Original Article

Alteration of Serum Soluble Endoglin Levels after the Onset of Preeclampsia Is More Pronounced in Women with Early-Onset

Chikako HIRASHIMA[1),*], Akihide OHKUCHI[1),*], Shigeki MATSUBARA[1)], Hirotada SUZUKI[1)], Kayo TAKAHASHI[1)], Rie USUI[1)], and Mitsuaki SUZUKI[1)]

It has been established that serum soluble endoglin (sEng) increases in women with preeclampsia. However, sEng levels have not been evaluated using a normal reference value specific to each gestational age. First, we established the normal reference value for sEng using 85 pregnant controls without preeclampsia, from whom serum samples were collected three times at 20–23, 27–30, and 36–38 weeks of gestation. Second, we evaluated the serum sEng levels after the onset of preeclampsia in 56 preeclamptic patients. In three women (3.5%) with normal pregnancies, sustained high sEng levels (>15 ng/mL) were observed. We calculated the reference value for sEng using the remaining 82 normal controls. The $\log_{10}$sEng was almost normally distributed at each gestational week during 20–38 weeks, and the mean $\log_{10}$sEng was represented as a quadratic curve of gestational week. The SD of $\log_{10}$sEng was represented as a linear equation of gestational week. The mean $\log_{10}$sEng significantly and gradually increased from 20–23 weeks to 27–30 weeks of gestation and then rapidly increased at 36–38 weeks of gestation. Ninety-three percent of preeclamptic women showed sEng≥95th percentile of the reference value. The $\log_{10}$sEng levels and the SD score (SDS) of $\log_{10}$sEng in women with early-onset preeclampsia (onset<32 weeks of gestation) were significantly higher than those in women with late-onset preeclampsia (onset≥32 weeks of gestation) (1.97±0.23 *vs.* 1.78±0.28, 9.94±2.61 *vs.* 4.47±2.06, respectively). In conclusion, alteration of serum sEng levels after the onset of preeclampsia was more pronounced in women with early-onset preeclampsia compared to those with late onset. (*Hypertens Res* 2008; 31: 1541–1548)

***Key Words*: soluble endoglin, early-onset preeclampsia, late-onset preeclampsia, pregnancy**

Introduction

Preeclampsia is characterized by the appearance of both hypertension and proteinuria during pregnancy. It occurs in about 2.5% of pregnant Japanese women (*1*) and results in substantial maternal and neonatal morbidity and mortality (*2*, *3*). Although some types of gene polymorphisms are associated with the occurrence of preeclampsia (*4*–*6*), the pathophysiology of preeclampsia remains uncertain despite significant research efforts.

Followed by the discovery of the increase of serum soluble fms-like tyrosine kinase 1 (sFlt1) before the occurrence of preeclampsia (*7*) and the discovery of the generation of preeclampsia-like symptoms after the administration of an sFlt1-virus vector to pregnant rats (*8*), a number of studies have consistently shown that an excess of sFlt1 is associated with preeclampsia (*9*, *10*). However, almost half of all women with

From the [1)]Department of Obstetrics and Gynecology, Jichi Medical University School of Medicine, Shimotsuke, Japan.

*These authors contributed equally to this work.

This work was supported by research grants (18591809) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Address for Reprints: Akihide Ohkuchi, M.D., Ph.D., Department of Obstetrics and Gynecology, Jichi Medical University School of Medicine, 3311–1 Yakushiji, Shimotsuke 329–0498, Japan. E-mail: okuchi@jichi.ac.jp

Received January 12, 2008; Accepted in revised form April 27, 2008.

late-onset severe preeclampsia (*9*) or almost half with term preeclampsia (*11*) displayed an sFlt1 value within the normal reference range (*9*, *11*), suggesting that other factors may be involved in the occurrence of preeclampsia.

Most recent studies have shown that soluble endoglin (sEng) plays an important role in the pathophysiology of preeclampsia (*12*, *13*). Endoglin (Eng) is a component of the receptor complex for transforming growth factor (TGF)-β, and it interacts efficiently with TGF-β (*14*). Preeclamptic placentas overexpress *sFlt1* and *Eng* mRNAs as well as sFlt1 and Eng proteins (*12*). Furthermore, recombinant sEng and sFlt1 induce a phenotype in pregnant rats similar to the clinical features of preeclampsia in humans (*12*). sEng inhibits TGF-β1 signaling and blocks TGF-β1–mediated nitric oxide synthetase activation in endothelial cells (*12*), suggesting that the increase in blood pressure in women with preeclampsia may be induced by the increase of circulating sEng concentration. A number of studies have reported that circulating sEng levels in women with preeclampsia after the onset of clinical disease are increased compared with those in normal pregnant women (*15–17*).

