Conclusion: The CBUN level would provide an estimate of adequate protein intake and the subsequent development of an ELBW infant.

1. Introduction

Human milk is recommended for the management of extremely low-birth-weight (ELBW) infants [1,2]. However, it needs to be supplemented with proteins and other nutrients because by itself, human milk cannot meet the high nutrient requirements of ELBW infants [3]. Human milk is usually fortified based on the nutritional recommendations such as those from the American Academy of Pediatrics (AAP) [4] or the European Pediatric Society of Gastroenterology and Nutrition (EPSGN) [5]. Compared to infants born at term, ELBW infants tend to have much higher nutritional requirements due to their poor nutrient store, rapid growth, severity of illness, and physiological immaturity [6,7]. It is well known that infants suffering from chronic lung diseases display poor weight gain as a result of inadequate nutrient intake [8]. These infants tend to have poor nutritional intakes due to fluid restrictions that are imposed due to their respiratory status. Furthermore, the nutrient content of human milk is not constant. A gradual reduction in the concentrations of the key components occurs during the first 2 months of lactation [9]. Therefore, a fixed level of human milk fortification may be inadequate for ELBW infants because of their variable nutritional demands. As recently advocated by Polberger et al. [10], individualized supplementation is recommended: however, this has not yet been popularized. Moro et al. [11] have proposed a method of adjusting the amount of human milk fortification based on corrected blood urea nitrogen (CBUN) levels. Since this monitoring method considers the infant's metabolic response in relation to protein intake, it may enable optimal nutritional supplementation in ELBW. At our neonatal intensive care unit (NICU), the human milk fortification method was not individualized according to the method described by Moro et al. The two types of fortification methods used were not adjusted based on the CBUN value; hence, the observed CBUN values varied. The purpose of this retrospective study was to evaluate whether the CBUN levels predicted the developmental outcome in ELBW infants at 36 months of post-conceptual age (PCA).

2. Materials and methods

Between 1996 and 1999, 178 ELBW infants born at <28 weeks of gestation were admitted to the NICU of the Osaka Medical Center for Maternal and Child Health. Of these, 32 infants died during the neonatal period. In this study, we excluded infants with neonatal factors, except for the nutritional factor, which could influence the cognitive and renal functions. Therefore, the exclusion criteria were death, major congenital anomalies, intraventricular hemorrhage (grades 3–4), meningitis, congenital hydrocephalus, cerebral infarction, administration of prostaglandin E1 (PGE1) inhibitors, intestinal perforation, and renal failure. A total of 79 infants were followed up; of these, 42 infants were either not assessed for developmental quotient (DQ) or not traceable at 36 months of PCA. The DQ was assessed only for the remaining 37 eligible infants at 36 months of PCA (Fig. 1).

Two clinical psychologists in our hospital assessed the DQ using the revised Kyoto Scale of Psychological Development [19] at approximately 36 months of PCA (range, 32–40 months of PCA). This examination has been stan-

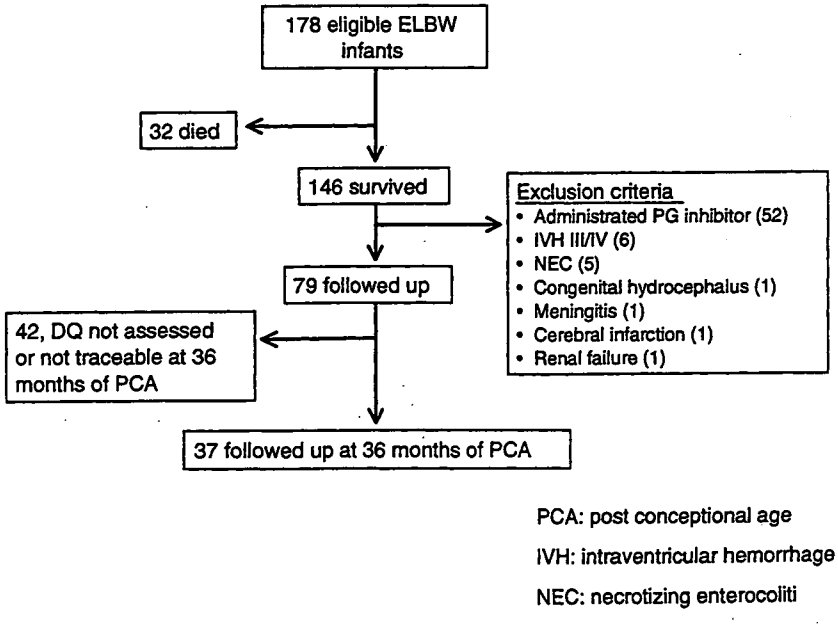

Figure 1 Formation of the study group that was followed up at the PCA of 36 months.

dardized and is widely used in Japan [20]. It has been modified from the Weschsler Intelligence Scale for Children Revised (WISC-R) [20] and it assesses all aspects of an infant's performance. The developmental performance of an infant is expressed as the developmental age for each behavioral area (postural-motor, cognitive–adaptive, and language–social areas) and all areas. The DQ is obtained by dividing the estimated developmental age by the chronological age and then multiplying the quotient by 100. The infants were divided into two groups based on their DQs at 36 months of PCA (DQ≥80 and DQ<80). At our center, DQ≥80 is defined as a value showing typical development in an infant [19]. Further, the clinical characteristics of infants were compared between the two groups.

The CBUN level was calculated using Moro's formula (BUN×0.5/serum creatinine level). It was determined at least once a week, and the area under the curve (AUC) of CBUN (mmol×day/l) between 28 and 84 days of life was calculated. The BUN values usually correlate with the protein intake after 4 weeks of life [12–14]. However, the rise in the BUN does not accurately reflect the protein load in premature infants during the first 4 weeks of life because the urea cycle at this age is not as developed as that in term infants [15]. Therefore, although nutrition is extremely important during the first 4 weeks of life [16–18], the CBUN levels cannot be used as an index of protein intake. The AUC of CBUN was calculated using ImageJ® software (ver. 1.32, NIH, Bethesda, Maryland, USA) after plotting one CBUN value every week; the CBUN value was obtained between 28 and 84 days of life. These values were plotted using Excel® software (Microsoft Corporation, USA) to evaluate the AUC of CBUN accurately.

The calorie and protein contents in human milk were estimated to be 0.69 kcal/ml and 1.3 g/dl, respectively. These values correspond to those observed in the milk at mid-lactation in Japanese women [21]. Differences between the milk of an infant's mother and donor human milk were not considered.

The study was approved by the local institutional review board, and an informed parental consent was obtained prior to the study.

2.1. Feeding strategy at our NICU

Table 1 summarizes the data of the nutritional content used in the human milk fortification method used at our NICU. We add either 3 g or 5 g of the fortifier, HMS-1® (Morinaga Milk Industry Co. Ltd., Japan) (protein 0.26 g/g of fortifier, energy 3.37 kcal/g), to 100 ml of human milk (HM) to achieve a target protein content of 3–4 g/kg/day and calorie intake of 120 kcal/kg/day [4,5]. The infants were fed HM+3 g/dl HMS-1® (3H) fortification when the amount of enteral feed was ≥150 ml/kg/day. The infants were fed HM+5 g/dl HMS-1® (5H) fortification when the amount of enteral feed was <150 ml/kg/day due to their condition. When the calorie intake was less than the target calorie intake, we further supplemented the milk with medium-chain triglyceride oil (approximately 2 ml/kg/day). When mother's milk was insufficient, we used donor milk during the first month and preterm formula later (Neomilk PM®, Bean Stalk Snow Co. Ltd., Japan) for feeding the ELBW infants; these were used because ELBW infants who fed on

Table 1 Variation in the nutritional content using the milk fortification protocol at our NICU

Feed intake	130 ml/kg/day				150 ml/kg/day				
	HM	HM+3H	HM+5H	18% PM	HM	HM+3H	HM+5H	16% PM	HMS-1™ (/g)
Protein (g/kg/day)	2.7	3.4	3.2	3.6	2.0	3.1	3.9	3.6	0.26 g
Fat (g)	4.8	4.8	4.1	4.6	5.6	5.6	5.6	4.7	0.8
Carbohydrate (g/kg/day)	10.0	12.2	12.5	11.0	11.6	14.1	15.8	14.4	0.56 g
Calories (kcal/kg/day)	89.7	102.9	111.6	98.8	103.5	118.7	128.8	114.0	3.37 kcal

HM: human milk.
HMS-1®: human milk fortifier used in Japan (Morinaga Milk Industry Co. Ltd., Japan).
HM+3H: fortified human milk+3 g/dl HMS-1®.
HM+5H: fortified human milk+5 g/dl HMS-1®.
16% PM: standard concentration of Neomilk PM® (Bean Stalk Snow Co. Ltd., Japan).

Table 2 Characteristics of the study population

	DQ≥80 (n=22)	DQ<80 (n=15)	p
Gestational age (weeks)	25.9±1.3	25.4±0.9	NS
Birth weight (g)	739.5±127.6	724.7±155.6	NS
Z score of birth weight	−0.7±0.6	−0.5±0.8	NS
Birth length (cm)	32.3±2.9	31.3±1.9	NS
Z score of birth length	−1.0±1.0	−0.8±0.9	NS
Birth head circumference (cm)	23.1±1.4	22.8±2.0	NS
Z score of birth head circumference	−0.7±0.5	−0.3±1.1	NS
Apgar score at 1 min	1–8(median 5)	1–8(median 3)	NS
Apgar score at 5 min	5–9(median 8)	1–9(median 6)	<0.01
Sex (no of males)	11	9	NS
Duration of artificial ventilation (days)	30.0±24.0	50.3±33.8	<0.05
Duration of O_2 supplementation (days)	71.1±56.9	127.6±112.6	<0.01
AUC of CBUN (mmol day/l)[a]	285.2±113.5	206.2±80.3	<0.05
Average CBUN (mmol/l)[b]	4.5±1.7	3.3±1.2	0.05
Human milk feeding ratio (HMFR) (2 months of life) (%)[c]	80.3±31.3	66.2±26.9	0.14

NS: not significant.
[a] Area under the curve of CBUN between 28 and 84 days of life.
[b] Average CBUN level between 28 and 84 days of life.
[c] Intake of human milk/intake of (human milk+formula).

formula milk are at a risk of developing necrotizing enterocolitis [22]. We adjust the concentration of the preterm formula as 16% and 18%; 16% concentration is equivalent to HM+3H fortification and 18% concentration is equivalent to HM+5H fortification.

