



Renin is activated in monochorionic diamniotic twins with birthweight discordance who do not have twin-to-twin transfusion syndrome

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birthweight discordance who do not have
twin-to-twin transfusion syndrome

双胎間輸血症候群ではない体重差がある一絨毛膜双胎においてレニン分泌は亢進している

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Key words: twin pair , discordancy , umbilical cord blood,
renin-angiotensin-aldosterone system, natriuretic peptide system

Original Article

**Renin is activated in monochorionic diamniotic twins with birthweight discordance
who do not have twin-to-twin transfusion syndrome**

Running title: Renin activation in monochorionic diamniotic twins

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All authors (K.F., I.M., A.M., T.Y., K.M., S.M., M.E., A.S., M.Mo., N.Y., H.Y., and M.Ma.)
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M.E., A.S., N.Y., and M.Ma. were neonatologists of the patients and were collected the
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hANP and BNP, and serum aldosterone levels. K.F., I.M., and M.E. performed statistical analysis of the data. M.Mo. and H.Y. were obstetricians and collected the cord blood samples of the patients.

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Abstract

Objective: To assess renin, aldosterone, human atrial natriuretic peptide (hANP), and brain natriuretic peptide (BNP) levels in cord blood from monochorionic diamniotic (MD) twins with a birthweight (BW) discordance that do not satisfy the criteria of twin-to-twin transfusion syndrome (TTTS).

Study design: Cord blood samples were obtained from 28 MD twins without TTTS. They were divided into two groups based on BW discordance as follows: large ($>7.5\%$) and small BW discordance ($\leq 7.5\%$). Cord blood renin, aldosterone, hANP, and BNP levels were measured.

Result: Renin levels in MD twins with a large BW discordance were significantly higher than those in MD twins with a small BW discordance, with no significant differences in aldosterone, hANP and BNP levels. A significant correlation was found between renin levels and BW discordance.

Conclusion: Renin is activated in MD twins with a BW discordance of more than 7.5%, even in non-TTTS.

Key words: twin pair; discordancy; umbilical cord blood; renin-angiotensin-aldosterone system; natriuretic peptide system

Introduction

Approximately 15% of monochorionic diamniotic (MD) twin pregnancies are complicated by twin-to-twin transfusion syndrome (TTTS), isolated discordant growth, twin anemia-polycythemia sequence, or congenital defects.^{1, 2} Despite the recent advances in perinatal management, TTTS is an important cause of neonatal death and handicap in infants.² TTTS is thought to arise from an imbalance of intertwin transfusion across vascular anastomoses in placentas, and leads to hypervolemia, polyuria, and polyhydramnios in the recipient, and hypovolemia, oliguria, and oligohydramnios in the donor.² However, although most of the placentas in MD twins have vascular anastomoses, approximately 85% of MD twins do not develop TTTS. Furthermore, most TTTS patients do not have significant intertwin hemoglobin discordance.³ The pathogenesis of TTTS is still unclear.

The renin-angiotensin-aldosterone (RAA) system, including renin and aldosterone, plays a major role in maintaining vascular tone, optimal salt and water homeostasis, and cardiac function. Its overactivity has been recognized to result in conditions of hypertension and left ventricular hypertrophy.⁴ The natriuretic peptide (NP) system includes human atrial natriuretic peptide (hANP) and brain natriuretic peptide (BNP), which have vasodilative effects and are oversecreted from the heart in patients with congestive heart failure.⁵ The RAA and NP systems have been shown to be activated in MD twins with TTTS, and they play an important role in the appearance of clinical manifestations.⁶⁻⁹ This phenomenon has resulted in determining obstetric interventions for TTTS in MD twins.^{8, 10}

The activation levels of the RAA and NP systems in MD twins before development of TTTS need to be determined. Furthermore, it is widely accepted that the birthweight (BW) discordance between MD twins is a common predictive factor for developing TTTS.¹¹ There may be hormonal activation in MD twins with BW discordance due to imbalanced blood flow between the twins, even in non-TTTS twins. Therefore, it is important to determine whether

the RAA and NP systems are activated in non-TTTS MD twins. However, there are no studies regarding the RAA and NP systems in MD twins without TTTS. The purposes of this study were 1) to assess cord blood levels of renin, aldosterone, hANP, and BNP in dichorionic diamniotic (DD) and MD twins that do not satisfy the criteria of TTTS, 2) to compare these hormone levels between smaller and larger MD twins, 3) to compare these hormone levels between small and large BW discordance in non-TTTS MD twins, and finally, 4) to investigate correlations between cord blood levels of renin and BW discordance in non-TTTS MD and DD twins.

