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Original Article

Adverse effects of hyperbilirubinemia on the development of healthy term infants

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Abstract

Background Indirect hyperbilirubinemia is a common problem during the neonatal period and may cause long-term abnormality or developmental delay.

Objective To evaluate the adverse effects of hyperbilirubinemia on the development of healthy term infants.

Methods This was a prospective cohort study on healthy term infants born in Sanglah Hospital, Denpasar. Mullen Scale Tests were performed at the ages of 3 and 6 months to assess subjects' development. Bivariate and multivariate analyses were conducted to examine the relationship between several dependent variables and developmental outcomes.

Results One hundred and twelve infants were enrolled in this study [56 with hyperbilirubinemia, 56 without hyperbilirubinemia; 58 (52%) male, 54 (48%) female]. Mean birth weight was 318.3 grams (SD 342.26) vs 3162.5 grams (SD 338.61). At the age of 3 months, below average category according to Mullen Scale Test was higher in infants with history of hyperbilirubinemia compared to those without hyperbilirubinemia, which was statistically significant for fine motor scale (17.9% vs 5.4%; respectively; P=0.039; RR 1.66; 95% CI 1.15;2.39). At 6 months of age, it was higher in infants with history of hyperbilirubinemia compared to those without hyperbilirubinemia and this was statistically significant for gross motor scale (19.6% vs 3.6%, respectively; RR 1.86; 95%CI 1.36; 2.56; P=0.008) and fine motor scale (17.9% vs 5.4%, respectively; RR 1.66; 95%CI 1.15; 2.39; P=0.039). Multivariate logistic regression test showed that only hyperbilirubinemia was correlated with gross motor scale delay at the age of 6 months (P=0.027; OR 5.97; 95%CI 1.22; 29.12).

Conclusion Healthy term infants with history of hiperbilirubinemia were associated with increased gross motor scale delay at the age of 6 months **[Pediatr Indones 2006;46:51-56].**

Keywords: healthy term infants, hyperbilirubinemia, gross motor scale delay, Mullen Test

Indirect hyperbilirubinemia is a common problem during the neonatal period. Neurological abnormalities such as athetoid-dystonic cerebral palsy, developmental delay, sensorineural hearing loss, intelligence deficit, and mental retardation are serious problems attributable to chronic bilirubin encephalopathy.[1-3] Ahdab-Barmada *et al* cited in Conolly[4] reported that the incidence of bilirubin encephalopathy in term infants was 2%. Most infants with bilirubin encephalopathy were asymptomatic and showed neurological problems or intelligence deficit several years later.[4,5]

Toxic effects of unconjugated bilirubin occur when unbound unconjugated bilirubin passes through the blood brain barrier (BBB) then binds to phospolipid and ganglioside of neuronal plasma membrane and finally causes neuronal death.[5-9]

There are several studies which involve the relationship between neonatal hyperbilirubinemia in term infants and developmental outcome. Yilmaz *et al*[1] reported a statistically significant relationship between neurological dysfunction and indirect serum

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bilirubin level of ≥20 mg/dl at the age of 10-72 months. Soorani-Lunsing *et al*[10] studied 20 healthy term infants with moderate hyperbilirubinemia and 20 controls, and found a significant increase of minor neurologic dysfunction throughout the first year of life.

Therefore, healthy term infants with history of hyperbilirubinemia require periodical follow up of physical growth along with motor and mental development.[11,12] Early detection and intervention should be done to optimalize developmental outcome.[11] The aim of the present study was to investigate whether there is a correlation between hyperbilirubinemia of healthy term infants and development at the age of three and six months.

Methods

This was a prospective cohort study conducted in the Child Health Department, Medical School, Udayana University, Sanglah Hospital, Denpasar, from December 2002-November 2003.

Subjects were selected by consecutive sampling from all healthy term infants until minimal sample (112 subjects) was completed. The study group consisted of term appropriate for gestational age (AGA) infants born at Sanglah Hospital or visited the outpatient clinic whose indirect serum bilirubin level ≥12 mg/dl on the third to fifth day of life. Control group consisted of healthy term AGA infants without hyperbilirubinemia, born in the same period and matched for birth weight. In jaundice infants, total and direct bilirubin measurements were performed using Diazo Method. Bilirubin levels of the control group were not measured considering that it was ethically unjustified to submit healthy nonjaundice infants for venous puncture merely to confirm their physiologic bilirubin status. Exclusion criteria were infants with one or more of the following conditions: low birth weight, any sign of acute bilirubin encephalopathy, sepsis, meningitis, asphyxia, congenital anomaly, intracranial bleeding, prolonged seizure, hypoglycemia, or refusal to participate. During follow-up, any subject with either prolonged hyperbilirubinemia, cholestasis, prolonged seizure/epilepsy, chronic diarrhea, malnutrition, and/or refused to participate further in the study would be considered as drop-out. All infants received treatment according to the guidelines of the Neonatology Division at Sanglah Hospital. This study was approved by the Ethics Committee at Medical School, Udayana University, Sanglah Hospital, Denpasar, and written parental consents were obtained.

