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Possible roles of bilirubin and breast milk in protection against retinopathy of prematurity

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Abstract

Aim—To explore the association of serum bilirubin level and breast milk feeding with retinopathy of prematurity (ROP) in preterm infants.

Methods—We conducted a case-control study to examine the independent and combined effects of serum bilirubin and breast milk feeding on ROP risk in infants <32 weeks gestation or with birth weight <1500 grams. Cases (66 infants with ROP) were matched with controls (66 infants without ROP) based on factors known to affect ROP risk.

Results—When analyzed using the paired t test, the peak bilirubin levels were lower in ROP cases than in controls (mean 7.2 vs 7.9 mg/dl; $P=0.045$). Using conditional logistic regression, we found a negative association between highest serum bilirubin level and risk of ROP (OR=0.82 per 1-mg/dl change in bilirubin ($P=0.06$)). There was no significant association between breast milk feeding and risk of ROP.

Conclusion—Bilirubin may help to protect preterm infants against ROP.

Keywords

bilirubin; breast milk; preterm; retinopathy of prematurity

Introduction

Retinopathy of prematurity (ROP) is a proliferative vascular disease of the retina that can lead to blindness in its severe stages. According to the National Eye Institute, 14,000-16,000 infants develop ROP in the United States each year, and 400-600 of these infants become legally blind. In this project, we explored potential avenues for prevention of ROP by studying the associations of serum bilirubin level and breast milk, individually and in combination, with ROP.

Breast feeding has been shown to protect infants in many different ways. Human milk contains antioxidants and enzymes that are known to decrease oxidative stress (1). Infants fed with human milk have been shown to score higher on developmental tests (2) and to have lower rates of necrotizing enterocolitis (3) and infection (4). Studies examining the potential role of breast milk feeding in prevention or amelioration of ROP have been inconclusive, with some showing protection and others showing no protection (5-9).

Although it has many benefits, breast milk is also known to increase the risk of hyperbilirubinemia (10). Unconjugated bilirubin is a product of hemoglobin breakdown. It is joined with glucuronic acid in the liver by the enzyme UDP-glucuronyl transferase (UDP-GT) to become conjugated bilirubin, a form the body can excrete. In neonates, this process is impaired because UDP-GT is developmentally regulated (11,12). Without proper management, jaundice can lead to kernicterus and hearing impairment.

Although elevated bilirubin can be harmful, bilirubin has been shown to be an antioxidant *in vitro* (13). The fact that bilirubin has been shown to provide protection against oxidative stress has led some investigators to study the association of serum bilirubin level with ROP. As with studies of breast milk, these studies have also provided inconsistent results (14-20). Previous studies of bilirubin and ROP have not reported what the infants were fed, and the studies of breast milk and ROP have not included bilirubin level in the analyses.

This retrospective case-control study examined the hypothesis that, in infants under 32 weeks gestation or less than 1500 grams, bilirubin and breast milk, independently and/or in combination, are protective against ROP. The study was designed to examine the associations of serum bilirubin level with ROP and breast milk with ROP. Associations, if found, would not establish that hyperbilirubinemia or breast milk is protective against ROP *per se* but might be used to generate further hypotheses and design further research to examine causality. If a link were found between hyperbilirubinemia and ROP or breast milk and ROP, this might ultimately affect management of hyperbilirubinemia and/or feeding method, with the potential to prevent many cases of ROP.

Methods

Study Population

The data from this study were taken from the University of Iowa Children's Hospital (UICH) Prematurity Study database. This database and the associated biorepository are used to study the complications of prematurity and focus on infants of less than 37 weeks gestation. The study team screens and tracks families, performs chart abstractions on both the mother and the baby, collects tissue samples, and conducts maternal interviews (21). Data for the database and samples for the biorepository are collected at the UICH, Magee-Womens Hospital (Pittsburgh, PA), the University of Rochester Medical Center (Rochester, NY), and Wake Forest University (Winston-Salem, NC). The Prematurity Study has the approval of the University of Iowa institutional review board for analysis of conditions related to preterm birth and prematurity.

