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Prevalence of Gilbert syndrome in parents of neonates with pathologic indirect hyperbilirubinemia

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BACKGROUND AND OBJECTIVES: The cause of hyperbilirubinemia cannot be found in about 45% of cases of neonatal jaundice. Gilbert syndrome (GS) is the most common congenital disease associated with bilirubin metabolism in the liver. Since the screening value of genetic tests cannot be fully determined until accurate data on the prevalence and penetrance of the GS genotype are known, we sought to estimate whether the prevalence of GS is higher in the parents of neonates with severe unexplained indirect hyperbilirubinemia.

DESIGN AND SETTING: Case-control study of parents of neonates with severe unexplained indirect hyperbilirubinemia admitted to a neonatal ward.

METHODS: We used the rifampin test (checked bilirubin before and 4 hours after administration of 600 mg rifampin) for diagnosis of GS in parents of 115 neonates with severe unexplained indirect hyperbilirubinemia. We compared the prevalence of GS in these parents with that of a control group of 115 couples referred for premarital counseling.

RESULTS: The 115 neonates were aged 5.2 (1.6) days (mean, standard deviation), all were breast-fed, and males constituted 56.5%. Mean total serum bilirubin (TSB) level was 20.96 (5.48) mg/dL. 14.8% were glucose 6 phosphate dehydrogenase (G6PD) deficiency was present in 14.8%, and 10.4% had A, B or O blood group (ABO) incompatibilities with their mothers. There was no difference in the prevalence of GS between parents of the group with hyperbilirubinemia (22.2%) and the control group (19.13%) (P=.42). Mean TSB in neonates with parents who had GS was more (about 3 mg/dL) than in neonates with normal parents (P=.004). Fathers had GS twice as often as the mothers among the parents of neonates with hyperbilirubinemia (P=.003), among the control group (P=.009) and among neonates (P=.014).

CONCLUSION: This study showed that GS cannot cause severe indirect hyperbilirubinemia by itself, but it may have a summative effect on rising bilirubin when combined with other factors, for example, G6PD. Our results showed that in GS, males are affected about twice as much as the females.

Jaundice is the most common and one of the most vexing problems that can occur in the newborn. Although most jaundiced infants are otherwise perfectly healthy, the condition is worrying because bilirubin is potentially toxic to the central nervous system. Approximately two-thirds of the more than 4 million neonates born annually in the United States become clinically jaundiced.[1] Clearly the number of jaundiced neonates who will develop sequelae of hyperbilirubinemia is substantially less than this. The challenges to the pediatrician are to determine which newborns may become or are already abnormally jaundiced and are therefore at risk for severe sequelae, and also to find the cause of jaundice.

Gilbert syndrome (GS) is a mild benign chronic or recurrent unconjugated hyperbilirubinemia with no evidence of liver disease or overt hemolysis.[2] GS is common, affecting approximately 6% to 9% of the general population, and both autosomal dominant and recessive patterns of inheritance have been suggested.[1] Typically, the indirect-reacting hyperbilirubinemia is not recognized until after puberty, and it manifests itself during fasting or illnesses. Many studies have shown that GS can play a ubiquitous role in the pathogenesis of neo-

natal hyperbilirubinemia.[1,2] Although most commonly diagnosed in young adulthood, GS can play a role in neonatal jaundice.[1] Some studies have revealed that the combination of the Gilbert genotype with other icterogenic factors such as breast feeding, G6PD deficiency, ABO incompatibility and pyloric stenosis dramatically increases a newborn's risk of hyperbilirubinemia and also complications such as bilirubin encephalopathy.[3-5] Maisele reported that "we do not know if GS plays an important role in the pathogenesis of extreme hyperbilirubinemia, and all the studies that have been done till now, were just done in mild-to-moderate hyperbilirubinemia. This suggests that it is a possibility worth investigating.[1]

Diagnosis of GS in adulthood is possible through history taking and physical examination and also by diagnostic tests such as the phenobarbital test, the nicotinic acid test and the rifampin test. However, these diagnostic tests have not been used in neonates until now due to the possible toxic effect of drugs in neonates. The diagnosis of GS in neonates is limited to specific genetic studies performed with special laboratory investigations in some developed countries. Although genetic testing may be helpful in individual patients, the screening value of such a genetic test cannot be fully determined until accurate data regarding the prevalence and penetrance of the GS genotype are known. Thus, genetic testing for GS cannot be routinely recommended.

