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Original Article

Circulating endothelial progenitor cells and placental abruption in women with preeclampsia

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ABSTRACT

Objective: Abnormalities in circulating angiogenic factors and endothelial progenitor cells (EPCs) have been reported in patients with preeclampsia and placental abruption. The objective of this study was to determine whether the number of EPCs is altered in patients with placental abruption.
Design: A case control study.
Setting: Hiroshima University Hospital in Japan.
Sample: Pregnant Japanese women with preeclampsia ($n = 27$) and those without any complications ($n = 15$).
Method: The EPC ($CD45^{low}CD34^{+}CD133^{+}$ cells) counts were examined using flow cytometry in peripheral blood collected from 27 women with preeclampsia and 15 normal pregnant women. Among the 27 women with preeclampsia, five subsequently developed placental abruption. All subjects were divided into three groups: normal pregnancy (NP, $n = 15$), preeclampsia without placenta abruption (PE, $n = 22$) and preeclampsia with placental abruption (PA, $n = 5$).
Main outcome measures: The EPC counts were measured in pregnant women with preeclampsia who subsequently developed placental abruption.
Results: The EPC count in the PE group significantly decreased in comparison to that observed in the NP group (620 cells/ml versus 1918 cells/ml, $P < 0.01$). In the PA group, the EPC count was found to markedly decrease in comparison to that observed in the PE group (221 cells/ml, $P < 0.05$).
Conclusions: The number of EPCs was found to significantly decrease in preeclamptic women who subsequently developed placental abruption.

Introduction

Placental abruption occurs in 0.5–2% of pregnancies [1,2] and is one of the most significant causes of both maternal and perinatal mortality and morbidity. However, the pathogenesis of placental abruption is unknown and no prediction markers have yet been established.

It has been suggested that placental abruption may be caused by abnormalities in trophoblast invasion and the subsequent rupture of maternal spiral arteries, thus causing retroplacental hemorrhage and premature separation of the placenta from the uterine wall [3,4]. Moreover, the histological findings of placental bed tissue from cases of

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placental abruption are marked by the frequent absence of trophoblast invasion in the spiral arteries, thus indicating similarities between placental abruption and preeclampsia [4]. Preeclampsia, other pregnancy-related hypertensive disorders, chronic hypertension and smoking are all regarded as important risk factors for placental abruption [2,5]. Abnormalities in circulating angiogenic factors have been reported in patients with preeclampsia and placental abruption [6–8]. In addition, decreases in the number of circulating endothelial progenitor cells (EPCs) in patients with preeclampsia have also been reported [9–12]. Therefore, we hypothesized that such alterations could be present in patients with placental abruption.

We examined in parallel the number of EPCs and the levels of PlGF and sFlt-1 in blood samples collected from females with preeclampsia who subsequently developed placental abruption, females with preeclampsia who did not develop placental abruption and normal pregnant females.

The aim of this study was to determine whether the number of EPCs is altered in patients with placental abruption and to evaluate the relationship between the number of EPCs and the levels of the angiogenic factors PlGF and sFlt-1.

Materials and methods

Subjects

We studied 27 females with preeclampsia and 15 normal pregnant females. All subjects received follow-up until delivery. Preeclampsia was defined as the development, after 20 weeks of gestation, of a blood pressure of 140/90 mmHg or higher and proteinuria of more than 300 mg/day. The control subjects were selected from normotensive pregnant women without any complications until termination. All of the women had singleton pregnancies. Out of 27 women with preeclampsia, five subsequently developed placental abruption. Placental abruption was diagnosed according to the clinical symptoms and pathological findings. All subjects were divided into three groups: normal pregnancy (NP; $n = 15$), preeclampsia without placenta abruption (PE; $n = 22$) and preeclampsia with placental abruption (PA; $n = 5$). The clinical characteristics of the three groups are shown in Table 1. The study protocol was approved by the Ethics Committee of Hiroshima University Graduate School of Biomedical Sciences. Written informed consent was obtained from all subjects before participation. The clinical management principles of preeclampsia were followed according to the 2009 Guidelines for the Care and Treatment of Hypertension in Pregnancy (Japan Society for the Study of Hypertension in Pregnancy).

Measurement of the number of EPCs

The number of EPCs was analyzed using flow cytometry. Briefly, samples of venous blood were placed in polystyrene tubes containing sodium EDTA (7 mg/ml). The EDTA-containing tubes were chilled promptly in an ice bath. Peripheral blood mononuclear cells (MNCs) were immediately isolated using Ficoll density gradient centrifugation (AXIS-SHIELD). After being thawed, 10^6 peripheral blood MNCs were incubated for 10 min with monoclonal antibodies against human fluorescein isothiocyanate-conjugated anti-CD45 (Miltenyi Biotech, Bergisch Gladbach, Germany), phycoerythrin-conjugated anti-CD133 (Miltenyi Biotech), allophycocyanin-conjugated anti-CD34 (Becton Dickinson Biosciences, San Jose, CA) and 7-amino-actinomycin D (Becton Dickinson Biosciences) for dead cell exclusion. To assess the background, isotype controls were used as negative controls based on the species and immunoglobulin G subclass of each antibody. After being incubated, the erythrocytes were lysed, and the remaining cells were washed with phosphate-buffered saline, fixed in 2% paraformaldehyde and then were analyzed on a fluorescence-activated cell sorting flow cytometer (FACSCalibur; Becton Dickinson Biosciences) according to the manufacturer's instructions. Each analysis

Table 1
Baseline and delivery characteristics of subjects and their infants.

	NP group ($n = 15$)	PE group ($n = 22$)	PA group ($n = 5$)	
Maternal characteristics				
Age (y)	30.3 ± 1.1	33.1 ± 1.2	32.3 ± 1.5	n.s.
No. of nulliparity [% of nulliparity]	10 [66.7%]	13 [59.1%]	2 [40.0%]	n.s.
Gestational age at sampling (day)	234.9 ± 5.4	241.1 ± 7.7	217.8 ± 13.7	n.s.
Gestational age at delivery (day)	260.3 ± 4.2	246.5 ± 7.2	224.8 ± 9.8*	*$P < 0.05$, vs NP group
Interval between sampling and delivery (day)	25.4 ± 5.1	5.5 ± 2.0*	7.0 ± 5.5*	*$P < 0.05$, vs NP group
Systolic blood pressure (mmHg)	107.8 ± 2.7	161.4 ± 2.7*	155.5 ± 8.4*	*$P < 0.05$, vs NP group
Diastolic blood pressure (mmHg)	60.6 ± 2.1	97.0 ± 2.3*	93.7 ± 7.5*	*$P < 0.05$, vs NP group
Infant characteristics				
Birth weight (g)	2624 ± 145	2143 ± 161	1758 ± 263*	*$P < 0.05$, vs NP group
Apgar score at one minute <4 – No. (%)	0 [0]	1 [4.5]	2 [40.0]**	**$P < 0.05$, vs PE group
Apgar score at five minutes <4 – No. (%)	0 [0]	0 [0]	1 [20.0]	
No. with SGA [% of SGA]	0 [0]	8 [36.4]**	0 [0]	**$P < 0.05$, vs NP group

NP, normal pregnancy; PE, preeclampsia; PA, placental abruption; SGA, small for gestational age.
Values are given as mean ± SE.
P values are given only for significant differences.
* Significantly different (Mann–Whitney *U* test).
** Significantly different (χ^2 test).