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Original article

Serum unbound bilirubin as a predictor for clinical kernicterus in extremely low birth weight infants at a late age in the neonatal intensive care unit

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Abstract

Background: This study aimed to evaluate peak serum total bilirubin (TB) and unbound bilirubin (UB) levels in preterm infants with clinical kernicterus (KI) who were diagnosed by clinical findings during infancy.

Design/subjects: For this multicenter retrospective study, 18 Japanese extremely low birth weight (ELBW) infants with clinical KI were included. Clinical KI was diagnosed based on the presence of motor developmental impairment with/without athetosis, and abnormal magnetic resonance imaging or brainstem auditory evoked potential findings during infancy. High and low TB or UB levels were defined as serum TB levels $\geqslant$ and $<$15 mg/dL or serum UB levels $\geqslant$ and $<$0.8 μg/dL, respectively. The clinical characteristics of KI preterm infants were analyzed. The proportion of infants with high or low serum TB levels and with high or low serum UB levels was then investigated. Sensitivity and specificity were calculated.

Results: In 18 KI infants, the median age when serum TB levels peaked was 28 days after birth. In eight KI infants with low serum TB levels, 88% of them had high serum UB levels. For comparison of the number of infants who had high or low serum TB and UB levels, the sensitivity was 90% and specificity was 13%.

Conclusions: Serum TB and UB levels peak at a later age than expected. Chronic serum UB monitoring may be helpful for identifying ELBW infants at risk for developing KI, even when they do not have high serum TB levels.

Keywords: Athetosis; Brainstem auditory evoked potentials; Jaundice; Magnetic resonance imaging; Motor impairment

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1. Introduction

Kernicterus (KI) in term infants is extremely rare in Japan because of the widespread use of a management guideline for hyperbilirubinemia and the initiation of phototherapy (PT) on the basis of guideline. However, some preterm infants have been diagnosed with KI, as shown by the presence of motor impairment with athetosis, abnormal magnetic resonance imaging (MRI) and/or brainstem auditory evoked potential (BAEP) findings during infancy (clinical KI) [1–4]. Most importantly, preterm infants with KI do not always have severe hyperbilirubinemia during their stay in the neonatal intensive care unit (NICU) [1–3,5–7]. A better predictive biomarker than serum total bilirubin (TB) is required to prevent the development of KI in preterm infants.

Clinical studies have suggested that serum unbound bilirubin (UB), which is a measure of bilirubin not bound to albumin, is a more sensitive predictor of low birth weight infants with "at risk" KI, infants with auditory impairment due to bilirubin-induced neurotoxicity, and extremely low birth weight (ELBW) infants with death or adverse neurodevelopmental outcomes than serum TB levels [8–12].

Our study aimed to evaluate peak serum TB and UB levels during the NICU stay in Japanese ELBW infants with clinical KI who were diagnosed by clinical findings during infancy, not autopsy.

2. Patients and methods

2.1. Subjects

For this multicenter retrospective study, we collected preterm infants <30 weeks' gestational age (GA), who were diagnosed with KI in their infancy from 2002 to 2012, and were measured both serum TB and UB levels during their NICU stay at Kobe University Hospital, Kobe Children's Hospital, Kakogawa West Municipal Hospital, Takatsuki General Hospital, Yodogawa Christian Hospital, Chibune General Hospital, and Juntendo University Hospital. Serum TB and UB levels were measured once a day until 7 days after birth, once every 2–3 days between 7 and 14 days of age. Indications for measurements of serum TB and UB levels, and treatment of phototherapy, intravenous albumin administration, or exchange transfusion were decided at the discretion of the responsible neonatologists after 14 days of age.

