# Bilirubin levels and severe retinopathy of prematurity in infants with estimated gestational ages of 23 to 26 weeks

*Mitchell H. DeJonge, MD, Annie Khuntia, BS, M. Jeffrey Maisels, MB,BCh, and Amtul Bandagi, MD*

Oxidative injury may contribute to the development of retinopathy of prematurity (ROP), and bilirubin may be a physiologically important antioxidant. Therefore we evaluated the relationship of ROP to bilirubin levels in 157 infants born at 23 to 26 weeks estimated gestational age. We found no definite association between bilirubin levels and severe ROP. (J Pediatr 1999;135:102-4)

Oxidative injury may play a role in the initiation and development of retinopathy of prematurity.[1] Bilirubin is a powerful antioxidant in vitro,[2] and there is a positive relationship between serum bilirubin levels and antioxidant activity in preterm and term infants.[3-5] There is also some suggestion of a physiologic role for bilirubin as an antioxidant in the human neonate.[5-7] Four studies have evaluated the relationship between bilirubin levels and ROP, and 3 showed no significant association.[8-11] We therefore evaluated this question in premature infants born at 23 to 26 weeks estimated gestational age, a population at particularly high risk for ROP.

*From the Department of Pediatrics, William Beaumont Hospital, Royal Oak, Michigan; and the Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan.*

Submitted for publication Dec 28, 1998; revision received Mar 15, 1999; accepted Apr 5, 1999.

Reprint requests: Mitchell H. DeJonge, MD, William Beaumont Hospital, 3601 W 13 Mile Rd, Royal Oak, MI 48073.



0022-3476/99/$8.00 + 0 9/22/99111

## METHODS

We reviewed the charts of all infants born at 23 to 26 weeks EGA in our hospital between January 1990 and December 1996 who survived until discharge. Gestational age was assigned by best obstetric estimate and supported by prenatal ultrasonography in 90% of cases. For each infant, we calculated the average of all bilirubin levels obtained on each day and the overall average bilirubin level for days 1 to 15 of postnatal life. Bilirubin levels were measured as clinically indicated, and with advancing age, fewer bilirubin levels were obtained. Infrequently, infants had 2 or 3 bilirubin levels drawn in a 24-hour period. We documented the length of time spent under phototherapy and also obtained information regarding 9 other factors thought to be important in the development of ROP including estimated gestational age at birth, birth weight, race, sex, number of days of mechanical ventilation, number of days of oxygen therapy, use of antenatal and postnatal steroids, and the presence of severe intracranial hemorrhage (grades III or IV) or periventricular leukomalacia. For the antenatal steroid category, infants whose mothers received less than 2 doses of betamethasone before delivery were categorized as "no," and all others were categorized as "yes." For postnatal steroids, infants given any length of steroid treatment were placed in the "yes" group. Postnatal steroids were used at the discretion of the attending neonatologist for treatment of bronchopulmonary dysplasia, and in general, infants received a 4- to 6-week course of dexamethasone.

| | |
|---|---|
| EGA | Estimated gestational age |
| ROP | Retinopathy of prematurity |

Phototherapy was initiated based on nursery guidelines, which were as follows: birth weight <1000 g; bilirubin >5 mg/dL for days 1 to 7 and >7 mg/dL after day 7; birth weight 1001 g to 1200 g; bilirubin >6 mg/dL for postnatal days 1 to 7 and >8 mg/dL after day 7. Guidelines were altered downward when clinically appropriate (eg, hemolysis).

We divided the population into 2 groups based on the infant's worst ROP examination results, as designated by one of two pediatric ophthalmologists, both of whom have extensive clinical experience with ROP. Each infant was examined initially at 4 to 6 weeks after birth and every 1 to 2 weeks thereafter, depending on the clinical findings. We classified those infants with ≤stage 3 disease (no surgical intervention) as "mild ROP" and those with ≥stage 3+ disease (laser and/or other retinal sparing surgery) as "se-

vere ROP." Statistical analysis was performed by using the Student *t* test (continuous variables) along with the Fisher exact test and the $\chi^2$ test (categorical variables) to evaluate the differences between the mild and severe groups. Stepwise logistic regression was performed by using only those variables found to be significant on univariate analysis to determine which variables were predictive of severe ROP. *P* values <.05 were considered to be significant for all statistical tests. The study was approved by the hospital human investigation committee.

## RESULTS

During the study period, 230 infants of 23 to 26 weeks EGA were born alive at our hospital, and 157 (68.3%) survived to discharge. We reviewed the charts of all surviving infants. One hundred fifteen infants (73.2%) comprised the mild ROP group: immature retinas (n = 34, 22%), stage 1 (n = 12, 8%), stage 2 (n = 49, 31%), stage 3 (n = 20, 13%). Forty-two (26.8%) infants had severe ROP (≥stage 3+). There were significant differences between the groups in EGA, birth weight, days of mechanical ventilation, days of oxygen therapy, and postnatal steroid use but no significant differences in the average duration of phototherapy or the average bilirubin level over the first 15 postnatal days (Table I). All infants received phototherapy, and none required an exchange transfusion. We performed logistic regression using only the variables found to be significant on univariate analysis. This model indicated that lower birth weight (odds ratio 0.995, 95% CI 0.992-0.998), an increased number of days of mechanical ventilation (odds ratio 1.018, 95% CI 1.004-1.038), and the administration of postnatal steroids (odds ratio 4.33, 95% CI 1.5-12.7) were significantly associated with severe ROP. On post hoc analysis, we did find that significantly more infants with severe ROP had average bilirubin levels <5 mg/dL for days 1 to 15 than did those with mild ROP (*P* = .039). No differences were found when levels of <4 mg/dL (*P* = .54) or <6 mg/dL (*P* = .74) were analyzed (Table II).

