主　論　文

Relationship Between the Changes in Placental Blood Flow Resistance Assessed by Doppler Technique and Maternal Serum Placental Aminopeptidases, Which Degrade Vaso-active Peptides, in Pre-eclampsia

超音波ドップラー法による胎盤血流抵抗とペプチドホルモンを代謝分解する母体血中胎盤性アミノペプチダーゼの変動の妊娠中毒症における相関

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Running title : S/D Ratio of Uterine or Umbilical Artery and Placental Aminopeptidases in Pre-eclampsia

Summary

Our study showed that there were statistically significant correlations between the systolic and diastolic ratio (S/D) of maternal uterine or umbilical artery and the levels of maternal serum aminopeptidase activities in pre-eclmapsia : kininase I was positively correlated with the S/D ratios, and placental leucine aminopeptidase (P-LAP)and aminopeptidase A were negatively correlated with the S/D ratios. It is known that the increased S/D ratios reflect the increased utero-placental blood flow resistance. Since our previous study showed that placental aminopeptidases degrade vasoaactive peptides such as oxytocin, angiotensin and bradykinin, which the fetus actively produces, our present study suggests that the increased vascular resistance in feto-placental circulation in pre-eclampsia is partly controlled by changes in vaso-active peptides, via degradation by placental aminopeptidases.

Key Words

Systolic and Diastolic (S/D) Ratio of Maternal Uterine or Umbilical Artery—Doppler Technique—Pre-Eclampsia —Placental Leucine Aminopeptidase (P-LAP)—Aminopeptidase A—Kininase I —Vasoactive Peptides—Utero-Placental Blood Flow Resistance.

Introduction

We developed the placental leucine aminopeptidase (P-LAP) test for evaluation of the placental function in 1976 (Mizutani, Yoshino and Oya 1976), and proved its clinical usefulness in predicting such as pre-eclampsia (Mizutani, Akiyama, Kurauchi, Taira, Narita and Tomoda 1985a). Recently we have shown that the placental aminopeptidase A (Mizutani, Yamada, Kurauchi, Ito, Narita and Tomoda 1987) and kininase I (Ito, Mizutani, Nomura, Kurauchi, Kasugai, Narita and Tomoda 1990) activities in maternal sera are also useful for monitoring the patients of pre-eclampsia.

On the other hand it is known that the doppler technique is a good index of placental flow resistance. Campbell, Diaz-Recasens, Griffin, Cohen-Overbeek, Pearce, Wilson and Teague (1983) and Trudinger, Cook, Jones and Gioles (1986) showed that pre-eclamptic patients are associated with an increase of the ratio of peak systolic to least diastolic (S/D ratio) of uterine or umbilical artery.

This study aims to examine the relationship between the S/D ratio of uterine or umbilical artery and placental aminopeptidases in maternal sera of pre-eclampsia and the pathophysiological role of placental aminopeptidases in the uteroplacental circulation is discussed.

Subjects and Methods

Patients attending the antenatal clinic and subsequently admitted to Nagoya University Hospital were the subjects of the present study. All women were 40 years of age or less, were sure of the date of their last menstrual period, and had a history of regular 28-to 30 day menstrual cycles. The patients in this study consisted of 5 mild pre-eclampsia (systolic pressure 140-170 mmHg; diastolic pressure 90-110 mmHg, together with slight edema or proteinuria) and 6 severe pre-eclampsia (systolic pressure over 170 mmHg and/or diastolic pressure over 110 mmHg, together with generalized edema or proteinuria). Table 1 shows the clinical characteristics of the patients grouped by the above criteria. All these patients were normotensive in the first half of pregnancy.

