. These tubes were agitated in this system that took approximately 3 min to measure platelet count, number of PA, size of immature platelet fraction (IPF), and mean platelet volume (MPV) simultaneously. This procedure was repeated three times for each CWB to determine changes over time in number of PA and platelet count at 30, 60, and 90 min after blood sampling. For the platelet count in CWB, the corrected platelet count for the dilution with 0.2 ml of sodium citrate solution was used. Platelet count determined in EDTA blood was used as the baseline platelet count, MPV, and IPF of each blood sample. The antenatal EDTA blood ($n = 154$, including 126 and 28 from women with singleton and twin pregnancies, respectively) after determination of these variables was centrifuged at 2000 *g* for 5 min at room temperature within 60 min after sampling and the plasma was stored at −20 °C until assay of sP-selectin using Human sP-selectin/CD62P Immunoassay® (R&D Systems Inc., Minneapolis, MN).

Data are presented as means ± SD. Statistical analyses were performed using the JMP® Pro11 statistical software package (SAS, Cary, NC). Differences in the means were tested using Wilcoxon's rank sum test between each group, and changes in variables within a group were compared using the Tukey–Kramer method. Pearson's product–moment correlation coefficient was used to measure linear correlations between two variables. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

3. Results

Neither maternal age nor fraction of nulliparous women differed significantly between the groups with singleton and twin pregnancies (Table 1). The sums of infant weight and placental weight were significantly greater in twin than in singleton pregnancies.

3.1. Baseline characteristics of platelet in EDTA blood between singleton vs. twin pregnancy

Baseline platelet count (in EDTA blood) increased significantly approximately 1 month postpartum in both singleton and twin pregnancies compared to the respective values in the 1st trimester (Fig. 1, A). Its level in the 3rd trimester of twin pregnancy was significantly reduced compared to that in singleton pregnancy. The MPV in the 3rd trimester was significantly greater in twin than in singleton pregnancies (Fig. 1, B). The IPF increased significantly within 1 week postpartum in both singleton and twin pregnancies compared to the respective values in the 1st trimester (Fig. 1, C).

3.2. Spontaneous platelet aggregation in CWB of singleton vs. twin pregnancy

The CWB exhibited an increase in number of PA over time after blood sampling in both singleton and twin pregnancies (Fig. 2). The degree of increase in PA count was lowest in the 1st trimester and greatest approximately 1 month postpartum in singleton as well as twin pregnancies. In singleton pregnancy, its degree appeared to increase with advancing gestation; the area under curve (AUC) was significantly greater in the 3rd trimester as well as at P2 (approximately 1 month postpartum) in comparison with that in the 1st trimester (Fig. 2, left). The number of PA counts in CWB of 2nd trimester at 60 min after sampling was significantly greater in twin than in singleton pregnancy (Fig. 3, left), resulting in a significantly greater net decrease in the platelet count of CWB at 30, 60, and 90 min after sampling in twin than in singleton pregnancy (Fig. 3, right).