METHODS

The parents of all neonates with jaundice who were admitted for intensive phototherapy or exchange transfusion to the neonatal ward of Nemazi Hospital, affiliated to Shiraz University of Medical Sciences, between March 2006 and March 2008 were considered as candidates for this case-control study. A routine hyperbilirubinemia workup, including complete blood count (CBC), reticulocyte count, peripheral smear, total and direct bilirubin levels of the neonates and the blood group of the mothers and neonates, was carried out. Inclusion criteria were birth weight more than 2000 g, gestational age more than 35 weeks and total serum bilirubin level more than the 95th percentile for age (hours).[6] Exclusion criteria were direct hyperbilirubinemia or known causes of indirect hyperbilirubinemia such as polycythemia, cephalhematoma, large ecchymosis, sepsis; known immune or nonimmune hemolytic anemia according to physical examination and routine hyperbilirubinemia workup (positive Coomb test and abnormal peripheral smear such as hereditary spherocytosis or eliptocytosis). All patients and controls were free of medications that cause abnormalities in glucuronidation, such as aspirin, coumarin, dopamine derivatives, acetaminophen and valproic acid, which may disturb the findings.

One hundred thirty neonates met the criteria and they and their parents were enrolled as the case group. All the parents agreed to participate after being provided with the details of the study and information regarding side effects of rifampin. The history of blood and liver diseases was obtained from all, and a physical examination was done to rule out chronic liver disease. Tests for complete blood count and reticulocyte count, as well as liver function tests (LFTs), were carried out in all the parents. Those parents with hemolytic or liver diseases (according to history, physical examination and laboratory workup) were excluded from the study.

We evaluated 230 individuals as the control group who were referred to us for premarital counseling or checkup. After describing the details of the study and the side effects of rifampin, most of them agreed to participate in this study. A history of blood and liver diseases was obtained from all of them, and physical examination was done to rule out any stigmata of liver disease. Tests for CBC and reticulocyte count, as well as liver function test, were carried out. Those with hemolytic or liver diseases were excluded from the study.

An overnight rifampin test was done for all parents and the control group. In this test, serum total and unconjugated bilirubin levels were measured from two samples — one before a single 600-mg oral dose of rifampin; and the second, 4 hours after drug administration while the parents were fasting. The overnight rifampin test was considered positive for a diagnosis of GS if increases in the serum total and unconjugated bilirubin levels were more than 2.4 and 1.3 mg/dL, respectively.[7] An increase in serum total bilirubin to more than 2.4 mg/dL has a 93.8% sensitivity and 93% specificity for the diagnosis of GS.[8] A rise in unconjugated bilirubin more than 1.3 mg/d has a 100% sensitivity and 100% specificity for the diagnosis of GS.[8] The data on the neonates were obtained from their hospital admission charts and also from the parents in a questionnaire.

LFTs, CBC and reticulocyte count and other routine workups were checked in Nemazi Hospital's laboratory. LFTs were checked with Autoanalyzer (Technicon- RA-1000, USA) and Parsazmoon biochemical kits (Iran). CBC was checked with a cell counter (Sysmex- K-800, Japan), and reticulocyte count was checked with supravital staining of peripheral blood smear manually. Bilirubin was measured by the diazo method. Unconjugated bilirubin was estimated as total bilirubin minus conjugated bilirubin. Normal total

serum bilirubin concentration using this technique is below 1 mg/dL (range, 0.2-1). Serum bilirubin in 99% of the general population is also below 1 mg/dL. It would have been more appropriate to select parents of neonates with no jaundice as controls in order to arrive at a definite conclusion, but accumulation of data in normal children in order to detect 230 neonates with normal bilirubin (considering high prevalence of hyperbilirubinemia in neonates) was difficult due to the high cost and time required. Also, the number of punctures for blood sampling to find 230 neonates with normal bilirubin was considered too invasive.