2.2. Statistical analysis

Data were retrospectively analyzed. The statistical analyses included the X^2 test, Mann–Whitney U test, and multiple logistic regression analysis. In all cases, StatView software (ver. 5.0, SAS institute Inc., USA) was applied.

3. Results

Table 2 lists the detailed characteristics of infants included as the study population. No significant differences were observed between the two groups with regard to the gestational age, birth weight, birth length, head circumference at birth, Apgar score at 1 min, sex, and human milk feeding ratio (HMFR, defined as intake of human milk/intake of (human milk+formula milk) during the first 2 months). Compared to the "DQ<80" group, the "DQ≥80" group displayed a higher Apgar score at 5 min, a shorter duration of artificial ventilation and O_2 supplementation, and a higher AUC of CBUN between 28 and 84 days of life. Table 3 shows the results of multiple regression analysis of the overall DQ scores ≥80 points at 36 months of PCA. After adjusting the gestational age, Z score of birth weight, sex, Apgar score at 5 min, and duration of ventilation, we observed that only the AUC of CBUN between 28 and 84 days of life influenced the overall DQ score at 36 months of PCA.

Table 3 Logistic multiple regression analysis for an overall DQ≥80 points at 36 months of PCA

	OR	95% CI	p
Gestational age (weeks)	0.71	0.20–2.53	0.60
Z score of birth weight	0.40	0.07–2.41	0.32
Sex (male)	0.20	0.03–1.63	0.13
Apgar score (5 min)	2.00	0.89–4.28	0.10
Duration of ventilation (days)	0.99	0.95–1.03	0.50
*AUC of CBUN (mmol day/l)	1.03	1.002–1.06	<0.05

(n=37, R^2=0.41)
OR: Odd's ratio.
CI: confidence interval.
* AUC of CBUN between 28 and 84 days of life.

Fig. 2 illustrates the mean calorie and protein intakes calculated every 2 weeks in both the "DQ≥80" and "DQ<80" groups. With the exception of protein intake between 2 and 4 weeks of life, no significant differences were observed between the groups with regard to the protein and calorie intakes.

Fig. 3 shows the average CBUN and serum creatinine levels estimated every 2 weeks after birth in the two groups, which were divided based on the overall DQ at 36 months of PCA. Although the average serum creatinine level did not differ, the CBUN level in the "DQ≥80" group was greater than that in the "DQ<80" group, except for the level during first 2 weeks of life.

There were no significant differences between infants with DQ≥80 and DQ<80 with regard to infants' growth at 36 months of PCA (weight (kg): 12.0±1.4 and 11.6±1.5, length (cm): 92.0±3.8 and 89.4±3.4, and head circumference (cm): 49.1±2.0 and 48.2±2.2, respectively).

4. Discussion

There are no indices to predict the optimal protein intake for ELBW infants. We could not clarify whether the CBUN value in ELBW infants used for estimating the protein intake predict their cognitive function later in life. On multiple regression analysis, we observed that DQ≥80 significantly correlated with the AUC of CBUN. This may suggest that a high CBUN value reflects adequate protein intake in ELBW

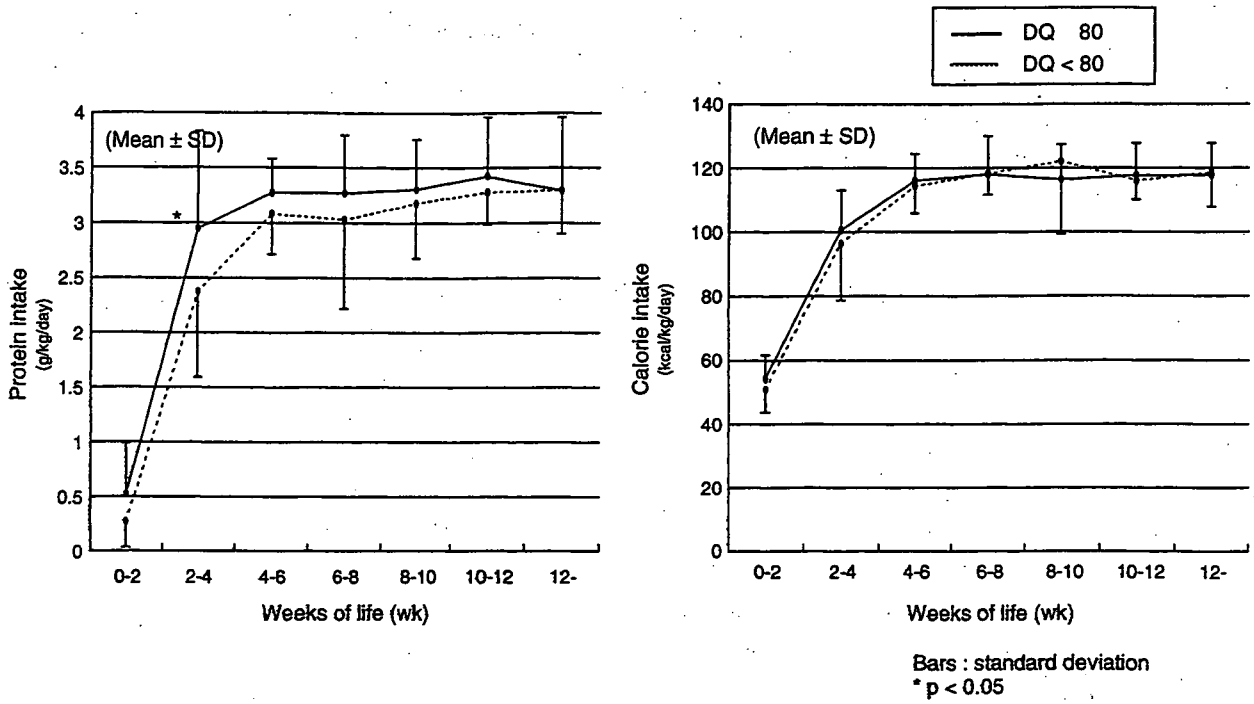

Figure 2 Comparison between the DQ≥80 and DQ<80 groups with regard to the calculated calorie and protein intakes.

infants. However, its clinical relevance is unclear because the Odd's ratio (OR) was 1.03 with 95% confidence interval (CI) of 1.002–1.06.

Only 37 of the 146 survivors (25.3%) were included in this study. Of the 79 infants who were followed up, 42 were either not assessed for the DQ or not traceable at 36 months of PCA. Of the 42 infants, the DQ was assessed in 34 infants after 36 months of PCA (from 4 to 9 years) and 8 infants moved to other areas or were not traceable. Furthermore, we believed that except for the nutritional factor, other factors that would affect BUN, although slightly, should be excluded. At our center, infants with patent ductus arteriosus (PDA) were treated with PGE1 inhibitor whenever possible, and they were often administered low-dose PGE1 inhibitor at approximately 1 month of life. Therefore, infants who were administered PGE1 inhibitors between 0 and 84 days of life were excluded from this study. Of the 146 survivors, 52 infants were administered PGE1 inhibitors for PDA, which was one of the exclusion criteria in this study.

As shown in Table 4, Moro et al. reported a method for adjusting the level of protein fortification that involved the addition of proteins and was dependent on the CBUN level. The CBUN level was corrected based on the normal serum creatinine level because the low glomerular filtration rate

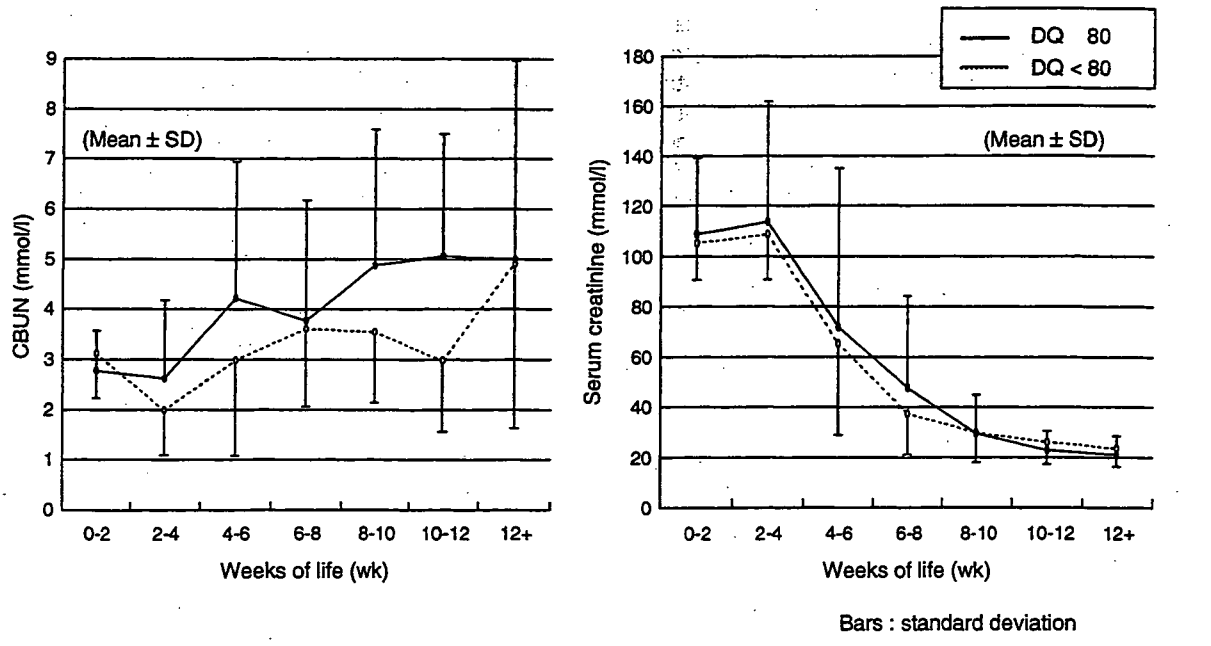

Figure 3 Comparison between the DQ≥80 and DQ<80 groups with regard to the average CBUN and serum creatinine level.