A pulsed and continuous-wave Doppler ultrasound unit RT3600 (Yokogawa Medical Co., Tokyo, Japan) with a 3.5 MHz transducer was used to record flow waveforms in the standard manner for investigative analysis (Campbell et al. 1983; Schulman 1987; Trudinger et al. 1987). Maximum systolic frequency (S), endo-diastolic frequency(D), time averaged maximum frequency and fetal heart rate were measured (Yamada, Kasugai, Ishizuka and Tomoda 1988). To evaluate the blood flow, S/D ratio is a good index. The umbilical artery was recognized by the characteristic shape of the velocity waveform on the oscilloscope. After observ-

ing several waveforms, we made measurements at three different waveforms. These three measurements were averaged and reported as the umbilical artery S/D ratio. The use of three different sites was necessarily to minimize the effect of variation of the S/D ratio in different parts of the cord. The waveform analyses were made under steady-state conditions (ie, no gross fetal body movements or fetal breathing). The maternal uterine artery waveform recorded from branches in the utero-placental bed, which had been located initially by ultrasonography. Several waveforms were visualized, and a minimum of three waveforms were measured and averaged for the reported urerine artery S/D ratio. S/D ratio measurements were performed in the morning (between 9:00-11:00h).

Serum samples were obtained from patients just before measurements of S/D ratio. Maternal serum placental leucine aminopeptidase (P-LAP) activity was measured according to our method : P-LAP activity was measured using L-leucyl-p-diethylaminoanilide as a substrate in the presence of 20 mM L-methionine (Mizutani, Yoshino and Oya 1976). The assay procedure for P-LAP and its precision were as described in our previous report (Mizutani, Noto, Inamoto, Sakura and Kawashima 1979). Maternal serum aminopeptidase A activity was assayed with L-aspartyl-β-naphthylamide as a substrate according to our method (Mizutani et al. 1987). Kininase I activity was assayed with L-Hippuryl-L-lysine (Bz-Gly-Lys) as a substrate by measurement of

the Bz-Gly released according to our method (Ito et al. 1990) . The assay precision of two enzyme activities were described in our previous reports. Student's t-test was used for the statistical analysis.

Results and Discussion

The activity of three enzymes of pre-eclmpsia were essentially similar to those which we reported previously (Mizutani et al. 1985a; Mizutani et al. 1987; Ito et al. 1990). Figure 1 shows S/D ratios of umbilical artery in patients in comparison with those in normal pregnant women (Hirose, Yamada, Kasugai, Ishizuka and Mizutani 1989). Four times out of nine measurements in severe pre-eclampsia showed higher S/D ratios than in normal pregnancy.

S/D ratios of umbilical and uterine artery, enzyme levels and the relationship between S/D ratios and enzyme levels are shown in figure 2-4. Our present study shows that there are statistically significant negative (P-LAP : Fig. 2, aminopeptidase A:Fig. 3) and positive (kininase I :Fig. 4) correlations between S/D ratio of umbilical and uterine arteries and maternal serum enzyme activities. Until now, there has never been any report with these kind of comparison. It is interesting that the S/D ratio in umbilical artery, which reflects utero-placental blood flow resistance (Trudinger et al. 1986), correlates with maternal serum placental enzyme levels in pre-eclampsia.

Although cardiac output increases 40% and circulatory blood volume 50% in the late stage of normal pregnancy, blood pressure remains stable. Since blood pressure is a function of cardiac output and peripheral vascular resistance, the decrease of vascular resistance in the whole body, especially in the uteroplacental circulation, which is reaching 800ml/min, is essential for the maintenance of normal blood pressure during pregnancy.

In regard to the mechanism for the maintenance of vascular resistance, it is reasonable to speculate that the vascular resistance and the volume of retroplacental blood pool are partly controlled by vaso-active substances such as peptide hormones : the concentration of bioactive peptides such as oxytocin, angiotensins and bradykinin regulated by the production from the fetal site (Melmon, Cline, Hughes and Nies 1968; Vallotton, Godard and Gaillard 1976; Ryan 1980) and the degradation by placental enzymes may directly affect the feto-placental circulation. Since the serum levels of placental aminopeptidases that are involved in the degradation of oxytocin (Mizutani, Sumi, Oka, Yamada, Kurauchi, Taira, Narita and Tomoda 1985c), angiotensins (Mizutani, Akiyama, Kurauchi, Taira, Narita and Tomoda 1985c) and bradykinin (Ito, Mizutani, Kurauchi, Kasugai, Narita and Tomoda 1989) seem to reflect the content of enzymes in the placenta, our present data (Fig.2-4) suggest that the increased vascular resistance in the feto-placental circulation in pre-eclampsia (Naeya 1989) is partly

controlled by changes in vaso-active peptides possibly derived from the fetus.