Clinical KI was diagnosed by criteria based on physical and neurological examinations, and laboratory findings during infancy, including the presence of motor impairment (dystonia, hypertonia, motor developmental delay, and/or disturbance of coordination or muscle tone) with/without athetosis, abnormal bilateral high intensity signals in the globus pallidi seen on T2-weighted images of brain MRI, and/or abnormal BAEP, such as bilateral no response or abnormal interwave separation at 90 dB [1,4]. Clinical KI infants were classified into three subtypes proposed by Shapiro [4]: classic KI, auditory KI, and motor KI. Classic KI refers to individuals with auditory neuropathy with/without hearing loss, neuromotor symptoms ± athetosis, oculomotor pareses, or dental enamel dysplasia. Auditory or motor KI refers to individuals with predominantly auditory symptoms with relatively minimal motor symptoms or predominantly motor symptoms with minimal auditory symptoms, respectively. Motor impairment and athetosis were diagnosed by a pediatric neurologist and brain MRI images were analyzed by a radiologist in each hospital.

2.2. Study methods

Clinical and laboratory findings in all of the enrolled infants were collected from medical charts. The clinical characteristics of preterm infants with KI were analyzed, including GA, birth weight (BW), the age when serum TB levels peaked, and serum TB and UB levels when serum TB levels peaked during the NICU stay. The cause and treatment of their hyperbilirubinemia were also analyzed. The number of infants with high or low serum TB levels and with high or low serum UB levels was determined in all KI infants, as well as KI infants who had peak serum TB levels at <28 days of age or ⩾28 days of age. Sensitivity and specificity were calculated in each group and values were compared between the <28 days of age and ⩾28 days of age groups. The collection and use of clinical data for this study were approved by the Ethical Committee of Kobe University Graduate School of Medicine.

2.3. Definitions of high and low serum TB and high and low serum UB levels

At the age when serum TB levels peaked in infants, high and low TB levels were defined as serum TB levels ⩾ and <15 mg/dL, respectively. High and low UB levels were defined as serum UB levels ⩾ and <0.8 μg/dL, respectively. These values are recommended for exchange transfusion based on our previous report [8].

2.4. Assay methods

Serum TB and UB levels were measured at the same time using a Food and Drug Administration-approved analyzer (UB Analyzer; Arrows Co., Ltd, Osaka, Japan) by spectrophotometry and the glucose oxidase–peroxidase method, respectively, as previously described [8,13–16]. Serum UB levels were measured using the single peroxidase concentration method, as recommended

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by the manufacturer, because this has a higher precision than the two peroxidase concentration method [13].

3. Results

3.1. Clinical characteristics of ELBW infants with clinical KI

Eighteen infants with clinical KI were collected from 7 NICUs. Table 1 shows the clinical characteristics. Eleven infants were classified as classical KI. Of these, one infant had dental enamel dysplasia and none had oculomotor pareses. Four and three infants had auditory and motor KI, respectively. Of the 18 KI infants, 7 (39%) had both specific abnormal brain MRI and BAEP findings. One female infant (#12) only had an abnormal BAEP finding, but she was diagnosed with auditory KI because she had dental enamel dysplasia.

Although we collected data on preterm infants <30 weeks' GA with clinically diagnosed KI, all preterm infants were born less than 28 weeks' GA, with BW less than 1000 g (median GA: 24 weeks, BW: 634 g). The median age when serum TB levels peaked was 28 days after birth. The median serum TB and UB levels at that age were 17.0 mg/dL and 1.67 μg/dL, respectively.

Most of infants (16/18, 89%) were the age ⩾14 days, and the latest age when serum TB levels peaked was 86 days. No treatment was provided to three (17%) infants (#s 4, 16, and 18). Except for these three infants, fifteen infants received treatments as soon as high serum TB and/or UB levels were detected (8: PT, 4: PT and exchange transfusion, 2: PT and intravenous albumin administration, 1: intravenous albumin administration alone). Only four (22%) infants received exchange transfusion. Causes of hyperbilirubinemia were as follows in order: idiopathic ($n = 11$, 61%), infection ($n = 4$, 22%), and others ($n = 3$, 17%).

3.2. Number of infants with high or low serum TB levels and with high or low serum UB levels at the age when serum TB levels peaked

A total of 56% or 89% of all KI infants had high serum TB or high serum UB levels, respectively. Of the eighteen KI infants, nine (50%) had both high serum TB and UB levels. On the other hand, eight had a low serum TB level (44%). In eight KI infants with low serum TB levels, there were seven (88%) infants with high serum UB levels. For comparison of the number of infants who had high or low serum TB and UB levels,

Table 1
Characteristics of extremely low birth weight infants with clinical kernicterus.

