

Is the serum L-arginine level during early pregnancy a predictor of pregnancy-induced hypertension?

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The objective of this study was to determine the concentration of serum L-arginine in healthy pregnant women and infant cord blood and to compare them with those in patients with pregnancy-induced hypertension (PIH). The serum concentration of L-arginine in normal pregnant women at early gestation ($n = 186$) was determined and analyzed based on maternal factors such as the age, pre-pregnancy body mass index (BMI), smoking and alcohol habits before pregnancy. Similarly, the concentration of cord blood of the newborns ($n = 142$) was also analyzed. These values were compared with those in the PIH group ($n = 21$). The potential risk factors for PIH were also estimated. The serum concentration of L-arginine at early gestation in normal pregnant women ($88.65 \pm 19.96 \mu\text{M}$) was not affected by the maternal age and BMI before pregnancy. A lower L-arginine concentration at early gestation ($<70 \mu\text{M}$) significantly elevated PIH risk [adjusted odds ratio (OR) = 4.26, 95% CI 1.29–14.50]. In addition, either women with large body mass before pregnancy ($\text{BMI} > 25 \text{ kg/m}^2$) or primipara women also showed a significant association with PIH risk [adjusted OR = 10.55 (2.95–40.68); 5.25 (1.72–19.15), respectively]. In conclusion, a lower L-arginine concentration at early gestation, overweight before pregnancy ($\text{BMI} > 25 \text{ kg/m}^2$) and primipara could predict to the development of PIH.

Key Words: L-arginine, pregnancy-induced hypertension, parity, cord blood

L-arginine is an important semi-essential amino acid with diverse functions in humans, and plays a critical role in producing nitric oxide (NO) as substrate. NO is one of the most important vascular signaling molecules, which relaxes and widens blood vessels and reduces platelet sensitivity to pro-aggregating agents. There has been an increasing attention being given to the role of L-arginine in the treatment and prevention of cardiovascular and cerebrovascular diseases.^(1–3) NO is thought to overcome oxidative stress, to prevent against aging, and endothelial dysfunction.^(4–7) In addition, increasing evidence has been reported indicating that L-arginine could promote insulin secretion and improve insulin sensitivity, and L-arginine is now being used in type-2 obese diabetic patients.^(8,9) L-arginine may also strengthen immunity, and has been considered to be able to remove infectious agent and carcinogenic substances from the body.

Pregnancy-induced hypertension (PIH) is known as an endothelial cell disorder associated with endothelial dysfunction, which is characterized by the enhanced formation of endothelin, reactive oxygen species (ROS) and increased sensitivity to angio-

tensin II, with decreased production of vasodilators such as NO and prostacyclin.^(10,11) It was already recommended that L-arginine be examined in clinical trials in high risk pregnancies ten years ago.⁽¹²⁾ Recently, a clinical study investigated the effect of supplementations during pregnancy with L-arginine and antioxidant vitamins, and demonstrated that the intervention reduced the incidence of preeclampsia in a Mexican population at high risk of the condition.⁽¹³⁾ With the increased need of NO to support the adaptive vasodilatation, the maternal L-arginine level has been shown to be deficient during pregnancy due to consumption by the fetus.^(14,15)

However, those data were obtained in specific races or in subgroups of populations that may have different dietary habit and the data on the Japanese population have been limited. Vadillo-Ortega *et al.*⁽¹³⁾ reported that L-arginine can be used to treat pregnant females with either a personal history of PIH or PIH in a first degree relative. However, more than half of all cases of preeclampsia occur in healthy first-time pregnancies.⁽¹⁶⁾ Therefore, if the serum L-arginine level in early gestation functions as a predictor of PIH, it would allow clinicians to more precisely detect appropriate candidates for L-arginine supplementation than previously reported markers. Thus, we conducted the present study to examine the maternal levels and changes in the L-arginine level throughout normal pregnancy, as well as to determine the cord blood concentration, and also to try to evaluate the associations of the L-arginine concentration with obstetrical complications such as PIH.

Subjects and Methods

Study participants. The study was approved by the Research Ethics Committee of Nagoya University of Medical Sciences (approval number: 648) and written informed consent was obtained from all study participants.

All subjects ($n = 221$) were recruited at a single clinic of obstetrics and gynecology clinic located in Nagoya, the Royal Bell clinic, from July 2012 to February 2013. These patients were new outpatients in early pregnancy (eight to 16 gestation weeks of gestation), and were followed up to the postpartum period. The subjects were registered in a database specially established for the clinic.

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Data collection. The information on all subjects from early pregnancy to delivery, which included the information such as the patient age, pre-pregnant state [body mass index (BMI), smoking and drinking], history of pregnancy and parity, and medical records regarding the blood pressure, weight and the results of biochemical examinations of the blood and urine during the whole period of pregnancy, was extracted from the database. Moreover, the general information about delivery and the newborn including the mode of delivery, gestational age at birth, neonatal sex, birth weight, placental weight, Apgar scores at 1 and 5 min after birth, umbilical blood gas analysis and so on were also selected simultaneously.

