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Etiology analysis and G6PD deficiency for term infants with jaundice

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Abstract
Background: Neonatal jaundice, characterized by yellow skin, sclera and conjunctiva due to hyperbilirubinemia. Bilirubin can cross the blood-brain barrier, leading to severe hyperbilirubinemia, acute bilirubin encephalopathy, nuclear jaundice, and even permanent brain damage. This study aims to analyses risk factors for baby hyperbilirubinemia and assess the role of G6PD deficiency in neonatal jaundice.
Method: The term infants with neonatal hyperbilirubinemia in Al elwyia teaching hospital for children and in Iraq from June 2018 to July 2022 were recruited for the retrospective analysis. All the infants underwent quantitative detection of the G6PD enzyme. The etiology was determined through laboratory tests and clinical manifestations.
Results: In a study of 1,119 term newborns, 435 had jaundice, with infection and G6PD deficiency being the main identifiable causes. Jaundiced newborns showed a significantly higher incidence of G6PD deficiency than non-jaundiced ones (19.54% vs. 10.23%, $p<0.001$), and those with G6PD deficiency had significantly lower hemoglobin levels. Among the jaundiced group, 35.63% had unknown causes, and six G6PD deficiency-related genotypes were identified.
Conclusion: In newborns with G6PD deficiency, infection, and neonatal hemolytic disease were identified as the main causes of hyperbilirubinemia and acute bilirubin encephalopathy. Specifically, Hemolytic factors in infants with G6PD deficiency may lead to reduced hemoglobin and increased bilirubin levels in jaundiced infants.

Keywords: Neonatal hyperbilirubinemia, neonatal jaundice, etiology, G6PD deficiency, hemolysis

Introduction
Neonatal jaundice is marked by yellowing of the skin, sclera, and conjunctiva due to hyperbilirubinemia. Bilirubin, able to cross the blood-brain barrier, may lead to severe hyperbilirubinemia, acute bilirubin encephalopathy, kernicterus, and potentially permanent brain damage [1]. In China, acute bilirubin encephalopathy among neonates poses a significant health concern, highlighting the importance of timely identification and management of jaundice risk factors to mitigate the occurrence and severity of hyperbilirubinemia and acute bilirubin encephalopathy [2]. Neonatal hyperbilirubinemia (NHB) can be caused by several factors, with infections being the primary cause, alongside perinatal and hemolytic factors [3, 4]. Genetic factors, such as hereditary conditions, also play a significant role in the development of neonatal hyperbilirubinemia [5]. This study conducted in a hospital in the western part of Guangdong province aimed to thoroughly explore the causes of jaundice in infants and assess the impact of glucose-6-phosphate dehydrogenase (G6PD) deficiency on the condition.

Method
This study focused on full-term infants with a gestational age over 37 weeks and a birth weight of at least 2,500 grams. All infants underwent quantitative detection of G6PD enzyme levels, and were admitted to the hospital at ages ranging from 1 to 28 days. Jaundice was identified based on the maximum total serum bilirubin (TSB) reaching or exceeding the 95th percentile according to the hour-specific TSB nomogram established by the Chinese Multicenter Study Coordination Group for Neonatal Hyperbilirubinemia in 2015. Severe hyperbilirubinemia was defined as TSB levels of 342 μmol/L or higher.

The study was conducted with an ethical waiver for written consent by the Ethics Committee of the People's Hospital of Yangjiang, as patient data was analyzed anonymously and blood samples were collected post clinical diagnosis. The research collected demographic and clinical data from medical records, covering variables such as infant sex, gestational age, birth weight, age and weight at admission, delivery mode, feeding pattern, and treatment measures. Key clinical causes of jaundice examined included hemolytic disease of the newborn (Specifically ABO and Rh incompatibility), G6PD deficiency, various infections (Like sepsis, pneumonia, and urinary tract infections), extravascular hemorrhages (Including intracranial and scalp hematomas, as well as gastrointestinal bleeding), congenital hypothyroidism, and breast milk jaundice-the latter diagnosed by exclusion in exclusively breastfed infants older than 14 days without other jaundice causes. Diagnostic criteria for ABO incompatibility hemolysis included two positive results out of three serological antibody tests: direct antiglobulin test, free antibody test, and antibody release test, with antibody elution test providing final confirmation. G6PD deficiency was confirmed by measuring the NADPH production rate, with a threshold of lower than 2,500 U/L categorizing an infant as G6PD-deficient. Molecular diagnosis involved collecting blood specimens from G6PD-deficient patients, DNA extraction, and amplification of the G6PD gene followed by detection of common G6PD mutations through the reverse dot blot method. Statistical analysis was conducted using SPSS 23.0, with continuous variables compared via the Mann-Whitney non-parametric test and categorical variables analyzed using the Chi-square or Fisher's exact test. A P-value less than 0.05 was considered statistically significant. This comprehensive methodological approach aimed to identify and quantify the various etiological factors contributing to neonatal jaundice in a specific hospital setting in Guangdong, with a particular focus on the role of G6PD deficiency.