Table 4 Moro's protein fortification method and its equivalent in our NICU method. Modified from Moro et al. [11]

Fortification level	CBUN (mmol/dl)	Added protein (g/dl)	[a]Total protein intake (g/kg/day)
+3	<1.2	1.20	3.75
+2	1.2–2.2	1.05	3.54
+1	2.3–3.4	0.93	3.35
0	3.5–4.5	0.79	3.14
−1	4.6–5.6	0.65	2.93
−2	5.6–6.8	0.52	2.73
−3	>6.8	0.38	2.52

[a] Amount of enteral feeding = 150 ml/kg/day.

observed in preterm infants leads to the elevation of BUN and is independent of the protein intake. The CBUN level was calculated using the formula BUN × 0.5/serum creatinine, where 0.5 is the normal serum creatinine concentration. Moro et al. concluded that this method was safe and it ensured adequate nutrient intake and growth. However, the developmental outcome in this fortification program was not evaluated. Although the human milk fortification method used at our NICU was fixed, a variation in the CBUN values was observed because of the infants' conditions. Maturation of metabolism and severity of illness may lead to considerable variation in the CBUN values. Therefore, the present retrospective study tested whether the CBUN values can be used to predict the developmental and anthropometric outcomes. Although the CBUN level was not used to predict the outcome of the anthropometric parameter in our study, the results may suggest that a high CBUN value reflects adequate protein intake in ELBW infants.

Renal function, fluid shift, or catabolism can affect the BUN level. Therefore, infants with renal diseases were excluded from the present study. Furthermore, no significant difference was observed in the serum creatinine levels between the two groups, and the CBUN level was corrected based on the serum creatinine levels. Therefore, renal factors hardly affected the CBUN values. Since no differences were observed between the two groups with regard to the amount of protein and calorie intakes (Fig. 3) and weight gain (data not shown), it was considered that fluid shift and catabolism did not significantly affect the BUN values.

The results showed that infants in the "DQ<80" group had a significantly lower Apgar score at 5 min and longer durations of artificial ventilation and O_2 supplementation. This indicates that these infants might be more ill than those in the other group, and the severity of illness during the neonatal period may affect the developmental outcome later in life. However, the AUC of CBUN in infants in the "DQ≥80" group between 28 and 84 days of life was higher than those in infants in the "DQ<80" group. The fixed fortification method used in this study might have led to inadequate protein intake in infants in "DQ<80" group, as indicated by their low CBUN values. Some studies on critically ill adults and children showed that they not only have higher nutritional requirements but also have a decreased capacity to maximize the use of different substrates [23]. Compared to healthy children critically ill children were recommended a high protein intake based on a higher protein turnover in this population [24]. On multiple regression analysis, only the AUC of CBUN between 28 and 84 days of life was related to the DQ at 36 months of PCA, whereas severity of illness was not significantly related to the DQ. The energy expenditure in infants was not analyzed. However, infants in the "CBUN<80" group might have required more nutrients due to their illness.

The actual individual protein intake could not be determined because the protein content of human milk was not analyzed. In this study, the protein and calorie contents in mother's milk and donor milk was found to be the same as that observed at mid-lactation in Japanese women [21] (calories and protein values in human milk are estimated to be 69 kcal/dl and 1.3 g/dl, respectively). Since the nutrient content of human milk is not always constant [25], the difference between the actual and the calculated protein and calorie intakes could not be calculated. Moreover, a fixed level of human milk fortification may be inadequate for ELBW infants because they have variable nutritional demands based on the severity of their illness and physiological immaturity. It was suggested that variable nutritional demands might have been the reason for the differences in the CBUN values in this study, although nutritional fortification was the same in both groups in this study. Cooke and Embleton suggested that the degree of fortification needed to sustain adequate growth might vary from day to day; therefore, preterm infants fed on current fortification regimens show less growth than those fed on a preterm infant formula [26]. However, routinely measuring the individual nutrient requirements and the content of human milk at bedside appears unlikely because of the effort and cost involved. Adjusting human milk fortification based on the CBUN values, as suggested by Moro at al., may rectify this problem.

Based on our small sample size and with limitations in the study design, we conclude that a low CBUN value is detrimental for the developmental outcome of an ELBW infant. However, we would argue that a CBUN value reflects inadequate rather than excessive dietary protein intake as suggested by the systematic review of the Cochrane library [27] and Lucas et al. [3]. ELBW infants are prone to suffer from malnutrition due to their rapid growth and risk of illness, and it is important to evaluate the nutritional state with reference to the physiological parameters. Adjustment of the protein intake based on the CBUN value, and not a fixed protein intake, may provide a method of human milk

fortification that meets the infant's nutritional requirements. Since our study was retrospective in nature, prospective studies that would estimate the correlation between the CBUN level during the neonatal period and the cognitive function in later life should be conducted.

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ARTICLE

Inhaled Nitric Oxide Therapy Decreases the Risk of Cerebral Palsy in Preterm Infants With Persistent Pulmonary Hypertension of the Newborn

Yuko Tanaka, MD[a], Tomoshige Hayashi, MD, PhD[b], Hiroyuki Kitajima, MD, PhD[a], Kiyoaki Sumi, MD[a], Masanori Fujimura, MD, PhD[a]

[a]Department of Neonatal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; [b]Department of Preventive Medicine and Environmental Health, Graduate School of Medicine, Osaka City University, Osaka, Japan

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ABSTRACT

OBJECTIVE. The aim was to determine whether inhaled nitric oxide therapy improves neurodevelopmental outcomes for infants with preterm persistent pulmonary hypertension of the newborn.

METHODS. We conducted a historical cohort study to compare the 3-year incidence of cerebral palsy in preterm singleton infants (<34 gestational weeks) with hypoxemic respiratory failure caused by persistent pulmonary hypertension of the newborn who received inhaled nitric oxide (16 patients) or 100% oxygen (15 patients) therapy. All neonates had clinical and echocardiographic evidence of pulmonary hypertension without structural heart disease.

RESULTS. The incidence of cerebral palsy among patients treated with inhaled nitric oxide was 12.5%, whereas that among patients treated with 100% oxygen was 46.7%. After adjustment for maternal fever (≥38°C) during delivery, birth weight, Apgar score at 5 minutes, high-frequency oscillatory ventilation, and surfactant therapy, inhaled nitric oxide therapy, compared with 100% oxygen therapy, was associated with a decreased risk of cerebral palsy in preterm infants with persistent pulmonary hypertension of the newborn.

CONCLUSION. Inhaled nitric oxide therapy decreases the risk of cerebral palsy in preterm infants with persistent pulmonary hypertension of the newborn.

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Key Words
preterm persistent pulmonary hypertension of the newborn, inhaled nitric oxide, cerebral palsy

Abbreviations
PPHN—persistent pulmonary hypertension of the newborn
NO—nitric oxide
INO—inhaled nitric oxide
CP—cerebral palsy
IQR—interquartile range
Fio_2—fraction of inspired oxygen

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Address correspondence to Yuko Tanaka, MD, Department of Neonatal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan. E-mail: yutanaka@msic.med.osaka-cu.ac.jp

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PERSISTENT PULMONARY HYPERTENSION of the newborn (PPHN) is a disease in which the pulmonary vascular resistance remains elevated during the neonatal period. Preterm PPHN, which is associated with a high risk of adverse health and neurodevelopmental outcomes, continues to be one of the most challenging conditions encountered in the NICU.

Nitric oxide (NO) is produced by vascular endothelial cells and plays an important role in increasing the blood flow to the lungs after birth.[1-5] Inhaled NO (iNO) causes selective pulmonary vasodilation in newborn lambs[1] and has been shown to have a short-term benefit by improving oxygenation in infants with hypoxemic respiratory failure caused by PPHN.[6] However, it is not clear whether, in preterm infants with PPHN, iNO therapy decreases the risk of cerebral palsy (CP), which is one of the most serious neurodevelopmental complications of preterm infants. We conducted a historical cohort study to compare the 3-year incidence of CP in preterm singleton infants with hypoxemic respiratory failure caused by PPHN who received either iNO or 100% oxygen therapy.