Materials and Methods

Study design and patient groups

This observational study was conducted under the approval of the ethical committee of Kobe University Graduate School of Medicine with written informed consent acquired from the parents of the patients. Of 91 twin pairs (182 newborn infants) who were born at Kobe University Hospital, which is a referral perinatal center in Kobe city, between April 2006 to June 2010, 68 twin pairs (136 newborn infants) whose cord blood was obtained, were enrolled in this study (gestational age: 36 weeks [27 to 37 weeks], BW: 2,169 g [658 to 2,968 g]). Patients with congenital or chromosomal anomalies, or patients whose cord blood could not be obtained, were excluded.

Sixty-eight twin pairs were separated into 28 MD twins (56 newborn infants) and 40 DD twins (80 newborn infants), who served as controls, by pathological findings of placentas after delivery. Monochorionic monoamniotic twins were not found during the study period. All of the 28 MD twins did not satisfy Quintero's diagnostic criteria of TTTS during pregnancies.¹² Smaller or larger MD twins were determined based on the BW of the twin pairs. Furthermore, 28 MD twin pairs (56 newborn infants) were divided into two groups

based on a 7.5% BW discordance: 30 newborns had a small BW discordance ($\leq 7.5\%$) and 26 newborns had a large BW discordance ($> 7.5\%$).

Clinical and laboratory data, including cord blood levels of renin, aldosterone, hANP, and BNP were compared between MD and DD twin groups, between smaller and larger MD twins, and between small and large BW discordance groups in MD twins.

Decision for delivery by caesarean section (CS)

The fetal condition was monitored conventionally by ultrasonography in combination with fetal heart rate monitoring and fetal biophysical profile. Indications for delivery by emergency CS were at the discretion of the attending obstetricians; indications included fetal deterioration and fetal growth arrest for at least 2 weeks. Abnormal doppler waveforms, such as reversed flow in the umbilical artery, were taken into consideration as indications for delivery by emergency CS. Cases without indications for delivery by emergency CS were delivered by elective CS at 36 to 37 weeks of gestation.

Clinical data of the patients

Clinical data were collected from the patient's record including gestational age, BW, Apgar scores (1 and 5 min), sex, maternal age, and the presence or absence of assisted reproductive technology, pregnancy induced hypertension (maternal systolic blood pressure > 140 mmHg and/or diastolic pressure > 90 mmHg during pregnancy), premature rupture of the membranes (rupture of the membranes more than 24 h before delivery), intrauterine growth restriction (BW less than the 10th percentile of mean BW at the same gestational age in Japanese newborns¹³), and neonatal asphyxia (Apgar scores ≤ 7 at 1 min after birth).

Calculation of BW discordance between twins

The BW discordance between twins was calculated using the following formula:

$$(\text{BW of the larger twin} - \text{BW of the smaller twin}) / \text{BW of the larger twin} \times 100.$$

Cord blood sample collection

All twin infants were delivered by CS. Umbilical venous cord blood samples were collected by obstetricians and were immediately given to neonatologists. They were then promptly ice-cooled and centrifuged. The plasma and serum samples were stored at -20°C until assay.

Measurements of plasma renin activity, hANP and BNP, and serum aldosterone levels

Plasma renin activity and serum aldosterone levels were measured by radioimmunoassay kits using Renin-Riabeal[®] (TFB, Tokyo, Japan) and SPAC-S Aldosterone[®] (TFB, Tokyo, Japan), respectively, as previously described.¹⁴ Plasma hANP and BNP levels were measured with a chemiluminescent enzyme immunoassay kit using MIO2 Shionogi ANP[®] (Shionogi, Osaka, Japan) and an immunoenzymometric assay kit using TOSOH AIA-PACK BNP[®] (TOSOH Corp, Tokyo, Japan), respectively, as previously described.¹⁵

Assays for renin activity, aldosterone, hANP, and BNP were not performed in 10, 5, 15, and 6 samples, respectively, because of an insufficient amount of cord blood. Therefore, renin activity, aldosterone, hANP, and BNP levels were measured in 126, 131, 121, and 130 samples, respectively.