Results

Among the 435 cases of jaundice, severe hyperbilirubinemia (TSB ≥342 μmol/L) was observed in 50 cases, 8 cases of which presented with acute bilirubin encephalopathy (2 males and 6 females), and 7 cases required exchange transfusions. The primary causes of acute bilirubin encephalopathy were G6PD deficiency (3 cases) and hemorrhage (3 cases), followed by infection (2 cases of sepsis and 1 case of pneumonia), ABO incompatibility hemolysis (2 cases), and congenital hypothyroidism (1 case) (Table 1). One female infant with acute bilirubin encephalopathy was admitted at the age of 17 days, who had congenital hypothyroidism combined with G6PD deficiency and had not undergone neonatal screening at birth (Table 1).

Of the 435 cases with jaundice, 19.54% (60 males, 25 females) were found to be G6PD-deficient, while only 10.23% (47 males, 23 females) of the 684 cases with normal bilirubin levels were G6PD-deficient. The prevalence of G6PD deficiency was significantly higher in infants with jaundice compared to their counterparts (p<0.001) (Table 2). The prevalence of G6PD-deficient in severe hyperbilirubinemia (TSB ≥ 342 μmol/L) and mild-medium jaundice (TSB < 342 μmol/L) was 30% (15 out of 50, 11 males, 4 females) and 18.18% (70 out of 385, 49 males, 11 females), respectively. A statistically significant difference was observed between the two groups (P = 0.041) (Table 3). In comparison, G6PD defi- ciency was present in 8.75% (42/480) of male adults during routine body check-ups.

Of the 435 infants with jaundice, one case was excluded due to the lack of hemoglobin data. The re- maining cases were grouped according to jaundice causes-normal, G6PD-deficient, ABO incompati- bility hemolysis, and ABO incompatibility hemolysis combined with G6PD-deficiency. The difference in total bilirubin levels among these four groups was compared (Table 4), and the peak bilirubin values were not statistically significant (P = 0.95). Among infants with jaundice, the hemoglobin levels of 80 G6PD-deficient infants were 146.85±24.88 g/L, which was significantly lower than that of infants with normal G6PD (156.30±22.07 g/L) (P = 0.001) (Table 5). Meanwhile, the hemoglobin levels of 27 infants with ABO incompatibility hemolysis were at 134.33±24.18 g/L, slightly lower than that of infants with G6PD deficiency (146.85±24.88 g/L) (P = 0.014).

A total of 65 blood samples from jaundiced infants with G6PD deficiency were analyzed for G6PD genotypes using reverse dot hybridization (10). Five types of gene mutations and one polymorphism were detected in infants with jaundice, namely c.95A > G, c.392G > T, c.1024C > T, c.1376G > T, c.1388G > A, and c.1311C > T (polymorphism). Furthermore, three kinds of compound heterozygous mutations were identified, namely c.871G > A/c.1311C > T, c.392G > T/c.1388G > A, and c.1376G > T/c.1311C > T (Table 6). Specifically, c.871G > A was consistently linked to 1311C > T in all nine cases (Table 6). Moreover, one G6PD-deficient infant developed acute bilirubin encephalopathy with compound heterozygous mutations of c.392G > T and c.1388G > A.

Table 1: Demographic and Clinical Characteristics of Infants with Acute Bilirubin Encephalopathy

S/N	Diagnosis	Sex	Age (day)	Gestational age (weeks)	Birth weight (g)	Delivery mode	Feeding method	G6PD (U/L)	TBIL (μmol/L)
1	Acute bilirubin encephalopathy	M	5	39 + 2	2700	Cesarean	Breast feeding	3457	493.5
2	Acute bilirubin encephalopathy	F	10	38 + 6	2700	Vaginal	Breast feeding	3425	401.7
3	Acute bilirubin encephalopathy	F	8	38 + 3	2900	Vaginal	Mixed feeding	2786	597
4	Acute bilirubin encephalopathy	M	6	38 + 1	3000	Vaginal	Breast feeding	774	634.9
5	Acute bilirubin encephalopathy	M	6	39 + 1	2700	Cesarean	Breast feeding	3457	500.9

Table 2: Comparison of G6PD Deficiency in Infants

Hyperbilirubinemia (n)	Normal bilirubin (n)	Total (n)	P value
G6PD deficiency	85	70	155
Normal G6PD	350	614	964
Total	435	684	1119

Table 3: G6PD Deficiency in Infants with Severe and Mild-Medium Jaundice

G6PD state	Severe hyperbilirubinemia	Mild-medium hyperbilirubinemia	Total	P value
G6PD deficiency	15	70	85	0.041
Normal G6PD	35	315	350	
Total	50	385	435	

Table 4: Comparison of Peak Bilirubin Levels in Infants with Hyperbilirubinemia

Group	Case (n)	TBIL (μmol/L)	H value	P value
Normal G6PD	322	278.65	0.35	0.95
G6PD deficiency	80	278.25		
ABO hemolysis	27	253		
ABO hemolysis/G6PD deficiency	5	267.8		