All infants' developmental outcomes were assessed at the age of 3 and 6 months by the Mullen Scales of Early Learning, American Guidance Service (AGS) Edition.[13] The Mullen Scales are designed for newborn infants through 68 months, and provide normative scores for five specific scales, i.e. gross motor, visual reception, fine motor, receptive language, expressive language, and a single composite score representing general intelligence. After scoring all items for each development sector, we computed the raw score to obtain T scores, percentile ranks, descriptive categories, and age equivalents. Finally, early learning composite scores were tabulated. Based on this score, we categorized the developmental score into five categories of very high, above average, average, below average, and very low. Infants were categorized as having developmental delay if descriptive categories fell in below average and very low.[13]

The statistical analysis was performed using SPSS 11.5. Comparison between study groups for discrete variables was performed with chi-square test, and used unpaired t-test for numeric variables. Relative risk (RR) was calculated to determine the association between independent variable (hyperbilirubinemia) and dependent variable (developmental category). Multivariate logistic regression analysis was used to evaluate several independent and dependent variables. Throughout the analysis, differences with P value <0.05 were considered statistically significant.

Results

During the study, there were 124 healthy term infants (62 with hyperbilirubinemia and 62 without hyperbilirubinemia). Twelve infants dropped out from this study (7 moved out, 2 prolonged hyperbilirubinemia, 1 cholestasis, and 2 with moderate malnutrition). As a result, 112 subjects were enrolled in the study (**Figure 1**).

Range of indirect serum bilirubin levels vary between 13.7-27.7 mg/dl (mean 19.5 (SD 3.89) mg/dl). Seventeen infants with hyperbilirubinemia received phototherapy. The mean indirect serum bi-

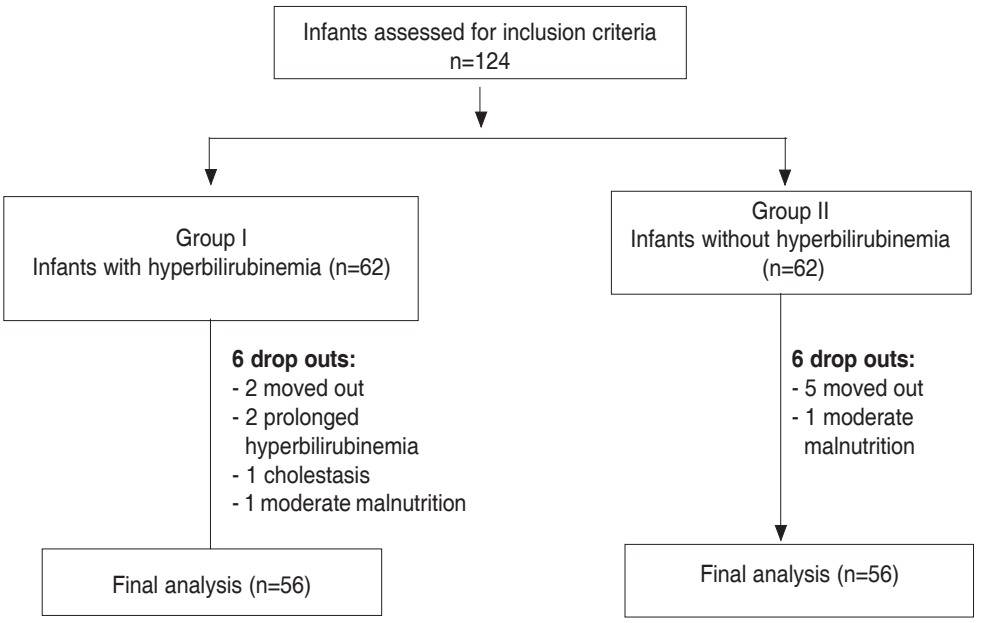

FIGURE 1. THE SCHEME OF ENROLLMENT, FOLLOW-UP, AND ANALYSIS OF SUBJECTS.

lirubin levels of infants who received phototherapy were significantly higher than those without phototherapy (**Table 1**).

