Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jmu-online.com
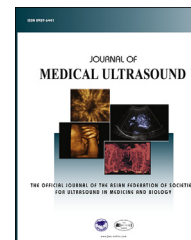
ORIGINAL ARTICLE

Volumetric Analysis of Gallbladder in Extremely Premature Infants

CrossMark

Takashi Imamura [1*], Maki Sato [1], Hayato Go [1], Kei Ogasawara [1],
Yuji Kanai [1], Mina Chishiki [1], Hajime Maeda [1],
Kentarou Haneda [1], Nozomi Kashiwabara [1], Aya Goto [2],
Nobuo Momoi [1], Mitsuaki Hosoya [1]

[1] Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima City, Japan,
and [2] Health Information and Epidemiology Integrated Center for Science and Humanities, Fukushima
Medical University School of Medicine, Fukushima City, Japan

Abstract *Background:* We hypothesized that gallbladder (GB) volume is affected by serial changes during the early infancy period in extremely premature infants.
Methods: We conducted a prospective study of extremely premature infants admitted to the neonatal intensive care unit of Fukushima Medical University Hospital, Fukushima City, Japan between January 2014 and December 2015. GB volume was measured by an abdominal ultrasound ellipsoid method between Day 0 and Day 56 after birth within 60 minutes before enteral feeding. We calculated GB volume (mL)/weight (kg), which was evaluated as GV/W.
Results: In total, 30 infants were included. The median gestational age of the infants was 26 weeks 5 days (range, 23 weeks 1 day—28 weeks 6 days), and the median birth weight was 731 g (range, 398—1220 g). The detection rate of GB decreased in the infants over time; the rates were $> 93\%$ between Day 0 and Day 7 and $< 77\%$ between Day 10 and Day 56 after birth. GV/W decreased in the infants over time. The median GV/W values were 0.18 (range, 0.05 —0.59) in infants on admission and constantly < 0.05 in those between Day 10 and Day 56 after birth. There was no correlation of GV/W with clinical variables after birth.
Conclusion: It is considered that GB volume is not affected by serial changes without nonfavorable course of enteral nutrition.
© 2017, Elsevier Taiwan LLC and the Chinese Taipei Society of Ultrasound in Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Neonatal mortality rates for extremely premature infants have decreased [1,2]. Most of the major advances in this remarkable improvement are attributable to specialized techniques, such as high-frequency ventilation, continuous positive airway pressure applications, prenatal corticosteroid treatment of the mother about to deliver, postnatal artificial surfactant treatment, and an expanding array of nutritional strategies, including new formulas, supplements to milk, and intravenous nutrient solutions [3]. Some infants are able to advance enteral nutrition quickly, particularly breastfed infants [4]. However, providing parenteral nutrition has risks, such as infection, thrombosis, and other complications associated with central venous access, and cholestasis [5]. Therefore, it is important to clarify the extent to which infants that reach full enteral nutrition at a rapid rate also benefit from parenteral nutrition [6].

The development of gallbladder (GB) function depends on the integrated maturation of digestive, absorptive, and motor functions [7]. Although an adequate bile acid level in the intestines enhances fat absorption, malabsorption of fat and intolerance to enteral feeding is particularly common in premature infants. Reference values for the evaluation of neonatal GB size provide insight into the normal GB dimensions of neonates under enteral nutrition [8,9]. Although Lehtonen et al [10] reported that the development of postprandial GB bile acid level in very premature infants is dependent on gestational age (GA), the most important determinant of the response of GB is bolus volume. Postnatal age, postconceptional age, and duration of enteral feeding are not important for the maturation of GB contractility. It is considered that the development of postprandial intestinal activity is mainly determined by the duration of enteral feeding and the increase in feed volume, which is dependent on the clinical condition of infants; infants in stable conditions are fed larger volumes earlier.

Recent laboratory findings suggest that GB secretion is induced by cytokines [9]. *In vitro* biliary epithelial cells respond to interleukin (IL) 6, resulting in the production and secretion of cytokines in an autocrine/paracrine manner [11,12]. Previous studies have demonstrated an association between higher levels of typical proinflammatory cytokines, such as IL-1β, -6, and -8, in the tracheal aspirate of the mechanically ventilated preterm infant and the later development of bronchopulmonary dysplasia (BPD) [13,14]. Yoon et al [14] also reported that the initiation of the inflammatory response can already occur *in utero*, i.e., in the case of chorioamnionitis (CAM). These conditions might contribute to an enhanced secretory function resulting in GB distension. However, to the best of our knowledge, volumetric analysis of GB in extremely premature infants has not been performed to determine correlations among clinical variables and over time after birth. Our objective was to evaluate GB volume by assessing serial changes during the early infancy period in extremely premature infants.