Sample collection. The subjects were registered in the database specifically established for the clinic, and respectively donated non-fasting venous blood, as previously reported.⁽¹⁷⁾ A total of 221 subjects in the study who continued visiting the clinic until delivery were included in the analysis in our study. Among of these patients, women with singleton pregnancies who passed all gestations without any complications and delivered a normal baby not eventually were defined as normal controls ($n = 186$). In the early period of gestation [mean \pm standard deviation (SD): 11.3 ± 1.3 weeks], blood samples were drawn from all subjects ($n = 186$), whereas in mid (25.4 ± 1.3 weeks) and late (37.4 ± 0.4 weeks) gestation, blood samples were obtained from 64 subjects at random, who were selected from among the 186 participants, respectively.

PIH was defined as a maternal systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure of ≥ 90 mm Hg with or without proteinuria (≥ 0.3 g/day or ≥ 1 + protein on ≥ 2 separate times, repeatedly) detected after 20 weeks of gestation, preeclampsia ($n = 4$) and gestational hypertension ($n = 17$), respectively. PIH subjects were detected in the samples from early ($n = 21$), mid ($n = 10$) and late ($n = 9$) gestation. In addition, superimposed preeclampsia ($n = 2$), abortion ($n = 6$), preterm delivery ($n = 4$), and gestational diabetes ($n = 2$) were also occurred in a few cases and were recorded.

Cord samples were obtained from the umbilical vein after delivery in the delivery room, simultaneously with sampling for the umbilical artery gas analysis. Samples of cord blood could be obtained from both normal pregnancies ($n = 142$) and PIH ($n = 14$) cases.

The vacuum blood collection tubes with the specimen were immediately kept at 4°C until isolation. The serum was isolated 16–24 h after blood collection to ensure a similar reserve time, and samples were thereafter stored at -80°C .

Measurement. The serum L-arginine concentration was quantitatively determined by using an L-arginine ELISA kit (Immundiagnostik AG, Germany). Samples were treated in strict compliance with the manufacturer's instructions, and the L-arginine concentration present in samples was determined directly from a dose response curve (based on a four-parameter-algorithm). The measurement of the concentrations in this study was performed in duplicate. The inter-assay coefficient variability (CV) was $< 6\%$, and the reliability was confirmed by control samples.

Statistical analysis. The differences between PIH cases and normal pregnancy controls were examined using the unpaired t test or Mann-Whitney U test for continuous variables with a normal or non-normal distribution and the chi-square test for categorical variables. Differences in the distributions of the serum L-arginine concentration based on the clinical characteristics of the expectant mothers and newborns in the normal pregnant women were detected with an unpaired t test or a one-way analysis of variance (one-way ANOVA) in cases with a normal distribution or by the Mann-Whitney U test or Kruskal-Wallis test for those with a non-normal distribution. Furthermore, multiple comparisons were sequentially carried out according to statistically significant results ($p < 0.05$) by using the Tukey/Steel-Dwass method for normal/non-normal distribution.

The changes in the serum L-arginine concentration throughout the three periods of pregnancy were estimated by the Friedman rank sum test, and the changes from early to mid gestation or mid to late gestation were determined by the Wilcoxon signed-rank test. The simple relationship of the cord blood L-arginine concentration with the maternal serum L-arginine concentration at various gestational periods was calculated using the Spearman coefficient of correlation. Moreover, the relationship of L-arginine concentration with systolic or diastolic blood pressure was also examined.

To assess the associations of a low serum L-arginine concentration ($< 70 \mu\text{M}$) with the incidence of PIH, the odds ratio (OR) and 95% confidence interval (CI) were estimated from unconditional logistic regression models, and were adjusted for potential confounding factors, such as the maternal age, pre-pregnancy BMI, smoking and parity. The reference group was determined to be that which had a serum L-arginine concentration $\geq 70 \mu\text{M}$. As potential risk factors for development of PIH, age more than 35 years old (index of advanced age at the first childbirth), large body mass (BMI $> 25 \text{ kg/m}^2$, the substitute of obesity) and smoking before pregnancy, and primipara were also simultaneously evaluated.

The statistical analyses in the present study were performed using the R version 3.0.2 software program, and with $p < 0.05$ regarded to indicate statistical significance.

Results

The clinical characteristics of the participants in this study are shown in Table 1. The percentage of patients with a BMI more than 25 kg/m^2 and the percentage of primipara women in the PIH group was significantly higher than that in the normal group ($p = 0.0003$ or $p = 0.0078$, respectively).

