

## Bilirubin-albumin binding capacity in term and preterm infants

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### Abstract

**Background** The risk of kernicterus remains a problem in jaundiced newborn especially in low birth weight infants. Kernicterus can develop at low bilirubin levels. Bilirubin-albumin binding plays an important role in its pathogenesis. Bilirubin albumin binding concentration can also be used as the cut-off point in the administration of phototherapy.

**Objective** To determine the pattern of albumin binding concentration in serum model in vitro and in serum of premature and term newborn infants from cord blood sample.

**Methods** This study was conducted in Installation of Maternal-Perinatal Dr. Sardjito Hospital from August-September 2004. Blood cord samples from 20 term and 17 preterm infants were analysed. Total bilirubin was measured spectrophotometrically and unbound bilirubin concentration was determined by horseradish peroxidase oxidation using UB-analyzer apparatus micromethod. Student t test and linear regression analysis were performed.

**Results** Bilirubin-albumin binding capacity of term infants showed a statistically significant difference compared to that of premature infants ( $18.9 \pm 2.1$  mg/dl vs  $10.2 \pm 3.6$  mg/dl,  $P < 0.001$ ). This cut-off level approximately reflected a value of unbound bilirubin of 1 mg/dl in term and 0.5 mg/dl in premature infants.

**Conclusions** There is a different pattern of bilirubin-albumin binding capacity between term and preterm infants which is higher in term infants. Bilirubin level of 19 mg/dl and 10 mg/dl in term and preterm newborn, respectively, can be used as cut-off point to perform more aggressive intervention, such as phototherapy, and to lower the risk of kernicterus. [Paediatr Indones 2007;47:32-34].

**Keywords:** bilirubin, albumin, kernicterus, phototherapy

Kernicterus remains a problem in jaundiced infants especially in low birth weight infants despite various diagnostic and therapeutic efforts intended to prevent this complication.<sup>1-3</sup> Kernicterus can develop at low bilirubin levels.<sup>1,4</sup> Bilirubin is bound to albumin in blood circulation transported to the liver. This suggests that deficiency of albumin which leads to an increasing of plasma unbound bilirubin is important in the pathogenesis of low bilirubin kernicterus. Many authors stated that serum bilirubin concentration alone is not sufficient for predicting brain damage and this has led to search for other risk factors for bilirubin encephalopathy. It is thought that the albumin molecule has limited bilirubin binding capacity and when it is completely saturated, free bilirubin concentration will definitely increase.<sup>5-7</sup> Several methods to measure bilirubin-albumin binding capacity of serum albumin as well as free bilirubin concentration have been developed.<sup>5</sup>

The aim of this study was to determine the pattern of albumin binding concentration in serum model in vitro and also in serum of premature and term newborn infants. We expect that this will explain

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how the possibility of low bilirubin level leads to kernicterus in premature infants.

## Methods

Our study was conducted at Installation of Maternal-Perinatal Dr Sardjito Hospital Yogyakarta from August-September 2004. This study involved 37 newborn infants, 20 of them were term and 17 of them were preterm infants. Inclusion criteria were: (1) gestational age  $>28$  weeks calculated from the first day of the last menstrual period. When it was uncertain, the gestational age was determined by physical and neurological examination; (2) appropriate in utero growth for gestational age; (3) no evidence of congenital anomalies or viral infections; (4) negative direct Coombs test; and (5) informed consent from one or both parents.

Materials of this study were: (1) Model serum (human albumin, Sigma) and bilirubin powder (Sigma) was added 0.1 N NaOH for diluting bilirubin. Concentrations of albumin in serum models were 2 g/dl, 2.5 g/dl, 3 g/dl, and 4 g/dl. (2) Umbilical cord blood was collected from the placental vessels after clamping the umbilical cord. The serum was separated and stored frozen until they were analyzed.

Bilirubin-albumin binding capacity measurement methods were as follow:

### 1. In vitro serum model

To measure the dissociation of bilirubin-albumin binding in different albumin concentrations (2 g/

dl, 2.5 g/dl, 3 g/dl, 3.5 g/dl, 4 g/dl) titrated by bilirubin and to measure total bilirubin serum and unbound bilirubin.