Materials and Methods

We conducted a prospective study of extremely premature infants who were admitted to the neonatal intensive care unit (NICU) of Fukushima Medical University Hospital, Fukushima City, Japan between January 2014 and December 2015. Some infants were considered not eligible for this study on the basis of the following predefined exclusion criteria: (1) death; (2) a history of intestinal operation; and (3) chromosome anomaly. Written informed consent was obtained from the parents, and this study was approved by the Ethics Committee of our hospital (No. 1872).

Definitions

Day 0 is defined as the day of birth. GA was determined from the last maternal menstrual period, obstetric history and examination, and prenatal ultrasound findings. Small for GA (SGA) was defined as a birth weight (BW) under the 10th percentile for GA in accordance with the gender-specific growth charts of Itabashi et al [15]. The histologic criterion based on Blanc's criteria [16] for CAM was the presence of accumulated leukocytes extending across the fetal membranes. The conventional view has been that BPD is caused primarily by oxidant- and ventilation-mediated injury [17]; severe BPD was defined as oxygen dependence (FiO$_2 \geq 0.3$) for ≥ 36 weeks postmenstrual age or the need for positive pressure respiratory management (positive pressure ventilation or nasal continuous positive airway pressure) during hospitalization [18]. Patent ductus arteriosus (PDA) ligation was performed on infants with symptomatic PDA, which shows a poor response to repeated indomethacin therapies. Intraventricular hemorrhage (IVH) was diagnosed on the basis of ultrasonography findings and graded in accordance with the definitions by Papile et al [19]. Sepsis was diagnosed when there was a systemic response to a possible infection; evidence of bacteremia or an infectious focus was not required [20]. Clinical infection was diagnosed on the basis of shock vital signs (> 1 of the following 3 signs: hypotension, oliguria, and increased demand for oxygen treatment), and C-reactive protein positivity (≥ 0.3mg/dL) after 7 postnatal days. Hypotension was defined as a systolic pressure of < 40 mmHg or a 20% reduction from the previous value, oliguria as a urine output of < 1 mL/kg/h during a 4-hour interval, and increased oxygen treatment as a rapid increase in the fraction of inspired oxygen by > 0.1 [21]. Periventricular leukomalacia (PVL) was diagnosed on the basis of magnetic resonance imaging findings [22]. Retinopathy of prematurity (ROP) was diagnosed by an ophthalmologist in our hospital and classified in accordance with the international classification of ROP [23]. Retinal photocoagulation was performed on infants whose ROP progressed to stage 3b. Full enteral feeding was defined as the successful intake of at least 100 mL/kg/d. These definitions were used consistently throughout the study period.

Nutritional management

Enteral feeding with very small amounts (< 0.1 mL) of breast milk was started as a part of routine management as soon as possible after birth in our NICU. Enteral feeding was performed every 3 hours, eight times a day, and the feed consisted of breast milk or milk formula (icreo, ICREO CO.,

LTD., Tokyo, Japan) administered via a gastric tube. The gastric tube (JF-C05040PUS, JMS, Ltd., Hiroshima, Japan) used was 5 FR × 40 cm. The feed volume was increased from 5 mL/kg/d to 10 mL/kg/d as the infant's tolerance to the feed increased. When they showed recurrent vomiting or formed large amounts of gastric residues (more than the bolus volume at a time), the volume of the feed was temporarily not increased or the feeding was discontinued. Parenteral nutrients with amino acids (AAs) (Pleamin-P Injection, Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) were continuously infused 0.5 g/kg/d on Day 1 after birth and increased up to 2.0 g/kg/d every 3 days (0.5 g/kg/d, 3 days; 1.0 g/kg/d, 3 days; 1.5 g/kg/d, 3 days). The infusion was discontinued within 3 days when enteral full feeding was reached. No fat supplement was administered intravenously during this study period.