Statistical analysis was carried out using SPSS (Chicago, USA, version 11.5). The paired t test, chi-square and Fisher exact tests were used for comparison. A *P* value less than .05 was considered significant. Quantitative data are mentioned as mean (standard deviation). The study was approved by the ethics committee of the Shiraz University of Medical Sciences. All the participants were provided with complete details of this study, and they filled and signed the informed consent form.

RESULTS

Among 130 neonates who entered this study, 15 neonates were excluded: 6 neonates were excluded because their parents did not refer for post-rifampin tests, 7 neonates had lab errors due to inadequate blood samples, and 2 neonates were excluded because one of their parents had signs and lab data suggestive of chronic liver disease or a hemolytic problem. Therefore, 115 neonates were included in the statistical analysis. Their age was 5.2 (1.6) days. Of the 115, 56.5% were male (65 neonates); and 43.5%, female (50 neonates). The mean serum total bilirubin on admission day was 20.96 (5.48) mg/dL. None developed prolonged jaundice (jaundice duration ≥3 weeks). While 83.5% of these neonates (96 neonates) received only intensive phototherapy, 13.9% (16 neonates) received intensive phototherapy and 1 exchange transfusion, 1.7% (2 neonates) received intensive phototherapy and two exchange transfusions, and just 1 neonate received intensive phototherapy plus three exchange transfusions. Among the neonates, 14.8% (17 neonates) had G6PD deficiency without hemolysis, and G6PD levels were sufficient in 85.2% of the neonates (98 neonates). In 103 (89.6%) neonates, blood groups (ABO) were compatible with their respective mothers, and 12 (10.4%) neonates had ABO incompatibility with their mothers. Hemolysis was not detected in these neonates and all had a negative Coombs test.

The results of the rifampin tests in parents, controls, and neonates is shown in **Tables 1 and 2**. Among the control group, 44 (19.1%) individuals had Gilbert syndrome, and 186 (80.9%) individuals did not. There was no statistical difference in the prevalence of GS between parents of neonates with unexplained severe indirect hyperbilirubinemia and those of the control group (*P*=.42). The mean of total serum bilirubin level in the neonates who had GS in one parent was significantly (3 mg/dL) higher than that in the neonates with unexplained severe indirect hyperbilirubinemia that had normal parents (*P*=.004). Among the neonates who had G6PD deficiency without hemolysis, mean total serum bilirubin in neonates who had GS in their parents was 25.16 mg/dL, and in those who had normal parents it was 20.7 mg/dL, but the number of neonates with G6PD deficiency was too small to carry out any standard statistical analysis.

Among the neonates who had ABO incompatibility with their respective mothers without any hemolysis, mean total serum bilirubin in the neonates who had GS in their parents was 20.33(3) mg/dL, and in neonates who had normal parents it was 20.97(5) mg/dL. The number of ABO-incompatible neonates was too small to carry out any meaningful statistical analysis.

Among the parents, 13.9% of the mothers and 30.4% of the fathers had GS, meaning that prevalence of GS was more in fathers of neonates with unexplained severe indirect hyperbilirubinemia (*P*=.003). Also, among the control group, 12.5% of the females and 25.6% of the males had GS, indicating that in a normal population, the prevalence of GS in males was more than that in females (*P*=.009). Among neonates with unexplained severe indirect hyperbilirubinemia, 24% of female neo-

Table 1. Results of rifampin tests in parents and controls.

	Parents with positive rifampin test	Controls with positive rifampin test	Total rifampin tests
Female	16 (13.9)	14 (25.5)	115
Males	35 (30.4)	30 (25.6)	115
Total	51 (22.6)	44 (19.1)	230

Data are number (%).

