学位論文

Developmental changes in urinary coproporphyrin ratio in premature infants

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Abstract

Background: Premature infants have a high concentration of conjugated bilirubin in their blood, although they have a poor glucuronide conjugation of bilirubin. This may be due to developmental changes in the function of ATP-binding cassette subfamily C member 2 (ABCC2), which is involved in the cellular export of conjugated bilirubin. In the present study, we examined the developmental changes in the UCP [I / (I+III)] (urinary coproporphyrin I / (urinary coproporphyrin I + urinary coproporphyrin III) ratio), a known biomarker for ABCC2 function, in premature infants.

Method: Twenty-one premature infants born between 25 and 32 weeks of gestation were included in the study. Urine samples were collected within 24 hours of birth, and at 1 week and 3-4 weeks after birth. The samples were analyzed by high-performance liquid chromatography to calculate UCP [I / (I+III)] in order to examine its association with postnatal age and corrected gestational age. Subjects were excluded if they had liver dysfunction, cholestasis, urinary tract infection, or chromosomal abnormalities.

Results: The average UCP [I / (I+III)] within 24 hours of birth, at 1 week, and at 3-4 weeks after birth was 0.84, 0.61, and 0.65, respectively. The UCP [I / (I+III)] within 24 hours of birth was significantly higher than that measured at 1 week or 3-4 weeks after birth. There was no significant correlation between UCP [I / (I+III)] and the corrected gestational age.

Conclusion: The UCP [I / (I+III)] was higher within 24 hours of birth. It decreased 1 week after birth and remained low without any significant changes for up to 4 weeks after birth.

Key words: urinary coproporphyrin, premature infants, developmental change, ATP-binding cassette subfamily C member 2, conjugated bilirubin

Introduction

Obstructive jaundice is common in premature infants as they have a poorly developed ability to excrete conjugated bilirubin, as well as premature liver anatomy characterized by a thin lumen within the bile canaliculi and poor hepatocyte structures. [1] Despite the developmentally poor glucuronide conjugation of bilirubin, [2] neonatal infants have a higher concentration of conjugated bilirubin in the blood than adults. [3, 4] To gain a better understanding of these phenomena, it is necessary to examine the conjugated bilirubin excretion ability within the developmental spectrum of premature infants and during the neonatal period. ATP-binding cassette subfamily C member 2 (ABCC2) is involved in the excretion of conjugated bilirubin from bile canaliculi, [5] but it is challenging to directly measure the function of ABCC2.

In addition to conjugated bilirubin, ABCC2 is involved in the excretion of both endogenous (i.e. coproporphyrin) and exogenous substances (i.e. drugs). Coproporphyrin is a metabolite originating from heme synthesis that exists in two isometric forms, coproporphyrin I and III. Coproporphyrin I is excreted mainly via the liver and constitutes approximately 70% of bile in adults. On the other hand, coproporphyrin III is excreted primarily in urine.[6] Dubin-Johnson syndrome, which is caused by mutations in the *ABCC2* gene, is characterized by an increased urinary coproporphyrin (UCP) I level as its excretion via the liver is reduced. Therefore, the ratio of UCP I and UCP III has conventionally been used as a diagnostic marker. [7] Furthermore, previous studies suggested that the ratio of UCP I over the sum of UCP I and UCP III {UCP [I / (I+III)]} is a useful biomarker of ABCC2 function. [8]

The UCP [I / (I+III)] in full-term healthy male newborns was reported to be almost 80% on day 1 after birth and decreased gradually to the level equivalent to that of

infants and adults by day 10. [9] Furthermore, the UCP [I / (I+III)]was reported to be approximately 59.4% in premature infants, [10] and the corrected gestational age was demonstrated to be negatively correlated with UCP [I / (I+III)] in infants with a corrected gestational age of 30-65 weeks. [11] In order to improve our understanding, we examined the developmental changes in UCP [I / (I+III)] in premature infants born before 32 weeks of gestation.