***Table I.*** Comparison of risk factors evaluated in the mild and severe ROP groups

| | Mild ROP | Severe ROP | *P* value |
|---|---|---|---|
| n | 115 | 42 | |
| EGA (wk) | 25.1 ± 0.9 | 24.3 ± 1.1 | .000 |
| Birth weight (g) | 814.6 ± 153.4 | 663.5 ± 150.2 | .000 |
| White | 95 (82.6) | 32 (76.2) | .103 |
| Male | 62 (53.9) | 23 (54.8) | .856 |
| Ventilator days | 39.6 ± 26.2 | 63.7 ± 28.4 | .000 |
| Oxygen days | 90.0 ± 51.4 | 131.0 ± 46.9 | .000 |
| Maternal steroids | 54 (47.0) | 22 (52.4) | .696 |
| Postnatal steroids | 58 (50.4) | 36 (85.7) | .000 |
| IVH ≥III or PVL | 5 (4.3) | 5 (11.9) | .315 |
| Phototherapy (h) | 83.3 ± 66.8 | 81.0 ± 44.2 | .808 |
| Mean bilirubin on days 1-15 (mg/dL) | 5.0 ± 0.8 | 4.7 ± 0.7 | .127 |

Data are presented as means ± SD or the number of subjects with percent in parentheses.
*IVH,* Intraventricular hemorrhage; *PVL,* periventricular leukomalacia.

***Table II.*** Mild versus severe ROP and average bilirubin levels for postnatal days 1 to 15

| | Serum bilirubin | | |
|---|---|---|---|
| Group | <4 mg/dL | <5 mg/dL | <6 mg/dL |
| Mild ROP (n = 115) | 12 (12%) | 58(50%)* | 108 (94%) |
| Severe ROP (n = 42) | 3 (7%) | 29(69%) | 39(93%) |

**P* = .039 versus severe ROP with bilirubin <5 mg/dL.

## DISCUSSION

ROP is a frequently seen but poorly understood morbidity in extremely low birth weight infants. A number of risk factors have been described, but as yet there is no common underlying mechanism that completely explains why ROP develops in premature infants. Oxygen radical formation during periods of hypoxia and re-oxygenation has been proposed as one possible etiology. This theory is intriguing and is especially important in light of the evidence that suggests that premature infants have reduced intracellular defenses against oxygen radicals.[1]

Bilirubin has been shown to be an antioxidant in both in vitro and in vivo studies.[2-7] Because of the potential relationship between ROP and oxidative injury and the potential role of bilirubin as a physiologic antioxidant, investigators have evaluated the relationship of bilirubin levels to ROP.

Heyman et al[8] compared 35 infants with ROP with control subjects of similar gestational age and found a relationship between lower bilirubin levels and an increased risk of ROP. However, 3 subsequent studies failed to show any relationship between bilirubin levels and ROP.[9-11] Major weaknesses of these studies include their small patient sample size and the inclusion of infants with gestational ages of up to 34 weeks, an age when ROP is virtually nonexistent.

Our study was designed to include only those infants at the highest risk for ROP. All of the data, including the 9

risk factors for ROP and the daily bilirubin levels, were collected prospectively and entered into our database. As expected, the 2 groups were significantly different in 4 well-known risk factors for ROP (EGA, birth weight, days of mechanical ventilation, and days of oxygen therapy). In addition, postnatal steroids were used significantly more frequently in the severe ROP group. When stepwise logistic regression was performed with these 5 factors, which were found to be significant on univariate analysis, the use of postnatal steroids was again found to be associated with severe ROP, together with lower birth weight and an increased number of ventilator days. The association of ROP with steroid use is intriguing and has been noted previously by Batton et al.[12] However, other reports have demonstrated either no relationship or a protective relationship of corticosteroids to ROP.[13,14]

We did not find a difference in the average bilirubin levels between the mild and severe ROP groups for days 1 to 15. When the groups were further subdivided into the individual stages of ROP, no significant differences in mean bilirubin levels were found. However, when the groups were arbitrarily divided by average bilirubin levels (<4 mg/dL versus ≥4 mg/dL, <5 mg/dL versus ≥5 mg/dL, or <6 mg/dL versus ≥6 mg/dL), we did find that a significantly higher proportion of infants with severe ROP had average bilirubin levels <5 mg/dL. This result is most likely a function of the fact that those infants with severe ROP were smaller and less mature and would therefore have received earlier and more aggressive phototherapy.

Given the fact that no association between bilirubin levels and severe ROP was found except on arbitrary post hoc analysis, it is unlikely that the level of bilirubin is an important factor in the development of severe ROP in infants with EGA of 23 to 26 weeks.

*The authors greatly appreciate the assistance of Mamtha Balasubramanian, MS, William Beaumont Hospital Research Institute Biostatistician.*

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