Statistical analysis

Data are expressed as the median (range) or number (%). Statistical analyses were performed with the Mann-Whitney nonparametric rank test, chi-square test, or Fisher's exact test as appropriate to compare data between the two groups. Furthermore, regression analysis was performed to linearly compare cord blood levels of renin and BW discordance in MD and DD twins. The regression equation and coefficient of determination (R^2) were also calculated. Differences and correlations were deemed statistically significant when $p < 0.05$.

Results

Clinical background in MD and DD twin groups

Clinical characteristics in the MD and DD twin groups are shown in Table 1. Gestational age, BW, Apgar scores and sex were similar in both groups. There were no significant differences in the incidence of pregnancy induced hypertension, premature rupture of the membranes, intrauterine growth restriction, and neonatal asphyxia. As expected, maternal age and the incidence of assisted reproductive technology were significantly higher in the DD twin group than those in the MD twin group ($p=0.01$ and $p<0.001$, respectively).

Comparison of cord blood levels of renin, aldosterone, hANP, and BNP between MD and DD twin groups

Cord blood levels of renin, aldosterone, hANP, and BNP were not significantly different between the MD and DD twin groups (Table 2).

Comparison of cord blood levels of renin, aldosterone, hANP, and BNP between smaller and larger MD twin groups.

When MD twins were divided into smaller and larger twin groups, cord blood levels of renin, aldosterone, hANP, and BNP were similar in both groups, with no significant differences (Table 3).

Clinical background of small and large BW discordance groups in MD twins

The clinical characteristics of the small and large BW discordance groups in MD twins are shown in Table 4. Gestational age, BW, Apgar scores, sex and maternal age were similar in both groups. There were also no significant differences in the incidence of assisted reproductive technology, pregnancy induced hypertension, premature rupture of the membranes, intrauterine growth restriction, and neonatal asphyxia among the groups.

Comparison of cord blood levels of renin, aldosterone, hANP, and BNP between small and large BW discordance groups in MD twins.

Cord blood levels of renin in the large BW discordance group were significantly higher than those in the small BW discordance group ($p < 0.001$, Fig. 1A). However, no significant differences were found in cord blood levels of aldosterone, hANP, and BNP between the groups (Fig. 1B-D).

Correlation between cord blood levels of renin and BW discordance in MD and DD twins.

As shown in Figure 2, cord blood levels of renin and BW discordance in MD twins were significantly correlated ($R^2 = 0.170$; $p < 0.01$). However, cord blood levels of renin and BW discordance were not correlated in DD twins ($R^2 = 0.00478$; $p =$ not significant [n.s.]).

Discussion

The present study has demonstrated for the first time that renin levels, but not aldosterone levels, in non-TTTS MD twins with a large BW discordance were significantly higher than those in non-TTTS MD twins with a small BW discordance, with no significant difference in clinical background between the groups. Furthermore, cord blood levels of renin and aldosterone were similar between larger and smaller MD twins. Our results are consistent with those in previous reports.^{6, 7, 10} Previous studies have found that both MD twin pairs (smaller and larger twin pairs) of TTTS with BW discordance had high renin and normal aldosterone cord blood levels.^{6, 7} Renin levels were high because renin is produced from the kidney of the donor (smaller) twin and it passes into the circulation of the recipient (larger) twin by intertwin transfusion along placental vascular anastomoses (paradoxical activation of the RAA system).^{6, 7} In addition, Galea et al. reported that renin mRNA is increased in the placenta of the recipient's territory compared with that in the donor's placenta in TTTS

patients.¹⁰ Renin is secreted from the placenta in recipient (larger) twins.¹⁰ On the other hand, the reason for normal aldosterone levels could be because of complex regulation of the secretion of aldosterone. Adrenal corticotrophin and concentrations of potassium and sodium are also related to the secretion of aldosterone, in addition to the RAA system.^{6, 7} We confirmed that there was no correlation between renin and aldosterone levels in our MD twin population (data not shown).