To our knowledge, the reference range of serum sEng concentration was not established in reported clinical studies on sEng in women with preeclampsia (*12*, *13*, *15–17*). Therefore, the deviation from the mean sEng levels in women with preeclampsia has not been analyzed. In our previous studies, we established reference values of serum sFlt1, PlGF, and sFlt1:PlGF ratio and found that almost all women with early-onset severe preeclampsia showed large deviations in the distributions of serum sFlt1 and PlGF levels from the normal reference value curves, whereas only half of the women with late-onset type showed such deviation (*9*). Because it has been reported that there is a significant correlation between sEng and sFlt1 levels (*15*, *17*), and that the difference in sEng levels between women with preterm delivery and matched controls was much larger than that between women with term delivery and matched controls (*13*), we hypothesized that the deviation in sEng levels from the normal reference value curve after the onset of preeclampsia, like the alterations in the sFlt1 level after the onset of preeclampsia (*9*), may be more pronounced in women with early-onset compared to late-onset preeclampsia.

In this study, we tried to construct a reference curve representing the 90% confidence interval (CI) for serum sEng levels throughout the second half of pregnancy. The second purpose of this study was to compare the serum sEng levels in women with early- and late-onset preeclampsia using the newly obtained reference value.

Methods

Subjects and Procedures

We obtained written informed consent from all women as well as the approval of the Ethics Committee of our institute. The study subjects consisted of two groups: one for constructing a reference range of serum sEng according to gestational age and the other for evaluating the serum sEng levels after the clinical manifestation of early- and late-onset preeclampsia. The study subjects were all Japanese women with singleton pregnancies. The first group consisted of 85 pregnant controls without preeclamspsia, from whom blood samples were collected three times at 20–23, 27–30, and 36–38 weeks of gestation; the samples were serially collected between April 2004 and April 2007. The second group consisted of 56 referred women with preeclampsia, from whom blood samples were collected as soon as possible after admission to our hospital due to the clinical manifestation of preeclampsia, between April 2000 and April 2007.

Blood samples were centrifuged at 4°C at 2,500 rpm for 15 min. Samples were stored at −20°C until use. Enzyme-linked immunosorbent assays (ELISAs) for human endoglin (DNDG00, R&D Systems, Minneapolis, USA) were performed in duplicate according to the manufacturer's instructions. The minimal detectable dose in the assays for sEng was 7 pg/mL, and the intraassay and interassay coefficients of variation for sEng were 3.2% and 6.5%, respectively.

We previously defined a reference value of serum sFlt1 during pregnancy (*10*) and previously measured the levels of serum sFlt1 in 34 preeclamptic women, 29 of whom had severe preeclampsia and had already been studied in our previous paper (*9*); however, five cases of mild preeclampsia were used for the first time in this study. We showed the difference of serum sFlt1 between women with early-onset preeclampsia and those with late-onset and analyzed the relationship between sEng and sFlt1 in women with preeclampsia.

Definitions of Preeclampsia and a Small-for-Gestational-Age Infant

We defined preeclampsia according to the definition and classification of pregnancy-induced hypertension (PIH) (2004) of the Japan Society for the Study of Hypertension in Pregnancy (JSSHP) (*18*). In brief, preeclampsia was defined as hypertension with proteinuria occurring after the 20th week of gestation. Superimposed preeclampsia was defined as chronic hypertension diagnosed prior to pregnancy or prior to the 20th week of gestation, with proteinuria emerging afterward. Superimposed preeclampsia was included in the category of preeclampsia in this study. Proteinuria was defined as 300 mg/d from 24 h urine collection. If only test tape was available, repeated semi-quantitative test results of 1+, which represented 30 mg/dL of protein or more, were considered to constitute a positive result. We defined preeclampsia in subjects whose systolic blood pressure (SBP) was 140–159 mmHg and/or whose diastolic blood pressure (DBP) was 90–109 mmHg as mild preeclampsia, and we defined preeclampsia in subjects whose SBP was equal to or over 160 mmHg and/or DBP was equal to or over 110 mmHg as severe preec-

