Original Article

Alterations in Placental Growth Factor Levels before and after the Onset of Preeclampsia Are More Pronounced in Women with Early Onset Severe Preeclampsia

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It has been established that the serum placental growth factor (PlGF) decreases and the soluble fms-like tyrosine kinase-1 (sFlt-1) increases in women with preeclampsia. However, there have been no studies on the relation between preeclampsia onset time and the changes in PlGF and sFlt-1. Furthermore, the PlGF and sFlt-1 levels have not been evaluated using their reference values specific to each gestational age. In this study we reevaluated the serum PlGF and sFlt-1 levels before and after the clinical manifestation of early and late onset severe preeclampsia using the new reference values developed in our recent longitudinal study. Blood specimens were obtained immediately after the clinical manifestation of severe preeclampsia in 34 referred women, and both before and after the clinical manifestation in 8 women receiving a routine checkup at our institute. Both women with early and those with late preeclampsia showed decreased PlGF and increased sFlt-1 levels compared to normotensive controls at 28 and 37 weeks (n=68). However, those with early onset preeclampsia had a higher incidence of low PlGF (<5th percentile on the reference values) and high sFlt-1 (≥95th percentile) than those with late onset (low PlGF: 93% *vs.* 55%; high sFlt-1: 100% *vs.* 60%). $\log_{10}$PlGF (r=0.574, p<0.001) and $\log_{10}$(sFlt-1/PlGF) (r=−0.556, p<0.001) were correlated with the week of onset of preeclampsia. Before the onset of preeclampsia, the incidence rate of low PlGF in the women with early onset preeclampsia was 100% (5/5), whereas that in the women with late onset preeclampsia was 0% (0/2) (p=0.048). Therefore, alterations in the PlGF levels both before and after the onset of preeclampsia may be more pronounced in women with early onset than those with late onset severe preeclampsia. (*Hypertens Res* 2007; 30: 151–159)

***Key Words*: early onset preeclampsia, late onset preeclampsia, placental growth factor, soluble fms-like tyrosine kinase-1, severe preeclampsia**

Introduction

Preeclampsia (pregnancy induced hypertension with proteinuria) is associated with maternal and infantile morbidity and mortality (*1*, *2*). The Japan Society for the Study of Hypertension in Pregnancy (JSSHP) classifies preeclampsia into early and late onset type according to whether the clinical manifestations occur before or after 32 weeks of gestation, respectively (*3*). The early onset preeclampsia may differ

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Table 1. Maternal and Infantile Characteristics in Women with Normal Pregnancy, Women with Early and Late Onset Severe Preeclampsia

	Women wtih			*p* value	Significant pairs among groups
	Normal pregnancy (group 1, *n*=65)	Early onset severe preeclampsia (group 2, *n*=14)	Late onset severe preeclampsia (group 3, *n*=20)		
Age (years)	28.8±3.9	31.5±5.1	29.3±6.9	n.s.	
Body height (m)	1.58±0.05	1.59±0.06	1.58±0.06	n.s.	
Prepregnancy body weight (kg)	52.1±7.2	57.5±9.8	57.6±8.4	0.008	1 *vs*. 2, 1 *vs*. 3
Prepregnancy body mass index (kg/m^2)	20.9±2.7	22.6±3.0	23.1±3.7	0.008	1 *vs*. 3
Nulliparity (*n* (%))	36 (55)	11 (79)	16 (80)	n.s.	
Gestational age at onset of preeclampsia (weeks)	—	28.1±2.6	36.3±2.2	<0.001	2 *vs*. 3
Gestational age at delivery (weeks)	39.5±1.1	29.8±3.2	37.2±1.9	<0.001	all pairs
Systolic blood pressure (mmHg)	—	177±16	178±12	n.s.	
Diastolic blood pressure (mmHg)	—	110±8	109±13	n.s.	
Urinary protein > 2 g/day (*n* (%))	—	8 (57)	8 (40)	n.s.	
Infant birth weight (g)	3,020±355	1,001±425	2,383±536	<0.001	all pairs
Infant birth weight (MoM)	−0.026±0.115	−0.258±0.122	−0.100±0.176	<0.001	1 *vs*. 2, 2 *vs*. 3
Small-for-gestational-age infant (*n* (%))	7 (11)	9 (64)	9 (45)	<0.001	1 *vs*. 2, 1 *vs*. 3

n.s., not significant; MoM, the multiple of the median.