Study subjects

The study included premature infants born between 25 and 32 weeks of gestation who were admitted to the Neonatal Intensive Care Unit (NICU) at Kochi Medical Care Center between July 1st 2017 and August 31st 2018. Subjects were included in the study if there were over 500 uL of residual urine samples after the urine examinations for routine clinical care such as test strip performed within 24 hours of birth, and at 1 week and 3-4 weeks after birth. Clinical data and laboratory data were extracted from medical records. Small for gestational age infant was defined as less than the 10th percentile of weight and height for gestational age using Japanese anthropometric charts for gestational age. Persistent pulmonary hypertension of the newborn was defined as the elevation pulmonary artery pressure and the presence of the right to left shunt through the ductus arteriosus using echocardiogram. Hyperglycemia was defined as blood sugar over 200mg/dL. Prolonged jaundice was defined as a visible jaundice persisting beyond 2 weeks of life.[12]

Subjects were excluded if they had liver dysfunction defined as alanine aminotransferase over 45 U/L, a direct bilirubin level of $\geq$ 1.5 mg/dL or cholestasis, urinary tract infection, or chromosomal abnormalities.

The study was approved by the Research Ethics Boards of Kagawa University Faculty of Medicine (H 29-028) and Kochi Health Science Center (161053). Parental informed consent was obtained on admission.

Methods

Reagents

Sodium acetate trihydrate (for automated amino acid analysis), acetic acid (super special grade), sodium carbonate (JIS special grade), distilled water (high-performance liquid chromatography (HPLC) -grade) and acetonitrile (HPLC-grade) were used. All these reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). ClinTest® (Recipe, Munich, Germany) was used for standard solution of coproporphyrin I and III.

Measurements

Urine samples were collected and sodium carbonate (Na_2CO_3) was immediately added to the samples in the dark to a final concertation of 5 g/L. The samples were frozen at -35°C and thawed on the day of the measurement. Hydrochloric acid (10μL) was added to 400μL of the sample, and the mixture was centrifuged. After centrifugation, 50μL of the supernatant was injected into the HPLC system. Coproporphyrin I and III were measured according to the protocol previously described by Respaud et al. [13] Alliance Separations Module 2695 (Waters Co. Ltd. Milford, USA) sample management system and pumps were used. Waters 2475 Multi λ Fluorescence Detector (Waters, Milford, USA) was used to detect fluorescent signals with an

excitation wavelength of 365 nm and emission at 624 nm. A symmetry C_{18} column (5 μm, 4.6 mm x 250 mm) (Waters Co. Ltd, Milford, USA) and a CTO-6A (Shimadzu, Kyoto, Japan) column oven were used with the temperature was set to 25°C. The mobile phase consisted of 0.015 M sodium acetate buffer (pH 4.0, solution A) and acetonitrile (solution B). Based on gradient elution of the mixture of solutions A and B, HPLC was run at 1.2 mL/min with 70% solution A (30% solution B), followed by a linear gradient for 10 minutes to reach the concentration of 53% solution A (47% solution B). Subsequently, the flow rate was changed to 1.3 mL/min and a linear gradient was run for 5 minutes to reach the concentration of 30% solution A (70% solution B). The flow rate was adjusted again to 1.2 mL/min and a linear gradient was run for 5 minutes to return to the original concentration of 70% solution A (30% solution B). This concentration was maintained until the measurements were complete.

The area under the curve (AUC) for UCP I and III were determined to calculate UCP [I / (I+III)]. Lastly, we examined the association between changes in UCP [I / (I+III)] and the postnatal age, as well as between UCP [I / (I+III)] and the corrected gestational age at the time of sample collection.

Statistical analysis

JMP13.0.0 was used for all statistical analyses. Multivariate analysis was performed for indomethacin, aminophylline, furosemide, sulbactam/ ampicillin, and amino acid concentrate in relation to UCP [I / (I+III)]. Repeated measures ANOVA with the Tukey post-hoc test was performed to determine the association between postnatal age and UCP [I / (I+III)]. Correlation and regression analyses were performed to clarify the association between the corrected gestational age and UCP [I / (I+III)].

Two-sided tests were used and $p<0.05$ was considered significant.