	GA (week)	BW (g)	Age of peak serum TB (days)	Peak serum TB levels (mg/dL)	Serum UB levels at the age of peak TB levels (μg/dL)	Treatment at the age of peak TB levels	Causes of hyperbilirubinemia	Type of KI	Motor impairment	Athetosis	Abnormal brain MRI	Abnormal BAEP
1	24	623	7	5.9	0.56	PT	Idiopathic	Classic	+	+	–	+
2	24	626	10	5.6	4.32	PT	Idiopathic	Classic	+	+	–	+
3	25	705	15	12.6	1.45	PT, glb	Infection	Classic	+	+	+	+
4	28	798	20	16.6	0.71	NT	Idiopathic	Classic	+	+	+	+
5	26	738	23	18.6	2.21	PT, ET	Intestinal atresia before operation	Classic	+	+	–	+
6	25	770	28	17.9	2.35	PT, Alb	After PDA repair	Classic	+	+	–	+
7	24	706	30	22.5	4.24	PT, ET	Idiopathic	Classic	+	+	+	+
8	23	590	35	20.2	1.33	PT	Idiopathic	Classic	+	+	+	+
9	24	587	36	25.1	3.00	Alb	Idiopathic	Classic	+	+	ND	+
10	25	845	42	11.8	1.14	PT	Infection	Classic	+	+	+	+
11	23	580	63	20.2	2.24	PT	Idiopathic	Classic	+	+	+	+
12	24	641	15	17.3	2.11	PT, ET	Hemolysis	Auditory	–	–	–	+
13	25	723	19	24.9	2.13	PT, ET	Idiopathic	Auditory	+	–	–	+
14	23	498	20	12.6	1.66	PT, Alb	Infection	Auditory	+	–	+	+
15	27	563	32	14.4	1.37	PT	Infection	Auditory	+	–	–	+
16	23	610	27	20.6	1.68	NT	Idiopathic	Motor	+	+	ND	–
17	25	772	28	10.8	0.86	PT	Idiopathic	Motor	+	+	–	–
18	23	558	86	11.1	1.06	NT	Idiopathic	Motor	+	+	+	–

Alb, intravenous albumin administration; BAEP, brainstem auditory evoked potentials; BW, birth weight; ET, exchange transfusion; GA, gestational age; glb, intravenous globulin administration; KI, kernicterus; MRI, magnetic resonance imaging; ND, not done; NT, no treatment; PDA, patent ductus arteriosus; PT, phototherapy; TB, total bilirubin; UB, unbound bilirubin.

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Table 2
Number of infants with high or low serum total bilirubin levels and with high or low serum unbound bilirubin levels at the age when serum total bilirubin levels peaked.

		Serum TB levels		
		High	Low	Total
A. All KI infants				
Serum UB levels	High	9	7	16 (89%)
	Low	1	1	2 (11%)
	Total	10 (56%)	8 (44%)	18 (100%)
Sensitivity = 90% and specificity = 13%				
B. KI infants who had peak serum TB levels at <28 days of age				
Serum UB levels	High	4	3	7
	Low	1	1	2
	Total	5	4	9
Sensitivity = 80% and specificity = 25%				
C. KI infants who had peak serum TB levels at ⩾28 days of age				
Serum UB levels	High	5	4	9
	Low	0	0	0
	Total	5	4	9
Sensitivity = 100% and specificity = 0%				

KI, kernicterus; TB, total bilirubin; UB, unbound bilirubin.

the sensitivity was 90% and specificity was 13% (Table 2A). When subgrouped by 28 days of age when serum TB levels peaked, specificity was lower in the ⩾28 days of age group (0%) than in the <28 days of age group (25%, Table 2B and C).

4. Discussion

KI used to be diagnosed by autopsy [6–8,17,18]. However, an increase in the survival rate of ELBW infants and advances in diagnostic technology, such as brain MRI and BAEP tests, have enabled KI to be clinically diagnosed in preterm infants in the last decade [1,4]. Okumura et al. and Shapiro proposed criteria for KI based on physical and neurological examinations and laboratory findings during infancy [1,4]. In this study, serum TB and UB levels during their NICU stay for the first time were evaluated in ELBW infants with KI who were diagnosed by clinical findings, not autopsy.