Continuous recording of fetal heart rate and uterine contraction patterns plays a central role in antepartum fetal monitoring (non-stress test, NST), and the NST is usually the primary means of fetal surveillance at pre-eclampsia. However not every case for monitoring the feto-placental function in pre-eclampsia by the NST can succeed. Our present data suggest the importance of placental enzyme levels in maternal sera for monitoring the feto-placental function in pre-eclmpsia.

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Table I Clinical characteristics of study groups (mean±SE)

Characteristics	Mild pre-eclampsia (n=5)	Severe pre-eclampsia (n=6)
Age (year)	29.4±4.63	28.3±4.92
Duration of pregnancy (week)	38.4±1.59	33.3±3.48
Onset of hypertension (week)	33.0±4.15	27.2±2.61
Highest blood pressure (week)	37.0±2.10	31.2±3.62
Weight of babies (g)	3194±634.9	1566±657.3
One-minute Apgar score	6.4±2.1	5.2±4.0
Placental weight (g)	551.0±52.76	388.3±195.4

Legend for figures

Fig. 1 Umbilical S/D ratios of pre-eclampsia in comparison with normal pregnant women (○——○, Ref. Hirose, Yamada, Kasugai, Ishizuka and Mizutani 1989).

Fig. 2 Relationship between umbilical S/D ratios and P-LAP acivities in pre-eclampsia.

Fig. 3 Relationship between umbilical S/D rations and amino-peptidase A activities in pre-eclampsia.

Fig. 4 Relationship between umbilical S/D ratios and kininase I activities in pre-eclampsia.

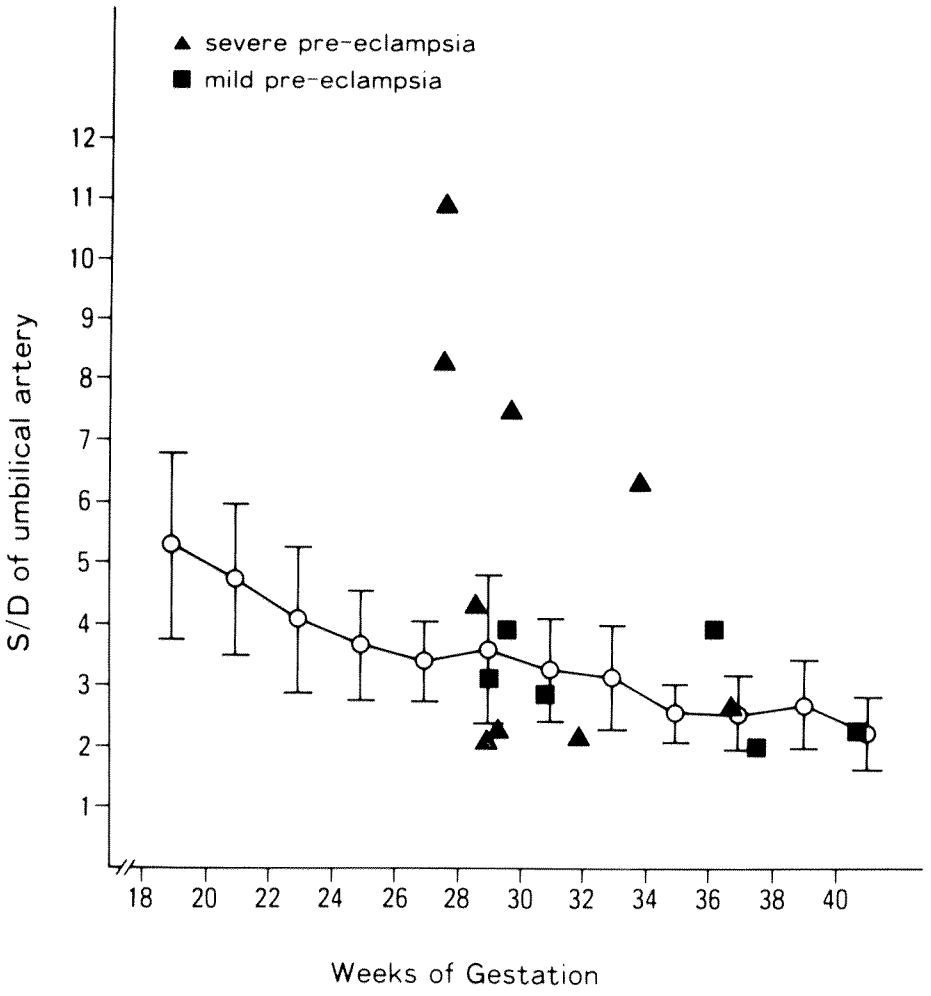
▲ severe pre-eclampsia
■ mild pre-eclampsia
S/D of umbilical artery
12
11
10
9
8
7
6
5
4
3
2
1
18
20
22
24
26
28
30
32
34
36
38
40
Weeks of Gestation

