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~~**Serum NT-proBNP levels in twin pregnancy**~~

Serum levels of N-terminal fragment of precursor protein brain-type natriuretic peptide (NT-proBNP) in twin pregnancy

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Short title: Behavior of NT-proBNP in twin pregnancies

Title: Serum levels of N-terminal fragment of precursor protein brain-type natriuretic peptide (NT-proBNP) in twin pregnancy

ABSTRACT

Twin pregnancy differs considerably from singleton pregnancy in many aspects and it is unknown how serum NT-proBNP level behaves in women with twin pregnancies. Serum NT-proBNP levels were determined longitudinally at gestational weeks (GW) 24 and 35 in normotensive women with 13 twin and 99 singleton pregnancies. The effects of maternal demographic characteristics on NT-proBNP levels were also analyzed. The serum NT-proBNP levels (pg/mL) in twin pregnancies, which were not different from those in singleton pregnancies at 24 GW (26 ± 15 vs. 40±27, respectively, $P = 0.0718$), increased significantly ($P = 0.0038$) and were significantly higher than those in singleton pregnancies at 35 GW (72 ± 49 vs. 34 ± 24, $P < 0.0001$). In the analysis including women with singleton pregnancies, the serum levels of NT-proBNP at 35 GW were significantly inversely correlated with pre-pregnancy body mass index (BMI, kg/m^2) and were significantly higher in nulliparous than multiparous women. Thus, women with twin pregnancy were likely to exhibit an increase in serum NT-proBNP levels in the late stage of pregnancy, especially in lean and nulliparous women. The relative greater blood volume expansion occurring in twin than in singleton pregnancies was considered to be responsible for this phenomenon.

Key words: NT-proBNP, volume expansion in pregnancy, twin pregnancy

Abbreviations: GW, gestational week; BMI, body mass index

INTRODUCTION

Twin pregnancy differs considerably from singleton pregnancy in many aspects, such as the duration of pregnancy [1], the degree of enhancement of coagulation-fibrinolysis [2], the prevalence of pregnancy-induced hypertension [3, 4], changes in plasma aldosterone and renin activity [5], and the degree of blood volume expansion [6]. These data suggest that pregnancy-induced changes in various biological and physiological parameters may be **higher** in women with twin pregnancies than in those with singleton pregnancies.

Brain-type natriuretic peptide (BNP) is synthesized in cardiac myocytes as the prohormone, proBNP. The active hormone, BNP, is cleaved and co-secreted in a 1:1 ratio from the myocytes along with its N-terminal propeptide (NT-proBNP). Pregnancy is characterized by the retention of sodium and an increase in the blood volume up to more than 50% in twin pregnancies [6]. NT-proBNP concentration has been shown to provide information similar to BNP, and the validity of the assay as a clinical tool is well documented [7]. BNP and NT-proBNP levels in the blood increase in response to volume or pressure overload in the non-pregnant state [8 – 10], as well as in the normal pregnant state [11] and in complicated pregnancies with cardiac and/or pulmonary diseases [12,13] or hypertensive disorder [13 – **22**]. However, neither NT-proBNP nor BNP level has been studied in women with twin pregnancy. Therefore, the present longitudinal study was performed to determine whether levels of NT-proBNP differ between normotensive women with singleton and twin pregnancies.

1. MATERIAL AND METHODS

This longitudinal study was conducted after being approved by the Institutional Review Board of Hokkaido University Hospital and written informed consent was obtained from all 112 participants consisting of 99 and 13 women with singleton and twin pregnancies, respectively. None of these 112 women developed hypertension during pregnancy and all gave birth between October 2009 and August 2011 at our institution. Postpartum hypertension was diagnosed when women exhibited hypertension (systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg) on **at least two occasions, recorded 6 hours apart after birth.**

Blood samples for determination of serum NT-proBNP levels were collected at 24 weeks of gestation (GW) and 35 GW from all 112 participants. Serum were stored at –40°C until assayed. Demographic characteristics and clinical laboratory data of the 112 women were obtained from medical charts.

Serum NT-proBNP levels were measured using an electrochemiluminescence immunoassay kit for NT-proBNP at SRL (Special Reference Laboratory Co., Tokyo). The intra- and interassay coefficients of variation were less than 5.0% for NT-proBNP. A laboratory threshold of 125 pg/mL was used to **rule out cardiac failure.**

Data are presented as means ± standard deviation. Statistical analyses were performed using the JMP8© statistical software package (SAS, Cary, NC). Differences in the means were tested using the Tukey–Kramer HSD (honestly significant difference) test between each group, (要 revision) and categorical variables were compared using Fisher's exact test. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

2. RESULTS

Women with twin pregnancies had a significantly shorter pregnancy, greater infant birth weight (total weight of both twins), and greater gain of the body mass index (BMI, kg/m^2) during pregnancy and were significantly more likely to exhibit hypertension transiently postpartum (postpartum hypertension) than women with singleton pregnancy (Table 1).