The distribution of the serum L-arginine concentration in the normal pregnant women and the cord blood at birth is shown in Table 2. The serum concentration of L-arginine in the mothers at early gestation was $88.7 \pm 20.0 \mu\text{M}$, which was similar with that of the cord blood, $87.0 \pm 22.6 \mu\text{M}$. The L-arginine level of the mothers at early gestation and the cord blood was not significantly affected by age or the life style before pregnancy (BMI, smoking and drinking). The parity was significantly associated with the concentration of L-arginine of cord blood ($p = 0.0071$), but the L-arginine level of the mothers at early gestation was not significantly affected by parity. The L-arginine concentration in the cord blood among the multipara cases with has delivered at least three times ($109.7 \pm 30.7 \mu\text{M}$) was significantly higher than that in primipara women ($81.6 \pm 22.5 \mu\text{M}$, $p = 0.017$ after multiple comparison). No significant difference was detected in the concentration of L-arginine in the cord blood based on the maternal age, BMI or smoking and drinking habits.

The L-arginine values at mid and late ($n = 64$) gestation of normal pregnancy are shown in Fig. 1. The L-arginine serum concentration was decreased in mid and late gestation ($p < 0.0001$), and that at mid gestation was not significantly different from that at late gestation. The concentration of L-arginine at mid and late gestation was significantly correlated with that of early gestation ($p = 0.0017$ and 0.0051 , respectively). The concentration of serum L-arginine at mid gestation only was significantly correlated with that of the cord blood ($p = 0.011$).

Whether the concentration of maternal L-arginine value at early gestation including the patient with PIH is associated with the characteristics of the newborns (gestational ages at birth, birth weight, placental weight, Apgar score and umbilical cord arterial blood gas values) was also determined (Table 3). For these analyses, we determined normal range as mean \pm SD, and the subjects were divided into the three groups according to the concentration of L-arginine; $< 70 \mu\text{M}$ ($< \text{Mean} - 1.0\text{SD}$, $n = 43$), $70 - 110 \mu\text{M}$ ($\text{Mean} - 1.0\text{SD} - \text{Mean} + 1.0\text{SD}$, $n = 128$), and $> 110 \mu\text{M}$

Table 1. The clinical characteristic of participants of the study participants

	Normal pregnancy (n = 186)	PIH (n = 21)	p value
Age (years)			
<35 (n, %)	132 (71.0)	10 (47.6)	
≥35 (n, %)	54 (29.0)	11 (52.4)	0.053
Median (rank)	31 (18–42)	35 (23–40)	
Mean (SD)	31.7 (4.8)	32.4 (5.0)	0.49
Pre-pregnancy BMI (kg/m ²)			
≤18.5 (n, %)	39 (21.0)	2 (9.5)	
≤25 (n, %)	131 (70.4)	11 (52.4)	
>25 (n, %)	16 (8.6)	8 (38.1)	0.0003
Median (rank)	19.9 (15.2–31.2)	21.5 (16.8–31.8)	
Mean (SD)	20.6 (2.9)	23.2 (4.5)	0.019
Parity (n, %)			
Primipara	80 (43.0)	16 (76.2)	
Multipara	106 (57.0)	5 (23.8)	0.0078
Pre-pregnancy smoking (n, %)			
No	142 (76.3)	16 (76.2)	
Yes	26 (14.0)	4 (19.0)	0.84
Missing value	18 (9.7)	1 (4.8)	
Pre-pregnancy drinking (n, %)			
No	99 (53.2)	15 (71.4)	
Sometimes	59 (31.7)	5 (23.8)	
Yes	8 (4.3)	0 (0.0)	0.33
Missing value	20 (10.8)	1 (4.8)	

Table 2. The values of L-arginine (μM) in the maternal serum at early gestation and in the cord blood based on the maternal factors

	Maternal serum at early gestation			Cord blood		
	n (%)	Mean ± SD	p value	n (%)	Mean ± SD	p value
Total subjects	186 (100)	88.7 ± 20.0		142 (100)	87.0 ± 22.6	
Age (years)						
≤25	17 (9.1)	90.1 ± 18.9		12 (8.5)	83.2 ± 19.2	
≤35	122 (65.6)	88.9 ± 20.3		95 (66.9)	87.4 ± 20.6	
>35	47 (25.3)	87.6 ± 19.8	0.89	35 (24.6)	87.3 ± 28.7	0.78
Pre-pregnancy BMI (kg/m ²)						
≤18.5	39 (21.0)	86.8 ± 21.1		28 (19.7)	87.6 ± 22.9	
≤25	131 (70.4)	88.9 ± 20.2		101 (71.1)	85.3 ± 22.2	
>25	16 (8.6)	91.2 ± 15.4	0.55	13 (9.2)	98.9 ± 23.8	0.12
Pre-pregnancy smoking						
No	142 (76.3)	87.5 ± 19.4		103 (72.5)	87.0 ± 22.6	
Yes	26 (14.0)	86.9 ± 20.2	0.87	21 (14.8)	83.2 ± 22.1	0.48
Missing value	18 (9.7)			18 (12.7)		
Pre-pregnancy drinking						
No	99 (53.2)	86.6 ± 19.2		74 (52.1)	86.4 ± 24.8	
Sometimes	59 (31.7)	89.2 ± 20.6		40 (28.2)	88.7 ± 21.4	
Yes	8 (4.3)	90.0 ± 12.9	0.67	8 (5.6)	85.5 ± 13.8	0.86
Missing value	20 (10.8)			20 (14.1)		
Parity						
0	80 (43.0)	88.3 ± 17.8		62 (43.7)	81.6 ± 22.5	
1	77 (41.4)	89.2 ± 22.6		58 (40.8)	88.2 ± 19.5	
2	21 (11.3)	88.6 ± 18.9		16 (11.3)	95.1 ± 24.6	
At least 3 times	8 (4.3)	87.1 ± 19.6	1.00	6 (4.2)	109.7 ± 30.7	0.0071*