Results

The study included 21 subjects (11 male) with a mean (± SD) gestation of 28.5 (± 1.7) weeks and birth weight of 1,051 (± 324) g (Table). Seventeen subjects required artificial ventilation, and among them, one subject had a severe complication with persistent pulmonary hypertension of the newborn. Subjects with small for gestational age infants, hyperglycemia and prolonged jaundice were five, two and one, respectively.

During the follow-up period, the following medications were administered to the subjects: indomethacin (n=20), aminophylline (n=18), doxapram (n=7), sulbactam/ ampicillin (n=18), miconazole (n=3), furosemide (n=16), hydrocortisone (n=10), dopamine (n=8), albumin (n=7), γ-globulin (n=5), factor XIII concentrate (n=3), insulin (n=2), fentanyl (n=1), and amino acid concentrate (n=15). Mean total serum bilirubin concentration(± SD) within 24 hours of birth, at 1 week, and at 3-4 weeks after birth was 2.2(± 0.4) mg/dL, 6.3(± 2.0), 5.2(± 2.8), respectively.

The mean UCP [I / (I+III)] (± SD) within 24 hours of birth, at 1 week, and at 3-4 weeks after birth was 0.84 (± 0.06), 0.61 (± 0.14), and 0.65 (± 0.15), respectively. The UCP [I/(I+III)] within 24 hours after birth was significantly higher than that measured at 1 week or 3-4 weeks after birth ($p<0.05$) (Figure 1). The UCP [I / (I+III)] was not consistent within each individual. No correlation was observed between the UCP [I / (I+III)] and total serum bilirubin concentration. No correlation was also observed between the UCP [I / (I+III)] and clinical factors (indomethacin, aminophylline, furosemide, sulbactam/ ampicillin, and amino acid concentrate) by multivariate analysis.

The corrected gestational age at 1 week and 3-4 weeks after birth was within the range of 26.0 and 35.7 weeks. There was no significant association between UCP [I / (I+III)] and corrected gestational age ($R^2 = 1.8x10^{-4}$, $p = 0.93$) (Figure 2).

Discussion

We identified 6 studies to date that examined urinary coproporphyrin excretion in newborns. For full-term infants, the UCP [I / (I+III)] changed from 0.87 to 0.34 between day 1 and day 10 after birth in birth weights between 2,840 and 4,500g. Furthermore, the UCP [I / (I+III)] on day 1 after birth was higher than that in newborns 10 days after birth, as well as in infants and adults. Thus, the UCP [I / (I+III)] of newborns 10 days after birth was equivalent to that of infants and adults. [9] The UCP [I / (I+III)] was approximately 0.68 in 4-10 day-old newborns who had hyperbilirubinemia not requiring treatment, [14] 0.59 in healthy newborns (< 3 days old) born between 36-42 weeks of gestation with birth weights of 2,140-4,670 g, [15] and 0.51 in 3-day-old newborns born at 39.5 weeks of gestation with birth weights of 3,328g. [16]

For preterm infants, in 17 male and 7 female premature infants with birth weights of 1.7kg, it was approximately 0.51. [10] Furthermore, urine samples were collected on different days after birth in newborns with a corrected gestational age of 30-65 weeks and the UCP [I / (I+III)] was demonstrated to be negatively correlated with the corrected gestational age. [11] The present study is the first to examine the change in UCP [I / (I+III)] over time in premature infants born at 28.5 (25.0-32.4) weeks of gestation.

Consistent with the study by Rocchi et al., [9] we found that the UCP [I / (I+III)] was higher within the first 24 hours of birth than at 1 week after birth. Wolkoff and Arias suggested that the change in UCP [I / (I+III)] within the first week after birth can be

explained by the developmental changes in coproporphyrin enzymes. [10] However, it is unlikely that such a marked change can be simply explained by the development of enzyme systems. During the adaptive changes of hepatic circulation in newborns, ductus venous patency functions as a shunt for the inferior vena cava and portal vein for up to 1 week after birth. [17] The probability of having a patent ductus venosus is higher in infants born at 28-32 weeks of gestation than in those born at 33-36 weeks of gestation, and 14% of the premature infants had patent venous ducts even after 3 weeks. [18] These findings suggest that the effect of patent ductus venous should be considered in order to improve our understanding of the mechanisms underlying the marked change in UCP [I / (I+III)]. Collectively, numerous factors in addition to the function of ABCC2 may affect the UCP [I / (I+III)] within 24 hours of birth. Thus, UCP [I / (I+III)] may not be useful as a biomarker of ABCC2 function during this period. However, coproporphyrin I is metabolized in a similar manner as conjugated bilirubin, even during the adaptive changes in hepatic circulation. Thus, UCP [I / (I+III)] may reflect the level of conjugated bilirubin in blood.