At the age of three months, below average category was higher in infants with history of hyperbilirubinemia compared to those without hyperbilirubinemia, which was statistically significant for fine motor (17.9% vs 5.4%; respectively; P=0.039; RR 1.66; 95%CI 1.15;2.39).

At 6 months, below average category was higher in infants with history of hyperbilirubinemia compared to those without hyperbilirubinemia, which was statistically significant for gross motor (19.6% vs 3.6%, respectively; RR 1.86; 95%CI 1.36; 2.56; P=0.008) and fine motor (17.9% vs 5.4%, respectively; RR 1.66; 95%CI 1.15; 2.39; P=0.039) **(Table 2)**.

Multivariate logistic regression test showed that only hyperbilirubinemia was correlated with gross motor scale delay at the age of 6 months (P=0.027; RO 5.97; 95%CI 1.22;29.12) (**Table 3**), yet not correlated with fine motor scale (P=0.079; RO 3.43; 95%CI 0.87;13.60).

Discussion

There are many instruments which determine child development, such as the Arnold Gessel Developmental Scale, Bayley Infant Neuro-developmental Scale, Denver II Screening test, and Mullen Test.[13-15] In this study, we used Mullen Test since it is easier to use and has better validity than any other test.[16]

The primary concern, with respect to exaggerated hyperbilirubinemia, is the potential for neurotoxic effects; however, general cellular injury may also occur. In vitro, bilirubin inhibits mitochondrial enzymes and can interfere with DNA synthesis, induces DNA-strand breakage, and inhibits protein synthesis and phosporylation.[5,8,17,18]

Many studies investigate the relationship between neonatal hyperbilirubinemia in healthy term infants and brain damage. One may relate the peak total bilirubin levels to hearing loss, another the duration of hyperbilirubinemia to the intelligence quotient (IQ), or the bilirubin binding capacity to abnormalities found on neurologic examination.[19]

Our study shows that the below average category of gross motor and fine motor scale according to Mullen Scales Test of infants with history of hyperbilirubinemia, was higher than those without hyperbilirybinemia at 6 months of age, which was statistically significant. However, after multivariate logistic regression test, we found that only hyperbilirubinemia has significant association with gross motor scale delay. Soorani-Lunsing *et al*[10] who studied 20 healthy non-hemolytic term newborns with

TABLE 1. BASELINE CHARACTERISTICS OF HEALTHY TERM INFANTS WITH AND WITHOUT HYPERBILIRUBINEMIA

Variable	With Hyperbilirubinemia (n=56)	Without Hyperbilirubinemia (n=56)
Peak ISB (mg/dL), mean (SD)	19.5 (3.89)	No data available
No phototherapy (n=39)	21.3 (3.22)	(no jaundice)
With phototherapy (n=17)	15.3 (1.06)*	
Birthweight (grams), mean (SD)	3181.3 (342.26)	3162.5 (338.61)
Gestational age (wk), mean (SD)	39.5 (1.14)	39.5 (0.95)
Methods of delivery, n (%)		
Operative delivery	11 (19.6)	6 (10.7)
Normal delivery	45 (80.4)	50 (89.3)
Sex, n (%)		
Male	25 (44.6)	33 (58.9)
Female	31 (55.4)	23 (41.1)
Mother's age (yr), mean (SD)	27.8 (5.35)	28.4 (4.97)
Mother's education, n (%)		
Primary school	7 (12.5)	9 (16.1)
Secondary school	9 (16.1)	13 (23.2)
High school	35 (62.5)	30 (53.6)
University	5 (8.9)	4 (7.1)
Father's education, n (%)		
Primary school	2 (3.6)	4 (7.1)
Secondary school	10 (17.9)	7 (12.5)
High school	35 (62.5)	37 (69.6)
University	9 (16.1)	6 (10.7)
Mother's occupation, n (%)		
Housewife	27 (48.2)	32 (57.1)
Government employee	8 (14.3)	7 (12.5)
Private employee	21 (37.5)	17 (30.4)
Father's occupation, n (%)		
Government employee	8 (14.3)	10 (17,9)
Private employee	48 (85.7)	46 (82,1)
Nutritional intake, n (%)		
Breastfeeding	14 (25.0)	22 (39.3)
Breastfeeding + formula	36 (64.3)	30 (53.6)
Formula only	6 (10.7)	4 (7.1)

ISB= indirect serum bilirubin, SD=standard deviation, *p*=probability.
*Mean ISB with phototherapy or with no phototherapy: t-test, P<0,001.

total serum bilirubin levels of 233-444 μmol/l (13.6-25.9 mg/dl) and 20 control infants, found a significantly higher presence of minor neurological dysfunction at the age of three and 12 months. In comparison to our study, it was similar to a moderate range of serum bilirubin concentrations (indirect serum bilirubin 13.7-27.7 mg/dl). Another study by Yilmaz *et al*[1] found that term infants with history of indirect serum bilirubin level <20 mg/dl did not show neurological dysfunction, while 11.5% (10/87) children with indirect serum bilirubin level ≥20 mg/dl showed neurologic dysfunction and abnormal Denver II Developmental Screening Test in three cases at 10-72 months.