METHODS

Study Subjects

A historical cohort study was performed that involved 61 consecutive patients without congenital anomalies who were admitted to the ICU at the Osaka Medical Center and Research Institute for Maternal and Child Health between January 1988 and December 1999 and who were singleton infants of <34 gestational weeks (median: 25.0 weeks; interquartile range [IQR]: 24.0–28.0 weeks) with hypoxemic respiratory failure caused by PPHN. Thirty of the 61 patients in the original cohort were excluded; 26 patients died within 3 years after birth (median: 1.0 day; IQR: 0.6–7.8 days) and 4 patients were monitored at other hospitals after discharge. The gestational age was estimated by means of menstrual dates and ultrasound scans obtained before 20 weeks of gestation. The scan date was preferred if the menstrual date was uncertain or if there was a discrepancy of >14 days between the 2 dates. All neonates had clinical and echocardiographic signs of pulmonary hypertension, without structural heart disease. Clinical evidence of pulmonary hypertension was defined as >5% difference between preductal and postductal oxygenation saturation or recurrent decreases (<85%) in arterial oxygen saturation over a period of 12 hours despite optimal treatment of the patient's lung disease. Echocardiographic evidence of pulmonary hypertension was defined as estimated peak systolic pulmonary-artery pressure that was >35 mm Hg or more than two thirds of the systemic systolic pressure, as indicated by the presence of a tricuspid regurgitate jet, a right-to-left patent ductus arteriosus shunt, or a right-to-left artery-level shunt. The study protocol was in accordance with the institutional guidelines for human research, and the patients' parents provided written informed consent for the diagnostic and therapeutic procedures that were required, which allowed the results of the examinations to be used in this study.

Data Collection

Dr Tanaka reviewed the infant and maternal records. Between January 1988 and September 1993, all preterm infants with PPHN received 100% oxygen therapy to treat their respiratory failure. In 1992, iNO therapy was reported to improve respiratory failure in patients with PPHN[6]; therefore, after institutional ethics committee approval was obtained, iNO therapy was given to all preterm infants with PPHN between October 1993 and December 1999. During the 2 time periods, the patients received similar treatments except for the iNO therapy or 100% oxygen therapy.

NO gas (Taiyo Toyo Sanso, Osaka, Japan) was delivered from an 800-ppm cylinder and was introduced into the afferent limb of the ventilator circuit near the endotracheal tube, which mixed the fixed flow of gas in the ventilator circuit. The flow was adjusted to yield the predetermined NO concentration. The iNO concentration was increased by 10 ppm at 30-minute intervals, with an upper limit of 30 ppm. The response to iNO therapy was evaluated as an increase in Pao_2 to >10 mm Hg. Infants who did not show a significant acute response continued to receive iNO therapy at 5 ppm for 12 hours; if there was still no satisfactory response, then the infants were weaned off iNO therapy. Infants who exhibited improvement continued to receive iNO at the minimal level found to be effective (attempts were made to decrease the concentration by reducing it by 5 ppm at 30-minute intervals). At that time, the fraction of inspired oxygen (FIo_2) and ventilation were reduced to prevent additional lung injury. When the FIo_2 could be decreased to ≤0.4, iNO therapy was terminated by slow weaning over several hours. Methemoglobin levels were measured before iNO therapy, 1 hour later, and then every 8 hours. If the methemoglobin level increased to >2%, then iNO therapy was discontinued. Infants were monitored for signs of increased bleeding (eg, pulmonary hemorrhage, gastrointestinal bleeding, or oozing from venipuncture sites). In addition, cranial ultrasonography was performed before the initiation, within 24 hours whenever possible and then every 24 hours after the initiation, and 24 hours after the final discontinuation of iNO therapy. The median duration of iNO therapy was 19.8 hours (IQR: 29.5–56.0 hours). The oxygenation index, calculated as [100 × FIo_2 × mean airway pressure (in centimeters of water)]/Pao_2 (in millimeters of mercury), was obtained within 1 hour before and at 1 hour after the initiation of inhalation therapy (iNO or 100% oxygen). The mothers' records were re-

viewed to determine the presence of maternal fever (≥38°C) during delivery, premature rupture of the membranes (≥24 hours), maternal bleeding, reason for delivery, and prenatal corticosteroid use. Neonatal data, which were obtained from the medical charts, included the number of gestational weeks at birth, gender, birth weight, Apgar scores at 1 and 5 minutes, use of iNO, type of ventilation (high-frequency oscillatory or intermittent mechanical ventilation), and surfactant therapy. All of the subjects' parents were Japanese. All surviving infants were scheduled to be seen by pediatricians at 3 years of age for a complete physical and neurologic examination. Necrotizing enterocolitis was diagnosed during surgery, at autopsy, or on the basis of a finding of pneumatosis intestinalis, hepatobiliary gas, or free intraperitoneal air on radiographs. Pulmonary hemorrhage was diagnosed if a blood-tinged tracheal aspirate was obtained. Intraventricular hemorrhage was graded 0 through 4, according to the highest grade on cranial ultrasonograms, by using the method described by Papile et al.[7] CP was defined as abnormal muscle tone in ≥1 extremity and abnormal control of movement and posture.

Statistical Analyses

Categorical variables were compared by using the χ^2 test or Fisher's exact test. Differences in the median values between the 2 groups were compared by using the Mann-Whitney *U* test. Univariate and multivariate logistic regression analyses were used to estimate the odds ratio for the incidence of CP. We calculated the 95% confidence interval for each odds ratio. We limited the number of independent variables in each model to avoid overfitting the data. *P* values were 2-tailed. A *P* value of <.05 was considered significant. Statistical analyses were performed by using the SPSS 10.0 software package (SPSS, Chicago, IL).

RESULTS

Of the 61 preterm infants with PPHN, 26 infants died within 3 years after birth. Mortality rates at 3 years after birth were similar for infants treated with iNO and those treated with 100% oxygen (44.1% vs 40.7%; $P = .791$). The incidences of outcomes in the iNO-treated and 100% oxygen-treated groups were 8.8% vs 29.6% for patent ductus arteriosus, 17.6% vs 25.9% for intraventricular hemorrhage (grade 3 or 4), 8.8% vs 0% for necrotizing enterocolitis, and 11.8% vs 16.0% for pulmonary hemorrhage.

During the 3-year follow-up period, the incidence of CP among patients treated with iNO therapy was lower than that among patients treated with 100% oxygen therapy (12.5% vs 46.7%; $P = .054$). The baseline clinical characteristics of the study patients according to type of inhalation therapy are summarized in Table 1. Patients who received iNO therapy had lower Apgar scores at 5 minutes and were given surfactant therapy more often, compared with those who received 100% oxygen therapy (Table 1). In univariate logistic analysis, use of iNO therapy was associated with a decreased incidence of CP (Table 2). Although the oxygenation index values before the start of inhalation therapy were similar for the 100% oxygen-treated group (median: 24.0; IQR: 16.0–35.4) and the iNO-treated group (median: 23.3; IQR: 16.0–45.0; $P = .695$), the oxygenation index 1 hour after the start of inhalation therapy was lower for patients treated with iNO (median: 4.7; IQR: 3.8–7.7) than for patients treated with 100% oxygen (median: 12.5; IQR: 7.6–18.5; $P = .013$).

We tested several regression models to assess the effect of iNO therapy on the incidence of CP in preterm infants with PPHN. After adjustments for maternal fever during delivery, birth weight, Apgar score at 5 minutes, high-frequency oscillatory ventilation, and surfactant therapy, iNO therapy was associated with a decreased risk of CP, compared with 100% oxygen therapy (Table 3).

DISCUSSION

Our data demonstrate that, for preterm infants with PPHN, the incidence of CP, during a 3-year follow-up

TABLE 1 Baseline Characteristics of Patients According to Treatment Group

	iNO (*N* = 16)	100% Oxygen (*N* = 15)	*P*
Maternal characteristics			
Maternal fever (≥38°C) during delivery, *n* (%)	2 (13.3)[a]	6 (40.0)	.215
Premature rupture of membranes (≥24 h), *n* (%)	4 (26.7)[a]	3 (20.0)	1.000
Maternal bleeding as reason for delivery, *n* (%)	10 (66.7)[a]	9 (60.0)	1.000
Prenatal corticosteroid therapy, *n* (%)	2 (13.3)[a]	5 (33.3)	.390
Infant characteristics			
Gestational age, median (IQR), wk	25.5 (25.0–28.8)	26.0 (24.0–30.0)	.589
Male, *n* (%)	8 (50.0)	8 (53.3)	.850
Birth weight, median (IQR), g	838 (628–1144)	818 (720–1400)	.363
Apgar score at 1 min, median (IQR)	4.0 (1.5–4.8)	4.0 (3.0–6.0)	.375
Apgar score at 5 min, median (IQR)	6.0 (5.0–7.0)	7.0 (7.0–8.0)	.010
High-frequency oscillatory ventilation, *n* (%)	14 (87.5)	9 (60.0)	.113
Surfactant used, *n* (%)	15 (93.8)	9 (60.0)	.037

[a] Data are missing for 1 infant.

TABLE 2 Baseline Characteristics of Patients According to Whether CP Developed After 3 Years

	CP		Crude Odds Ratio (95% Confidence Interval)	P[a]
	Yes (n = 9)	No (n = 22)		
Maternal characteristics				
Maternal fever (≥38°C) during delivery, n (%)	3 (37.5)[b]	5 (22.7)	2.04 (0.36–11.67)	.423
Premature rupture of membranes (≥24 h), n (%)	2 (22.2)	5 (23.8)[b]	0.91 (0.14–5.90)	.925
Maternal bleeding as reason for delivery, n (%)	5 (62.5)[b]	14 (63.6)	0.95 (0.18–5.08)	.954
Prenatal corticosteroid therapy, n (%)	3 (37.5)[b]	4 (18.2)	2.70 (0.45–16.26)	.278
Infant characteristics				
Gestational age, median (IQR), wk	26.0 (25.0–28.0)	26.0 (24.0–29.8)	0.97 (0.74–1.29)[c]	.848
Male, n (%)	5 (55.6)	11 (50.0)	1.25 (0.26–5.94)	.779
Birth weight, median (IQR), g	796 (752–880)	914 (638–1303)	0.52 (0.15–1.81)[d]	.307
Apgar score at 1 min, median (IQR)	3.0 (1.0–7.0)	4.0 (3.0–5.0)	0.99 (0.66–1.50)	.978
Apgar score at 5 min, median (IQR)	7.0 (4.0–9.0)	7.0 (6.0–7.0)	0.82 (0.55–1.23)	.336
iNO used, n (%)	2 (22.2)	14 (63.6)	0.16 (0.03–0.98)	.048
High-frequency oscillatory ventilation, n (%)	8 (89)	15 (68)	3.73 (0.39–35.84)	.254
Surfactant used, n (%)	7 (77.8)	17 (77.3)	1.03 (0.16–6.62)	.976

[a] P values for univariate logistic analyses.
[b] Data are missing for 1 infant.
[c] Per 1-week increase.
[d] Per 500-g increase.