Table 2. Serum Levels of PlGF, sFlt-1, and PlGF/sFlt-1 Ratio in Women with Normal Pregnancy, Women with Early and Late Onset Severe Preeclampsia

	Women with				*p* value	Significant pairs among groups
	Normal pregnancy		Early onset severe preeclampsia	Late onset severe preeclampsia		
	(group 1-1, *n*=65)	(group 1-2, *n*=65)	(group 2, *n*=14)	(group 3, *n*=20)		
Gestational age at measurement (weeks)	28.3±0.9	37.5±0.6	28.5±2.6	36.8±2.0	<0.001	1-1 *vs*. 1-2, 1-1 *vs*. 3, 1-2 *vs*. 2, 2 *vs*. 3
$\log_{10}$PlGF (pg/ml)	2.78±0.24	2.51±0.27	1.84±0.28	2.20±0.26	<0.001	all pairs
$\log_{10}$sFlt-1 (pg/ml)	2.60±0.22	3.05±0.20	3.60±0.21	3.50±0.22	<0.001	all pairs but not 2 *vs*. 3
$\log_{10}$(sFlt-1/PlGF)	−0.18±0.27	0.53±0.33	1.76±0.41	1.29±0.26	<0.001	all pairs

PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

from the late onset type not only in its high perinatal morbidity but also in its risk factors (*4*). For example, it has been reported that women with early onset preeclampsia show a greater incidence of infant growth restriction (*5*, *6*) and infant morbidity (*7*) than those with the late onset type. Thus it is the early, rather than the late onset type preeclampsia, to which our efforts at detection, prevention and therapy should be focused.

It has been shown that preeclamptic women have lower serum/plasma levels of placental growth factor (PlGF; an angiogenic protein) (*8–12*) and higher levels of soluble fms-like tyrosine kinase-1 (sFlt-1; a circulating anti-angiogenic protein) (*8*, *10–16*) compared to non-preeclamptic controls; this phenomenon has been considered to be closely associated with the pathogenesis/pathophysiology of preeclampsia (*8*). PlGF, acting synergistically with vascular endothelial growth factor (VEGF), may be necessary for the maintenance of endothelial cell health during pregnancy (*17–21*). The sFlt-1 is an anti-angiogenic protein that antagonizes VEGF and PlGF (*21*). Low PlGF and high sFlt-1—in other words, an imbalance of these two angiogenic/anti-angiogenic proteins—may cause endothelial dysfunction, leading to various manifestations of preeclampsia, including hypertension and proteinuria (*21*).

Although an imbalance of sFlt-1/PlGF may play important roles in the pathogenesis of preeclampsia, a majority of the studies focusing on the relationship between preeclampsia and these two proteins (*8–10*, *12*, *13*, *15*, *16*) have not taken