2.1.Difference in serum NT-proBNP level between singleton and twin pregnancies

The serum NT-proBNP level (pg/mL) in twin pregnancies was not different from that in singleton pregnancies at 24 GW (26 ± 15 vs. 40±27, respectively, $P = 0.0718$). **The serum NT-proBNP level in twin pregnancies** increased significantly ($P = 0.0038$) and became significantly higher than that in singleton pregnancies at 35 GW (72 ± 49 vs. 34±24, $P < 0.0001$), while that in singleton pregnancies did not change over the same period (Fig. 1). Thus, women with twin pregnancies showed a significant increase in serum NT-proBNP level in the late stage of pregnancy: 10 (77%) of the 13 women with twin pregnancies exhibited an increase of more than 20% from the baseline value at 24 GW, while such an increase was observed in only 25 (25%) of the 99 women with singleton pregnancies ($P = 0.0002$).

2.2.Factors associated with NT-proBNP levels at 35 weeks of gestation

The correlations of NT-proBNP levels at 35 GW with 8 continuous variables, i.e., maternal age, systolic and diastolic blood pressures, gestational week at delivery, body mass indices (BMI) before pregnancy and at delivery, BMI gain during pregnancy, and infant birth weights (sum of twins for twin pregnancy), were analyzed. The serum levels of NT-proBNP at 35 GW were significantly and negatively correlated with **both pre-pregnancy BMI (Fig. 2, left) and BMI at delivery (Fig. 2, right) in analysis including the 112 women with singleton and twin pregnancies. Similar correlations were observed in analysis of the 99 women with singleton pregnancies only** (R =

–0.349, P = 0.00043 for pre-pregnancy BMI; R = –0.273, P = 0.00616 for BMI at delivery). Among the 98 women with singleton pregnancies (pre-pregnancy BMI data were missing for one subject), those with pre-pregnancy BMI below (n = 49) and above (n = 49) the median (20.57 kg/m^2) showed serum NT-proBNP levels (pg/mL) of 40 ± 25 and 27 ± 21, respectively (P = 0.0051): corresponding figures (median value of pre-pregnancy BMI for women with twins: 20.27 kg/m^2) were 97 ± 62 (n = 6) and 50 ± 22 (n = 7), respectively (P = 0.0885) in the 13 women with twin pregnancies, suggesting that women with a lower BMI are likely to exhibit an increase in NT-proBNP level in the late stage of pregnancy.

Parity appeared to influence the serum NT-proBNP level (pg/mL) at 35 GW: in the analysis including all 112 women, those in the 69 nulliparous women were significantly higher than those in the 43 multiparous women (43 ± 34 vs. 31 ± 22, P = 0.0439); corresponding figures were 38 ± 26 vs. 27 ± 18 (P = 0.0238) for singleton pregnancies and 94 ± 61 vs. 53 ± 28 (P = 0.1472) for twin pregnancies, respectively. In the 13 women with twin pregnancies, the serum NT-proBNP levels (pg/mL) were significantly higher in the 3 women with both nulliparity and a pre-pregnancy BMI of < 20.27 kg/m^2 (median value for twin pregnancies) than in the other 10 women (143 ± 44 vs. 51 ± 25, P = 0.0005).

The serum NT-proBNP levels (pg/mL) did not differ between women who did and did not develop postpartum hypertension in either singleton (24 ± 18 vs. 35 ± 24, respectively, P = 0.257) or twin (77 ± 66 vs. 70 ± 44, respectively, P = 0.831) pregnancies.

3. DISCUSSION

The results of the present study indicated that women with twin pregnancies are likely to show an increase in serum NT-proBNP level in the late stage of pregnancy in the absence of hypertensive disorders. Elevated NT-proBNP levels in the late stage of pregnancy were not associated with subsequent appearance of postpartum hypertension, but appeared to be associated with nulliparity and a lower pre-pregnancy BMI.