Table 2. Results of rifampin tests in neonates by rifampin test results in parents.

Negative test in both parents	73 (63.5)
Positive test in one parent	33 (28.7)
Positive test in both parents	9 (7.8)

Data are number (%).

nates and 46.2% of male neonates had one or both of parents with GS, meaning that the number of male neonates whose parents had GS was more than that of the female neonates whose parents had GS (P=.014).

DISCUSSION

We studied 115 neonates with indirect hyperbilirubinemia; their bilirubin was higher than the 95th percentile for their age. They needed either intensive phototherapy or exchange transfusion. Serum bilirubin levels was 20.96 (5.48), indicating severe jaundice.[6] They did not have any explained cause of hyperbilirubinemia in their routine workup6 (e.g., hemolysis, sepsis) and all were breastfed.

In previous studies, there are some controversies about the causal relationship of GS with neonatal indirect hyperbilirubinemia. Several investigations have shown that neonates who are homozygous for the variant 7/7 uridine diphosphate glucoronyle transferase (UGT) gene promoter (affected gene in Gilbert syndrome) show a more rapid rise in their total serum bilirubin (TSB) levels[9] and higher TSB levels 96 hours after birth.[10] Of neonates with a TSB concentration greater than 13 mg/dL, 26.8% were homozygous for the variant 7/7 promoter versus 12.2% of those whose TSB levels were ≤13 mg/dL.[11] In a study on the Scottish population, 31% of the primarily breast-fed newborns with TSB levels greater than 5.8 mg/dL after 14 days of life were homozygous for the 7/7 GS promoter genotype as compared with only 6% of the control group with acute jaundice.[12] Of the 17 breast-fed Japanese infants with prolonged jaundice, 16 had at least one mutation of the UGT1A1 gene, primarily of the G71R type.[13] Another study showed GS was significantly more common in hyperbilirubinemic neonates in the first week of life, even though neither the peak of bilirubin nor the day on which the highest value was found differed according to genotype.[5] In contrast, one study investigated whether a TATA box polymorphism in the promoter of the UGT exon I, the most commonly detected DNA polymorphism in GS, is a contributory factor in unexplained pathologic jaundice. Thirty-seven neonates who had unexplained prolonged jaundice and 35 healthy nonjaundiced neonates were enrolled in the study. They concluded that TA 7/7 and TA 6/7 genotypes are not rare in their population and that the presence of those polymorphisms alone does not play a significant role in the etiology of unexplained pathologic or prolonged neonatal hyperbilirubinemia.[14] Another study in Spain showed that there was a relationship between neonatal jaundice and Gilbert syndrome.[15]

Maisele et al in 2006 reported that "we do not know if GS plays an important role in the pathogenesis of extreme hyperbilirubinemia" and the author suggested that it was a possibility worthy of investigation.[1] In all of the previous studies, a diagnosis of GS was done through genetic study and genotype determination. Although genetic testing may be helpful in individual patients, the screening value of such a genetic test cannot be fully determined until accurate data regarding the prevalence and penetrance of the GS genotype are known.[16] Thus, genetic testing for GS cannot be routinely recommended.[16] In our study, due to unavailability of genetic test and genetic data about the prevalence and penetrance of GS genotype in our country, we could not use this test for screening the patients. Thus, we used the rifampin test in addition to a history and physical examination in parents for diagnosis of GS. The rifampin test is highly sensitive and specific.[8] In one study, the rise in unconjugated bilirubin more than 1.3 mg/dL 4 hours after using 600 mg rifampin was 100% sensitive and 100% specific.[7] This test has not been used in neonates until now, so we used it in parents of neonates to evaluate the footprint of GS in the neonates. This was an interesting point of this study, because it can be a sensitive and specific way to study GS in neonates.