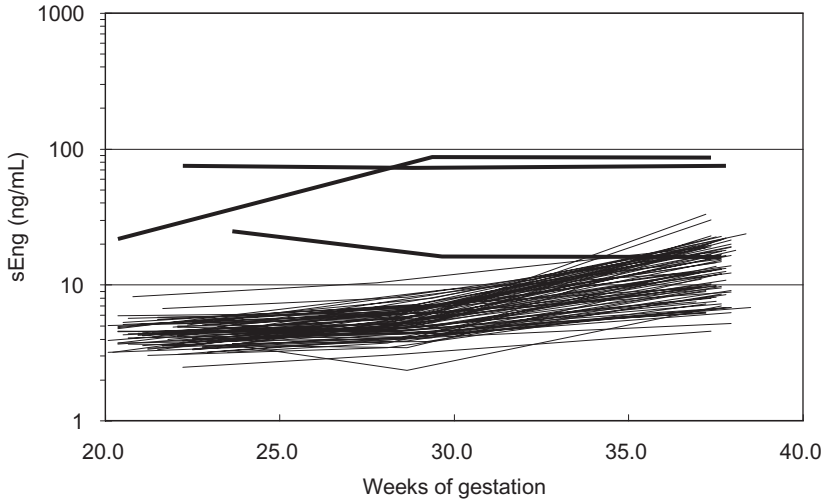

Fig. 1. *Individual changes in the serum concentration of soluble endoglin (sEng) at 20–23 weeks, 27–30 weeks, and 36–38 weeks of gestation. Blood samples were collected from 85 pregnant control women without preeclamspsia. Thick lines represent three women who showed sustained high sEng levels (> 15 ng/mL) during 20–38 weeks of gestation. Thin lines represent the remaining 82 women, the majority of whom showed constant increases in sEng during 20–38 weeks of gestation.*

lampsia. We defined preeclampsia whose onset was earlier than 32 weeks of gestation as early-onset preeclampsia, and we defined preeclampsia whose onset was at 32 weeks of gestation or later as late-onset preeclampsia. We diagnosed women with the following three findings—serum lactate dehydrogenase levels > 1.5-fold the upper limit in our institute, serum aspartate aminotransferase levels > 1.5-fold the upper limit in our institute, and low platelet (platelet counts $< 10 \times 10^4/\mu L$)—as having hemolysis, elevated liver enzyme, and low platelet (HELLP) syndrome, respectively. We defined an small-for-gestational age (SGA) infant as one having a birth weight below the 10th percentile for the Japanese population (*19*). The multiple of the median (MoM) for birth weight was 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.

Table 1. Clinical Profiles of the Normal Pregnant Controls without Preeclampia

Total numbers	82
Age (years)	31.5±4.4
Nullipara	41 (60%)
SBP at 16–23 weeks	115±12
DBP at 16–23 weeks	68±9
SBP at 26–29 weeks	114±11
DBP at 26–29 weeks	68±8
SBP at 36–38 weeks	116±11
DBP at 36–38 weeks	70±8
Gestational age at delivery (weeks)	39.6±1.1
Preterm delivery	0 (0.0%)
Birth weight (g)	3,098±352
Low birth weight infants	4 (4.9%)
Small-for-gestational-age infants	5 (6.1%)
Cesarean section	14 (17%)

SBP, systolic blood pressure; DBP, diastolic blood pressure.

Statistical Analysis

We calculated the standard deviation scores (SDS) of $\log_{10}$sEng and $\log_{10}$sFlt1 as $(A - B)/C$ (where A is the raw value, B is the mean, and C is the SD). We defined high sEng and high sFlt1 as concentrations ≥95th percentile of the reference values at a given gestational week. The results are presented as the mean±SD. For both sEng and sFlt1, statistical testing was conducted after logarithmic transformation because it was proven that the data for serum sEng and sFlt1 after logarithmic transformation in pregnant controls closely resembled normal distributions using normal proportion-proportion (P-P) plots and the Kolmogonov-Smirnov test. The unpaired *t*-test or paired *t*-tests were used to compare two continuous variables if appropriate. The χ^2 test or Fisher's exact test was used to compare the categorical data. The fitted curve estimation was performed using built-in software within the SPSS software package (version 13.0J for windows). A level of $p < 0.05$ was considered statistically significant.

Results

In 85 women, serial serum data were examined throughout the second half of pregnancy. Individual changes in the serum

Table 2. The Level of the Logarithm of Serum sEng in 82 Women Who Were Serially Examined in the Three Periods

	20–23 weeks (period 1)	27–30 weeks (period 2)	36–38 weeks (period 3)	Significant pairs among periods
Weeks of gestation	21.8±1.2	28.9±0.7	37.5±0.4	all pairs
log_{10}sEng	0.628±0.067	0.729±0.110	1.099±0.192	all pairs

log_{10}sEng, logarithm of soluble endoglin.