Measurements

Ultrasonography was performed employing a 12 MHz small-parts transducer (EPIQ7: Philips Ultrasound, Bothell, WA, USA). Maximal GB dimensions were measured by a single investigator on Day 0, Day 2, Day 5, Day 7, Day 10, Day 14, Day 21, Day 28, Day 35, Day 42, Day 49, and Day 56 after birth within 60 minutes before enteral feeding. GB volume was examined by the ellipsoid method [24]: $1/6 \times \pi \times$ maximum length $\times$ lateral diameter^2. The planimetric ellipsoid size of GB was measured on the basis of its maximal longitudinal ultrasonographic image (Figures 1A and 1B). Each parameter was measured three times consecutively, and the mean maximum length and width were calculated. Body weight was measured once a week during endotracheal ventilation, and every day after endotracheal ventilation was discontinued. GB data were standardized using body weight. Finally, we calculated GB volume (mL)/weight (kg), which was evaluated as GV/W. "Undetected" was indicated when the investigator was unable to detect GB within 5 minutes of examination.

Laboratory examination was performed on Day 0, Day 7, Day 14, Day 28, and Day 56 after birth as a part of routine management in our NICU.

Data collection and statistical analysis

The data shown below were collected from perinatal case records. The continuous variables were as follows: GA, BW, umbilical artery hydrogen ion concentration (UmApH), 5-minute Apgar score, duration of endotracheal ventilation, days to first enteral feeding after birth, days to successful enteral intake of 50 mL/kg/d, days to full enteral feeding, duration of AA administration, days to reach 1500 g of weight, and levels of gamma-glutamyl transpeptidase (γGTP), total bile acid (TBA), total bilirubin (TB), direct bilirubin (DB), and amylase (AMY). The categorical variables were as follows: sex, birth number, SGA, delivery type, antenatal steroid usage, CAM, respiratory distress syndrome (RDS), severe BPD, PDA ligation, IVH grade 3 or 4, sepsis, necrotizing enterocolitis (NEC), PVL, and ROP requiring coagulation therapy. All univariate analyses were performed using Dr. SPSS II for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$. The correlations of GV/W and perinatal factors with continuous variables were determined using Spearman's rank correlation. The correlation was applied to 99 analyses, and the final candidate selection was done by filtering for the Bonferroni correction for multiple comparisons ($p < 0.0005$). The associations of GV/W and perinatal factors with categorical variables were determined by the Mann–Whitney U test where appropriate, and data are presented as median with range. The test was applied to 60 analyses, and the final candidate selection was done by filtering for the Bonferroni correction for multiple comparisons ($p < 0.0008$).

Results

Patients

Forty extremely premature infants (≤ 28 weeks of GA) were admitted to our hospital during the study period. Ten infants were excluded on the basis of the predefined criteria. Of the 30 extremely premature infants included, the
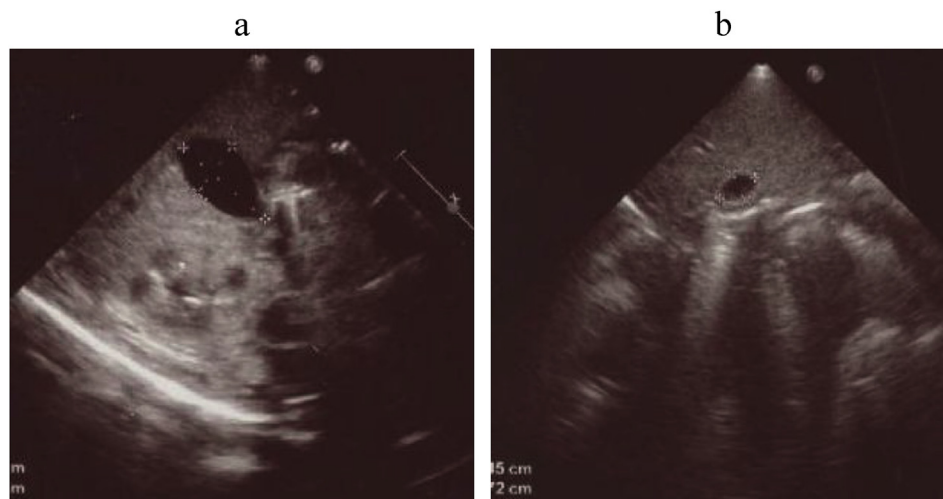