### 2. Cord blood serum

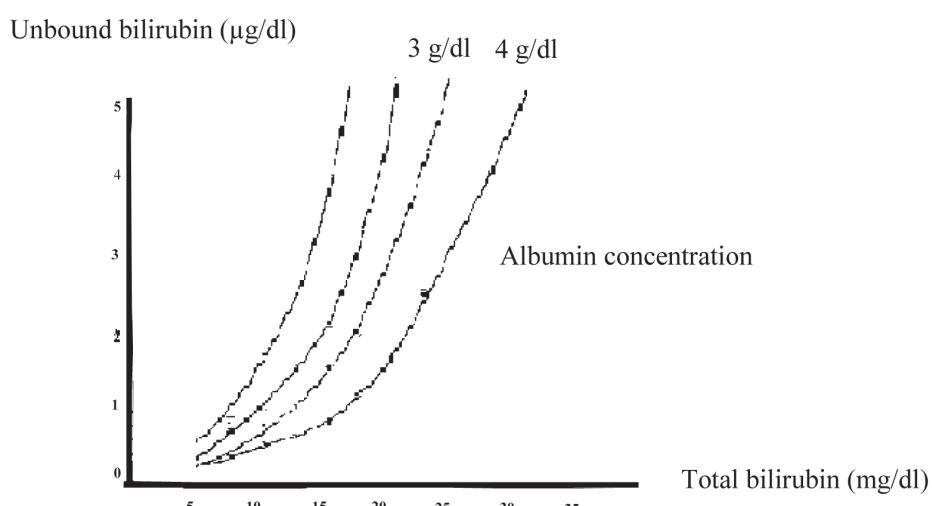
Albumin concentrations were measured by a standard colorimetric method. Total bilirubin concentration was measured spectrophotometrically. The apparent unbound bilirubin concentration was determined by horseradish peroxidase oxidation using UB-analyzer apparatus micromethod.<sup>7</sup> Titration curves were prepared by adding small amount of an alkaline bilirubin (Sigma, St Louis) solution to aliquot of serum. Bilirubin titration was used to determine bilirubin-albumin binding by measuring unbound bilirubin. Bilirubin binding capacity was determined from the curve which was presented as an acceleration of unbound bilirubin serum.

Data was expressed as mean $\pm$ SD and compared using the student *t* test. Curve linear and linear regression analyses were performed. For all statistical tests the null hypothesis was considered to be rejected if  $P<0.05$ .

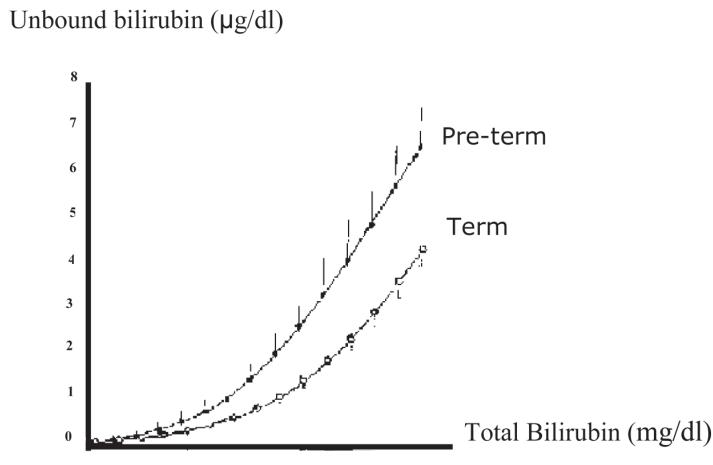
## Results

Titration curves were obtained from serum containing adult albumin. There were various patterns and binding capacities in all sorts of albumin serum concentration.

Mean(SD) bilirubin-albumin binding capacity was 18.9 (2.1) mg/dl in term infants and 10.2 (3.6)



**Figure 1.** Titration curve of bilirubin-albumin binding capacity in serum model



**Figure 2.** Titration curve of bilirubin-albumin binding capacity in cord blood serum

mg/dl in preterm infants. It was statistically significant. **Figure 2** shows titration curves of serum from term and preterm infants; those were the direct plot of apparent unbound bilirubin concentration against total bilirubin.

## Discussion

Bilirubin-albumin binding capacity is not the only factor that influences the development of bilirubin encephalopathy. Blood brain barrier and other central nervous system components are also playing roles in its pathogenesis.<sup>8</sup> Another condition which is also important is chemical blood changes in the first days of life especially in preterm infants beside tendency to be medicated.<sup>9</sup>

Our results were similar to those of previous study<sup>6</sup> as follows: (1) Albumin concentration influenced bilirubin-albumin binding capacity; (2) Bilirubin-albumin binding capacity was higher in term-infants than that of preterm infants; (3) Mean bilirubin-albumin binding capacity in term infants was 18.9 mg/dl equal to unbound bilirubin 1 mg/dl, and mean bilirubin-albumin binding capacity in preterm infants was 10.2 mg/dl equal to unbound bilirubin 0.5 mg/dl. This result can be used as a limit value for administration of phototherapy in both groups.

Based on bilirubin-albumin binding capacity concentration, we suggest that *cut-off points* for

performing phototherapy are 19 mg/dl (1 mg/dl unbound bilirubin) in term infants and 10 mg/dl (0.5 mg/dl unbound bilirubin) in preterm infants.

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