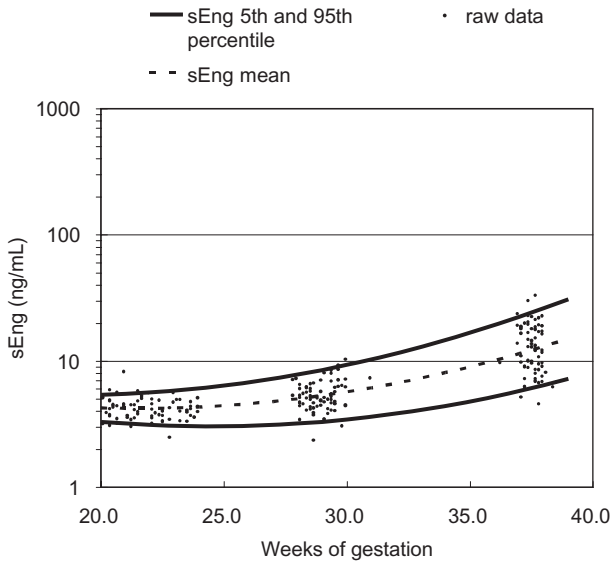

Fig. 2. *Quadratic curves representing the mean and the 5th and 95th percentiles for the serum level of soluble endoglin (sEng) from 20 to 38 weeks of gestation. The solid curves represent the 5th and 95th percentiles of the reference values, and the dotted curve represents the mean. Small dots represent the 82 women examined in this study. The mean at each week could be represented by a quadratic curve, and the SD at each week could be represented by a linear function. Therefore, the 95th percentile of log_{10}sEng was represented by a quadratic curve ($y = 0.0018\,x^2 - 0.0663\,x + 1.342$, y: 95th percentile of log_{10}sEng, x: weeks of gestation).*

concentration of sEng at 20–23 weeks, 27–30 weeks, and 36–38 weeks of gestation were plotted (Fig. 1). Three women (3.5%) showed sustained high sEng levels (>15 ng/mL) during 20–38 weeks of gestation, although these three women showed neither hypertension nor proteinuria during the pregnancy. The majority of the remaining 82 women showed constant increases in sEng levels during 20–38 weeks of gestation. The characteristics of the remaining 82 women are shown in Table 1. All women gave birth at ≥37 weeks of gestation, and the rate of SGA infants was 6.1%. The levels of blood pressure during pregnancy were all within normal ranges. The average of log_{10}sEng levels in the 82 normal pregnant women significantly and gradually increased from 20–23 weeks to 27–30 weeks, and increased rapidly at 36–38 weeks of gestation (Table 2).

In three cases for which data were excluded, high values in the 20–23 weeks of gestation did not regress toward the mean in the 27–30 weeks of gestation, suggesting that these three values did not occur incidentally but held high value constantly. Using normal P-P plots, we checked the distribution of sEng levels at 20–23, 27–30, and 36–38 weeks of gestation, and we found that the data after logarithmic transformation closely distributed normally. In addition, the data distribution of log_{10}sEng in 82 cases after excluding the three cases more closely resembled a normal distribution at 20–23 weeks, at 27–30 weeks, and at 36–38 weeks of gestation compared with the data distribution of log_{10}sEng in 85 cases, including the three cases. Therefore, we calculated the reference value for serum sEng using the 82 normal controls after excluding three women with sustained high sEng levels. The distribution of log_{10}sEng during the second half of pregnancy could be represented by a quadratic curve ($y = 0.0018\,x^2 - 0.0773\,x + 1.454$, y: mean log_{10}sEng, x: weeks of gestation, $p < 0.001$). We hypothesized that the middle value at each week on the quadratic curve was equal to the mean of log_{10}sEng at each week because the data distribution of log_{10}sEng at each week were close to normal distribution using normal P-P plots when the sample size at each week was at least 10. As the results of Levene statistics of log_{10}sEng at 20–38 weeks were significant ($p < 0.001$), and as the SD tended to increase according to gestational week, we tried to find the best fitted function by which the SD at each week could be represented. We found that the SD at each week could be represented by a linear function ($y = 0.00667\,x - 0.0682$, y: SD of log_{10}sEng, x: weeks of gestation, $p < 0.001$). Thus, the 95th percentile of log_{10}sEng was defined as mean log_{10}sEng + 1.645 SD ($y = 0.0018\,x^2 - 0.0663\,x + 1.342$, y: 95th percentile of log_{10}sEng, and x: weeks of gestation) (Fig. 2).

Women with early-onset preeclampsia gave birth to infants approximately 7 weeks earlier than those with late-onset preeclampsia (Table 3). Women with early-onset preeclampsia gave birth to an SGA infant more frequently than women with late-onset, and they had significantly lower MoM birth weights compared with those with late-onset. There were no significant differences in the frequency of nulliparity, severe preeclampsia, HELLP syndrome, or the levels of SBP/DBP, although the frequency of urinary protein ≥2 g/d in women with early-onset preeclampsia was higher than that in women

Table 3. Maternal and Infantile Characteristics in Women with Early and Late Onset Preeclampsia