Figure 1 Sagittal sonographic section abdominal right upper quadrant. Pear-shaped cystic mass at a location in gallbladder (GB) in an infant with 26 weeks of gestational age on (a) Day 2 and (b) Day 49 after birth. The planimetric ellipsoid size of GB was measured on the basis of its maximal longitudinal image.

following were their GAs: three at 23 weeks, four at 24 weeks, four at 25 weeks, nine at 26 weeks, two at 27 weeks, and eight at 28 weeks. None of the infants have hepatobiliary diseases (biliary atresia, cholestatic hepatitis, and sludge or stone formation in the GB).

Clinical characteristics of 30 infants

Table 1 shows the perinatal characteristics of the 30 infants. Females were 46.7% and consisted of 22 (73.3%) singletons and eight (27.7%) twins. The median GAs of the infants was 26 week 5 days (range, 23 week 1 day—28 week 6 days). The median BW of the infants was 731 g (range, 398—1220 g). In total, 26 (86.7%) infants were delivered by cesarean section, and 23 (76.7%) infants were born to mother with antenatal steroid usage. Eleven (36.7%) infants experienced CAM.

Clinical courses

Table 2 shows the clinical courses of the 30 infants. The median duration of endotracheal ventilation of the infants was 33 days (range, 0—113 days). Seven (23.3%) infants experienced severe BPD, and two (6.7%) infants underwent PDA ligation. The median days to first and full enteral feedings after birth were 1 day (range, 0—4 days) and 13 days (range, 9—22 days), respectively. Moreover, the median number of days to reach 1500 g weight was 60 (range, 35—105 days).

Measurement of GB

Figure 2 shows the detection rate of GB in the infants between Day 0 and Day 56 after birth. The rate decreased in the premature infants over time. The rate between Day 0 and Day 7 after birth was > 93%, whereas that between Day 10 and Day 56 after birth was < 77%.

Table 1 Characteristics of 30 infants.

Characteristic	$n = 30$
Sex (female)	14 (46.7)
Singleton (birth No.)	22 (73.3)
GA (wks)	26 wk 5 d (23 wk 1 d—28 wk 6 d)
BW (g)	731 (398—1220)
SGA	6 (20.0)
Cesarean section (delivery type)	26 (86.7)
Birth place, in born	30 (100)
UmApH	7.346 (6.658—7.414)
5-min Apgar score	6 (0—8)
Antenatal steroid usage	23 (76.7)
Chorioamnionitis	11 (36.7)

BW = birth weight; GA = gestational age; SGA = small for gestational age; UmApH = umbilical artery hydrogen ion concentration.
SGA was defined as a birth weight under the 10th percentile for GA in accordance with the gender-specific growth charts of Itabashi.
Data are presented as n (%) or median (range).

Table 2 Clinical courses of 30 infants.

Courses	$n = 30$
Duration of endotracheal ventilation (d)	33 (0—113)
RDS	25 (83.3)
Severe BPD	7 (23.3)
PDA ligation	2 (6.7)
IVH grade 3 or 4	4 (13.3)
Sepsis	3 (10.0)
NEC	1 (3.3)
PVL	0 (0)
ROP requiring coagulation therapy	13 (43.3)
Days to first enteral feeding after birth (d)	1 (0—4)
Days to successful intake of 50 mL/kg/d (d)	9 (6—18)
Days to full enteral feeding (d)	13 (9—22)
Duration of AA administration (d)	16 (8—60)
Days to reach 1500 g weight (d)	60 (35—105)

Data are presented as n (%) or median (range).
AA = amino acid; BPD = bronchopulmonary dysplasia; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; PDA = patent ductus arteriosus; PVL, periventricular leukomalacia; RDS = respiratory distress syndrome; ROP = retinopathy of prematurity.

Figure 3 shows GV/W in the infants between Day 0 and Day 56 after birth. GV/W decreased in the premature infants over time. The median GV/W value of the infants on admission was 0.18 (range, 0.05—0.59), whereas that of the infants between Day 14 and Day 56 after birth was constantly < 0.05.

