Combination of ABO blood group incompatibility and glucose-6-phosphate dehydrogenase deficiency: effect on hemolysis and neonatal hyperbilirubinemia

M Kaplan 1,3, HJ Vreman 4, C Hammerman 1,3, C Leiter 2, B Rudensky 2, MG MacDonald 5 and DK Stevenson 4

Department of Neonatology 1, and Hematology Laboratory 2, Shaare Zedek Medical Center, Hebrew University, Hadassah Medical School 3, Jerusalem, Israel, and Department of Pediatrics 4, Stanford University Medical Center, Stanford, CA, and Division of Neonatology 5, Childrens’ National Medical Center, George Washington University School of Medicine and Health Sciences, Washington DC, USA

The incidence (%) of hyperbilirubinemia (serum bilirubin $\geq 257 \text{ mol/l}$) was similar in neonates with a combination of ABO incompatibility and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (45%), with ABO incompatibility alone (54%), or G-6-PD deficiency (37%), alone (ns). Carboxyhemoglobin values, corrected for inspired CO, were similarly elevated in all three groups (0.87 ± 0.32%, 0.82 ± 0.29%, 0.76 ± 0.18%, respectively, ns), but correlated with bilirubin only in those with ABO incompatibility alone. ABO-incompatible/G-6-PD-deficient neonates, compared with those with either condition alone, are not at increased risk for hemolysis or hyperbilirubinemia.

Both ABO blood group incompatibility and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency are frequently associated with increased bilirubin production and neonatal hyperbilirubinemia. We asked whether newborns with a combination of these two conditions would have a higher rate of hemolysis and hyperbilirubinemia than neonates with only one of these conditions.

Methods
Clinical survey
Male Sephardic Jewish term newborns, at high risk for G-6-PD deficiency (1), were surveyed prospectively for hyperbilirubinemia. They were screened at birth for G-6-PD deficiency, blood group, Rh typing, and direct Coombs’ test. Excluded were neonates with a positive direct Coombs’ test due to any condition other than ABO incompatibility. Neonates were observed clinically for jaundice, with serum total bilirubin determinations as necessary, until stabilization of serum total bilirubin values. Phototherapy was provided if serum total bilirubin values exceeded 257 $\text{mol/l}$ and exchange transfusion was performed if bilirubin values remained $>342 \text{ mol/l}$ despite phototherapy.

COHb and CO sample collection
Midway into the study, carboxyhemoglobin (COHb) measurements, a reliable index of bilirubin production (2), became available for some of the subjects, following establishment of a collaboration with Stanford University. A blood sample was drawn on the third day of life, with a simultaneous serum total bilirubin determination. To prevent any possible effect of maternal smoking (3), sampling was performed between 48 and 72h of life. Blood was collected into custom-made tubes (4, 5), which were refrigerated and shipped on ice to Stanford University. A sample of room air was collected simultaneously. The protocol was approved by the Institutional Review Board of the Shaare Zedek Medical Center.

Laboratory analysis
G-6-PD screening was performed using a commercial kit (No. 400K, Sigma Diagnostics, St Louis, MO, USA) and confirmed in those with an abnormal screening test by quantitative enzyme testing using Kit No. 202 (Sigma Diagnostics). Serum total bilirubin was measured using an automated analyser (Astra 8, Beckman Instruments, Brea, CA, USA) by a modified diazo reaction method. Blood COHb concentration, expressed as a percentage of
total hemoglobin (tHb), was determined by gas chromatography and corrected (COHbc) for ambient CO. tHb was measured using a manual cyanmethemoglobin method (Kit No. 525, Sigma Diagnostics) (4, 5).

**Definitions and patient categories**

“Hyperbilirubinemia” was defined as a serum total bilirubin \( \geq 257 \mu mol/l \). ABO incompatibility was defined as any blood group A or B neonate, of blood group O mother. Because there is a spectrum of disease, the more severe cases not always detected by a direct Coombs’ test, results of direct Coombs’ testing were not included in criteria for ABO incompatibility. Any other combinations of maternal/neonatal blood groups were defined as ABO compatible.