*P value of multiple comparisons: The L-arginine concentration of the umbilical blood of multipara patients with more than 3 deliveries was significantly higher than that in primipara patients ($p = 0.017$).

(>Mean + 1.0SD, $n = 36$). The Apgar score at 1 and 5 min in the group with the value <70 μM was higher than that in the group with a value > 110 μM (9.0 ± 0.6 vs 8.6 ± 0.8 , $p = 0.019$ and 9.8 ± 0.4 vs 9.6 ± 0.6 , $p = 0.06$, respectively). Moreover, the

umbilical artery pH in the group with a value <70 μM showed a higher tendency than that in the other groups (7.37 ± 0.05 vs 7.34 ± 0.06 , $p = 0.08$). However, these values were within the normal range. No other parameters of the newborns were found

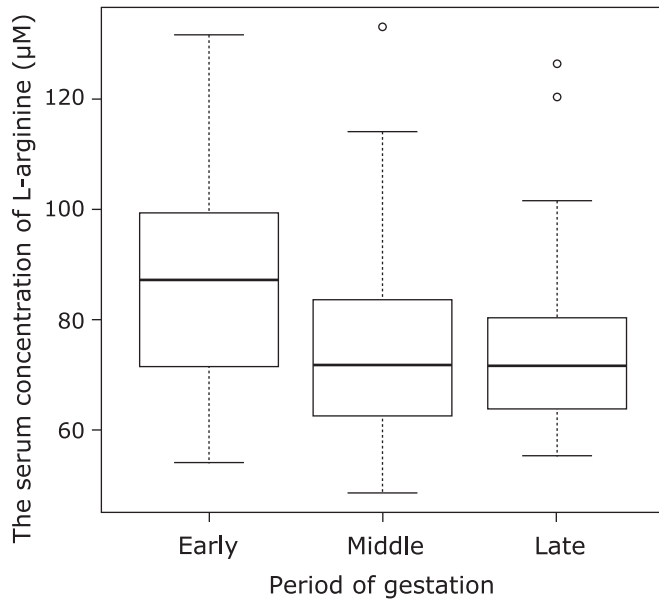


Fig. 1. The Maternal serum concentration of L-arginine during the three gestational periods. Data are shown as a box plot. The bottom and top of each box show the 25th and 75th percentile, respectively. The line in the middle of each box indicates the 50th percentile. Whiskers above and below each box illustrate the upper and lower adjacent values. Open dots mean outside values. The mean \pm SD of the L-arginine concentration were: $88.65 \pm 19.96 \mu\text{M}$ in early pregnancy, 74.52 ± 16.54 in mid pregnancy and 74.77 ± 14.16 in late pregnancy.

to be related to the concentration of L-arginine.

Similarly, the concentration of L-arginine value in the cord blood was also investigated by dividing the patients into three groups (Table 4). Although there were no significant differences in either the birth weight or placental weight, both the gestational age at delivery and the Apgar score at 1 min after birth were found a difference ($p = 0.09$ and $p = 0.07$, respectively), not reached the statistical significance.

The concentration of L-arginine in the male newborns was $86.9 \pm 22.1 \mu\text{M}$ ($n = 78$), which was not significantly different from that of female newborns, $87.2 \pm 23.4 \mu\text{M}$ ($n = 64$). In terms of the mode of delivery, the values of cord L-arginine in cases with forced delivery, including those of forceps or vacuum delivery and emergency Cesarean section ($n = 31$, $83.2 \pm 19.3 \mu\text{M}$) were lower than those after normal vaginal delivery ($n = 91$, $85.6 \pm 24.1 \mu\text{M}$) or elective Cesarean section ($n = 20$, $99.4 \pm 16.4 \mu\text{M}$) ($p = 0.027$).

Table 5 shows the clinical features of the subjects with a normal pregnancy and PIH. In the PIH group, the highest blood pressure during pregnancy was significantly higher than that in normal pregnancy (Table 5, $p < 0.0001$), but the gestational age at birth, mode of delivery, placental and birth weight, Apgar score and umbilical artery pH were not significantly different from those in normal pregnancy (Table 5).