Serum BNP levels increase in response to volume or pressure overload in the non-pregnant state [8–10] as well as the normal pregnant state resulting in approximately twofold higher levels than in non-pregnant women [11], and pregnancies complicated with cardiac and/or pulmonary diseases [12, 13] and preeclampsia [13–**19**]. These studies regarding BNP levels during normal singleton pregnancies indicated that BNP levels, once elevated to approximately twofold those in non-pregnant state, do not change during pregnancy until term. Although NT-proBNP level, a more stable biomarker in the blood than BNP, has also been shown to be elevated in women with preeclampsia [**20 – 22**], to our knowledge there have been no previous studies regarding either NT-proBNP or BNP levels in otherwise

healthy women with twin pregnancies. The results of the present study suggested that twin pregnancy should be added to the list of conditions in which increased NT-proBNP levels are anticipated that includes cardiopulmonary diseases [7,12,13], infection [**23, 24**], and hypertensive disorders [**20 – 22**] during pregnancy.

We speculated that the relatively greater volume overload occurring in twin compared with singleton pregnancy may be responsible for the increase in NT-proBNP in the late stage of twin pregnancy. Blood volume expansion continues until delivery [**25**] and the blood volume of 3200 – 3800 mL in non-pregnant state increases to 4800 – 5000 mL in the late stage of singleton pregnancy [6, **25**] and up to 5800 mL in the late stage of twin pregnancy [6]. This extra blood volume expansion occurring in twin pregnancy (approximately 900 mL) may induce elevation of NT-proBNP level. Serum BNP levels are approximately twofold higher in healthy pregnant women with singleton pregnancy than in non-pregnant counterparts [11]. Moderate increases in extravascular lung water levels and pulmonary vascular permeability are seen even in singleton pregnancy suggesting an increased risk of pulmonary edema in pregnant women [**26**] and a further increased risk of pulmonary edema is seen in twin pregnancy; 7 of 18 obstetric patients with pulmonary edema were twin pregnancies [**27**], although twin pregnancies account for only 1.0% – 2.0% of all pregnancies. Thus, women with twin pregnancy shown to have elevated NT-proBNP levels in the late stage of pregnancy may have a higher risk for the development of pulmonary edema as do women with preeclampsia [**28**] in whom higher NT-proBNP levels have been documented [**20 – 22**]. Preeclampsia accounted for 28% of 88 cases of obstetric pulmonary edema [**28**], while the prevalence of preeclampsia is less than 5.0%.

Nulliparity and lower pre-pregnancy BMI were associated with increased serum NT-proBNP levels in this study. Although BMI at delivery was also **negatively** correlated with serum NT-proBNP level at 35 GW, BMI at delivery may have reflected that before pregnancy. With regard to the effect of parity on NT-proBNP level during pregnancy, previous reports indicated no difference [**29**] or a slightly but significantly higher level in multiparous than in nulliparous women [**20**]. The reason for these conflicting results is not yet clear. Previous studies indicated inverse relationships between BMI and the levels of BNP and/or NT-proBNP in non-pregnant individuals [**30, 31**], consistent with the results of the present study. However, the reason why women with lower BMI show increased likelihood of elevated NT-proBNP level remains unknown.

In conclusion, this longitudinal study of 112 normotensive women with singleton and twin pregnancies determining NT-proBNP levels at 24 and 35 weeks of gestation indicated that women with twin pregnancies were likely to exhibit increased serum NT-proBNP levels in the late stage of pregnancy. The greater degree of blood volume expansion occurring in

twin than singleton pregnancies is considered to be responsible for this phenomenon.

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DISCLOSURE

None of the authors have a conflict of interest.

VITAE

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FIGURE LEGENDS

Fig. 1: Changes in the serum NT-proBNP levels in singleton (○) and twin (●) pregnancies

The serum NT-proBNP levels were examined longitudinally in 13 and 99 women with twin and singleton pregnancies at gestational weeks (GW) 24 (24.9 ± 1.4 for twins and 24.7 ± 0.9 for singletons) and 35 (34.8 ± 0.8 for twins and 35.5 ± 1.0 for singletons).

*, $P < 0.0001$ vs. value in women with singleton pregnancies; †, $P = 0.0038$ vs. value at 24 GW.

Fig. 2: Correlations of serum NT-proBNP levels at gestational week 35 with demographic characteristics

Among 8 continuous variables shown in Table 1, the serum levels of NT-proBNP at gestational week (GW) 35 were significantly and negatively correlated with pre-pregnancy BMI and BMI at delivery. These significant correlations were also confirmed in analysis of the 99 women with singleton pregnancies.

○, Singleton pregnancy; ×, Twin pregnancy.