Our study has limitation that sample collection age is 4 weeks of less. Different from the report by Rocchi et al., [9] we found that as a biomarker of ABCC2 function, the UCP [I / (I+III)] did not decrease to the level in infants and adults 1 week to 3-4 weeks after birth. Furthermore, in contrast to the report by Kunikata et al., [11] we demonstrated that there was no association between the corrected gestational age (26-35.7 weeks) and UCP [I / (I+III)]. This suggests that there is little change in the function of ABCC2 for up to 3-4 weeks after birth. Of note, although the activity of hepatic bilirubin-UDP-glucuronosyl transferase in infants born at around 30 weeks of gestation is only 0.1% that of adults, [2] these infants have a high level of unconjugated bilirubin

that serves as the substrate of UDP-glucuronosyl transferase. As conjugated bilirubin is also excreted by ABCC2, there may be some level of competition with coproporphyrin in terms of its excretion. Although none of the subjects in our study were administered drugs that can function as substrates of ABCC2,[19,20] it is important to consider not only anatomical factor but also the possible influence of drugs because newborns are often administered many types of drugs.

Premature infants have a higher level of conjugated bilirubin than adults, [4] and often develop obstructive jaundice due to premature development. [1] Considering the function of ABCC2, these characteristics may be a result of an underdeveloped liver excretion system that is insufficient to excrete the amount of conjugated bilirubin being produced. The results of this study also will be an important foundational source of the next clinical study, when conducting the study of comparison of UCP [I / (I+III)] in term infants. In addition to the function of ABCC2, future studies on the excretion of conjugated bilirubin should examine how endogenous and exogenous substances excreted via the same pathway influence the excretion of conjugated bilirubin, and whether the pathway is involved in the excretion of optical isomers of bilirubin via the liver.

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Disclosure

The authors disclose no financial relationships relevant to this article.

Conflict of interest

The authors have no conflicts of interest to disclosure.

Author contribution

HO and SI designed the study; YN and HO performed experiments; YN collected sample and analyzed data; YN wrote the manuscript; HO, SI and TK gave technical support and conceptual advice. All authors read and approved the final manuscript.

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Legend of figures

Figure 1. Developmental changes in UCP [I / (I+III)] in premature infants

Each open circle and error bar represent the mean ± SD. The UCP [I / (I+III)] within 24 hours after birth was significantly higher than that measured at 1 week or 3-4 weeks after birth ($p<0.05$). There was no significant change in the UCP [I / (I+III)] measured at 1 week after birth or 3-4 weeks after birth. UCP; urinary coproporphyrin.

Figure 2. Correlation between UCP [I / (I+III)] and the corrected gestational age

The graph presents the relationship between the corrected gestational age and UCP [I / (I+III)] measured at 1 week and 3-4 weeks after birth. There was no correlation between the variables. UCP; urinary coproporphyrin.