As shown in Table 6, although no significant difference was found in the distribution of maternal L-arginine concentration at the respective gestational periods in comparison with the normal group, the cord blood L-arginine level showed a tendency to be lower in the PIH group ($79.0 \pm 21.9 \mu\text{M}$) than in the normal pregnancy group ($87.0 \pm 22.6 \mu\text{M}$).

The potential risk factors for the development of PIH are summarized in Table 7. After adjusted analysis, pre-pregnancy overweight (BMI $>25 \text{ kg/m}^2$; adjusted OR = 10.55, 95% CI = 2.95–40.68) and nulliparity (adjusted OR = 5.25, 95% CI = 1.72–

Table 3. The characteristics of newborns based on the values of L-arginine in the maternal serum at early gestation

	<70 μM ($n = 43$)		70–110 μM ($n = 128$)		>110 μM ($n = 36$)		<i>p</i> value
	Mean (SD)	Median (rank)	Mean (SD)	Median (rank)	Mean (SD)	Median (rank)	
Gestational ages at delivery (days)	274.6 (8.1)	275 (260–291)	276.6 (7.8)	276 (261–293)	274 (7.1)	275 (257–285)	0.22
Birth weight (g)	3,082 (295)	3,124 (2,380–3,616)	3,081 (325)	3,105 (2,244–3,852)	3,026 (441)	3,014 (2,086–4,210)	0.78
Placental weight (g)	586 (100)	594 (376–841)	590 (112)	576 (333–987)	575 (112)	562 (375–804)	0.83
1 min Apgar score	9.0 (0.6)	9.0 (8–10)	8.8 (0.5)	9.0 (7–10)	8.6 (0.8)	9.0 (6–10)	0.008*
5 min Apgar score	9.8 (0.4)	10 (9–10)	9.6 (0.6)	10 (8–10)	9.6 (0.6)	10 (8–10)	0.06
Umbilical artery pH	7.36 (0.05)	7.37 (7.21–7.45)	7.34 (0.06)	7.34 (7.14–7.53)	7.33 (0.07)	7.35 (7.13–7.48)	0.08
Umbilical artery BE	-1.6 (1.6)	-2.0 (-5–1)	-2.4 (2.4)	-2.0 (-12–2)	-2.8 (3.1)	-2.0 (-13–1)	0.18

*Multiple comparison: The 1 min Apgar score in the lowest group (maternal serum L-arginine $<70 \mu\text{M}$) was significantly higher than that in the highest group ($>110 \mu\text{M}$) ($p = 0.019$).

Table 4. The characteristics of newborns based on the values of L-arginine in the cord blood

	<70 μM ($n = 37$)		70–110 μM ($n = 97$)		>110 μM ($n = 22$)		<i>p</i> value
	Mean (SD)	Median (rank)	Mean (SD)	Median (rank)	Mean (SD)	Median (rank)	
Gestational ages at delivery (days)	278.6 (8.7)	280 (257–291)	275.8 (7.7) (7.7)	276 (259–293)	274.3 (8.2)	275 (261–289)	0.09
Birth weight (g)	3,130 (325)	3,148 (2,380–3,672)	3,080 (349)	3,078 (2,086–3,852)	3,083 (408)	3,136 (2,392–4,210)	0.76
Placental weight (g)	568 (119)	562 (333–987)	596 (106)	592 (375–887)	584 (96)	574 (459–807)	0.38
1 min Apgar score	9.0 (0.5)	9 (8–10)	8.8 (0.6)	9 (6–10)	8.8 (0.5)	9 (7–9)	0.07
5 min Apgar score	9.8 (0.4)	10 (9–10)	9.7 (0.6)	10 (8–10)	9.5 (0.7)	10 (8–10)	0.39
Umbilical artery pH	7.34 (0.07)	7.35 (7.20–7.48)	7.35 (0.06)	7.35 (7.19–7.49)	7.34 (0.06)	7.35 (7.16–7.44)	0.89
Umbilical artery BE	-2.2 (1.9)	-2 (-6–0)	-2.1 (2.2)	-2 (-10–2)	-2.2 (3.1)	-2 (-13–1)	0.77