TABLE 3 Multivariate Models for Determining the Incidence of CP

Variable	Odds Ratio (95% Confidence Interval) for CP	P[a]
Model 1		
iNO therapy vs 100% oxygen therapy	0.08 (0.01–0.85)	.036
Maternal fever (≥38°C) during delivery	1.07 (0.15–7.52)	.947
Model 2		
iNO therapy vs 100% oxygen therapy	0.12 (0.02–0.84)	.032
Birth weight, per 500-g increase	0.38 (0.09–1.57)	.181
Model 3		
iNO therapy vs 100% oxygen therapy	0.06 (0.05–0.70)	.025
Apgar score at 5 min	0.59 (0.32–1.09)	.092
Model 4		
iNO therapy vs 100% oxygen therapy	0.08 (0.01–0.61)	.014
High-frequency oscillatory ventilation vs intermittent mechanical ventilation	10.5 (0.85–130.51)	.067
Model 5		
iNO therapy vs 100% oxygen therapy	0.12 (0.02–0.82)	.031
Surfactant therapy	2.67 (0.33–21.68)	.359

[a] P values for multivariate logistic analyses.

period, among patients treated with iNO therapy showed a trend toward being lower than that among patients treated with 100% oxygen therapy. After adjustments for maternal fever during delivery, birth weight, Apgar score at 5 minutes, high-frequency oscillatory ventilation, and surfactant therapy, iNO therapy was associated with a decreased risk of CP, compared with 100% oxygen therapy.

Previous research had not shown that iNO therapy reduces the risk of CP in preterm infants with hypoxemic respiratory failure. Bennett et al[8] reported that, in a randomized, controlled study of 42 preterm infants who were thought to be at high risk of developing chronic lung disease, the rates of CP at 30 months of age were similar in the iNO-treated and control groups. In addition, Mestan et al[9] conducted a double-blind, randomized, controlled trial of 138 preterm infants with respiratory failure and found that, although iNO therapy improved cognitive neurodevelopmental outcomes, the rates of CP at 2 years of age were similar in the iNO-treated and control groups. Those results are not consistent with our findings. The study groups in the previous studies were not limited to preterm infants with PPHN, whereas we included only preterm infants with PPHN in the present study. Therefore, the reduced risk of CP with iNO therapy may be limited to such cases. However, because the incidence of CP in the control group was low in the previous studies (14% in the study by Bennett et al[8] and 10% in the study by Mestan et al[9]), compared with our study (47%), the beneficial neurodevelopmen-

tal outcomes associated with iNO treatment might have been underestimated in the previous studies; the incidence of CP in the iNO-treated group was 0% in the study by Bennett et al[8] and 9% in the study by Mestan et al.[9]

We did not identify the reasons why iNO therapy decreased the risk of CP in preterm infants with PPHN. However, an in vitro study using rat brain slices showed that hypoxia induced white matter damage mainly through oxidation.[10] In comparison with gray matter, white matter contains larger amounts of fat and iron, which are involved in free radical production, and a smaller amount of glutathione, which is an antioxidant; this suggests that white matter has greater susceptibility to oxidative stress. In addition, blood flow to cerebral white matter is extremely low in premature newborns,[11] which indicates that cerebral white matter is particularly vulnerable to hypoxia in preterm infants. These results suggest that hypoxia easily can induce white matter damage in preterm infants. We demonstrated that, although the oxygenation index values before the start of inhalation therapy were similar in the iNO-treated and 100% oxygen-treated groups, the oxygenation index 1 hour after the start of inhalation therapy was lower for preterm infants with PPHN treated with iNO, compared with infants treated with 100% oxygen. Therefore, iNO therapy may decrease the risk of CP in these infants through the resolution of hypoxia during a critical phase of neurodevelopment. Alternatively, iNO therapy may affect the brain directly by stimulating neuronal maturation.[12–14] However, there is no clear evidence that iNO affects brain development directly.

Our study has some potential limitations. First, we did not conduct a randomized, placebo-controlled trial, for ethical reasons; we conducted a historical cohort study to compare the 3-year incidence of CP in preterm infants with PPHN who received either iNO or 100% oxygen therapy. After adjustment for multiple potential confounding variables, iNO therapy was associated with a decreased risk of CP. In addition, the type of inhalation therapy was determined on the basis of the time of each subject's admission. Therefore, the selection of the type of inhalation can not introduce bias. Second, in our study, we enrolled only preterm infants with PPHN. Therefore, it is not clear whether iNO therapy, compared with 100% oxygen therapy, would decrease the risk of CP in preterm infants with hypoxemic respiratory failure not caused by PPHN. Third, we analyzed a limited number of patients. Therefore, other variables, such as early gestational age, low birth weight, presence of maternal fever during delivery,[15,16] premature rupture of membranes of long duration,[16–20] maternal bleeding, reason for preterm delivery,[21,22] and low Apgar scores at birth,[16,17,22] which are thought be risk factors for CP, might not have been identified as being useful for predicting CP in this study. In addition, use of surfactant therapy and use of high-frequency oscillatory ventilation were not associated with a decreased incidence of CP in preterm infants with PPHN. These results might be attributable to a limited number of study subjects. In particular, the use of high-frequency oscillatory ventilation had a high odds ratio, compared with intermittent mechanical ventilation. Therefore, to generalize the results of this study, studies involving a large number of patients are essential. Finally, because we conducted a historical cohort study, we could not explore unknown risk factors for CP.

CONCLUSIONS

Our results provide evidence that iNO therapy, compared with 100% oxygen therapy, decreases the risk for CP in preterm infants with PPHN. iNO therapy may protect brain white matter during a critical phase of neurodevelopment and thus reduce the risk of CP in these infants. Additional studies are needed to clarify the mechanism through which iNO therapy decreases the risk of CP in preterm infants with PPHN.

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DROTRECOGIN ALFA (ACTIVATED) IN CHILDREN WITH SEVERE SEPSIS: A MULTICENTRE PHASE III RANDOMISED CONTROLLED TRIAL

Background: Drotrecogin alfa (activated) (DrotAA) is used for the treatment of adults with severe sepsis who have a high risk of dying. A phase 1b open-label study has indicated that the pharmacokinetics and pharmacodynamics of DrotAA are similar in children and adults. We initiated the RESOLVE (REsearching severe Sepsis and Organ dysfunction in children: a gLobal perspectiVE) trial to investigate the efficacy and safety of the drug in children.
Methods: Children aged between 38 weeks' corrected gestational age and 17 years with sepsis-induced cardiovascular and respiratory failure were randomly assigned to receive placebo or DrotAA (24μg/kg/h) for 96 h. . . .
Findings: 477 patients were enrolled: 237 received placebo, and 240 DrotAA. Our results showed no significant difference between groups. . . .
Interpretation: Although we did not record any efficacy of DrotAA in children with severe sepsis, serious bleeding events were similar between groups and the overall safety profile acceptable, except in children younger than 60 days. However, we gained important insights into clinical and laboratory characteristics of childhood severe sepsis, and have identified issues that need to be addressed in future trials in critically ill children.

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Original Article

Utility of a new transcutaneous jaundice device with two optical paths in premature infants

FUMIHIKO NAMBA AND HIROYUKI KITAJIMA

Department of Neonatology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

Abstract

Background: Hyperbilirubinemia may cause dysfunction of the central nervous system of newborn infants. Recently, a new transcutaneous bilirubin device has been developed, which is not limited by maturity or melanin concentration of the skin. However, there have been few reports limiting the subjects to preterm and very low-birthweight (VLBW) infants.

Methods: Transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) were measured within 1 h of time lag in 50 premature infants. TcB was measured with the new jaundice device on the forehead. TSB samples were measured by direct colorimetry. The correlation coefficient and regression line were calculated.

Results: The results showed a good correlation between TcB and TSB. However, the correlation tended to be worse with infants whose birthweights were lower than 1000 g, or whose gestational ages at birth were shorter than 28 weeks.

Conclusion: TcB and TSB have a close correlation, and TcB tends to be higher than TSB. The Minolta transcutaneous jaundice device could be used as a screening instrument, leading to the avoidance of invasive blood samplings for preterm and VLBW infants. However, in patients whose birthweights are lower than 1000 g or whose gestational ages are shorter than 28 weeks, care must be taken when using the transcutaneous jaundice device because of low reliability in these patients.

Key words hyperbilirubinemia, neonatal jaundice, preterm infant, transcutaneous jaundice device, very low-birthweight infant.

Hyperbilirubinemia may cause dysfunction of the central nervous system of newborn infants such as bilirubin encephalopathy, and is known to be one of the causes of cerebral palsy. It has been possible to screen hyperbilirubinemia with a transcutaneous bilirubin device in mature infants.[1–6] Recently, a new transcutaneous bilirubin device with two optical paths (JM-103; Minolta, Osaka, Japan) has been developed, which is not limited by maturity or melanin concentration of the skin. However, there have been few reports limiting the subjects to preterm and very low-birthweight (VLBW) infants.[7–10] We, therefore, studied the utility of the transcutaneous bilirubin with two optical paths in preterm and VLBW infants. This study was performed to confirm the correlation between the transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) of preterm and VLBW infants. The results of this study may contribute to establish a way to control serum bilirubin levels with minimal invasion for preterm and VLBW infants, therefore, improving the quality of their developmental care.