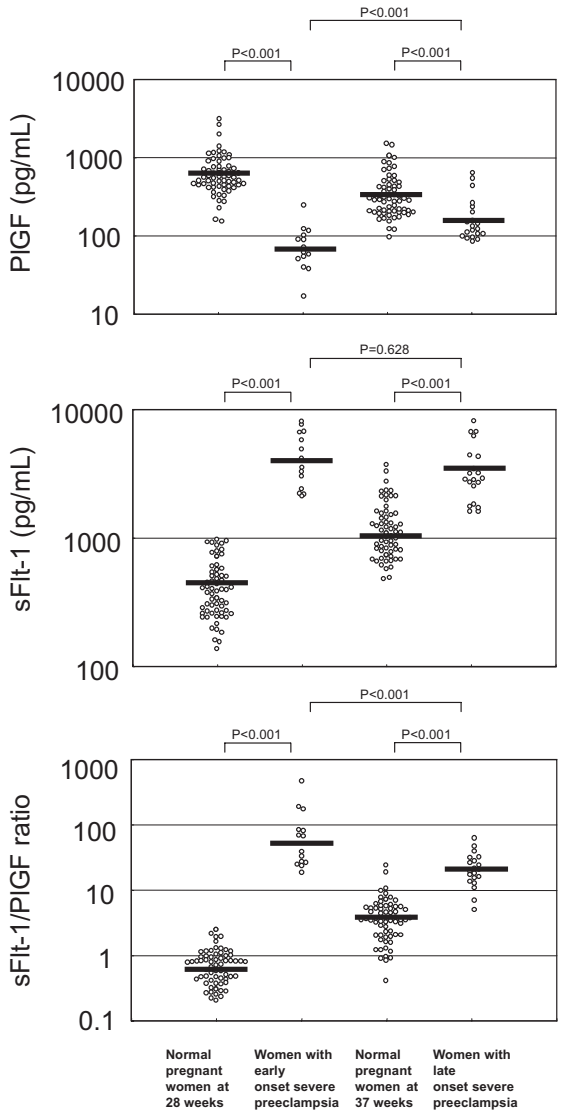

Fig. 1. *The fractions of serum PlGF and sFlt-1 levels, and the sFlt-1/PlGF ratio in normal pregnant women at 28 and 37 weeks of gestation, and women with early and late onset preeclampsia. Each circle depicts the mean of individual data of duplicate measurements. Horizontal bars indicate the mean of each group. Serum samples of early and late preeclampsia were measured at 28±3 and 37±2 weeks of gestation, respectively. Women with early onset preeclampsia showed significantly lower levels of PlGF, higher levels of sFlt-1, and higher levels of the sFlt-1/PlGF ratio than the normotensive controls at 28 weeks of gestation (*$p < 0.001$ *for each). Women with late onset preeclampsia also showed significantly lower levels of PlGF, higher levels of sFlt-1 and higher levels of the sFlt-1/PlGF ratio than the normotensive controls at 37 weeks of gestation (*$p < 0.001$ *for each). The PlGF levels and sFlt-1/PlGF ratios were significantly different between women with early and late onset severe preeclampsia (*$p < 0.001$ *for each), whereas the sFlt-1 levels were not different between the two groups (*$p = 0.628$*).*

the onset time (early *vs*. late) into account. However, there have been a few notable exceptions. Levine *et al*. reported that the serum levels of PlGF were lower, and that those of sFlt-1 were higher, in women with early onset preeclampsia before the onset of preeclampsia (*11*). Our group also showed that the levels of PlGF were lower than the 5th percentile at 16–20 weeks in all 4 women who later developed early onset preeclampsia in 148 cohorts (*22*). Chaiworapongsa *et al*. reported that the sFlt-1 levels after the onset of preeclampsia were much higher in women with early onset preeclampsia than in those with the late onset type (*14*). Based on these evidences, we hypothesized that the alterations of the serum levels of both PlGF and sFlt-1 before and after the onset of clinical disease may be more pronounced in women with early onset preeclampsia than those with the late onset type.

The level of PlGF and sFlt-1 changes according to the gestational age; for this reason, we must obtain reference values specific to each gestational age in order to determine whether a given PlGF or sFlt-1 value is normal for a particular gestational age (*22*, *23*). However, almost all the previous studies have used only fixed values for PlGF and sFlt-1 instead of employing gestational age–specific reference values (*8–21*). Recently, we constructed reference values for serum PlGF and SFlt-1 based on a longitudinal analysis of 148 cohorts of pregnant women at various gestational stages (*22*).

The first purpose of this study was to determine the serum PlGF and sFlt-1 levels in women with early and late onset severe preeclampsia and to compare them to those of normotensive pregnant controls at 28 and 37 weeks of gestation, using the newly developed reference values. The second purpose was to analyze the relationship between the onset time and three parameters: serum PlGF, serum sFlt-1, and the sFlt-1/PlGF ratio. The third purpose was to obtain basic data as to whether or not PlGF or sFlt-1 measurement before the onset of preeclampsia may be of some help in predicting the later occurrence of preeclampsia, especially the early onset type.

Methods

We obtained written informed consent from all women and the approval of the Ethics Committee of our institute. Study subjects were confined to women with severe preeclampsia and consisted of two groups. The first group consisted of 34 women with singleton infants who were referred to our hospital from 2002 to 2005 due to the diagnosis of severe preeclampsia. The control subjects were 65 women with normal blood pressures throughout pregnancy (normotensive controls) who were recruited from the previous longitudinal cohort study (*22*). We collected blood specimens at around 28 and 37 weeks in the control subjects, and immediately after admission in the subjects with severe preeclampsia. The second group consisted of 8 women with severe preeclampsia who we followed in our institute from the first trimester of their pregnancies; that is, before the onset of preeclampsia. Between April 2000 and August 2005, we collected the blood

Table 3. Standard Deviation Scores of Serum PlGF, Serum sFlt-1, and PlGF/sFlt-1 Ratio in Women with Normal Pregnancy, Women with Early and Late Onset Severe Preeclampsia