The UICH High-Risk database, managed by its High-Risk Infant Follow-Up Program, was also consulted to obtain additional information about these subjects. This database includes data for all infants who have been admitted to the UICH Neonatal Intensive Care Unit. For High-Risk Program infants also enrolled in the Prematurity Study, data from both databases were obtained and merged. The hospital's electronic medical record systems (INFORMM, version 005:000:287, Hospicomm, Philadelphia, PA; Epic Hyperspace, version Spring 2008 IU 5, Verona, WI) were queried to complete any missing information. All data were entered into the Progeny 7 database, a pedigree software program with a customizable database program for data management and pedigree analysis (Progeny software, version 7.6. South Bend, IN).

From the infants in the database who were born from 2000 to 2009 at less than 32 weeks gestation or with birth weight below 1500 grams, infants with ROP were identified and matched, wherever possible, with control infants without ROP. These paired infants were used for statistical analysis.

Data Collected

The predictor variables in this study were highest serum bilirubin level (mg/dl) and feeding status at time of hospital discharge. Feeding status categories were breast milk in any amount and infant formula only. Breast milk feeding included the feeding of either maternal or banked donor milk. The outcome variable was the presence of ROP of any stage. According to UICH protocol, infants born at less than 32 weeks or less than 1500 grams are routinely examined for ROP by a pediatric ophthalmologist. For infants born at or before 27 weeks gestation, the first examination is conducted at 32 weeks. For infants born after 27 weeks gestation, the examination is conducted at 5 weeks chronological age. Gestational age, birth weight, race, time on supplemental oxygen, gender, and use of phototherapy were collected for matching case and control infants. Infant race was determined by maternal self-report.

Case-Control Matching

Using Statistical Analysis Software (SAS Institute Inc. Statistical Analysis Software: version 9.2. Cary, NC), control infants were matched with ROP infants using the greedy case-control matching method (22) at a one-to-one ratio. With this method, each case infant is paired with a control infant that best matches it (based on distances in multivariate space) and set aside; a control infant is matched with only one case infant. Matching was based on gestational age, birth weight, race, time on oxygen, gender, and use of phototherapy, in that order of importance. Gestational age was matched to within 2 weeks, birth weight was matched to within 50 grams, and time on oxygen was matched to within 10 days. Race, gender, and phototherapy status (used or not used) were the same for case and control.

Statistical Analysis

The data were analyzed in two ways. First, conditional logistic regression was used to examine the association between bilirubin and/or feeding status and ROP. Odds ratios and 95% confidence intervals were calculated for the association of bilirubin level and/or feeding status with ROP. P-values were computed using the Wald chi-square test. $P < 0.05$ was considered statistically significant.

The second approach was to compare the peak serum bilirubin level and the use of breast feeding between ROP infants and their paired controls. If the peak bilirubin level were found to be higher in the control infants than in the ROP infants, this finding would be consistent with a protective effect of bilirubin against ROP. Similarly, a higher prevalence of breast feeding in controls versus cases would indicate a protective effect of breast feeding. The specific tests used were paired t-tests for peak bilirubin and McNemar's test (equivalent to a paired sign test) for breast feeding.

Results

Of the 739 infants in the database who were born from 2000 to 2009 with gestational age less than 32 weeks or birth weight below 1500 grams, 235 developed ROP. For 66 of these infants, it was possible to identify a suitable matched control infant. These 66 pairs of infants were used for analysis. The summary statistics of cases and controls show that the case-control matching was effective because variables (excluding predictor variables) lacked significant differences between cases and controls (Table 1).

Using conditional logistic regression, there was a borderline-significant negative association between highest total bilirubin level and risk of ROP (OR = 0.82 per 1-mg/dl change in bilirubin [95% CI 0.66, 1.01; $P = 0.06$]), without adjusting for whether infants received

breast milk (Table 2). After controlling for breast milk feeding, the negative association was virtually unchanged between peak serum bilirubin level and risk of ROP.

Using a paired t-test, we found a statistically significant difference between peak bilirubin levels in infants with (mean 7.16 mg/dl) and without (7.87 mg/dl) ROP ($P = 0.0453$), suggesting a protective effect of bilirubin against ROP (Table 3). With the same method, the association between feeding status and ROP was not statistically significant.