Correspondence: Fumihiko Namba, MD, 840, Murodocho, Izumi, Osaka, 594–1101, Japan. Email: n_fumihiko@mrj.biglobe.ne.jp

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Methods

Selection of study cases

Patients were selected with the consent of their families from infants admitted into Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, from September 2003 to March 2004. During this period, infants were admitted into the Neonatal Intensive Care Unit. The criteria for selection were: birthweight <1500 g, or gestational age <34 weeks, and birthweight ≥500 g. The criteria for exclusion were: (i) day 0–3 (if gestational age <26 weeks); (ii) intraventricular hemorrhage (IVH); (iii) bleeding tendency; (iv) platelet count <50 000/mm^3; (v) hemolytic jaundice; (vi) hydrops fetalis; and (vii) patient judged to be inadequate as subject by doctor. In the criteria for selection, birthweight ≥500 g

was set because the extremely low-birthweight infants (ELBW; birthweight <1000 g) with birthweight lower than 500 g were considered to have a poor survival prognosis. In the exclusion criteria, number i was set because the acute phase of infants whose gestational ages were under 26 weeks was considered to be at high risk for IVH, and numbers ii, iii and iv were set because the measurement, putting probe on the forehead of infants, might lead to IVH. Number v was set because the correlation between TcB and TSB was adversely affected by the presence of hemolytic disease, and number vi was set because the values tend to appear lower than the true values when there is peripheral circulatory failure. Finally, a total of 50 patients were selected for this study according to the criteria (Fig. 1). Their gestational ages were 30.4 ± 3.2 weeks, birthweights were 1281 ± 476 g and the measurement age was 6.1 ± 4.7 days of life (mean ± standard deviation, respectively).

Methods for measuring transcutaneous bilirubin and total serum bilirubin

This study protocol is approved by the ethics committee of Osaka Medical Center and Research Institute for Maternal and Child Health.

The measurements were not conducted while phototherapy was underway, because TcB tended to decrease earlier than TSB during phototherapy in preterm and VLBW infants, as is known among mature infants (data not shown). TcB and TSB were measured within 1 h of each other. We thought that only one data set per infant was allowed in theses analyses, but we measured several sets per infant because the results would be nearly the same for our restricted criteria. The correlation coefficient and regression line were calculated with 95% prediction interval.

TcB was measured with the Minolta jaundice device JM-103 by nurses. It was calibrated by a reading-checker before this study started. The measurement probe was put on infants' foreheads. TcB measurement was not taken at the sternum because the measurement might lead to thoracic deformity by its flexibility and might affect the circulatory system in premature infants. Two measurements were made with this device, and TcB was defined as their average. It has been already reported that TcB and TSB are closely correlated in mature infants.[7,11,12] The new Minolta jaundice device JM-103 has two optical pathways (long and short). The previous edition of the device JM-102 had only one optical pathway. JM-103 can determine the difference of optical concentration between the two optical paths, which leads to determining the real difference of optical concentration of the subcutaneous tissue only, deducting factors concerning the epidermis and dermis. JM-103 can, therefore, minimize the effect of melanin pigment and maturity of the skin, and provide a value representing the real serum bilirubin concentration.[10]

TSB with capillary samples was measured by direct colorimetry with the PHOTO B-H METER V (Sanko Junyaku, Tokyo, Japan) by technicians in the laboratory. We usually take samples with capillary because capillary sampling from infants can reduce volumes of blood.

Arterial and venous blood samplings (64 and 14 samples, respectively) were performed by doctors and capillary blood sampling (273 samples) by nurses. The distribution of samples is shown in Figure 2. We confirmed the accuracy of the bilirubin measurement performed at our laboratory with a standard solution. The standard solution was made by a method recommended by the American Association for Clinical Chemistry and the National Committee for Clinical Laboratory Standards. The measurement values were compared with the standard values, and the measurement device showed significant correlation (the squared correlation coefficient was 1.00 and regression line was $y = 0.9996x - 0.08$; Fig. 3).

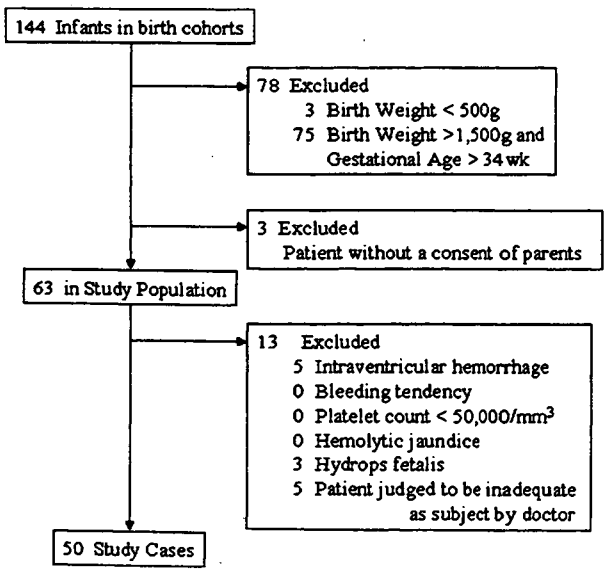

Fig. 1 Selection of study cases. A total of 50 patients were selected for this study according to the criteria.

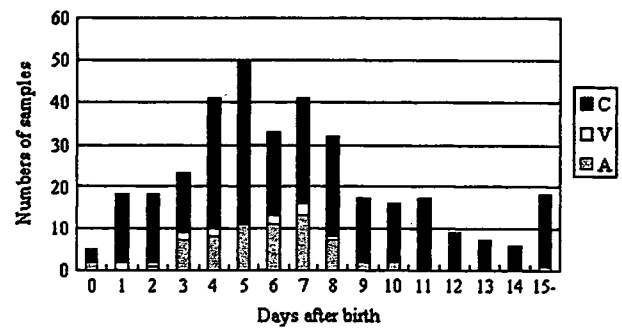

Fig. 2 Distribution of samples. C, capillary blood sampling (273 samples); V, venous blood sampling (14 samples); A, arterial blood sampling (64 samples).

Results

Correlation between transcutaneous bilirubin and total serum bilirubin

Figure 4 shows a correlation between TcB and TSB in 50 preterm and VLBW infants. The vertical axis shows TcB and the horizontal axis shows TSB. The squared correlation coefficient was 0.69 and regression line was y =0.929x + 1.467, which shows a good correlation in 50 patients; 351 samples.

We next set birthweights and gestational ages on the horizontal axis and examined the differences between TcB and TSB. The differences tended to become smaller with heavier birthweights and longer gestational ages (data not shown). Actually, in the infants whose birthweights were more than 1000 g, the squared correlation coefficient was 0.74 and regression line was y =0.953x + 1.337 (Fig. 5). In the infants whose gestational ages were longer than 28 weeks, the squared correlation coefficient was 0.72 and regression line was y =0.970x + 0.983 (Fig. 6). It is clear that both limitations for birthweight and gestational age make the correlation closer. In contrast, in infants, whose birthweights were lower than 1000 g or whose gestational ages were shorter than 28 weeks, the correlations became worse (the squared correlation coefficients were 0.37 and 0.39, and regression lines were y =0.724x + 2.859 and y =0.878x + 2.296, respectively; Figs 5,6).

We also examined whether small-for-gestational-age affected the result. It is clear that this factor did not affect the differences between TcB and TSB (Fig. 7).

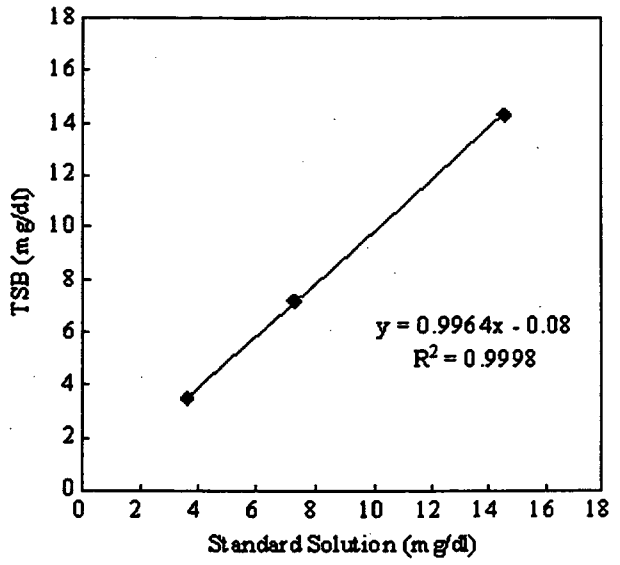

Fig. 3 Accuracy of the serum bilirubin measurement. The measurement values of total serum bilirubin (TSB) were compared with the standard values and the measurement device showed significant correlation.

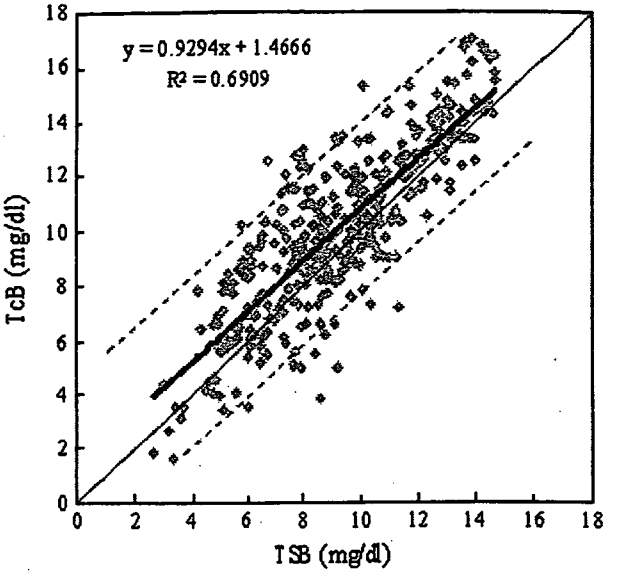

Fig. 4 Relationship between transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) in 50 preterm and very low-birthweight infants. TcB and TSB showed a close correlation.