Fig. 1

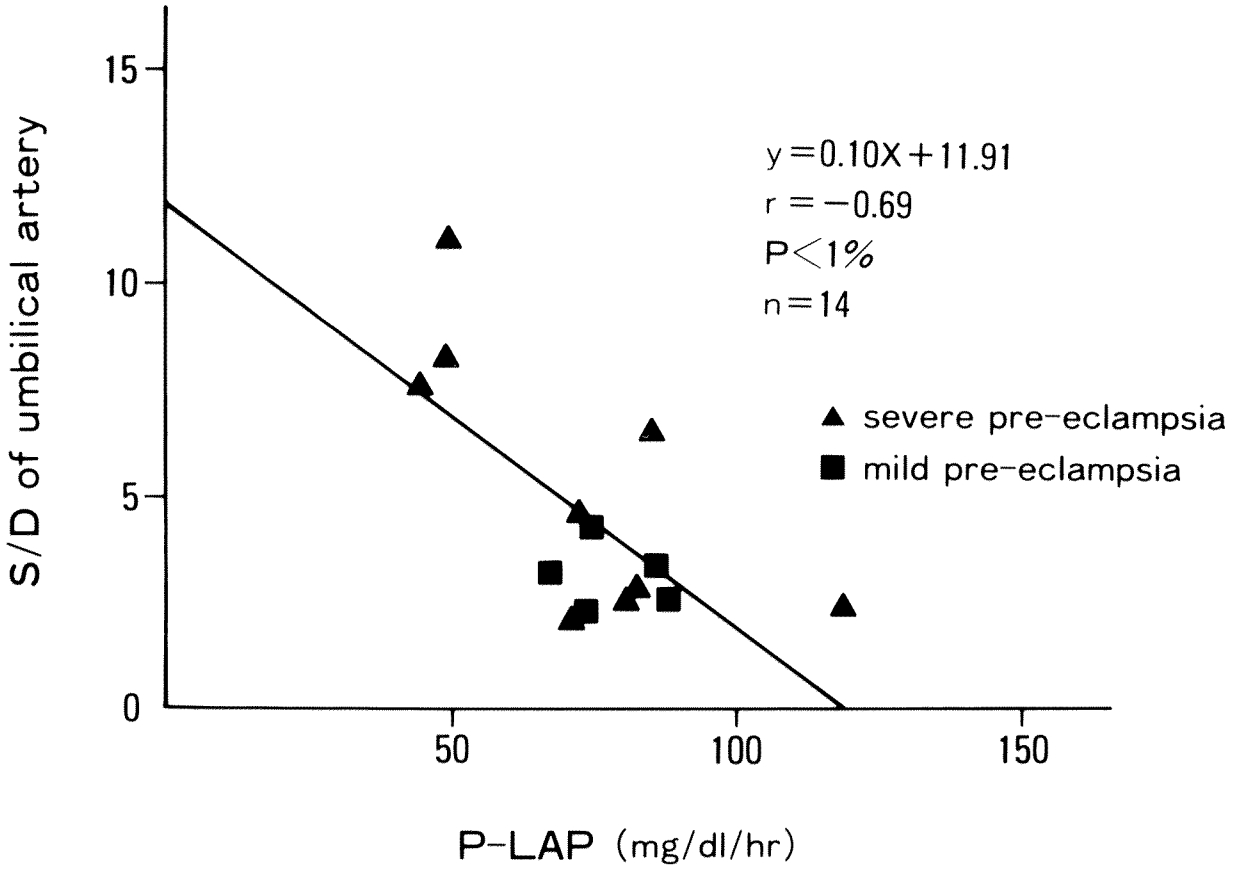
15
10
5
0
S/D of umbilical artery
y=0.10X+11.91
r=−0.69
P<1%
n=14
▲ severe pre-eclampsia
■ mild pre-eclampsia
50
100
150
P-LAP (mg/dl/hr)