Relationships between GV/W and perinatal variables

Table 3 shows the relationships between GV/W and perinatal variables. Determined by the Spearman's rank
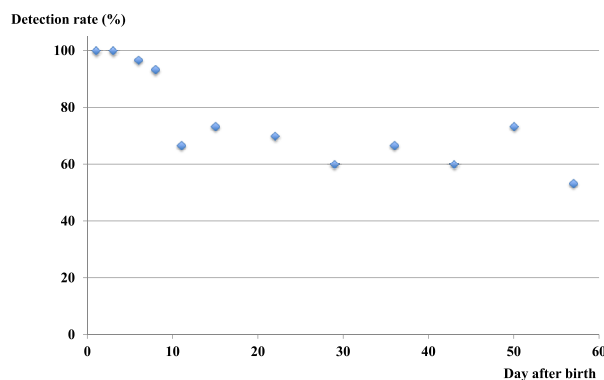

Figure 2 Detection rate (%) of gallbladder (GB) in the infants between Day 0 and Day 56 after birth: 100% on Day 0, 100% on Day 2, 96.7% on Day 5, 93.3% on Day 7, 66.7% on Day 10, 73.3% on Day 14, 70.0% on Day 21, 60.0% on Day 28, 66.7% on Day 35, 63.3% on Day 42, 76.7% on Day 49, and 56.7% on Day 56. Days to first enteral feeding and successful intake of 50 mL/kg/d after birth were 1 (0—4) and 9 (6—18), respectively. Finally, a day to full enteral feeding was 13 (9—22). The detection rate of GB gradually decreased with the progress of enteral feeding in the extremely premature infants over time.

Table 3 Relationships between gallbladder volume/weight (GV/W) and perinatal variables determined by univariate analysis. Determined by the Spearman's rank correlation with Bonferroni correction, GV/W on Days 0 and 14 were significantly associated with days to reach 1500 g weight and γGTP level on Day 14, respectively ($p = 0.001$, $rs = 0.65$ and $p = 0.001$, $rs = -0.75$). GV/W on Day 21 was also significantly associated with birth number determined using the Mann—Whitney U test with Bonferroni correction ($p = 0.001$).

| Day after birth	0		2		5		7		10		14		21		28		35		42		49		56	
											GV/W													
Variable	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p
Gestational age (wk)	−0.31	0.10	−0.21	0.26	−0.13	0.51	0.06	0.75	−0.13	0.59	−0.15	0.50	−0.55	0.01	−0.3	0.23	−0.24	0.31	−0.19	0.44	0.04	0.87	0.26	0.34
Birth weight (g)	−0.46	0.01	−0.32	0.09	−0.46	0.01	−0.3	0.12	−0.1	0.69	−0.38	0.08	−0.41	0.07	−0.23	0.35	0.09	0.69	−0.50	0.03	−0.28	0.22	−0.43	0.10
First enteral feeding after birth (d)	0.06	0.75	−0.09	0.63	−0.03	0.88	0.09	0.63	−0.15	0.53	−0.19	0.41	0.33	0.15	0.11	0.68	0.47	0.04	0.34	0.61	−0.12	0.59	−0.07	0.80
Successful enteral intake of 50 mL/kg/d (d)	0.14	0.46	0.06	0.75	0.29	0.04	0.51	0.01	0.10	0.69	0.23	0.30	0.22	0.34	0.09	0.74	0.40	0.08	0.27	0.27	−0.05	0.81	−0.19	0.48
Full enteral feeding (d)	0.18	0.35	0.10	0.59	0.31	0.11	0.42	0.03	0.14	0.57	0.29	0.20	0.22	0.33	0.10	0.70	0.43	0.06	0.26	0.27	−0.06	0.79	−0.25	0.34
Duration of endotracheal ventilation (d)	0.44	0.01	0.30	0.11	0.25	0.20	0.06	0.75	0.17	0.49	0.31	0.17	0.47	0.03	0.13	0.61	−0.17	0.48	−0.08	0.74	0.11	0.63	−0.14	0.60
Weights reached 1500 g (d)	0.65	0.001*	0.52	0.01	0.51	0.02	0.26	0.24	0.11	0.67	0.27	0.31	0.57	0.01	0.47	0.09	0.25	0.34	0.68	0.01	0.33	0.18	0.30	0.29
γGTP (U/L)	−0.19	0.32					−0.27	0.18			−0.75	0.001*			0.16	0.54							0.16	0.65