Fig. 1

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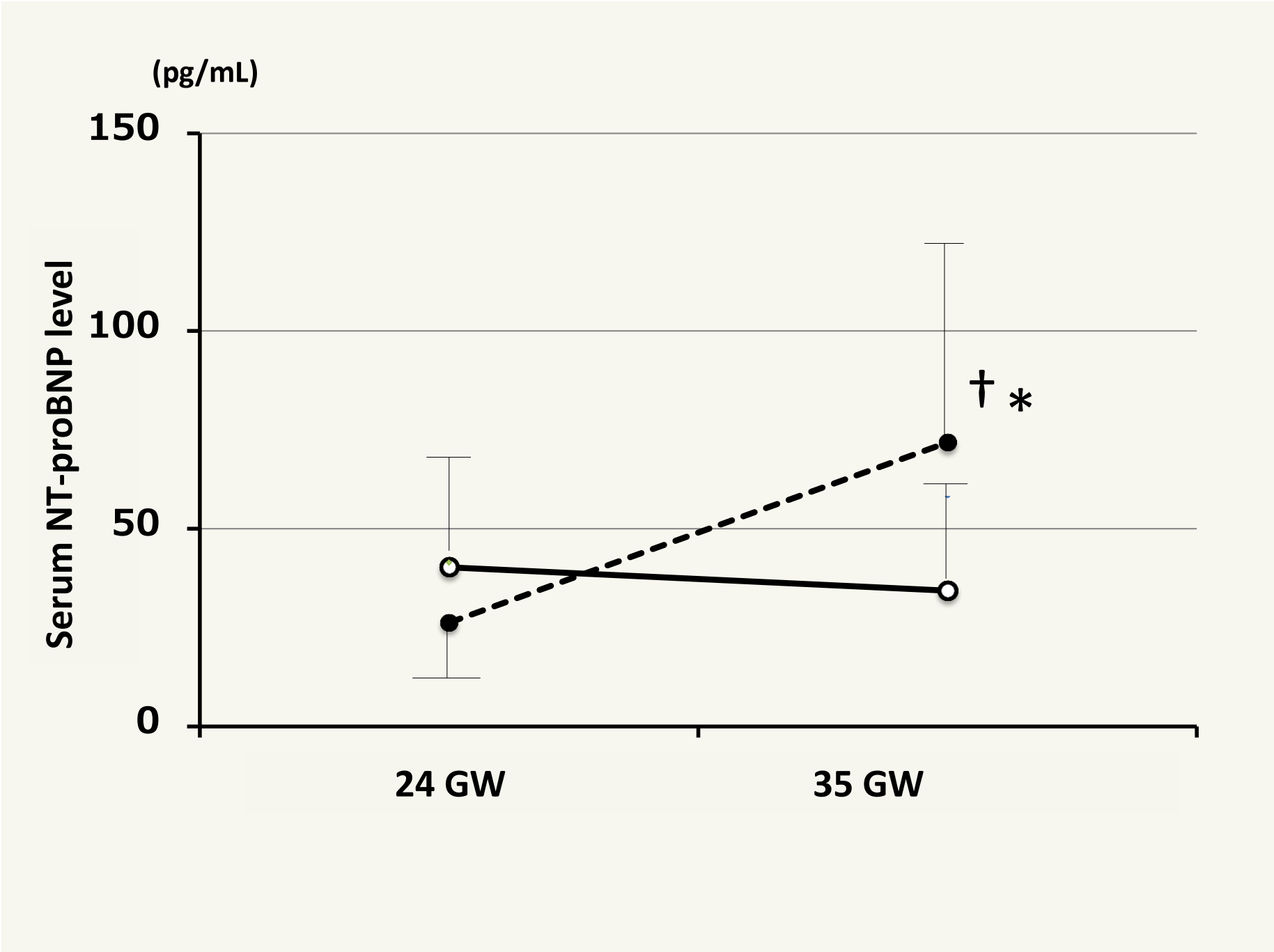