Fig. 2

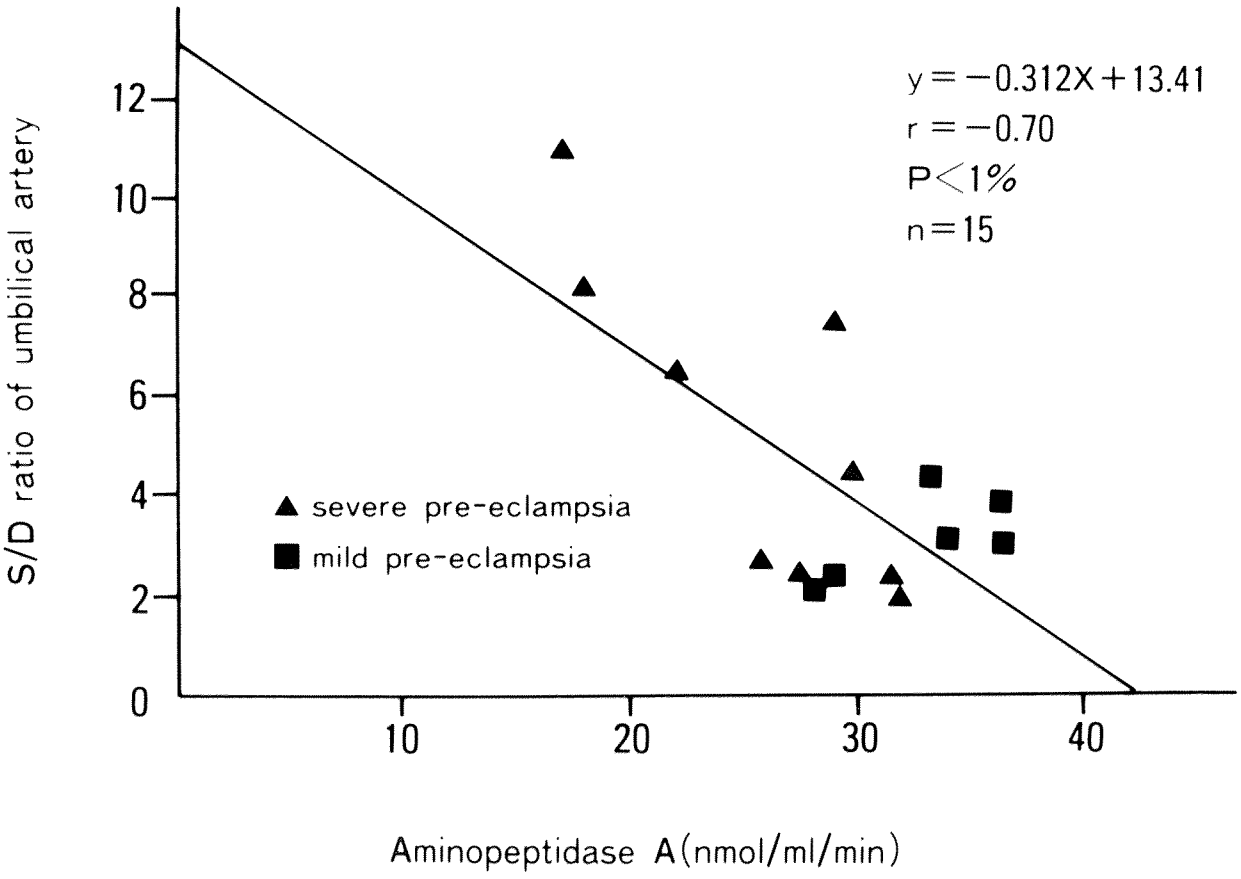
y = −0.312X + 13.41
r = −0.70
P < 1%
n = 15
▲ severe pre-eclampsia
■ mild pre-eclampsia
S/D ratio of umbilical artery
0
2
4
6
8
10
12
10
20
30
40
Aminopeptidase A(nmol/ml/min)