	Women with				*p* value	Significant pairs among groups
	Normal pregnancy		Early onset severe preeclampsia	Late onset severe preeclampsia		
	(group 1-1, *n*=65)	(group 1-2, *n*=65)	(group 2, *n*=14)	(group 3, *n*=20)		
Gestational age at measurement (weeks)	28.3±0.9	37.5±0.6	28.5±2.6	36.8±2.0	<0.001	1-1 *vs*. 1-2, 1-1 *vs*. 3, 1-2 *vs*. 2, 2 *vs*. 3
$\log_{10}$PlGF SDS	0.26±0.99	−0.12±1.11	−3.52±1.11	−1.51±1.52	<0.001	all pairs but not 1-1 *vs*. 1-2
$\log_{10}$sFlt-1 SDS	−0.09±0.92	−0.05±0.89	4.26±0.89	2.10±1.14	<0.001	all pairs but not 1-1 *vs*. 1-2
$\log_{10}$(sFlt-1/PlGF) SDS	−0.24±0.92	0.02±1.15	6.31±1.45	2.87±1.16	<0.001	all pairs but not 1-1 *vs*. 1-2

PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; SDS, standard deviation score.

Table 4. Incidence Rates of Serum PlGF <5th Percentile, Serum sFlt-1 ≥95th Percentile, and sFlt-1/PlGF Ratio ≥95th Percentile in Women with Normal Pregnancy, Women with Early and Late Onset Severe Preeclampsia

	Women with				*p* value	Significant pairs among groups
	Normal pregnancy		Early onset severe preeclampsia	Late onset severe preeclampsia		
	(group 1-1, *n*=65)	(group 1-2, *n*=65)	(group 2, *n*=14)	(group 3, *n*=20)		
Gestational age at measurement (weeks)	28.3±0.9	37.5±0.6	28.5±2.6	36.8±2.0	<0.001	1-1 *vs*. 1-2, 1-1 *vs*. 3, 1-2 *vs*. 2, 2 *vs*. 3
PlGF <5th percentile (*n* (%))	2 (3.1)	2 (3.1)	13 (93)	11 (55)	<0.001	all pairs but not 1-1 *vs*. 1-2
sFlt-1 ≥95th percentile (*n* (%))	0 (0.0)	4 (6.2)	14 (100)	12 (60)	<0.001	all pairs but not 1-1 *vs*. 1-2
sFlt-1/PlGF ≥95th percentile (*n* (%))	2 (3.1)	3 (4.6)	14 (100)	18 (90)	<0.001	1-1 *vs*. 2, 1-1 *vs*. 3, 1-2 *vs*. 2, 1-2 *vs*. 3

PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

specimens from 291 pregnant women at around 20 and 28 weeks, including these 8 women who developed preeclampsia; 3 of these 8 cases were already reported in the previously published article (*22*).

Severe preeclampsia was defined as a systolic blood pressure (SBP) ≥160 mmHg or a diastolic blood pressure (DBP) ≥110 mmHg on 2 occasions recorded 24-h apart in association with proteinuria (≥2 g/day) (*3*). Preeclamptic women were divided into two groups: a group with early onset preeclampsia (appearance of both hypertension and proteinuria <32 weeks of gestation) and a group with late onset preeclampsia (≥32 weeks) (*3*). We defined a growth-restricted infant as one having a birth weight below the 10th percentile for the Japanese population (*24*). The multiple of the median (MoM) for birth weight was also calculated as $(A - B)/B$ (where A is the infant's birth weight, and B is the median birth weight at the corresponding gestational week), representing the degree of deviation from the median.

Blood samples were obtained immediately or not more than 2 weeks after the onset of the clinical manifestation of preeclampsia for the first group, and they were taken both before and after the manifestation of preeclampsia for the second group. Samples were centrifuged at 4°C at 2,500 rpm for 15 min. Serum aliquots were stored at −70°C until being assayed. Enzyme-linked immunosorbent assays for human PlGF and sFlt-1 (R&D Systems, Minneapolis, USA) were performed in duplicate, as previously described (*22*). The minimal detectable doses in the assays for PlGF and sFlt-1 were 7 and 5 pg/ml, respectively. The inter- and intra-assay coefficients of variation were 10.9% and 5.6% for PlGF, and 7.6% and 3.3% for sFlt-1, respectively.

We calculated the standard deviation scores (SDS) of $\log_{10}$PlGF, $\log_{10}$sFlt-1, and $\log_{10}$(sFlt-1/PlGF) as $(A - B)/C$ (where A is the raw value, B is the mean, and C is the SD). We defined low PlGF and high sFlt-1 as a PlGF concentration <5th percentile and an sFlt-1 concentration ≥95th percentile

of the reference values at the given gestational week, respectively (*22*). The results are presented as the mean±SD. For PlGF, sFlt-1 and the sFlt-1/PlGF ratio, statistical testing was conducted after logarithmic transformation. For multiple group comparisons, the homogeneity of variance was assessed using the Levene test. We used one way analysis of variance (ANOVA) to test for the overall differences among groups, followed by Gabriel's method to compare the separate group means when the Levene test was not significant, and followed with the Dunnett-T3 method to compare those when the Levene test was significant. The χ^2 test or Fisher's exact test was used to compare the incidence of the discrete variables. The correlation between the two continuous variables was assessed by regression analysis. All analyses were performed with the SPSS software package (version 13.0J for Windows). A level of $p<0.05$ was considered statistically significant.