Figure 1

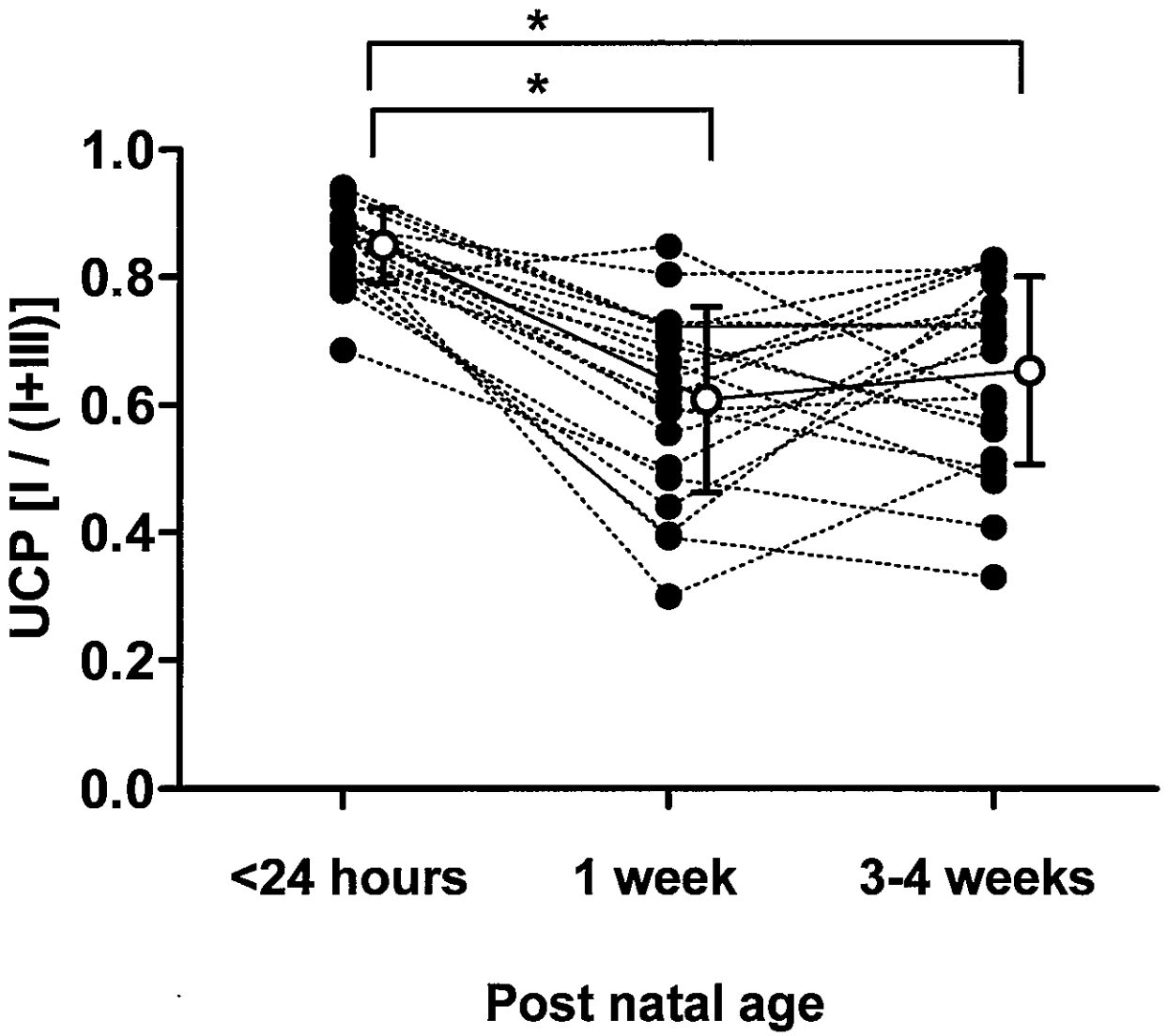
*
*
1.0
0.8
0.6
0.4
0.2
0.0
UCP [I / (I+III)]
<24 hours
1 week
3-4 weeks
Post natal age