Our study showed that there was no difference in the prevalence of GS between the parents of neonates with severe unexplained indirect hyperbilirubinemia and the normal population (P=.42), but the mean of total serum bilirubin levels of the neonates who had GS in their parents was nearly 3 mg/dL higher than that of the neonates who had normal parents (P=.004). That is, GS cannot cause severe indirect hyperbilirubinemia by itself, but it may have a summative effect to increase bilirubin when combined with other factors. Factors such as breastfeeding,[13,17] G6PD deficiency,[18] ABO incompatibility between neonates and their respective mothers, and pyloric stenosis can associate with GS to cause overt jaundice.[1]

The pathogenesis of the hyperbilirubinemia present in approximately 30% of neonates affected by G6PD deficiency is an unsolved problem, with only three studies addressing the issue. Aldascon[19] indicated that GS did not account for the hyperbilirubinemia occurring in some neonates with G6PD deficiency. Furthermore, their results suggest that hemolysis is not the major event in the pathogenesis of hyperbilirubinemia in these patients.[19] However, a remarkable interaction between G6PD deficiency and GS was demonstrated by Kaplan and associates.[18] In this study of Israeli infants, neither the presence of the variant UGT promoter (for GS) by itself nor G6PD deficiency alone had a significant effect on the incidence of hyperbiliru-

binemia, but there was a significant increase in hyperbilirubinemia in G6PD-deficient infants who also had the variant UGT promoter. It is interesting, however, that in Italian G6PD-deficient neonates, homozygosity for the variant 7/7 promoter did not increase the risk of hyperbilirubinemia.[19]

In our study, 14.8% of the neonates with severe unconjugated hyperbilirubinemia had G6PD deficiency, but no sign of hemolysis. The number of patients with G6PD deficiency was too small to undertake evaluation of any valuable relationship between GS and G6PD deficiency, but grossly, the mean bilirubin of neonates with G6PD deficiency who had parents with GS was 25.16 (8) mg/dL, while the mean bilirubin of G6PD-deficient neonates who had normal parents was 20 (5) mg/dL.

Kaplan investigated whether uridine diphosphate glucoronyle transferase (UGT) gene promoter polymorphism (GS) would increase hyperbilirubinemia in direct Coombs negative ABO-incompatible neonates, as seen in other combinations with this condition. They showed that GS is a determining factor for neonatal hyperbilirubinemia in ABO incompatibility.[3] In our study, 10.4% of the neonates with severe unexplained hyperbilirubinemia had ABO incompatibility with their respective mothers, and they did not have hemolysis or a positive direct Coombs test. The number of neonates with ABO incompatibility was insufficient to make any meaningful comparison. The mean total bilirubin level in ABO-incompatible neonates who had parents with GS was 20.33 (3.05) mg/dL; and in neonates who had normal parents, it was 20.97 (5.34) mg/dL.

Our study showed that GS was more prevalent in the fathers of neonates with unexplained severe unconjugated hyperbilirubinemia than in their mothers (P=.003). Also, in our control group, which was a normal population, it was more common in males than in the females (P=.009), and most of the neonates with parents having GS were male (P=.014). The male-female ratio was 2.14 in parents, 2.04 in normal population and 1.92 in neonates with severe unexplained indirect hyperbilirubinemia. It seems that generally in GS, males are affected about twice as often as females. The cause of this relationship is unknown and needs further investigation.

All of the neonates in this study were breastfed, so any association of GS and breastfeeding in these neonates could not be evaluated. Moreover, our study showed that the prevalence of GS in Iran was more than that reported in previous studies.[1] This may be due to more consanguinous marriages, variability in method of screening (rifampin test), and high genetic susceptibility in Iran.

In summary, we showed that GS alone cannot cause severe indirect hyperbilirubinemia, but it may have a summative effect to increase bilirubin when combined with other factors, for example, G6PD deficiency, and other unknown problems. Our results showed that in GS males are affected about twice as often as females; however, the cause of this preponderance is unknown and needs further investigation. Also, GS was found to be more prevalent in Iran.

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