Results

Group 1: Retrospective Observation (or Data after the Manifestation of Preeclampsia)

Women with early onset preeclampsia gave birth to infants approximately 7 and 10 weeks earlier than those with late onset preeclampsia or the normotensive women, respectively (Table 1). Although women with both the early and late onset disease gave birth to growth restricted infants more frequently than the normotensive women, infants whose mothers had early onset preeclampsia had significantly lower MoM birth weights compared to those whose mothers had late onset preeclampsia and those with mothers in the normotensive control group. There were no significant differences in SBP, DBP, or the frequency of severe proteinuria between the early and the late onset preeclampsia.

Serum samples of early and late preeclampsia were measured at 28±3 and 37±2 weeks of gestation, respectively (Table 2, Fig. 1). Both types of preeclampsia affected the PlGF and sFlt-1 levels. Women with early onset preeclampsia showed lower levels of PlGF, higher levels of sFlt-1 and higher levels of the sFlt-1/PlGF ratio than the normotensive controls at 28 weeks of gestation ($p<0.001$ for all). Women with late onset preeclampsia also showed lower levels of PlGF, higher levels of sFlt-1, and higher levels of the sFlt-1/PlGF ratio than the normotensive controls at 37 weeks of gestation ($p<0.001$ for all). Besides, at a glance, we can see that the alterations in mean PlGF levels, sFlt-1 levels, and sFlt-1/PlGF ratio in women with early onset severe preeclampsia may be much pronounced than those with late onset severe preeclampsia. The $\log_{10}$PlGF SDS, $\log_{10}$sFlt-1 SDS and $\log_{10}$(sFlt-1/PlGF) SDS were all significantly different between women with early and late onset severe preeclampsia (Table 3). When plotting the values of PlGF and sFlt-1 through the pregnancy weeks on the reference value curves (*22*), 93% and 100% of the values of PlGF and sFlt-1, respectively, deviated from this curve in the early onset type; conversely, in the late onset type, only 55% and 60% of the values of PlGF and sFlt-1, respectively, deviated from this curve; the remaining 45% and 40% of the values of PlGF and sFlt-1, respectively, were within the normal value (Table 4, Fig. 2). All these data indicate that alterations in PlGF and sFlt-1 levels after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. However, the abnormal rates of the sFlt-1/PlGF ratio were not significantly different between women with early and those with late onset preeclampsia (100% *vs*. 90%), indicating that alterations in sFlt-1/PlGF ratios after the onset of preeclampsia are not more pronounced than those in PlGF