Figure 2

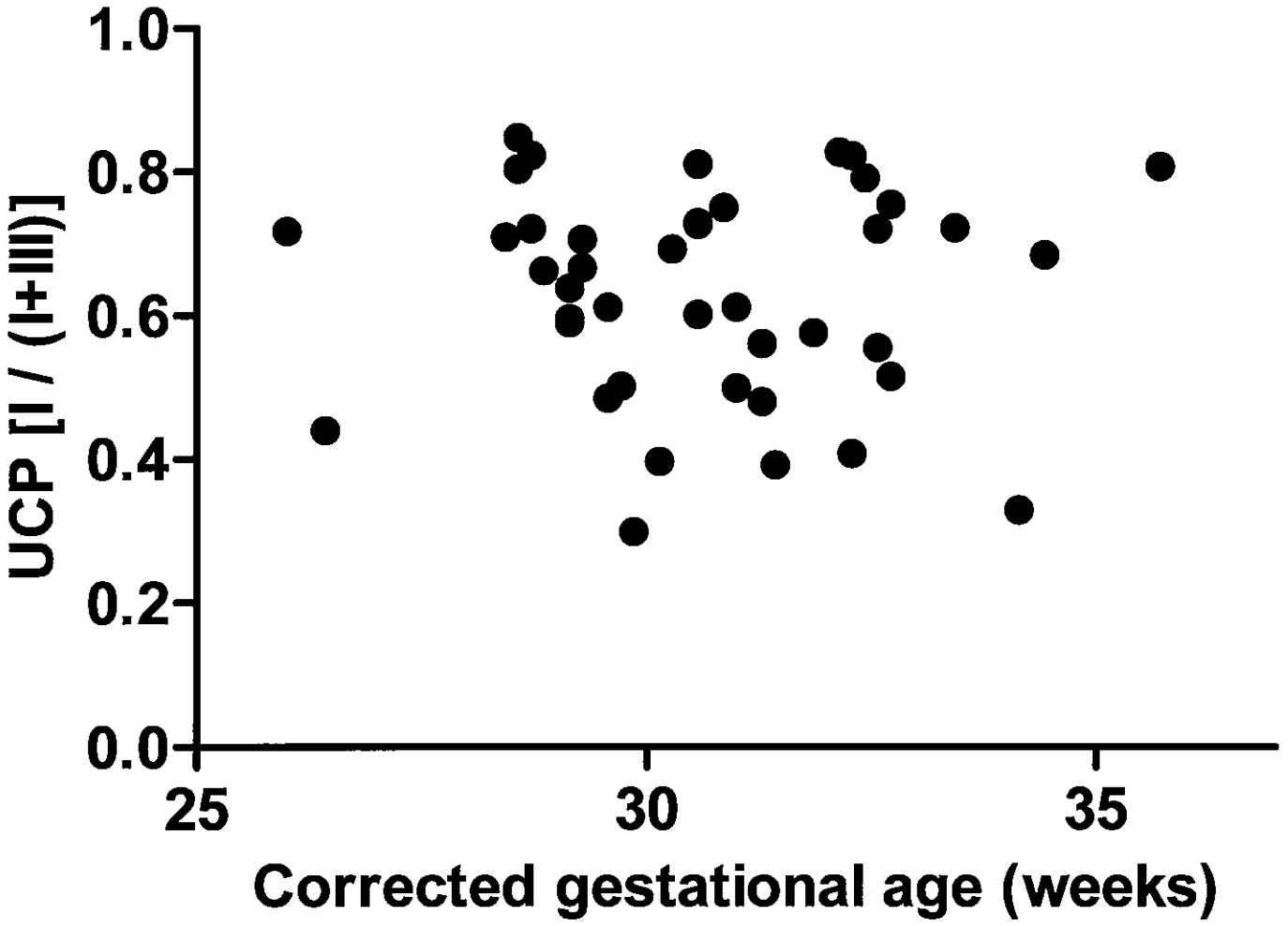
1.0
0.8
0.6
0.4
0.2
0.0
UCP [I / (I+III)]
25
30
35
Corrected gestational age (weeks)

Table. Patients characteristics

	N=21
Gestational age (weeks)	28.5 (1.7)
Birth weight (g)	1051 (324)
Male/Female	11/10
SGA infants	5
The use of mechanical ventilation	17
PPHN	1
Hyperglycemia	2
Prolonged jaundice	1

values indicated mean (SD). SGA, small for gestational age; PPHN, pulmonary hyper tension of newborn
SGA is defined as less than the 10th percentile of weight and height for gestational age using Japanese anthropometric charts for gestational age. PPHN was defined as the elevation pulmonary artery pressure and the presence of the right to left shunt through the ductus arteriosus or foramen ovale using echocardiogram. Hyperglycemia defined as blood sugar over 200mg/dL. Prolonged jaundice is defined as a visible jaundice persisting beyond 2 weeks of life.