Fig.2

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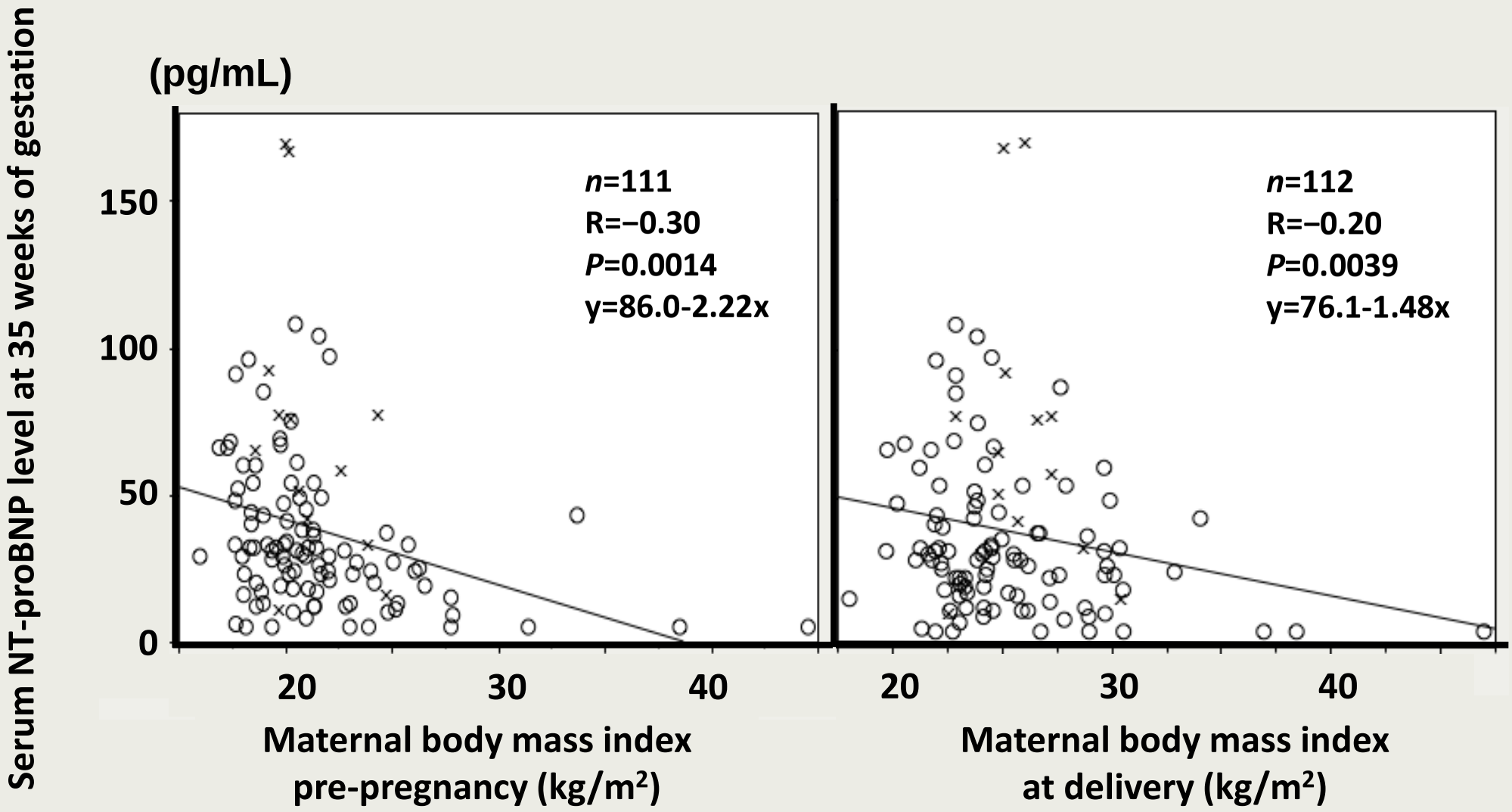

Table 1. Clinical backgrounds of 112 study subjects

	Singleton pregnancy	Twin pregnancy	*P*-value
No. of women	99	13	
Age (years old)	33.3 ± 4.6	32.2 ± 3.3	0.3861
Nulliparity	63 (63.6%)	6 (46.2%)	0.2230
Blood pressure (mmHg)			
Systolic	110 ± 12	112 ± 15	0.5800
Diastolic	65 ± 10	65 ± 12	0.7798
Postpartum hypertension	7 (7.1%)	4 (30.8%)	0.0213
Gestational week at delivery	38.4 ± 1.6	36.6 ± 0.9	0.0001
Body mass index (kg/m^2)			
Pre-pregnancy	21.6 ± 4.3	21.2 ± 2.1	0.7391
At delivery	25.3 ± 4.2	26.0 ± 2.2	0.5523
Gain during pregnancy	3.6 ± 1.9	4.9 ± 1.1	0.0215
Infant birth weight (g)	2963 ± 377	4583 ± 625*	< 0.0001

*, Sum of weights of both twins