	All women wtih preeclampsia ($n=56$)	Onset of preeclampsia		p value
		Early onset ($n=25$)	Late onset ($n=31$)	
Age (years)	31.8±5.6	32.7±4.8	31.1±6.2	0.312
Body height (m)	1.58±0.06	1.59±0.06	1.58±0.05	0.522
Prepregnancy body weight (kg)	57.4±11.1	55.3±10.8	59.1±11.3	0.204
Prepregnancy body mass index (kg/m^2)	23.0±4.3	21.9±3.8	23.8±4.6	0.105
Nullipara	38 (68%)	16 (64%)	22 (71%)	0.774
Gestational age at onset of preeclampsia (weeks)	32.2±4.3	28.2±2.6	35.5±1.8	<0.001
Gestational age at delivery (weeks)	33.8±3.9	30.3±3.1	36.5±1.6	<0.001
Severe preeclampsia	50 (89%)	24 (96%)	26 (84%)	0.210
HELLP syndrome	3 (5.4%)	1 (4.0%)	2 (6.5%)	1.000
Maximum SBP (mmHg)	172±28	180±19	166±33	0.058
Maximum DBP (mmHg)	108±13	110±15	105±12	0.154
Urinary protein >2 g/d (%)	30 (54%)	18 (72%)	12 (39%)	0.017
Infant birth weight (g)	1,761±776	1,049±380	2,336±474	<0.001
Infant birth weight (MoM)	−0.150±0.174	−0.259±0.103	−0.062±0.171	<0.001
Small-for-gestational-age infants	30 (54%)	21 (84%)	9 (29%)	<0.001

HELLP, hemolysis, elevated liver enzyme, and low platelet; SBP, systolic blood pressure; DBP, diastolic blood pressure; MoM, the multiple of the median.

Table 4. Serum Levels of sEng in Women with Early and Late Onset Preeclampsia

	All women with preeclampsia ($n=56$)	Onset of preeclampsia		p value
		Early onset ($n=25$)	Late onset ($n=31$)	
Gestational age at measurement (weeks)	33.1±4.1	29.2±2.8	36.2±1.5	<0.001
$\log_{10}$sEng (pg/mL)	1.87±0.27	1.97±0.23	1.78±0.28	<0.001
$\log_{10}$sEng SDS	6.92±3.58	9.94±2.61	4.47±2.06	<0.001
sEng≥95th percentile	52 (93%)	25 (100%)	27 (87%)	0.120

sEng, soluble endoglin; SDS, SD score.

with late-onset.

Both early- and late-onset preeclampsia affected the serum sEng levels. Women with early-onset preeclampsia showed higher levels of $\log_{10}$sEng than women with late-onset (Table 4). Moreover, it clearly appears that the deviation of sEng level from the normal reference curve in women with early-onset preeclampsia may be more pronounced than that in women with late-onset (Fig. 3). This finding was supported statistically by the almost twofold difference in the average $\log_{10}$sEng SDS between the two groups (Table 4). The incidence rate of sEng≥95th percentile in women with early-onset preeclampsia was 100%, and that in women with late-onset was 87%, suggesting that the increased sEng levels may be associated with the clinical symptoms, such as hypertension or proteinuria, in most women with preeclampsia, especially in women with early-onset.

In women with early-onset preeclampsia, the levels of $\log_{10}$sEng in mild type ($n=1$) and severe type ($n=24$) were almost the same (Fig. 3) (mean levels: 1.99 *vs*. 1.97±0.23). In women with late-onset preeclampsia, those with mild type ($n=5$) and severe type ($n=26$) were not significantly different (mean levels: 1.62±0.33 *vs*. 1.81±0.27, $p=0.177$). Thus, serum levels of sEng may not be significantly associated with the severity of preeclamspsia.

We analyzed the effect of the onset of preeclampsia on the levels of sFlt1 in 34 women. Women with early-onset preeclampsia showed higher levels of $\log_{10}$sFlt1 than women with late-onset (Table 5). There were almost twofold differences of the average $\log_{10}$sFlt1 SDS between the two groups (Table 5). The incidence rate of sFlt1≥95th percentile in women with early-onset preeclampsia was 100%, and that in women with late-onset was 65%. In addition, the incidence rate of both sFlt1≥95th percentile and sEng≥95th percentile in women with early-onset preeclampsia was 100%, whereas that in women with late-onset was 60%. Thus, alteration of both sFlt1 and sEng was a characteristic of early-onset preeclampsia. There was a positive correlation between the levels of $\log_{10}$sFlt1 and $\log_{10}$sEng in 34 preeclamptic women