In our study, gross motor abnormalities are regarded as minor forms of chronic bilirubin enchepalopathy.[4,10] Bilirubin enchepalopathy is caused by neuronal injury in subcortical areas, such as basal ganglia, dentate nucleus of the cerebellum, and various nuclei in the brain stem.[4,5] Newman and Klebanoff,[2] who analyzed data from the American Collaborative Perinatal Project, reported that total

Table 2. The results of child development at the age of 6 months in term infants with and without history of hyperbilirubinemia

Descriptive category	With Hyperbilirubinemia n (56)	Without Hyperbilirubinemia n (56)	P	Relative risk (95%CI)
Gross motor, n (%)				
Below Average	11 (19.6)	2 (3.6)	0.008	1.86 (1.36;2.56)*
Average	43 (76.8)	45 (80.4)		
Above Average	2 (3.6)	9 (16.1)		
Fine motor, n (%)				
Below Average	10 (17.9)	3 (5.4)	0.039	1.66 (1.15;2.39)
Average	46 (82.1)	53 (94.6)		
Visual reception, n (%)				
Below Average	9 (16.1)	4 (7.1)	0.140	1.46 (0.96;2.21)
Average	47 (83.9)	52 (92.9)		
Receptive language, n (%)				
Below Average	7 (12.5)	3 (5.4)	0.185	1.46 (0.93;2.29)
Average	49 (87.5)	53 (94.6)		
Expressive language, n (%)				
Below Average	4 (7.1)	2 (3.6)	0.401	1.36 (0.75;2.47)
Average	52 (92.9)	54 (96.4)		
Cognitive, n (%)				
Below Average	9 (16.1)	3 (5.4)	0.140	1.46 (0.96;2.21)
Average	47 (83.9)	53 (94.6)		

CI: confidence interval; *p*: probability
*CI and *p* value of gross motor = below average compared with average + above average

Table 3. Relationship between several independent variables (hyperbilirubinemia, methods of delivery, mother's age, mother's occupation, and nutritional intake) and gross motor scale delay as a dependent variable at the age of 6 months

Variable	P	OR	95%CI
Hyperbilirubinemia	0.027	5.97	1.22;29.12
Methods of delivery	0.164	2.72	0.67;11.09
Mother's age	0.362	1.06	0.93; 1.20
Mother's occupation			
Housewife	0.807		
Government employee	0.836	0.87	0.23;3.31
Private employee	0.609	1.85	0.18;19.59
Nutritional intake			
Breastfeeding	0.801		
Breastfeeding + formula	0.508	2.03	0.25;16.62
Formula only	0.673	1.49	0.23;9.52

CI: confidence interval; *p*: probability; OR: odds ratio

serum bilirubin levels exceeding 342 μmol/l in infants with birth weight ≥2500 grams were associated with an increase of minor motor problems, such as mild hipotonia, non-specific gait abnormalities at the age of 7 years. Grimmer *et al,* cited from Soorani-Lunsing *et al,*[10] reported a follow-up study of 16 term neonates with total serum bilirubin levels of 340-510 μmol/l and 18 case controls found that at the age of 5-15 years showed higher incidence of choreiform dyskinesia. Long term consequences of moderate hyperbilirubinemia for motor, cognitive, and behavioral development should be investigated at school age.[2]

Delay of gross motor development was still found in 39 infants with history of hyperbilirubinemia who had received phototherapy according to procedures. This finding indicates that the brain had been exposed to unconjugated bilirubin for 2 hours or more at the initiation of phototherapy.[8]

One of the limitations of this study was that we did not perform unbound bilirubin measurement for a more accurate evaluation of bilirubin toxicity. Another was that Mullen Test examinations were done only two times (at the age of three and 6 months). The observation was brief.

In conclusion, healthy term infants with history of hyperbilirubinemia were associated with the increase in gross motor developmental delay at the age of 6 months.

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