Table 5. The clinical outcome of normal pregnancy vs PIH

	Normal pregnancy (n = 186)	PIH (n = 21)	p value
Systolic blood pressure (mmHg)*			
Mean (SD)	119.5 (10.1)	151.4 (10.0)	<0.0001
Diastolic blood pressure (mmHg)*			
Mean (SD)	67.7 (9.2)	92.3 (4.9)	<0.0001
Gestational age at birth (days)			
Mean (SD)	275.6 (7.7)	276.9 (8.5)	0.29
Mode of delivery, (n, %)			
Normal vaginal delivery	123 (66.1)	12 (57.1)	
Emergency delivery [†]	37 (19.9)	7 (33.3)	
Scheduled CS	26 (14.0)	2 (9.5)	0.35
Placental weight (g)			
Mean (SD)	584 (106)	606 (136)	0.51
Newborn gender, (n, %)			
Female	82 (44.1)	10 (47.6)	
Male	104 (55.9)	11 (52.4)	0.94
Birth weight (g)			
Mean (SD)	3,068 (337)	3,102 (382)	0.67
1 min Apgar score			
Median (rank)	9.0 (6–10)	9.0 (8–10)	
Mean (SD)	8.8 (0.6)	8.9 (0.6)	0.96
5 min Apgar score			
Median (rank)	10.0 (8–10)	10.0 (8–10)	
Mean (SD)	9.7 (0.5)	9.6 (0.6)	0.43
Umbilical artery pH			
Median (rank)	7.35 (7.13–7.53)	7.35 (7.14–7.44)	
Mean (SD)	7.34 (0.06)	7.33 (0.06)	0.33
Umbilical artery BE			
Median (rank)	-2 (-13–2)	-1 (-12–0)	
Mean (SD)	-2.3 (2.4)	-2.1 (2.8)	0.38

*The highest blood pressure during pregnancy. [†]Including forceps, vacuum delivery and emergency Cesarean section (CS).

Table 6. The maternal serum and cord blood L-arginine (μM) of normal pregnancy vs PIH

L-arginine	Normal pregnancy	PIH	p value
Early gestation			
n	186	21	
Median (rank)	87.4 (51.7–145.7)	79.4 (49.5–132.0)	
Mean (SD)	88.7 (20.0)	85.5 (24.2)	0.39
Middle gestation			
n	64	10	
Median (rank)	71.9 (48.5–133.3)	73.2 (59.2–114.3)	
Mean (SD)	74.5 (16.5)	78.3 (16.7)	0.55
Late gestation			
n	64	9	
Median (rank)	71.8 (55.5–126.5)	75.6 (67.1–117.1)	
Mean (SD)	74.8 (14.2)	80.5 (15.7)	0.23
Cord blood			
n	142	14	
Median (rank)	85.7 (32.8–148.1)	72.6 (45.2–128.2)	
Mean (SD)	87.0 (22.6)	79.0 (21.9)	0.21

19.15) were significantly associated with the development of PIH. Advanced maternal age (≥ 35 years old, adjusted OR = 2.67, 95% CI = 0.88–8.19) and smoking before pregnancy (adjusted OR = 3.41, 95% CI = 0.79–13.53) were shown a trend to be related with PIH, but no statistical significances were found. Although the maternal serum L-arginine at early gestation in the PIH group was

not significantly different from that in normal pregnant women (Table 6), the ratio of the group with a value $<70 \mu\text{M}$ at early gestation in the PIH group was higher than that in the normal pregnancy group (33.3% vs 19.4%, Table 7). A lower serum concentration of serum L-arginine at early gestation ($<70 \mu\text{M}$) was also shown to be a risk factor for PIH (adjusted OR = 4.26,

Table 7. The risk factors associated with the development of PIH

	Normal pregnancy (n, %)	PIH (n, %)	Crude OR (95% CI)	Multi-adjusted OR* (95% CI)
L-arginine concentration in early gestation (μM)				
≥ 70	150 (80.6)	14 (66.7)	1 (Ref)	1 (Ref)
< 70	36 (19.4)	7 (33.3)	2.08 (0.74–5.40)	4.26 (1.29–14.50)
Age (years)				
< 35	132 (71.0)	10 (47.6)	1 (Ref)	1 (Ref)
≥ 35	54 (29.0)	11 (52.4)	2.69 (1.07–6.82)	2.67 (0.88–8.19)
Pre-pregnancy BMI (kg/m^2)				
≤ 25	170 (91.4)	13 (61.9)	1 (Ref)	1 (Ref)
> 25	16 (8.6)	8 (38.1)	6.54 (2.30–18.06)	10.55 (2.95–40.68)
Parity				
Multipara	106 (57.0)	5 (23.8)	1 (Ref)	1 (Ref)
Primipara	80 (43.0)	16 (76.2)	4.24 (1.59–13.40)	5.25 (1.72–19.15)
Pre-pregnancy smoking				
No	142 (76.3)	16 (76.2)	1 (Ref)	1 (Ref)
Yes	26 (14.0)	4 (19.0)	1.37 (0.37–4.08)	3.41 (0.79–13.53)

*Adjusted for every confounding factors such as L-arginine concentration in early gestation, age, pre-pregnancy BMI, smoking and parity but itself.