GV/W (ml/kg)
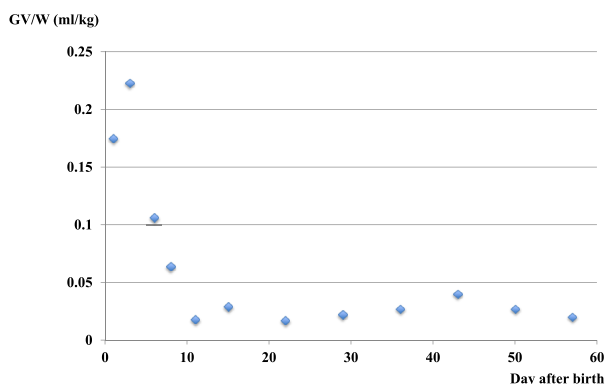
Figure 3 Gallbladder volume/weight (GV/W) in infants between Day 0 and Day 56 after birth. The following were their median GV/W values (range): 0.18 (0.05—0.59) on Day 0, 0.22 (0.01—0.90) on Day 2, 0.11 (0.02—2.61) on Day 5, 0.06 (0.01—0.82) on Day 7, 0.02 (0.01—0.65) on Day 10, 0.03 (0.01—0.45) on Day 14, 0.02 (0.01—0.44) on Day 21, 0.02 (0.01—0.53) on Day 28, 0.03 (0.01—1.04) on Day 35, 0.04 (0.01—0.38) on Day 42, 0.03 (0.01—0.41) on Day 49, and 0.02 (0.01—0.26) on Day 56. Days to first enteral feeding and successful intake of 50 mL/kg/d after birth were 1 (0—4) and 9 (6—18), respectively. Finally, a day to full enteral feeding was 13 (9—22). The GV/W gradually decreased with the progress of enteral feeding in the extremely premature infants over time.

correlation with Bonferroni correction, GV/W on Day 0 and Day 14 were significantly associated with days to reach 1500 g weight and γGTP level on Day 14, respectively ($p = 0.001$, $rs = 0.65$ and $p = 0.001$, $rs = -0.75$, respectively). GV/W on Day 21 was also significantly associated with birth number determined using the Mann—Whitney U test with Bonferroni correction ($p = 0.001$). GV/W did not correlate with the following perinatal variables: sex, GA, BW, SGA, delivery type, birth place, UmApH, 5-minutes Apgar score, antenatal steroid usage, CAM, duration of endotracheal ventilation, RDS, severe BPD, PDA ligation, IVH grade 3 or 4, sepsis, NEC, PVL, ROP requiring coagulation therapy, days to first enteral feeding after birth, days to successful enteral intake of 50 mL/kg/d, days to full enteral feeding, duration of AA administration, and levels of TBA, TB, DB, and AMY between Day 0 and Day 56 after birth.

Discussion

To the best of our knowledge, this is the first study in which GB volume in extremely premature infants was evaluated by assessing serial changes during the early infancy period. In this study, we obtained large amounts of neonatal GB data under both enteral nutrition and total parenteral nutrition (TPN). Moreover, we observed that GB in extremely premature infants is fully distensible at birth; however, both the detection rate of GB and GV/W decreased with the progress of enteral feeding in the premature infants over time. Although extremely premature infants easily develop clinical complications, there was no correlation between GV/W and clinical variables after birth in this study.