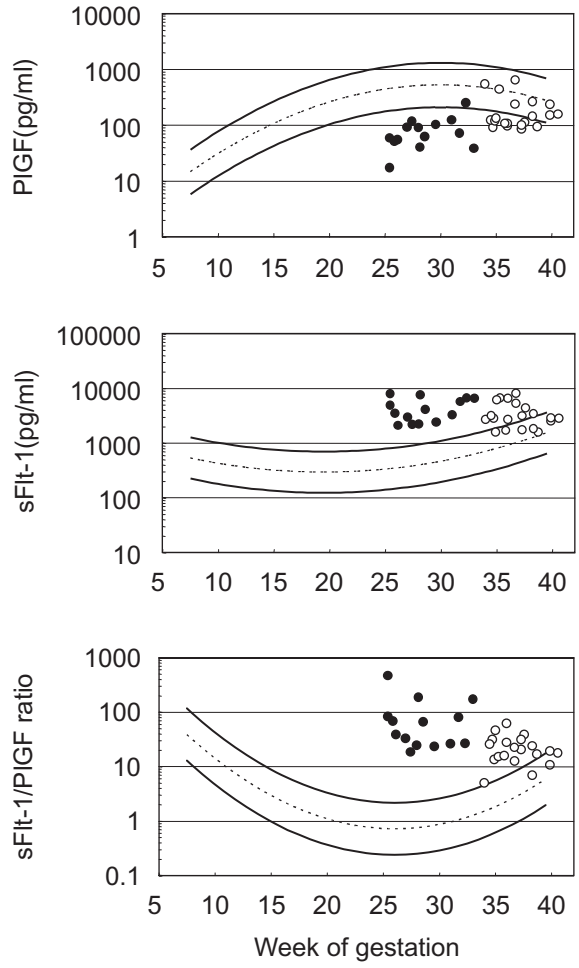

Fig. 2. *Serum levels of PlGF and sFlt-1, and the sFlt-1/PlGF ratio immediately after the clinical manifestation in women with early and late onset severe preeclampsia. The solid curves represent the 5th and 95th percentiles of the reference values, and the dotted curves represent the mean. Closed circles, data after the onset of preeclampsia in women with early onset preeclampsia (*n = *14). Open circles, data after the onset of preeclampsia in women with late onset preeclampsia (*n = *20).*

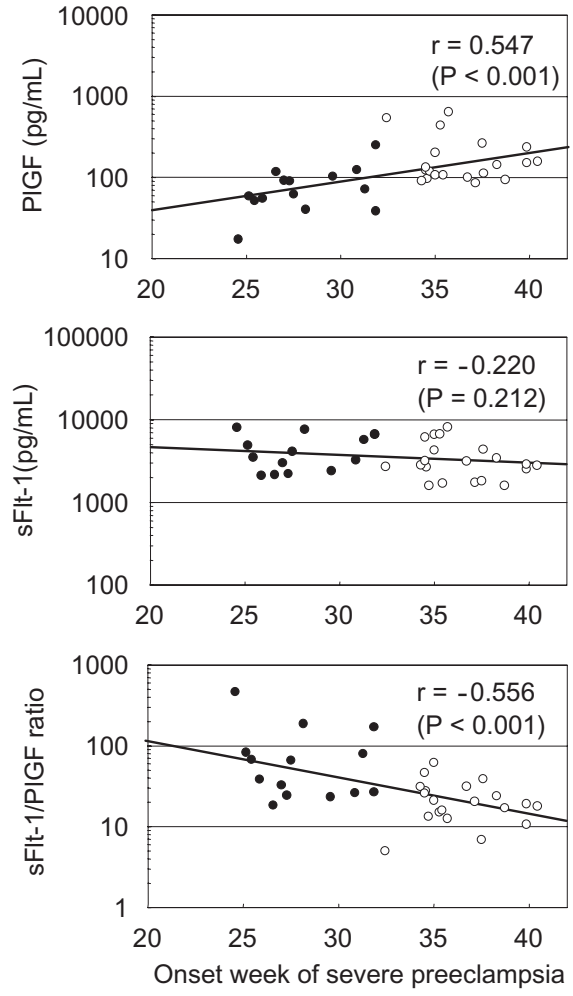

Fig. 3. *Relationship among the week of onset of severe preeclampsia, the serum PlGF level, the serum sFlt-1 level, and the sFlt-1/PlGF ratio immediately after the clinical manifestation. Closed circles, women with early onset severe preeclampsia; open circles, women with late onset preeclampsia. The serum levels of PlGF increased with an increase of the week of onset of severe preeclampsia (*r = *0.547,* p < *0.001). The serum levels of sFlt-1 were not correlated with the week of onset of severe preeclampsia (*r = *−0.220,* p = *0.212). The sFlt-1/PlGF ratios decreased with an increase of the week of onset of severe preeclampsia (*r = *−0.556,* p < *0.001).*