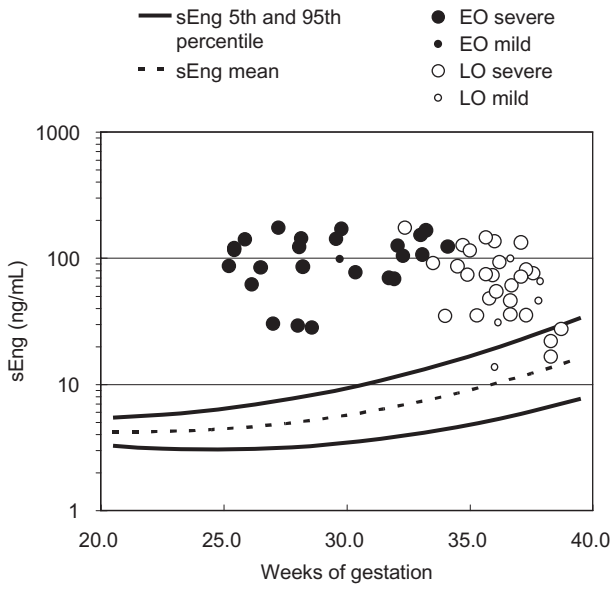

Fig. 3. *Serum levels of soluble endoglin (sEng) in 56 preeclamptic women. Blood samples were collected as soon as possible after admission to our hospital due to the clinical manifestation of preeclampsia. The solid curves represent the 5th and 95th percentiles of the reference values, and the dotted curve represents the mean. Large closed circles, data in women with severe early-onset (EO) preeclampsia (n = 24); small closed circles, data in women with mild EO preeclampsia (n = 1); large open circles, data in women with severe late-onset (LO) preeclampsia (n = 26); and small open circles, data in women with mild LO preeclampsia (n = 5). The serum level of sEng was not significantly affected by the severity of preeclamspsia.*

($r = 0.581$, $p < 0.001$).

In normal controls, there was weak inverse correlation between $\log_{10}$sEng at 20–23 weeks and SBP at 16–23 weeks ($r = -0.273$, $p = 0.013$), although there were no correlations between other combinations. In women with preeclampsia, the $\log_{10}$sEng levels correlated with neither SBP nor DBP ($r = -0.105$ and $r = -0.036$, respectively).

Discussion

In the present study, we established the normal reference value of the serum concentration of sEng throughout the second half of pregnancy. We also made two important observations. First, we found that there were several women with persistently high sEng levels during the second half of pregnancy who did not show clinical manifestation of preeclampsia, *i.e.*, hypertension or proteinuria. Second, after the onset of preeclampsia, women with early-onset showed high sEng levels compared with those with late-onset; in addition, the deviation of sEng levels from the reference value in women with early-onset preeclampsia was more pronounced than that in women with late-onset.

We calculated the reference value for serum $\log_{10}$sEng using the 82 normal controls after excluding three women with sustained high sEng levels. The results of Kolmogonov-Smirnov analysis supported our judgment, that is, the exclusion of three cases with extremely high values, and the logarithmic transformation of the data. The concentration of sEng gradually increased from 20–23 weeks to 27–30 weeks, and it rapidly increased at 36–38 weeks of gestation. Thus, the 95th percentile of $\log_{10}$sEng as an arbitrary cut-off value for abnormality should be changed according to the gestational period. In short, from 20 to 27 weeks, a level of sEng of >8 ng/mL is abnormal; from 28 to 30 weeks, >10 ng/mL is abnormal; from 31 to 33 weeks, >15 ng/mL is abnormal; from 34 to 35 weeks, >20 ng/mL is abnormal; from 36 to 37 weeks, >25 ng/mL is abnormal; and at 38 weeks, >30 ng/mL is abnormal. Salahuddin *et al*. (*16*) reported that the serum level of 24.8 ng/mL in women with preeclampsia in the third trimester (34.6±3.1 weeks) had the highest sensitivity (90%) and specificity (95%). The sEng value of 24.8 ng/mL at 34 weeks of gestation corresponded to the value greater than the 95th percentile in our data. The serum level of the 95th percentile in women with early- and late-onset preeclampsia in our study had sensitivities of 100% and 87%, respectively, which are sufficiently as high as the sensitivity reported by Salahuddin *et al*. (*16*). Therefore, the cut-off values using the 95th percentile of the reference value curve of serum sEng levels might be appropriate for the prediction of preeclampsia in a future prospective study.

Although, to our knowledge, the reference range of sEng has not been established in previous clinical studies on serum/plasma sEng levels in women with preeclampsia (*13*, *15–17*, *20*, *21*), the mean levels of serum/plasma sEng during certain periods of pregnancy in normotensive women have been reported. Levine *et al*., after randomly selecting 120 normotensive women at 10–42 weeks, performed a cross-sectional analysis within 4-week intervals of gestational age (*13*). The mean levels of sEng among controls were stable until 33–36 weeks of gestation, after which levels of sEng increased by an average of 0.69 ng/mL/week until labor or delivery (*13*). However, in our study, the sEng levels between 20–23 weeks and 27–30 weeks were significantly different. This difference might have resulted from the method of the collection of normal samples: our samples were collected longitudinally, whereas Levine *et al*. collected samples cross-sectionally at 10–42 weeks (*13*), resulting in a statistical weakness in finding small differences at 20–30 weeks in the study by Levine *et al*. Regarding the concentration of sEng around 20 weeks of gestation, Rana *et al*. (*20*) reported that the sEng level (mean±SEM) at 17–20 weeks was 5.2±0.1 ng/mL; Levine *et al*. (*13*) reported that the mean sEng level at 17–20 weeks in controls was 5.8 ng/mL; the mean level of sEng at 20 weeks in our study was 4.2 ng/mL. Thus, the levels of sEng around 20 weeks in three studies, including our study, were almost