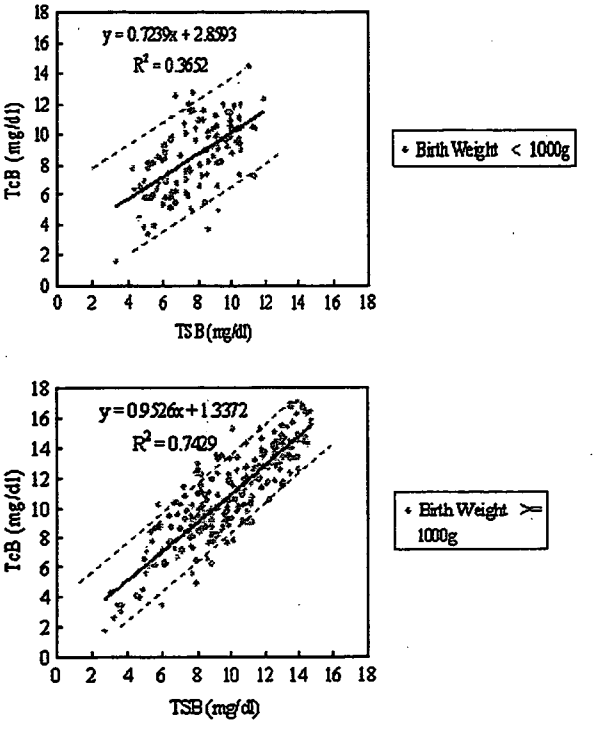

Fig. 5 Relationship between transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) in birthweight <1000 g and ≥1000 g. In the infants whose birthweights were more than 1000 g, the squared correlation was closer. In contrast, in infants whose birthweights were under 1000 g, the correlations became worse.

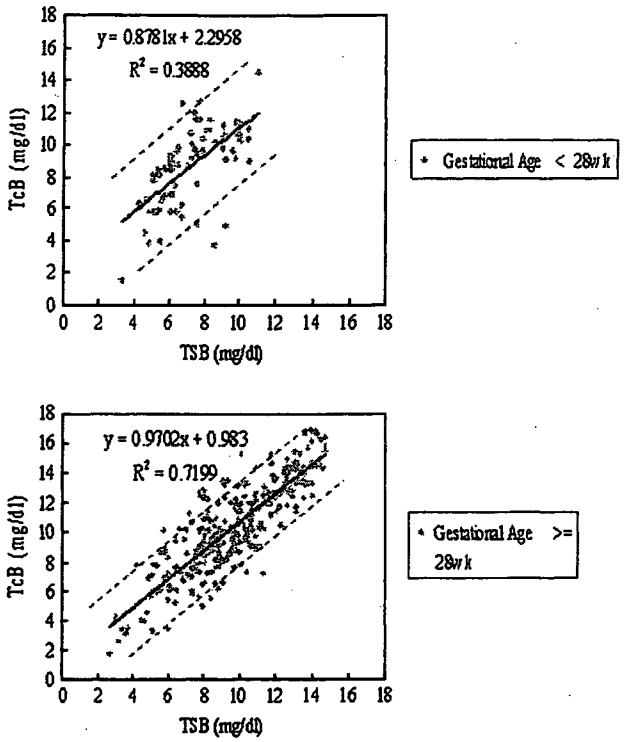

Fig. 6 Relationship between transcutaneous bilirubin (TcB) and total serum bilirubin (TSB) in gestational age <28 weeks and ≥28 weeks. In the infants whose gestational ages were longer than 28 weeks, the correlation was closer. In contrast, in infants whose gestational ages were shorter than 28 weeks, the correlation became worse.

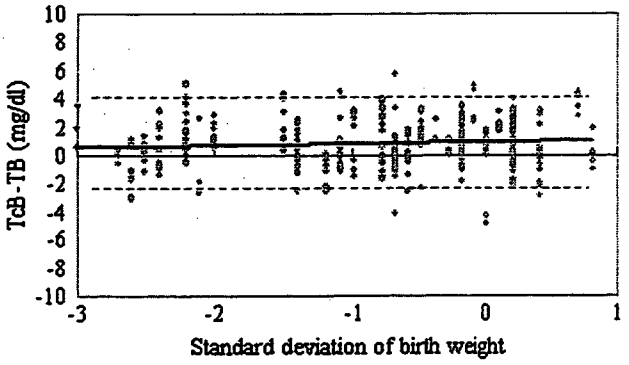

Fig. 7 Effect of small for gestational age (SGA) on difference between transcutaneous bilirubin (TcB) and total serum bilirubin (TSB). SGA do not affect the differences between TcB and TSB.

Discussion

Hyperbilirubinemia may cause dysfunction of the central nervous system of newborn infants. Hyperbilirubinemia of mature infants can be controlled to prevent this dysfunction by using transcutaneous jaundice devices. But preterm and VLBW infants still need invasive blood sampling to control their hyperbilirubinemia. Blood sampling is painful and this stress to infants may have short and long-term negative effects. Recently, the Minolta transcutaneous jaundice device JM-103 was developed. We know that by using this instrument TcB shows good correlation with TSB, but there have been few reports limiting its subjects to preterm and VLBW infants. For example, Yamanishi *et al.* included 20 low birthweight infants (birthweight <2500 g) in 24 infants (1999) and 14 low birthweight infants in 69 (2001).[10] Yasuda *et al.* included 22 preterm infants (gestational age<35 weeks) in 77 (2003).[7]

There were many factors contributing to the correlation between TcB and TSB. We used three methods for sampling: arterial, venous and capillary sampling. It has been previously reported by Schlebusch *et al.* that the determinations of bilirubin in capillary plasma and serum of venous blood samples showed good precision and accuracy.[13] Chance *et al.* reported that the difference of bilirubin values between artery and capillary sampling could be ignored clinically in neonates.[14] We, therefore, ignored the difference of TSB from three samplings and investigated the correlation.

Our study showed close correlation between TcB measured with this device and TSB in preterm and VLBW infants. Recently, Karolyi *et al.* reported that with the previous edition of the Minolta jaundice device, the squared correlation coefficient was 0.46 in VLBW infants.[9] From this report, we can understand that the new Minolta jaundice device is more accurate. However, this does not make the new device more suitable for screening of hyperbilirubinemia in ELBW infants. The correlation becomes closer only in infants whose birthweights are more than 1000 g and gestational ages longer than 28 weeks.

The correlation between TcB and TSB was not so close in ELBW or preterm infants with gestational age shorter than 28 weeks. This is not only due to the immaturity of the skin but also to the accuracy of the measurement techniques. For example, the skin area for probes is not large and is limited for accurate measuring, mainly because of their small size, and the many tubes they need for a respirator or other devices make the techniques difficult for accurate measurement of TcB. To solve these kinds of problems, averaging many measurement values using the average calculating function of the Minolta transcutaneous jaundice device JM-103 might improve the correlation.

From this study, we concluded the following. i) In preterm and VLBW infants, measurement of TcB is safe and accurate. This procedure can, therefore, be used clinically for screening of hyperbilirubinemia. ii) However, in patients whose birthweights are lower than 1000 g or whose gestational ages are shorter than 28 weeks, we must be careful of using the transcutaneous jaundice device because of low reliability in these patients.

Further study is needed to improve the accuracy and reliability of TcB for screening of hyperbilirubinemia in ELBW infants.

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●原　　著●

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Allgrove症候群の臨床像を呈した超低出生体重児の1例

*1大阪府立母子保健総合医療センター　新生児科，*2北里大学医学部　小児科

釼持　学*1*2・北島博之*1・藤村正哲*1

Key words：Allgrove syndrome, Extremery low birth weight infant, Adrenocortical insufficiency, Alacrima, Achalasia

要　　旨

在胎30週0日，出生体重666gで出生し，日齢5に副腎不全を発症，乳児期に無涙症，アカラシアを合併した超低出生体重児を経験した。小児には，それぞれ稀な病態を合併したことから，Allgrove症候群と診断した。Allgrove症候群は，通常はACTH不応症の合併であり，我々の症例は典型例とは異なっていた。また，自律神経系を含む，神経系の異常を伴い，4A症候群とする報告も近年多く見られるが，本症例も，視神経委縮があり，これを充たすものであった。現在までに，Allgrove症候群は常染色体劣性遺伝であることがわかっており，責任遺伝子も同定されている。我々の症例も遺伝子解析を施行したが，同定することができなかった。これまでの報告をみても，乳児期に診断されているものは認めず，また，超低出生体重児に合併したものも認めない。

緒　　言

Allgrove症候群は1978年にAllgroveらにより初めて報告された[1]。ACTH不応症（ACTH unresponsiveness），無涙症（Alacrima），そしてアカラシア（Achalasia）を3徴とする疾患で，3つの頭文字をとって3A症候群とも呼ばれている[2]。稀な疾患であり，本邦での報告は散見する程度である[3]～[6]。

今回，我々は子宮内発育遅延のために，早産・超低出生体重児で出生し，新生児期早期に副腎不全で発症，乳児期にAllgrove症候群の3徴全てを認めた症例を経験した。この3徴候は，いずれも小児では稀な病態である。これまでAllgrove症候群を乳児期に診断した例は報告がなく，また，超低出生体重児例も見当たらないため，若干の文献的考察を加え治療経験を報告する。