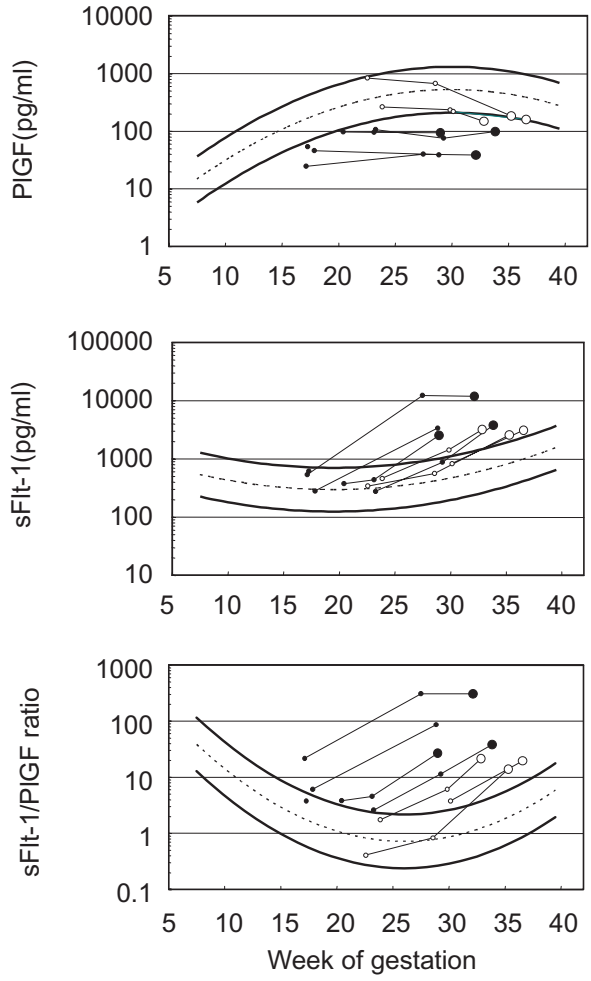

Fig. 4. *Serum levels of PlGF and sFlt-1, and the sFlt-1/PlGF ratio before and after the clinical manifestation in women with early onset preeclampsia and late onset preeclampsia who were prospectively observed from mid-gestation. The solid curves represent the 5th and 95th percentiles of the reference values, and the dotted curves represent the mean. Closed circles, data in women with early onset preeclampsia (*n = *5): large closed circles, data after the clinical manifestation; small closed circles, data before the clinical manifestation. Open circles, data in women with late onset preeclampsia (*n = *3): large open circles, data after the clinical manifestation; small open circles, data before the clinical manifestation. The values from the same women are indicated by connected lines between the circles.*

and sFlt-1 levels.

The onset time also affected the PlGF and sFlt-1 levels (Fig. 3). Combining all the data (early and late), linear regression analyses indicated that $\log_{10}$PlGF (r=0.547, p<0.001) and $\log_{10}$(sFlt-1/PlGF) (r=−0.556, p<0.001), but not $\log_{10}$sFlt-1 (r=−0.220, p=0.212), were correlated with the onset week of preeclampsia. The lower the $\log_{10}$PlGF and the higher the $\log_{10}$(sFlt-1/PlGF), the earlier the preeclampsia occurred.

Group 2: Prospective Observation (or Data before the Manifestation of Preeclampsia)

We measured the serum levels of PlGF and sFlt-1 in 7 women at around 20 weeks of gestation before the occurrence of severe preeclampsia (Fig. 4). In all 5 women with early onset preeclampsia, the PlGF levels were low, whereas in 2 women

with late onset preeclampsia, the PlGF values were ≥5th percentile, making a striking contrast (Fisher exact test: $p=0.048$). However, the sFlt-1 levels at this stage were all <95th percentile in women with both early and late onset preeclampsia. The sFlt-1/PlGF ratios at this stage were high in 80% of the women with early onset preeclampsia, whereas, those in 2 women with late onset preeclampsia were <95th percentile. The difinite difference for the serum level of PlGF between women with early and late onset severe preeclampsia at around 20 weeks was also seen at around 28 week. In 6 women (3 early onset and 3 late onset), blood samples were collected at around 28 weeks of gestation before the clinical manifestation of preeclampsia. The PlGF levels were decreased in all 3 cases with the early type, whereas the PlGF levels in women with the late type were all ≥5th percentile. However, the sFlt-1 levels were increased in 67% and 33% of the women with early and late onset preeclampsia, respectively; the sFlt-1/PlGF ratios were increased in 100% and 67% of women with early and late onset preeclampsia, respectively. Thus, the sFlt-1/PlGF ratios at around 28 weeks of gestation before the onset of severe preeclampsia were increased in 83% of cases; whereas the sFlt-1 levels were increased in only half the cases at the same time point.

Discussion

In the present study, we have made three important observations. First, after the onset of the disease, almost all women with early onset severe preeclampsia showed deviation of the distributions of serum PlGF and sFlt-1 levels from the normal reference value curve, whereas only half the women with the late onset type showed such deviation; the remaining half showed normal values. Second, serum $\log_{10}$PlGF and $\log_{10}$(sFlt-1/PlGF), but not $\log_{10}$sFlt-1, were correlated with the week of onset of severe preeclampsia. Third, before the onset of the disease, the serum PlGF levels in women with early onset severe preeclampsia were low, whereas those in women with late onset type were within the normal range.