Table 5. Serum Levels of sFlt1 in Women with Early and Late Onset Preeclampsia

	All women with preeclampsia ($n=34$)	Onset of preeclampsia		p value
		Early onset ($n=14$)	Late onset ($n=20$)	
Gestational age at measurement (weeks)	33.2±4.2	28.9±2.9	36.1±1.6	<0.001
$\log_{10}$sFlt1 (pg/mL)	3.54±0.25	3.65±0.20	3.46±0.26	0.025
$\log_{10}$sFlt1 SDS	3.06±1.61	4.41±0.82	2.12±1.33	<0.001
sFlt1≥95th percentile	27 (79%)	14 (100%)	13 (65%)	0.026
Both sFlt1 and sEng≥95th percentile	26 (76%)	14 (100%)	12 (60%)	0.011

sFlt1, soluble fms-like tyrosine kinase 1; SDS, SD score; sEng, soluble endoglin. We previously defined a reference value of serum sFlt1 during pregnancy (*10*) and previously measured the levels of serum sFlt1 in 34 preeclamptic women, in whom 29 women with severe preeclampsia were already used in our previous papers (*9*), however, 5 cases of mild preeclampsia were firstly used in this study.

identical, indicating good reliability of the ELISA kit for sEng.

To our knowledge, the existence of persistently high sEng levels during the second half of pregnancy in women with normotensive blood pressure level has not been reported. We have observed that there are some women with later occurrence of preeclampsia in whom the levels of sEng increased early from the second trimester until the occurrence of preeclampsia (unpublished data). Therefore, these women with persistently high sEng levels might have been women with high risk for the occurrence of preeclampsia who fortunately did not develop the clinical manifestation of preeclampsia before delivery. However, in one woman showing persistently high sEng levels, the levels of sEng were constantly >70 ng/mL during 20–38 weeks, which was higher than the mean sEng level in women with late-onset preeclampsia (60 ng/mL). Therefore, it may be true that there are some women with high sEng levels during the second half of pregnancy who never develop preeclampsia. To test this hypothesis, we should observe a large prospective pregnant cohort in the future.

In our study, the mean sEng level in women with early-onset preeclampsia was almost 1.5-fold higher than in women with late-onset (93 ng/mL *vs*. 62 ng/mL). Levine *et al*. (*13*) reported that the mean sEng level in women with preterm preeclampsia was almost 1.5-fold higher than that in women with term preeclampsia (46 ng/mL *vs*. 31 ng/mL). Staff *et al*. (*17*) also reported that the median sEng level in women who delivered before 34 weeks of gestation was almost twofold higher than in women who delivered from 34 weeks and onward (76 ng/mL *vs*. 40 ng/mL). Thus, preeclamptic women with earlier onset or with preterm delivery tended to have high sEng levels.

In addition, in our study, we demonstrated that the deviation of sEng levels from the normal reference value curve in women with early-onset preeclampsia was more pronounced than that in women with late-onset. The mean SDS in women with early-onset preeclampsia was almost twofold larger than that in women with late-onset (9.9 *vs*. 4.5). Levine *et al*. (*13*) reported that the mean sEng level in women with preterm preeclampsia was almost fivefold larger than that in matched controls, whereas the mean sEng level in women with term preeclampsia was only twofold larger than in matched controls, although they did not consider the effect of SD. Thus, in women with earlier-onset preeclampsia or with preterm delivery, the increase in sEng levels was more pronounced than in those with later onset or with term delivery.

The exact mechanism of the action of sEng in preeclampsia is far from clear (*22*). At present, one important pathologic role of sEng in the pathophysiology of preeclampsia was clarified by Venkatesha *et al*. (*12*); that is, sEng blocks the TGF-β1–mediated activation of endothelial nitric oxide (NO) synthase (eNOS) by inhibiting TGF-β1 binding and signaling in endothelial cells. Since several studies have supported the hypothesis that decreased biologically available NO is central to the pathogenesis of hypertension in women with preeclampsia (*23*), the inhibition of eNOS activation by intravascular sEng suggests a molecular basis for the elevated blood pressure (*12*). Most recently, one of the molecular mechanisms of the increases of both sFlt1 and sEng in preeclamptic women was elucidated by Cudmore *et al*. (*24*); that is, heme oxygenase-1 (HO-1) is involved in negative regulation of both sFlt1 and sEng release, and it has already been reported that protein levels of HO-1 in preeclamptic placenta are decreased (*25*). The concentrations of sFlt1 and sEng in women with preeclampsia were positively correlated in our data and in previous reports (*12*, *15*, *17*), supporting the experimental results of Cudmore *et al*. (*24*).

Acknowledgements

We are indebted to Maiko Yuzawa for the daily collection of blood samples and for centrifuging and storing samples.