Both logistic regression and the paired t-test produced the same conclusion – that elevated serum bilirubin may have a protective effect against ROP.

There was no significant association between breast milk feeding and risk of ROP independent of bilirubin level (OR = 0.67 [95% CI 0.34, 1.31]; $P = 0.24$ by conditional logistic regression; $P = 0.237$ by McNemar's test). After adjusting for peak bilirubin level, there was still no association of breast feeding with ROP risk.

Discussion

In this study, we investigated the individual and combined effects of bilirubin and breast milk on ROP risk. We hypothesized protection against ROP based on the antioxidant properties of breast milk (23) and bilirubin (13). Previous studies focused on the independent effects of breast feeding and bilirubin on ROP have produced conflicting results (5-9,14-20).

Studies examining the protective effect of breast feeding against ROP have also had inconsistent results (5-9). Hylander *et al.* found that human milk feedings were independently correlated with a reduced risk for ROP (5), and Okamoto *et al.* (7) and Porcelli and Weaver (9) both found that infants with severe ROP had been fed significantly smaller amounts of human milk. However, Furman *et al.* (6) and Heller *et al.* (8) found no association between human milk feeding and ROP risk. Several studies have shown that a high serum level of bilirubin is protective against ROP (14,17), whereas other studies have found no association between serum bilirubin level and ROP (15,16,18,19). All the above studies used similar sample frames, control matching variables, and methods of analysis.

Unlike these previous studies, the current study examined the effects of the two predictor variables both singly and in combination. The results of this study are similar to the results of Furman *et al.* (6), Heller *et al.* (8), Heyman *et al.* (14), and Canto *et al.* (17). We found no association between breast milk feeding and ROP but found a significant association between high bilirubin and ROP. These findings support the hypothesis that elevated bilirubin has a protective effect on ROP but not the hypothesis that human milk feeding protects against ROP.

There are several potential mechanisms for the protective effect of bilirubin against ROP. Barañano *et al.* (24) found that bilirubin becomes oxidized to biliverdin when it acts as an antioxidant. They also found that biliverdin can be converted back into bilirubin *in vivo* and used again as an antioxidant. This cycling between bilirubin and biliverdin provides antioxidant protection against oxidative stress, a presumed cause of ROP. Katz and Robison (25) found that normal retinal vascularization was halted after birth, presumably in an effort to minimize oxidative injury resulting from the sudden increase in atmospheric oxygen that a preterm infant experiences after birth.

Any potential intervention that might reduce the risk of ROP could have significant health and economic benefits. The cost of ROP screening and treatment presents a large financial

burden, particularly for the growing fraction of U.S. families without adequate health insurance, and the impact of impaired vision from ROP on quality of life is immeasurable.

Decreasing the risk of ROP would reduce healthcare costs but, more importantly, would save the vision of many infants. According to the World Health Organization, ROP is the third leading cause of avoidable blindness in the developed world. Bilirubin level can be controlled through its treatment or lack thereof. If, in fact, bilirubin is protective against ROP, it is reasonable to speculate that less aggressive treatment of hyperbilirubinemia might reduce the risk of ROP. However, a recent large trial found no impact of aggressive versus conservative phototherapy on the rate of severe ROP, although more aggressive phototherapy was associated with a reduced rate of neurodevelopmental impairment (26).

The strengths of this study include the fact that the effects of bilirubin and feeding method on ROP were evaluated in combination. Bilirubin and breast milk feeding are interdependent, given the known tendency of human milk feeding to increase serum bilirubin. This interplay must be taken into account in interpreting epidemiologic studies designed to examine the impact of either factor.

The use of case-control matching in this study helped to limit the impact of confounding variables. Because case and control infants were matched by both gestational age and birth weight, maturational differences in organ development and antioxidant systems do not explain the results of the study. Infants were matched based on time on supplemental oxygen to control for differences in both degree of lung disease and oxidative stress resulting from oxygen exposure. Because several studies have shown a higher risk of ROP among males, infants under phototherapy, and certain racial groups (27-30), matching cases and controls by gender, phototherapy use, and race eliminated the effects of these factors in the analysis.