The newborns were divided into the following groups: Group A (Study group): ABO incompatible, G-6-PD deficient; Group B (ABO control group): ABO incompatible, G-6-PD normal; and Group C (G-6-PD-deficient control group): ABO compatible, G-6-PD deficient. Those neonates in whom COHbc, tHb and third day serum bilirubin testing were performed are designated subgroups. COHbc values of some of the neonates in subgroup C have been reported (6), but were deemed essential for comparison with the study group.

**Data analysis**

\( \chi^2 \) analysis, Fisher’s exact test, Student’s \( t \)-test or linear regression analysis, were utilized, as appropriate, significance being defined as \( p < 0.05 \).

**Results**

The incidence of hyperbilirubinemia was similarly high in the ABO-incompatible/G-6-PD-deficient study group A (13/29, 45%), the ABO-incompatible control group B (44/82, 54%) and G-6-PD control group C (45/121, 37%), ns. Four neonates in the G-6-PD-deficient control group C required exchange transfusion. The incidences of hyperbilirubinemia were similar in the subgroups of babies in whom COHbc values were obtained.

COHbc determinations were not significantly higher in the study subgroup A (0.87 ± 0.32%, \( n = 20 \)) than in control subgroup B (0.82 ± 0.29%, \( n = 44 \)), with a trend to being higher than those of subgroup C (0.76 ± 0.18%, \( p = 0.08, n = 46 \)). No significant differences were noted in third day serum total bilirubin values [193 ± 70 \( \mu \)mol/l (mean ± SD); 224 ± 82 \( \mu \)mol/l; 205 ± 80 \( \mu \)mol/l], or in tHb values (179 ± 23 g/l; 181 ± 28 g/l; and 178 ± 23 g/l), for subgroups A, B and C, respectively. Linear regression analysis showed that COHbc and serum bilirubin values did not correlate in the ABO-incompatible/G-6-PD-deficient subgroup (A) or in the ABO-compatible/G-6-PD-deficient subgroup (C), but did in the ABO-incompatible/G-6-PD-normal subgroup (B) (Fig. 1).

**Discussion**

Contrary to expectations, we found that neonates with combined ABO incompatibility and G-6-PD deficiency had a similar incidence of hyperbilirubinemia as neonates with only one of these conditions. For those babies in whom it was available, COHbc values and third day serum total bilirubin values, were also similar. The COHbc values were all significantly higher.
than non-hyperbilirubinemic, G-6-PD normal neonates sampled concurrently with the present study (0.53 ± 0.13%, p < 0.05) (6). Previous studies, although not prospective, also did not find an additive effect of ABO blood group incompatibility on G-6-PD deficiency (7–9).

Further analysis of this phenomenon is afforded by our study of COHbc values, a technique not previously used to study possible interaction between the two conditions. The significant correlation between COHbc and serum total bilirubin values in the ABO incompatible, G-6-PD normal subgroup B implies that increased bilirubin production is a major element in the pathogenesis of hyperbilirubinemia due to ABO incompatibility. In contrast is the lack of correlation seen in the G-6-PD-deficient subgroups A and C. These graphs imply that increased bilirubin production is not a major factor in the pathogenesis of G-6-PD-associated hyperbilirubinemia, and that alternative mechanisms (10) play a greater part in the pathogenesis of the jaundice than in ABO incompatibility. Different and independently acting icterogenic mechanisms were most likely responsible for the lack of a cumulative effect of ABO incompatibility and G-6-PD deficiency.

Acknowledgments.—This study was supported in part by a grant from the General Research Fund at the Shaare Zedek Medical Center, and at Stanford University by the National Institutes of Health grants HD14426 and RR00070, the Mary L. Johnson Research Fund, the Hess Research Fund, and the Providence Foundation.

References

Received July 23, 1997. Accepted in revised form Dec. 12, 1997