Bajoria et al. showed high concentrations of hANP in the cord blood of recipient (larger) twins of TTTS.⁸ Van Mieghem et al. showed that BNP levels are increased in the amniotic fluid of recipient twins of TTTS.⁹ Therefore, since larger TTTS twins might have high levels of hANP and BNP, we compared hANP and BNP levels between smaller and larger MD twins. We found that cord blood levels of hANP and BNP were not significantly different between larger and smaller MD twins. Furthermore, there were no significant differences in cord blood levels of hANP and BNP between MD and DD twins. We speculate that the reason that there was no increase in hANP and BNP cord blood levels, is that MD twins in our study had not developed TTTS and they did not have congestive heart failure, because these hormones are mainly secreted from the heart.⁵

We also found a correlation between BW discordance and renin activation in MD twins, but not in DD twins. Several reports have confirmed that BW discordance in MD twins is a common predictive factor for developing TTTS or neonatal mortality.^{11, 16, 17} Fetal growth discordance in MD twins without TTTS can have different etiologies, such as unequal placental sharing, and imbalanced function and blood flow in the placenta. Our results suggest that there may be renin activation due to imbalanced blood flow between the twins in MD twins without TTTS. Recently, there have been some reports on the correlation between BW discordance in MD twins with high activities of some hormones such as insulin-like growth factor-1, ghrelin, leptin, and adiponectin.¹⁸⁻²⁰ Renin should also be included as one of these

hormones. These previous reports¹⁸⁻²⁰ defined a discordant twin as a discordant rate >15%. In our study, we divided MD twins into two groups depending on a discordant rate of 7.5% for the following reasons: 1) we set our baseline to half that of the 15% discordant rate according to definition of discordant twins for the previous reports¹⁸⁻²⁰, to clarify the process of development of TTTS, and 2) we adjusted the patient numbers in each group equally, as the median discordant rate of the total cohort was nearly 7.5%.

We enrolled sufficient numbers of DD twins to serve as controls. The clinical background in DD twins was similar to that in MD twins, except for maternal age and the rate of assisted reproductive technology, which is consistent with a previous report.²¹ Median cord blood levels of renin, aldosterone, hANP, and BNP in MD twins were similar to those in DD twins, and had similar values as for singletons in our center for perinatal care (p = n.s., data not shown). Although there have been many studies regarding hormonal activity in MD twins, particularly related to TTTS,^{6-9, 18-20} there have been few studies using DD twins as controls. MD twins that have not developed TTTS have usually served as controls for studies comparing them with MD twins with TTTS. Our results suggest that caution is advised when MD twins without development of TTTS are used as controls, because some hormones including renin are activated in MD twins with BW discordance, even in non-TTTS.

Recently, it has been widely accepted that there is a certain MD twin group that has a poor prognosis, but it does not satisfy the criteria of TTTS, such as selective intrauterine growth restriction, which is defined as growth restriction of one of the twins, and it mainly manifests as fetal weight discordance.^{22, 23} Kondo et al. have reported a case of MD twins in which one twin had cardiac hypertrophy without TTTS, while the other fetus had selective growth restriction without any signs of TTTS.²⁴ The RAA system has significant influence on the fetal/neonatal heart function.²⁵ In enrolled MD twins for this study, the incidence of patients with circulatory collapse, who were treated by inotropic agents, was significantly higher in

the large BW discordance group (7/26, 27%) than those in the small BW discordance group (2/30, 7%). Because renin was activated in our MD twins with large BW discordance, RAA activation may serve as an etiology of circulatory collapse after birth in MD twins with BW discordance. In addition, some reports of animal studies have shown that intrauterine growth-restricted offspring exhibited an enhanced sensitivity to angiotensin in spite of normal expressions of intrarenal angiotensin.^{26, 27} RAA activation in the pathogenesis of TTTS may be applied to not only TTTS patients, but also to MD twins with BW discordance and with selective intrauterine growth restriction.

The limitation of this study is that we could not divide the subjects by a more detailed BW discordance, such as 15%, and 25%, because of the relatively small numbers of MD twin patients and control subjects. Further studies using larger twin patient sets and another definition of BW discordance are required. Additionally, we were unable to analyze the data of amniotic fluid volume because of broad dispersion at some examinations or missing data. However, most of the amniotic fluid volumes of MD twin patients in our study were within the normal range since there were no MD twins that satisfied the criteria of TTTS.¹² In conclusion, this study showed for the first time that renin is activated in MD twins with BW discordance even in non-TTTS.

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Conflict of Interest

The authors have no conflicts of interest to declare.

Figure legends

Figure 1. Comparison of cord blood levels of renin (A), aldosterone (B), hANP (C), and BNP (D) between small and large BW discordance groups in MD twins. Cord blood levels of renin, aldosterone, hANP, and BNP are expressed as the median (range). Cord blood levels of renin in the large BW discordance group were significantly higher than those in the small BW discordance group ($p<0.001$). There were no significant differences in cord blood levels of aldosterone, hANP, and BNP between the groups. *n.s.*, not significant.