Many studies have shown that KI in preterm infants does not involve severe hyperbilirubinemia (high serum TB levels) during the neonatal period [1–3,5–7]. However, serum TB levels are the most popular for identifying infants at risk for developing KI worldwide. Therefore, Sugama et al. reported that assessing the risk of KI in preterm infants may be difficult in the neonatal period [2]. In our cohort, we found that 44% of KI infants had a low serum TB level. Interestingly, even if KI infants did not have high serum TB levels, approximately 90% of them had high serum UB levels. In our cohort, we also found that serum TB levels peaked at a later age than expected (median age: 28 days of age). Furthermore, the specificity was 0% in KI infants who had peak serum TB levels at ⩾28 days of age compared with infants who had high or low serum TB and UB levels. This result suggested that serum TB and UB levels were not proportional. Even when serum TB levels were low, serum UB levels were high. Because serum UB was a better marker than serum TB, especially at ⩾28 days of age, serum UB levels should be monitored for a long time during the NICU stay in ELBW infants.

In KI infants with low serum TB levels, only one infant (#1) did not have a high serum UB level. This finding indicates that serum UB levels may not be a perfect marker for predicting KI. However, in this case (#1), serum TB levels peaked at 7 days after birth. Case #1 had serum TB and UB levels monitored until 14 days of age. We speculate that this case (#1) might develop hyperbilirubinemia later in life in the NICU.

The reason for occurrence of clinical KI in infants in Japan could be due to failure of management of hyperbilirubinemia in preterm infants. In the last decade, during the first 1–2 weeks of life, treatment for hyperbilirubinemia has been strictly performed according to an hour-specific nomogram in all preterm infants. However, after 2 week of age, Japanese neonatologists have not always managed and treated hyperbilirubinemia. Indeed, only 4 of the KI infants in our study cohort were treated by exchange transfusion. Three KI infants had no treatment, even when serum TB or UB levels were high. Therefore, KI, which results in peak serum TB levels later in life, has developed in Japan. Hyperbilirubinemia management guidelines for preterm infants after 2 week of age are urgently required to reduce the development of KI in Japan.

With regard to development of hyperbilirubinemia in newborns, the presence of risk factors, such as infection, acidosis, and blood type incompatibility are important [4]. In our study cohort, four KI infants had high serum UB levels without high serum TB levels when infection

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occurred. Therefore, when preterm infants are infected when they are in the NICU, serum UB levels should be measured and they should be treated by PT and/or exchange transfusion. In approximately 60% of KI infants, the cause of hyperbilirubinemia was idiopathic. Japanese newborns often develop idiopathic hyperbilirubinemia because of the G71R mutation of the *uridine diphosphate glucuronyltransferase 1A1* (*UGT1A1*) gene [19]. The *UGT1A1* gene G71R mutation contributes to prolonged jaundice in Japanese term newborns [20]. Further studies on the association between the G71R mutation of the *UGT1A1* gene and hyperbilirubinemia in preterm infants are needed in Japan.

A limitation of this study is that it was a retrospective cohort study. Serum albumin and direct bilirubin levels when serum TB levels peaked were not always measured. Although bilirubin neurotoxicity depends on the amount and duration of hyperbilirubinemia [4,21], we could not investigate the durability of high serum UB and TB levels. Because ELBW infants may be at risk for developing KI and adverse neurodevelopmental outcomes [1,11,22,23], further prospective studies with a large number of ELBW infants using a unified measurement protocol of serum TB and UB levels are required to determine whether serum UB levels are a good predictor of developing KI and adverse neurodevelopmental outcomes.

In conclusion, serum TB and UB levels peak at a later age than expected in ELBW infants with clinical KI. Measurement of serum UB levels during the NICU stay may be helpful for identifying ELBW infants at risk for developing clinical KI when they do not have high serum TB levels, especially at $\geqslant$28 days of age.

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