Women with early onset preeclampsia showed more pronounced alterations in the PlGF and sFlt-1 levels after the onset of preeclampsia than those with the late onset type. Their distributions of PlGF and sFlt-1 levels did not overlap with the normal reference values, except in one case. These data suggest that decreased PlGF concomitant with increased sFlt-1 may be a phenomenon highly characteristic of early onset preeclampsia. Many previous reports (*8–16*) have indicated decreased PlGF and increased sFlt-1 in women with preeclampsia, but most of these reports did not take the onset time (early *vs*. late) into account, with a few exceptions (*11*, *14*). Chaiworapongsa *et al*. (*14*) showed the tendency for the alterations of the sFlt-1 levels after the onset of preeclampsia to be more pronounced in early onset preeclampsia compared to the late onset type (*14*). We focused our attention on the distribution of PlGF and sFlt-1 in the early and late onset type in this study. The data obtained here basically agree with those obtained by Chaiworapongsa *et al*. (*14*). Quite recently, while we were revising this article, one report (*25*) appeared that was targeted at the determination of the relationship between PlGF and sFlt-1 and early *vs*. late onset preeclampsia. Although after the onset of preeclampsia, women with both early and late preeclampsia showed lower PlGF and higher sFlt-1 levels than the normotensive controls, the alterations of these two substances were more pronounced in the early type than the late type. In this report (*25*), the researchers dealt with not only severe preeclampsia, but also moderate cases, and 34 weeks, not 32 weeks, was adopted for the definition of early *vs*. late. Even with these differences in the study population, our present data showed complete agreement with those in this recent study (*25*).

The serum $\log_{10}$PlGF and $\log_{10}$(sFlt-1/PlGF), but not $\log_{10}$sFlt-1, were correlated with the week of onset of severe preeclampsia. The higher the $\log_{10}$(sFlt-1/PlGF), the earlier the preeclampsia occurred. This observation suggests the possible clinical usefulness of $\log_{10}$(sFlt-1/PlGF). Although some previous researchers suggested that low serum PlGF (*11*, *26–28*) and high sFlt-1 (*11*, *14*, *29–31*) before the onset of the disease may be potentially useful for predicting the subsequent development of preeclampsia, they did not make mention of the sFlt-1/PlGF ratio (*11*, *14*, *26–31*). The sFlt-1/PlGF ratios at around 28 weeks of gestation before the onset of severe preeclampsia were increased in 83% of cases; whereas the sFlt-1 levels were increased in only half the cases at the same time point. Therefore, the serum sFlt-1/PlGF ratio may be one candidate for the prediction of the future development of both early and late onset preeclampsia. Further extensive study, however, may be needed to determine its clinical usefulness (*32*).

We believe that the change of PlGF may precede that of sFlt-1 in early onset preeclampsia for the following two reasons. First, the data obtained from the first study group showed that the $\log_{10}$PlGF, and not $\log_{10}$sFlt-1, was closely associated with the week of onset of preeclampsia, including the early type. The lower the $\log_{10}$PlGF, the earlier the preeclampsia occurred. Second, the data obtained from the second study group indicated that before the onset of the disease at around 20 weeks, all women with the early type showed low levels of PlGF, whereas none showed high levels of sFlt-1. The increase in sFlt-1 followed the decrease in PlGF, and finally, the concomitant decrease in PlGF and increase in sFlt-1 occurred in almost all cases of early onset preeclampsia. Although at present we do not know the biological/pathophysiological mechanism responsible for this phenomenon, it is still worthy of note.

Although at present we do not know the cause-effect relationship between the decreased PlGF/increased sFlt-1 and the occurrence of preeclampsia, placental hypoxia in this disease may play a role. A previous study indicated that one morphological characteristic of the preeclamptic placenta is an impaired trophoblastic invasion to the maternal spiral arteriole/placental bed (*33*), which may disturb the uterine arterial/

placental circulation, leading to the utero-placental hypoxia (*34*). Hypoxia has been shown to induce a decreased production of PlGF and an increased production of sFlt-1 in placental trophoblast cells (*35*, *36*). It was also reported that the abnormalities of the uterine artery flow velocity waveforms in mid-gestation were more frequently observed before the onset of preeclampsia in women with early onset preeclampsia than those with the late onset type (*37*). In the present study, we observed that the alteration of PlGF and sFlt-1 was exaggerated in the early type compared to the late type. Further study is needed to determine whether these two types of diseases have different pathophysiology/etiology or can be considered parts of a single clinical entity with a continuous spectrum. A large clinical study is underway in our laboratory to clarify the pathophysiological meaning and its clinical application of PlGF and sFlt-1 in women with preeclampsia, especially the early onset type preeclampsia. Although this study was limited by the small sample size and potentially biased study populations, the data obtained here should be useful for understanding the pronounced alterations of the PlGF levels before and after the onset of preeclampsia in women with early onset severe preeclampsia

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