95% CI = 1.29–14.50, Table 7) after adjusted for the other potential risk factors including age, pre-pregnancy BMI and parity. The best threshold is calculated as 64.9 μM (sensitivity: 89.2%, specificity: 28.6%), from the ROC curve (Area under the curve: 0.57, 95% CI: 0.41–0.71), which was insufficient. In addition, the number of subjects under 65 μM was very small ($n = 6$), which was unavailable for statistical analysis. The normal range is often determined as Mean \pm 2SD, which was also unworthy for statistical analysis because of wide distribution of samples in this study. Thus, we determined the lower normal limits of serum L-arginine as 70 μM (sensitivity: 80.6%, specificity: 33.3%), which was corresponding to mean $-$ 1.0SD.

The correlative relationship of maternal L-arginine concentration with systolic or diastolic blood pressure were respectively measured, but no significant correlation was found ($p = 0.94$ or $p = 0.59$, respectively).

In cases with superimposed preeclampsia ($n = 2$), preterm birth ($n = 4$), and abortion ($n = 6$), the L-arginine value of the maternal serum at early gestation (67.7 ± 6.7 , 69.6 ± 4.3 , and 74.9 ± 15.0 μM , respectively) was lower than that of normal pregnancy (88.7 ± 20.0 μM), but the number of these cases was too small to be analyzed statistically.

Discussion

In the present study, the mean value of serum L-arginine at early gestation was 88.7 μM . This value was not affected by age, the pre-pregnancy BMI, pre-pregnancy habits including alcohol or tobacco use or the parity. There was a previous report that the serum L-arginine level at early gestation in low BMI pregnant women was not significantly different from that in normal BMI pregnant women.⁽¹⁸⁾ There have been no previous reports that have investigated the effects of alcohol on the serum L-arginine level. However, the serum L-arginine in male smokers was reported to be significantly higher than in that in male non-smokers.⁽¹⁹⁾ This inconsistency might be related to the difference of gender, the number of cigarettes smoked, or the years of smoking.^(13,20) The mean values in the present study were similar to or higher than those in several previous studies of other races. Taking in account the differences in the measurement techniques, this result suggested that a depletion of L-arginine was not be detected in healthy Japanese pregnant women. However, the serum L-arginine concentration in normal pregnancies was significantly decreased

in mid gestation compared to early gestation, but remained unchanged until the end of gestation. We don't know the precise reason. However, the decrease from early to mid gestation in the maternal L-arginine level might be dependent on due to consumption by the fetus and the increased need of NO to support the adaptive vasodilatation.^(14,15) Others reported that nitric oxide synthase (NOS) expression was increased in human placenta during mid and late gestation.⁽²¹⁾ In addition, there is a report that expression of arginase was decreased in late gestation compared to early gestation.⁽²²⁾ Those complicated gestational change of consumption and degradation of L-arginine might determine L-arginine concentration. In contrast, the serum asymmetric dimethylarginine (AMDA) level was reported to trend to increase during normal pregnancy.⁽²³⁾ AMDA inhibits endothelial-NOS. These results suggested that the NO production may be decreased throughout gestation. Exteriorization of this trend might be related with to the onset of preeclampsia after the second trimester.

Of note, 20.8% ($n = 43$) of the total subjects ($n = 207$) had a value of L-arginine < 70 μM at early gestation in all normal pregnancies, and the gestational ages at birth, birth weight and placental weight were not significantly affected by the concentration of serum L-arginine at early gestation. It has previously been demonstrated that an arginine-deficient diet results in the birth of small-for-gestational age babies in rats.⁽²⁴⁾ However, data for human are lacking and evidence supporting the use of arginine supplementation in cases of fetal growth retardation remains conflicting.⁽²⁵⁾ Our data were restricted to the value at early gestation and included a largely normal pregnancy population. Thus, the value of L-arginine at early gestation does not appear to play a role in the gestational ages of the infants at birth or in the infant birth weight. We initially speculated that pregnant females with an L-arginine level of < 70 μM may exhibit poor outcomes with respect to their neonates. However, unexpectedly, the mean values of both the 1 and 5 min Apgar score and the umbilical artery pH, which are assessments of the newborn's well-being, in the population with an L-arginine value < 70 μM at early gestation were significantly higher than those in the population with higher values. Moreover, the 1 min Apgar score in the population with a value < 70 μM in the cord blood was also significantly higher than that in the population with a value ≥ 70 μM . The low concentration of L-arginine in the cord blood might improve the 1 min Apgar score by not depressing the blood pressure of the fetus; however, it cannot be concluded that an L-arginine level of < 70 μM in early

gestation is associated with the amelioration of the 1 min Apgar score and umbilical artery pH. These findings should be interpreted carefully, as the differences are in the clinically normal range. To clarify the relationship between the newborn's well being and the low maternal and fetal serum concentrations of L-arginine, a large number of newborns with a wider range of prognoses should be analyzed. In addition, it may be necessary to monitor arginine supplementation in normal pregnant patients with an L-arginine level of $<70 \mu\text{M}$ in early gestation so as not to exceed an L-arginine serum concentration of $110 \mu\text{M}$.