References

1. Ohkuchi A, Iwasaki R, Suzuki H, *et al*: Normal and high-normal blood pressures, but not body mass index, are risk factors for the subsequent occurrence of both preeclampsia and gestational hypertension: a retrospective cohort study. *Hypertens Res* 2006; **29**: 161–167.

2. Nagaya K, Fetters MD, Ishikawa M, *et al*: Causes of maternal mortality in Japan. *JAMA* 2000; **283**: 2661–2667.
3. Hecher K, Campbell S, Doyle P, Harrington K, Nicolaides K: Assessment of fetal compromise by Doppler ultrasound investigation of the fetal circulation. Arterial, intracardiac, and venous blood flow velocity studies. *Circulation* 1995; **91**: 129–138.
4. Maruyama A, Nakayama T, Sato N, Mizutani Y, Furuya K, Yamamoto T: Association study using single nucleotide polymorphisms in the estrogen receptor beta (ESR2) gene for preeclampsia. *Hypertens Res* 2004; **27**: 903–909.
5. Chappell S, Morgan L: Searching for genetic clues to the causes of pre-eclampsia. *Clin Sci* (*Lond*) 2006; **110**: 443–458.
6. Molvarec A, Vér A, Fekete A, *et al*: Association between estrogen receptor α (ESR1) gene polymorphisms and severe preeclampsia. *Hypertens Res* 2007; **30**: 205–211.
7. Levine RJ, Maynard SE, Qian C, *et al*: Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; **350**: 672–683.
8. Maynard SE, Min JY, Merchan J, *et al*: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003; **111**: 649–658.
9. Ohkuchi A, Hirashima C, Matsubara S, *et al*: Alterations in placental growth factor levels before and after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. *Hypertens Res* 2007; **30**: 151–159.
10. Hirashima C, Ohkuchi A, Arai F, *et al*: Establishing reference values for both total soluble fms-like tyrosine kinase 1 and free placental growth factor in pregnant women. *Hypertens Res* 2005; **28**: 727–732.
11. Chaiworapongsa T, Romero R, Kim YM, *et al*: Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. *J Matern Fetal Neonatal Med* 2005; **17**: 3–18.
12. Venkatesha S, Toporsian M, Lam C, *et al*: Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006; **12**: 642–649.
13. Levine RJ, Lam C, Qian C, *et al*, CPEP Study Group: Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006; **355**: 992–1005.
14. Lebrin F, Deckers M, Bertolino P, Ten Dijke P: TGF-beta receptor function in the endothelium. *Cardiovasc Res* 2005; **65**: 599–608.
15. Masuyama H, Nakatsukasa H, Takamoto N, Hiramatsu Y: Correlation between soluble endoglin, vascular endothelial growth factor receptor-1, and adipocytokines in preeclampsia. *J Clin Endocrinol Metab* 2007; **92**: 2672–2679.
16. Salahuddin S, Lee Y, Vadnais M, Sachs BP, Karumanchi SA, Lim KH: Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endoglin in hypertensive diseases of pregnancy. *Am J Obstet Gynecol* 2007; **197**: 28.e1–28.e6.
17. Staff AC, Braekke K, Johnsen GM, Karumanchi SA, Harsem NK: Circulating concentrations of soluble endoglin (CD105) in fetal and maternal serum and in amniotic fluid in preeclampsia. *Am J Obstet Gynecol* 2007; **197**: 176.e1–176.e6.
18. Sato K: A proposal for a new definition and classification of "Pregnancy Induced Hypertension (PIH)" (2004), in Japan Society for the Study of Toxemia of Pregnancy (ed): Historical Perspective of Study of Pregnancy-Induced Hypertension in Japan. Tokyo, Medical View Co, 2005, pp 54–87.
19. Ogawa Y, Iwamura T, Kuriya N, *et al*: Birth size standards by gestational age for Japanese neonates. *Acta Neonatologica Japonica* 1998; **34**: 624–632.
20. Rana S, Karumanchi SA, Levine RJ, *et al*: Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. *Hypertension* 2007; **50**: 137–142.
21. Robinson CJ, Johnson DD: Soluble endoglin as a second-trimester marker for preeclampsia. *Am J Obstet Gynecol* 2007; **197**: 174.e1–174.e5.
22. Lopez-Novoa JM: Soluble endoglin is an accurate predictor and a pathogenic molecule in pre-eclampsia. *Nephrol Dial Transplant* 2007; **22**: 712–714.
23. Lowe DT: Nitric oxide dysfunction in the pathophysiology of preeclampsia. *Nitric Oxide* 2000; **4**: 441–458.
24. Cudmore M, Ahmad S, Al-Ani B, *et al*: Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation* 2007; **115**: 1789–1797.
25. Ahmed A, Rahman M, Zhang X, *et al*: Induction of placental heme oxygenase-1 is protective against TNFalpha-induced cytotoxicity and promotes vessel relaxation. *Mol Med* 2000; **6**: 391–409.