Figure 2. Correlation between cord blood levels of renin and BW discordance in MD (A) and DD (B) twins. Cord blood levels of renin and BW discordance in MD twins were significantly correlated. No correlation was found in DD twins. R^2 , coefficient of determination. *n.s.*, not significant.

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Table 1. Clinical background in MD and DD twins.

		MD twin n=56	DD twin n=80	P value
Gestational age (weeks)		36 (29-37)	36 (27-37)	n.s.
Birth weight (g)		2,232 (1,030-2,968)	2,122 (658-2,904)	n.s.
Apgar score	1 min	8 (2-9)	8 (3-9)	n.s.
	5 min	9 (1-10)	9 (7-10)	n.s.
Male (%)		22/56 (39%)	37/80 (46%)	n.s.
Maternal age (years)		30 (23-39)	33 (22-51)	0.01
Assisted reproductive technology (%)		4/28 (14%)	31/40 (78%)	< 0.001
Pregnancy induced hypertension (%)		2/28 (7%)	4/40 (10%)	n.s.
Premature rupture of the membranes (%)		4/28 (14%)	5/40 (13%)	n.s.
Intrauterine growth restriction (%)		5/56 (9%)	17/80 (21%)	n.s.
Neonatal asphyxia (%)		13/56 (23%)	17/80 (21%)	n.s.

Data are expressed as median (range) or number (percent); *MD*, monochorionic diamniotic; *DD*, dichorionic diamniotic; *n.s.*, not significant

Table 2. Comparison of cord blood levels of renin, aldosterone, hANP, and BNP between MD and DD twins.

	MD twin		DD twin		P value
	n	Cord blood level	n	Cord blood level	
Renin (ng/ml/h)	53	6.6 (1.8-20.0)	73	4.6 (0.5-19.0)	n.s.
Aldosterone (pg/ml)	55	590 (124-1200)	76	539 (152-1580)	n.s.
hANP (pg/ml)	53	61 (15-310)	68	52 (18-239)	n.s.
BNP (pg/ml)	56	30 (6-249)	74	27 (6-279)	n.s.

Data are expressed as median (range); *MD*, monochorionic diamniotic; *DD*, dichorionic diamniotic; *hANP*, human atrial natriuretic peptide; *BNP*, brain natriuretic peptide; *n.s.*, not significant

Table 3. Comparison of cord blood levels of renin, aldosterone, BNP, and hANP between smaller and larger MD twins.

	Smaller twin		Larger twin		P value
	n	Cord blood level	n	Cord blood level	
Renin (ng/ml/h)	26	7.1 (1.9-19.0)	27	6.3 (1.8-20.0)	n.s.
Aldosterone (pg/ml)	27	641 (124-1200)	28	547 (173-1120)	n.s.
hANP (pg/ml)	25	61 (15-310)	28	66 (17-254)	n.s.
BNP (pg/ml)	28	28 (6-180)	28	32 (10-249)	n.s.

Data are expressed as median (range); *MD*, monochorionic diamniotic; *hANP*, human atrial natriuretic peptide; *BNP*, brain natriuretic peptide; *n.s.*, not significant

Table 4. Clinical background of small and large BW discordance groups in MD twins.

		Small BW discordance n=30	Large BW discordance n=26	P value
Gestational age (weeks)		36 (29-37)	36 (29-36)	n.s.
Birth weight (g)		2,255 (1,142~2,638)	2,108 (1,030~2,968)	n.s.
Apgar score	1 min	8 (2-9)	8 (2-9)	n.s.
	5 min	9 (8-10)	9 (1-10)	n.s.
Male (%)		12/30 (40%)	10/26 (38%)	n.s.
Maternal age (years)		29 (23-39)	32 (24-39)	n.s.
Assisted reproductive technology(%)		2/15 (13%)	2/13 (15%)	n.s.
Pregnancy induced hypertension(%)		1/15 (7%)	1/13 (8%)	n.s.
Premature rupture of the membranes(%)		1/15 (7%)	3/13 (23%)	n.s.
Intrauterine growth restriction(%)		1/30 (3%)	4/26 (15%)	n.s.
Neonatal asphyxia (%)		6/30 (20%)	7/26 (27%)	n.s.

Data are expressed as median (range) or number (percent); *MD*, monochorionic diamniotic; *BW*, birthweight; *n.s.*, not significant