On the other hand, the rate of women who developed PIH in the population of with a L-arginine level $<70 \mu\text{M}$ tended to be higher than that in the population with a value $\geq 70 \mu\text{M}$. That result was not statistically significant, but the adjusted OR for the development of PIH in the population with an L-arginine value $<70 \mu\text{M}$ of L-arginine at early pregnancy was 4.26 (95% CI: 1.29–14.50). In addition, no significant correlation between maternal serum L-arginine and systolic/diastolic blood pressure was detected. It suggested that maternal serum L-arginine would decrease prior to blood pressure elevation in PIH patients.

Moreover, we simultaneously evaluated the relative risk factors such as advanced maternal age (≥ 35 years old), overweight before pregnancy ($\text{BMI} > 25 \text{ kg/m}^2$), smoking before pregnancy, and parity. In this study, overweight before pregnancy (adjusted OR = 10.55, 95% CI = 2.95–40.68) or primipara (adjusted OR = 5.25, 95% CI = 1.72–19.15) was also found to be significantly associated with the risk of PIH after the multiple logistic regression analysis, which were consistent with the previous reports.^(26–28) Those results suggested that a combination of low maternal L-arginine level at early gestation, overweight before pregnancy ($\text{BMI} > 25 \text{ kg/m}^2$) and primipara could be comprehensive criteria for predicting PIH. Several papers have reported that at late gestation, maternal serum L-arginine in PIH patients is lower than that in normal pregnancies.^(29–32) However, this is the first study to evaluate serum L-arginine level in early pregnancy in cases of PIH vs normal controls. Consequently, the L-arginine levels were found to be reduced in early pregnancy in cases of superimposed preeclampsia, preterm birth or abortion, although these findings could not be analyzed statistically due to the small number of patients. Thus, a low serum L-arginine at early gestation might be associated with the development of obstetrical complications, and such patients should be closely watched.

The cord blood L-arginine level was not affected by the maternal age or the pre-pregnancy drinking and smoking. The L-arginine value trended to be increased, when the pre-pregnancy BMI exceeded 25. Similar to the maternal serum L-arginine level, the birth weight and placental weight were also not affected by the cord blood L-arginine level. However, the gestational age at delivery was higher in the low concentration group ($<70 \mu\text{M}$) than in the high concentration group with regard to the cord blood L-arginine. Hence, the cord blood L-arginine level may decrease as the gestational ages increase. To confirm it, the analysis for the cord blood L-arginine in the premature infants should be performed.

In the PIH group, cord blood L-arginine was at a lower level than that in normal pregnancy, which was similar to the finding of previous reports.^(29,33) It was also previously reported that arginase,

which degrades L-arginine, is increased in the maternal serum in PIH cases.⁽²⁰⁾ The low concentration of the cord blood of patients with PIH might therefore be caused by the increased expression of arginase. In addition, the cord blood L-arginine level in primipara cases was significantly lower than that in multipara cases with more than three deliveries. The placental expression of arginase may be increased in primipara patients, subsequently resulting a decreased the cord L-arginine. The finding of a lower expression of arginase in the multipara placenta is reasonable, as the proportion of primipara females is higher among PIH patients. In addition, although one report found that the AMDA level in the cord blood is not affected by the number of parity,⁽³⁴⁾ there are no reports regarding the relationship between parity and the cord blood L-arginine level. The effects of a low concentration of cord blood L-arginine remain unknown. However, it is known that offspring exposed to preeclampsia *in utero* often develops cardiovascular disease, including hypertension,^(35,36) the value of fetal L-arginine might be involved in this process with regard to the epigenetics.

Our study is associated with a number of limitations. Almost all samples were obtained from normal pregnancies. The data from a large number of preterm births or small-for-gestational-age infants might help to clarify the effects of the L-arginine concentration on the gestational age at birth or on the birth weight, respectively. Moreover, the number of PIH patients was also limited in the present study. In this study, we determined the lower normal limits of serum L-arginine as $70 \mu\text{M}$ (sensitivity: 89.2%, specificity: 28.6%) for the deviation of samples in this study. The number of subjects under $65 \mu\text{M}$, which was calculated as the cutoff value by ROC curve, was too small ($n = 6$) to analyze statistically. Thus, the cutoff value of serum L-arginine could be fluctuant at different population. An analysis with a larger number of PIH patients might provide a more precise cut-off value at early gestation for predicting the development of PIH.

In conclusion, the maternal and fetal L-arginine levels in normal pregnancy were determined. The maternal L-arginine level was decreased from early to mid gestation. The maternal L-arginine at early gestation was not affected by the maternal age or pre-pregnancy BMI. The cord blood L-arginine level was not affected by the gender of newborns. The birth weight was also not affected by the maternal or fetal L-arginine values. However, a low maternal L-arginine level at early gestation was related to the development of PIH with other risk factors such as overweight before pregnancy ($\text{BMI} > 25 \text{ kg/m}^2$) and primipara. More research is required to determine further strategies for selecting appropriate candidates for arginine supplementation.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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