別刷請求先：〒228-8555　神奈川県相模原市北里1-15-1
北里大学病院小児科
釼持　学

症　　例

在胎週数：30週0日，

出生体重：666g（－2.9SD），身長32.5cm（－2.8SD），頭囲22.6cm（－2.1SD），Apgar score 1分値4点，5分値7点，帝王切開で出生した女児。

家族歴：母19歳，父20歳。同胞なし。その他，特記することなし。

妊娠・分娩経過：0経妊，0経産。妊娠24週5日に子宮内発育遅延，羊水過少を指摘され当センター産科に紹介され，精査のため入院となった。羊水検査にて染色体異常なし。奇形なし。母体は妊娠中毒症や高血圧も既往になく，また自己抗体などの特異抗体の上昇も認めなかった。

妊娠28週頃より児の発育不良が著明となり妊娠30週で予定帝王切開となった。胎盤は重量300g，多発梗塞を認め，胎盤機能不全を疑わせる所見であった。

入院時所見（図1）：全身の色素沈着を認めた。背部を中心に点状出血を認めた。顔貌に特異的所見なく，明らかな外表奇形も認めなかった。外性器は正常女性

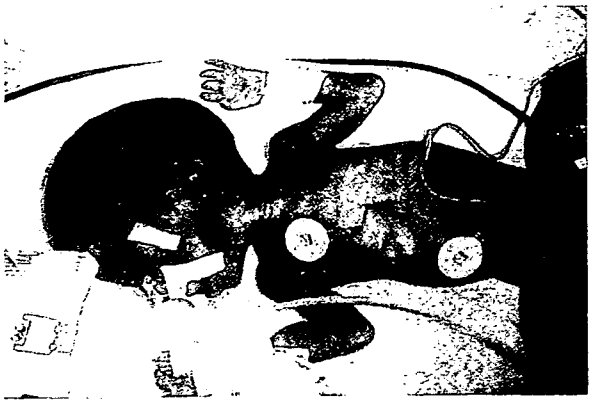

図1　出生後数日

型であった。筋トーヌスは正常で，反射，その他神経学的に異常を認めなかった。

出生後の経過（**図2**）：呼吸窮迫症候群と診断し，人工肺サーファクタント補充療法を行った。子宮内発育遅延に伴うものと考える血小板低値，凝固系の延長を認め，直ちに血小板，新鮮凍結血漿の輸注を行った。出生時より総ビリルビン値が上昇しており光線療法を開始した（入院時検査所見：**表1**）。生後18時間に施行した頭部超音波で，左脳室内出血（Papile分類Grade Ⅱ）を認めた。日齢1，動脈管開存症に対してインドメタシン投与を行い，効果を認めた。日齢5に，血圧低下（収縮期血圧28mmHg），低血糖（35mg/dℓ）が出現した。この時の血清はNa123mEq/ℓ，K4.4mEq/ℓであり副腎機能低下症を疑い，ハイドロコルチゾン投与を行い，血圧，血糖値ともに上昇した。超低出生体重児であることから，一過性の可能性を考えハイドロコルチゾンは2回投与で一旦中止した。しかし，日齢11に同様の症状が出現した。症状が繰り返されることに加え，日齢5に施行した血液検査所見でACTHの異常高値，コルチゾール低下，アルドステロン低下（**表2**）を伴っていたことより，副腎不全と診断し，ハイドロコルチゾン持続投与を開始した。投与開始後，症状は速やかに改善を認めた。ハイドロコルチゾンは日齢30に内服に変更した。

生後1ヶ月頃より両側角膜の混濁が出現した。Schirmerテストの結果，無涙症と診断した。乾燥による角膜潰瘍と診断し，乾燥予防のために点眼を繰り返し行ったが，潰瘍は徐々に進行していった。また，新生児期より両側の視神経に委縮を認めた。

生後3ヶ月，右上腕の病的骨折を認めた。児の体重を考慮し，保存的治療を選択したが，骨折していたにも関わらず児の体動が激しく骨折端が皮膚を貫き開放

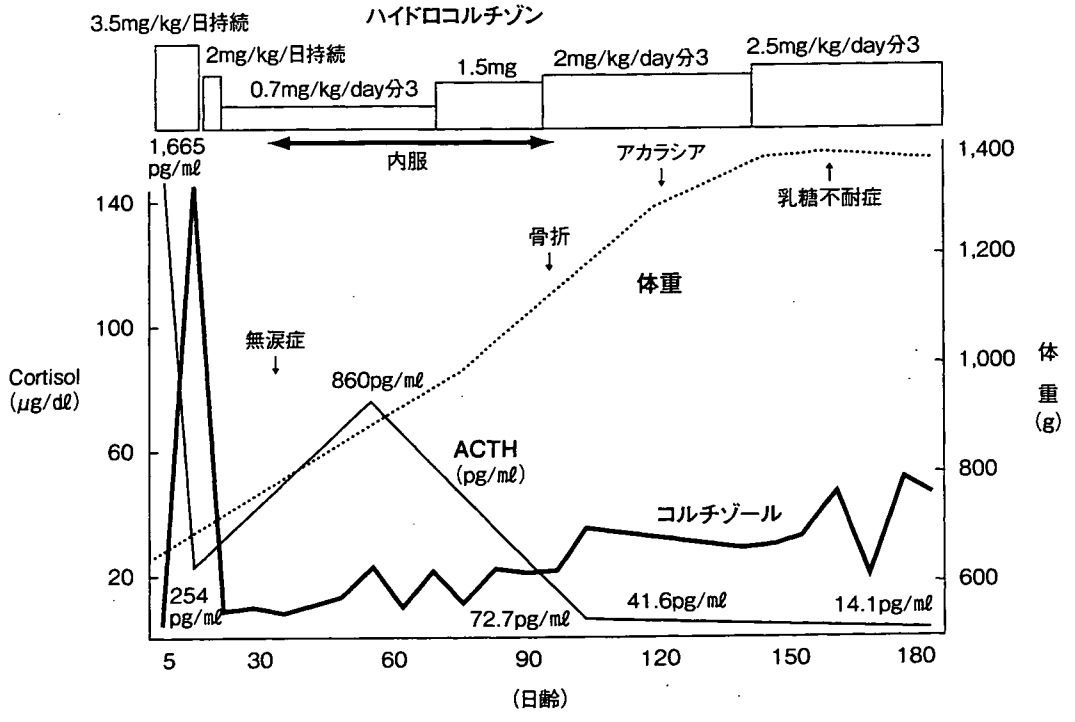

図2　経過

表1　入院時の検査結果

WBC	26,800/μℓ	CRP	＜0.1mg/dℓ
RBC	298万/μℓ	Na	139mEq/ℓ
Hb	14.9g/dℓ	K	4.4mEq/ℓ
Ht	44.2%	Cl	105mEq/ℓ
Plt	1.7万/μℓ	Ca	9.8mg/dℓ
PT	43%	TP	4.5g/dℓ
PTT	＞100sec	T-bil	5.32mg/dℓ
Fib	82mg/dℓ	GOT	27 IU/ℓ
BS	66mg/dℓ	GPT	4 IU/ℓ

表2　副腎ホルモン値

	日齢5	日齢56	日齢117
ACTH（pg/mℓ）	1,665.6	860	41.6
Cortisol（μg/dℓ）	4.19	22.7	10.5
Aldosteron（pg/mℓ）	25	—	143
17-OH-P（ng/mℓ）	0.4	—	—
Na（mEq/ℓ）	123	134	139
K（mEq/ℓ）	4.4	3.9	3.7
BS（mg/dℓ）	35	145	110

創となった。静脈麻酔での鎮静をし，固定を行った。このため内服が困難になり，ハイドロコルチゾン静注に変更した。

同時期より誤嚥性肺炎を繰り返すようになり，生後4ヶ月，精査のため上部消化管造影，食道内圧測定を施行した。上部消化管造影では，胃への流入障害，食道の拡張像を認めた。食道内圧測定では，食道蠕動波の亢進と，嚥下時の下部食道括約筋の弛緩が認められなかった。以上のことからアカラシアと診断した。診断後，経口投与は中止し，経管栄養に変更したが，誤嚥のエピソードは続いたため，生後5ヶ月より十二指腸チューブを挿入した。母乳栄養は初期のみで，低出生体重児用ミルクを投与した。しかし，生後4ヶ月に感染性腸炎罹患後，乳糖不耐症となり，ラクトレスミルクに変更した。体重増加は，アカラシアの症状が出現したころから不良であった。

体重増加不良と，角膜潰瘍の悪化があり入院継続となっていた。生後7ヶ月，突然，多量の上部消化管出血し，ショックとなり死亡した。病理解剖は承諾が得られず行っていない。

考　察

近年，Allgrove症候群については，常染色体劣性遺伝性疾患であり，その責任遺伝子は，染色体12q13に位置するAAAS遺伝子の転座であることが証明されている[7]。AAAS遺伝子は536個のアミノ酸からなる蛋白をコードしている。この蛋白はALADIN（alacrima, achalasia, adrenal insufficiency neurologic disorder）と命名され，WD-repeat蛋白ファミリーに属するとされているが，その機能は現在のところ不明である[8)9)]。本症例も遺伝子解析を行ったが，変異の同定はできなかった。両親の検索も行ったが変異は同定されなかった。本症例の遺伝子変異が同定されなかったことに関し文献的に検討したが，我々が知るかぎりでは，現在のところ変異遺伝子の保有率などに関しては不明であった。一部に他の責任遺伝子を指摘している報告もあり[15]，今後，症例が蓄積され検討が進むことを期待する。

Allgrove症候群の副腎機能障害は，ACTH不応症によるものが一般的である。ACTH不応症では，ア