Limitations of this study are the small sample size and the lack of availability of certain data. The precision of estimations of various variables in the population was compromised by the small sample size. Also, the majority of the infants in the database lacked data concerning one or more of the matching variables.

This study would have been more powerful had certain additional data been available. The information on bilirubin levels would have been strengthened by having data on the area under the curve of serum bilirubin versus time, since the highest total bilirubin provides a limited view of bilirubin exposure. The use of phototherapy as a matching criterion would have been more powerful had the total dosage or duration of phototherapy been available. Studies have shown that phototherapy has a negative impact on the antioxidant defense system in newborns with hyperbilirubinemia (12). Feeding data would have been stronger had they reflected the feeding method used during the period before ROP diagnosis and during bilirubin data collection. The data available for this analysis included the feeding method at the time of discharge, which was not necessarily the same as during earlier periods. Finally, this study examined associations but cannot establish a causal link of either exposure with ROP risk.

Further studies should utilize data on the severity of ROP and not just the presence of ROP to examine for evidence of a dose-response relationship between exposure (bilirubin and breast milk) and ROP risk.

Future studies might also investigate what level of bilirubin over what period or whether specific intakes of breast milk are needed to confer protection against ROP. This study has shown that elevated serum bilirubin may have a protective effect against ROP, but it did not show the degree of hyperbilirubinemia needed to make a difference in ROP. It is known that extreme hyperbilirubinemia can lead to kernicterus. Ultimately, intervention studies would

be required to further test the hypothesis that elevated serum bilirubin reduces the risk of ROP in preterm infants.

In conclusion, the results of this study suggest that bilirubin may help to protect preterm infants from ROP. While no association was found in our study between breast milk feeding and ROP risk, this question warrants further investigation with stronger epidemiological methods. Breast milk feeding provides important, well-documented benefits despite the lack of association of breast feeding with ROP risk in our analysis. The current study in no way contradicts these known benefits of breast feeding.

It is critically important to continue the search for ways to reduce the risk of the potentially disabling effects of ROP.

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Table 1

Characteristics of the study population

Case Subjects (ROP, n = 66)				
Variable	Mean	Standard Deviation	Minimum	Maximum
Gestational age (weeks)	27.0	2.0	23.0	31.0
Birth weight (grams)	962	273	458	1727
Peak serum bilirubin (mg/dL)	7.2	1.6	4.8	12.4
Time on supplemental oxygen (days)	62.2	39.2	0	133
Gender (M=0, F=1)	0.45	0.50	0	1.00
Phototherapy (no=0, yes=1)	1.00	0	1.00	1.00
Feeding (formula=0, milk=1)	0.45	0.50	0	1.00
Control Subjects (no ROP, n = 66)				
Variable	Mean	Standard Deviation	Minimum	Maximum
Gestational age (weeks)	27.2	2.0	23.0	31.0
Birth weight (grams)	960	272	440	1730
Peak serum bilirubin (mg/dL)	7.9	2.6	2.2	18.5
Time on supplemental oxygen (days)	62.9	39.4	0	131
Gender (M=0, F=1)	0.45	0.50	0	1.00
Phototherapy (no=0, yes=1)	1.00	0	1.00	1.00
Feeding (formula=0, milk=1)	0.56	0.50	0	1.00

Table 2

Results of conditional logistic regression on the independent and combined effects of bilirubin and breast milk on ROP

	Odds Ratio	95% CI	P-value
Bilirubin and ROP	0.82	[0.66, 1.01]	0.06
Bilirubin and ROP (adjusted for breast milk)	0.81	[0.65, 1.01]	0.06
Breast milk and ROP	0.67	[0.34, 1.31]	0.24
Breast milk and ROP (adjusted for bilirubin)	0.65	[0.32, 1.31]	0.23

Table 3

Results of paired t-test to examine the independent effects of bilirubin and breast milk on ROP

	t	95% CI	P-value
Bilirubin and ROP*	-2.04	[-1.41, -0.02]	0.045
Breast milk and ROP [†]	-1.19	[-0.28, 0.07]	0.24

* Case bilirubin value minus control bilirubin value

[†] Case feeding value minus control feeding value