Lehtonen et al [10] observed the dependence of development of postprandial GB contractility in very premature infants (GA, 24—37 weeks) on GA with a maturational turning point of 32 weeks of gestation. However, we observed that the GV/W of the infants was not associated with GA between Day 0 and Day 56 after birth. Moreover, some clinical complications such as CAM, symptomatic PDA, and long duration of endotracheal ventilation were also not associated with GV/W of the infants. Although GV/W on Day 0 was associated with days to reach 1500 g weight, the duration of AA administration was not associated with GV/W between Day 0 and Day 56 after birth. These results indicate that GV/W is more useful for establishing the degree to which the neonatal GB contracts in response to favorable course of enteral nutrition. TPN is effective for supplying energy and nutrients to support growth in premature neonates [25]. Some recent evidence has demonstrated that inadequate nutrition in premature infants in the 1st week results in growth retardation and may lead to permanent detrimental effects [26]. Therefore, parenteral nutrition is required until full enteral nutrition can be established [27,28]. The secretion of cholecystokinin (CCK) is chiefly stimulated by fat, proteins, and AAs, and minimally by glucose [29—31]. CCK plays an important role in regulating basal GB tone and postprandial GB contraction [32]. A common feature of premature neonatal management is the administration of parenteral nutrients prior to the enlargement of GB [33]. In particular, premature infants are often nourished by parenteral hyperalimentation leading to prolonged inactivation of GB. Jawaheer et al [34] examined the effects of continuous parenteral feeding and bolus enteral feeding on GB contractility in premature neonates. They found that GB was significantly larger in parenterally fed infants than in enterally fed infants. Interestingly, the detection rates of GB and GV/W were also approximately the same after the discontinuation of AA infusion and the increase in enteral feeding volume in this study. These results indicate that enteral fat, proteins, and AAs may be more potent contracts of GB than intravenous glucose, AAs, and fat in extremely premature infants. It is considered that the method of delivering nutrients is the reason which gradually decreased the detection rate of GB in the study and which may have an important role in modulating GB functions. As none of the infants had hepatobiliary complications, such as cholestasis, sludge and stone formation, or thickening of GB wall in this study, it is necessary to include these complications that are also induced after TPN in the evaluation in future studies.

This study has several limitations. First, although the infants underwent abdominal ultrasonography within 60 minutes before enteral feeding, GB volume may easily change. The features of GB were not always similar; curving, constriction, and narrowing of GB were sometimes observed, which may be more correctly measured by a single or biplane method [24]. Second, intravenous glucose and AAs theoretically have different effects on CCK secretion and GB contractility. MacGregor et al [31] reported that GB contraction is impaired by glucose infusion, whereas CCK secretion is stimulated by AA administration. All infants were given carbohydrate alone or in combination with AAs in this study, which might have affected our results. Finally, this study was performed with a small number

of infants. Moreover, the detection rate of GB decreased in the premature infants over time. Owing to the above reasons, it was difficult to perform further analysis, such as multivariate median regression analyses.

In conclusion, we were able to better understand GV/W in extremely premature infants by assessing serial changes in GB volume during the early infancy period. Although transient GB dilatation or poor contraction during the early postnatal period may be categorized as a normal physiologic state, we observed that both the detection rates of GB and GV/W decreased with the progress of enteral feeding in the premature infants over time. It is considered that GB volume is not affected by serial changes without a nonfavorable course of enteral nutrition. Further longitudinal and epidemiologic studies of larger populations are necessary to clarify the physiology of GB functions in extremely premature infants.

Acknowledgments

References

[1] Patel RM. Short- and long-term outcomes for extremely preterm infants. Am J Perinatol 2016;33:318—28.

[2] Shah PS, Lee SK, Lui K, et al. The international network for evaluating outcomes of very low birth weight, very preterm neonates (iNeo): a protocol for collaborative comparisons of international health services for quality improvement in neonatal care. BMC Pediatr 2014;14:110.

[3] William WH. Strategies for feeding the preterm infant. Neonatology 2008;94:245—54.

[4] Ehrenkranz RA. Early nutritional support and outcomes in ELBWI infants. Early Hum Dev 2010;86:21—5.

[5] Hughes CA, Talbot IC, Ducker DA, et al. Total parenteral nutrition in infancy: effect on the liver and suggested pathogenesis. Gut 1983;24:241—8.

[6] Morisaki N, Belfort MB, McCormick MC, et al. Brief parenteral nutrition accelerates weight gain, head growth even in healthy VLBWs. PLoS One 2014;9:e88392.

[7] Ho ML, Chen JY, Ling UP, et al. Gallbladder volume and contractility in term and preterm neonates: normal values and clinical applications in ultrasonography. Acta Paediatr 1998; 87:799—804.

[8] Bowen A. Ultrasound of the normal neonatal gallbladder. Diagn Imaging Clin Med 1984;53:231—6.

[9] Schmidt B, Roth B, Stutzer H, et al. Prospective volumetric analysis of gallbladders in critically Ill newborns: the impact of nutrition. Neonatology 2007;92:201—4.

[10] Lehtonen L, Svedstrom E, Kero P, et al. Gall bladder contractility in preterm infants. Arch Dis Child 1993;68:43—5.