Fig. 3

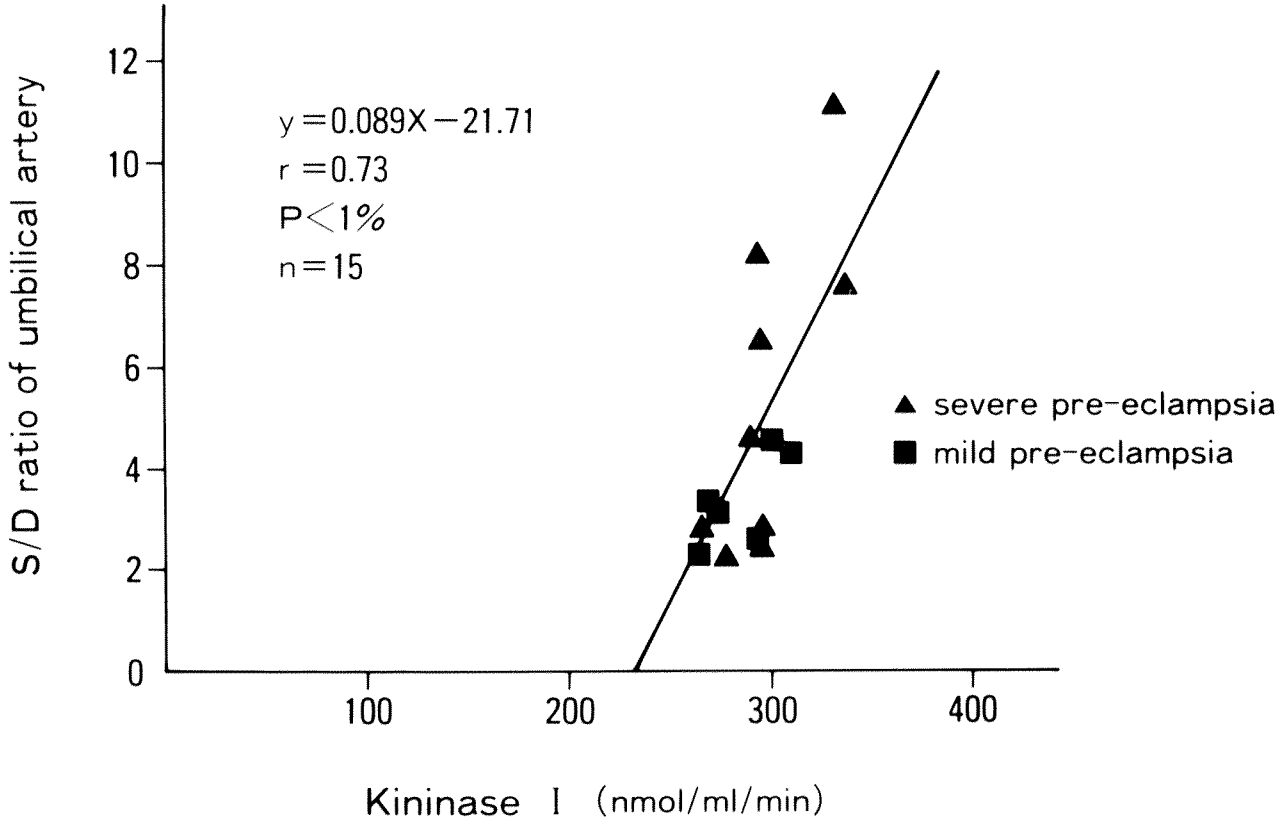
S/D ratio of umbilical artery
12
10
8
6
4
2
0
y=0.089X−21.71
r=0.73
P<1%
n=15
100
200
300
400
Kininase I (nmol/ml/min)
severe pre-eclampsia
mild pre-eclampsia

Fig. 4