[11] Savard CE, Blinman TA, Choi HS, et al. Expression of cytokine and chemokine mRNA and secretion of tumor necrosis factor-alpha by gallbladder epithelial cells: response to bacterial lipopolysaccharides. BMC Gastroenterol 2002;2:23.

[12] Yokomuro S, Lunz 3rd JG, Sakamoto T, et al. The effect of interleukin-6 (IL-6)/gp130 signalling on biliary epithelial cell growth, in vitro. Cytokine 2000;12:727—30.

[13] Speer CP. Pulmonary inflammation and bronchopulmonary dysplasia. J Perinatol 2006;26:S57—64.

[14] Yoon BH, Romero R, Jun JK, et al. Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. Am J Obstet Gynecol 1997;177:825—30.

[15] Itabashi K, Miura F, Uehara R, et al. New Japanese neonatal anthropometric charts for gestational age at birth. Pediatr Int 2014;56:702—8.

[16] Blanc WA. Pathology of the placenta, membranes, and umbilical and in bacterial, fungal, and viral infections in man. Monogr Pathol 1981;22:67—132.

[17] Northway Jr WH, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease: bronchopulmonary dysplasia. N Engl J Med 1967;276:357—68.

[18] Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163:1723—9.

[19] Papile LA, Burstein J, Burstein R, et al. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1500 gm. J Pediatr 1978;92:529—34.

[20] Saez-Llorens X, McCracken Jr GH. Sepsis syndrome and septic shock in pediatrics: Current concepts of terminology, pathophysiology, and management. J Pediatr 1993;123:497—508.

[21] Masumoto K, Kusuda S, Aoyagi H, et al. Comparison of serum cortisol concentrations in preterm infants with or without late-onset circulatory collapse due to adrenal insufficiency of prematurity. Pediatr Res 2008;63:686—90.

[22] Kuzmanic-Samija R, Resic B, Tomasovic M, et al. West syndrome with periventricular leukomalacia: ten year clinical study. Coll Antropol 2008;32:105—11.

[23] Flynn John T, An international classification of retinopathy of prematurity. II. The classification of retinal detachment. The international Committee for the Classification of the Late Stages of Retinopathy of Prematurity. Arch Ophthal 1987;105: 906—12.

[24] Folland ED, Parisi AF, Moynihan PF, et al. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. A comparison of cineangiographic and radionuclide techniques. Circulation 1979;60: 760—6.

[25] Matos C, Avni EF, Gansbeke D, et al. Total parenteral nutrition (TPN) and gallbladder diseases in neonates. Sonographic assessment. J Ultrasound Med 1987;6:243—8.

[26] Ehrenkranz RA. Early, aggressive nutritional management of very low birth weight infants: what is the evidence? Semin Perinatol 2007;31:48—55.

[27] Liu MY, Chen YY, Hu SH, et al. The influence of aggressive parenteral nutrition to preterm and very low birth weight infants. Glob Pediatr Health 2015;2. 2333794x14567192.

[28] Smallpeice V, Davies PA. Immediate feeding of premature infants with undiluted breast-milk. Lancet 1964;2:1349—52.

[29] de Boer SY, Masclee AA, lam WF, et al. Hyperglycemia modulates gallbladder motility and small intestinal transit time in man. Dig Dis Sci 1993;38:2228—35.

[30] Lucas A, Bloom SR, Aynsley-Green A. Metabolic and endocrine events at the time of the first feed to human milk in preterm and term infants. Arch Dis Child 1978;53:731—6.

[31] MacGregor IL, Deveney C, Way LW, et al. The effect of acute hyperglycemia on meal-stimulated gastric, biliary and pancreatic secretion, and serum gastrin. Gastroenterology 1976;70:197—202.

[32] Liddle RA, Goldfine ID, Rosen MS, et al. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. J Clin Invest 1985;75:1144—52.

[33] Arad I, Peleg O, Udassin R, et al. Gallbladder distention in premature neonates receiving parenteral nutrition. J Perinat Med 1989;17:337—40.

[34] Jawaheer G, Pierro A, Lloyd DA, et al. Gall bladder contractility in neonates: effects of parenteral and enteral feeding. Arch Dis Child Fetal Neonatal Ed 1995;72:F200—2.