Table 5: Comparison of Hemoglobin Levels in Infants with Jaundice

Group	Case (n)	HB (g/L)	95% CI	F value	P value
Normal G6PD	322	156.30±22.07	153.88–158.72	11.48	<0.001
G6PD deficiency	80	146.85±24.88	141.31–152.39		
ABO hemolysis	27	134.33±24.18	124.77–143.90		
ABO hemolysis/G6PD deficiency	5	133.40±23.58	102.12–164.68		

Table 6: G6PD Genotypes Distribution in 65 Cases of G6PD Deficient Infants with Hyperbilirubinemia

Action	Hemi zygote (n)	Heterozygote (n)	Homozygote (n)	Total (n)	Percentage (%)
Wild type	1	1	0	2	3.1
c.95A > G	5	0	0	5	7.7
c.392G > T	3	0	0	3	4.6
c.1024C > T	2	1	0	3	4.6
c.1311C > T	1	0	0	1	1.5
c.1376G > T	10	3	1	14	21.5
c.1388G > A	21	3	0	24	36.9
c.871G > A/c.1311C > T	6	3	0	9	13.8
c.392G > T/c.1388G > A	0	1	0	1	1.5
c.1376G > T/c.1311C > T	0	3	0	3	4.6
Total	49	15	1	65	100

Discussion

The pathogenic factors of neonatal hyperbilirubinemia are multifaceted, and different cases can have a single or mixed etiology. Common causes include extravascular hemorrhage, hemolytic diseases, infection, G6PD deficiency, breastfeeding, and maternal disease factors. Furthermore, the etiological com- position of neonatal hyperbilirubinemia can vary in different regions. Among the 435 full-term infants with hyperbilirubinemia in our study, the main causes were combined factors (22.3%), infection (16.32%), G6PD deficiency (9.66%), hemorrhage (8.05%), hemolytic diseases (3.45%), breast milk jaundice (2.53%), other factors (2.07%), and congenital hypothyroidism (0.23%). A study conducted in the eastern region of China reported that the top five pathogenic factors of neonatal unconjugated hyperbilirubinemia were combined factors (16%), infections (15%), breast milk jaundice (11%), hemorrhage (10%), hemolytic diseases (7%), and G6PD deficiency (1%) [4]. In comparison, the cohort in our study had a higher prevalence of G6PD deficiency, likely due to the high prevalence of G6PD deficiency in the population of western Guangdong province. Nevertheless, infections, hemolytic diseases, and hemorrhage remained the main pathogenic factors of neonatal jaundice in our study. In this study, the prevalence of G6PD deficiency was 19.54% in the jaundiced group, while it was 10.23% in the control group with normal bilirubin levels. In Chaozhou of eastern Guangdong province, among 882 neonates with hyperbilirubinemia, 74 cases (8.39%) were G6PD-deficient [5], and in Fujian province, the prevalence of G6PD deficiency among neonates with hyperbilirubinemia was 7% [6]. As such, the prevalence of G6PD deficiency among neonates with hyperbilirubinemia in the western part of Guangdong is higher compared to that in eastern Guangdong and Fujian province. Multiple reports have demonstrated that G6PD-deficient infants have a significantly higher predisposition to neonatal jaundice and are more susceptible to acute bilirubin encephalopathy [2, 4, 7]. In our study, the prevalence of G6PD deficiency was 30% in infants with severe jaundice and 18.18% (70/385) in infants with mild-medium jaundice. Of the neonates with acute bilirubin encephalopathy, 37.5% (3 in 8) were G6PD-deficient. Moreover, the frequency of G6PD deficiency in Yangjiang males was 8.75%, which was higher than that of the entire Guangdong province [8]. In the voluntary Kernicterus Registry in the United States, 20.8% of 125 affected newborns were G6PD-deficient, while the male frequency of G6PD deficiency was estimated to be 0.5%–2.9% [9]. Our study, along with previous re- search, indicates that G6PD-deficient infants are predisposed to neonatal jaundice, and even to kernicterus. In 2004, the American Academy of Pediatrics (AAP) suggested that G6PD deficiency should be con- sidered as a high-risk factor for jaundice in newborns ≥35 weeks old, and should therefore be evaluated in the diagnosis and treatment of jaundiced newborns [10]. In clinical practice, given the relatively high prevalence of G6PD deficiency in this population, G6PD screening for all local newborns is required. Furthermore, transcutaneous bilirubin monitoring is strongly recommended when an infant with G6PD deficiency is

discharged. Early detection is beneficial for prompt treatment, which is in accordance with the AAP guidelines. These improvements in neonatal care could decrease neonatal morbidity and mortality in this region. G6PD deficiency is the most prevalent inherited enzyme deficiency disease, yet the mechanism of neonatal hyperbilirubinemia resulting from G6PD deficiency remains incompletely understood [4]. Previously, it was believed that jaundice in infants with G6PD deficiency was mainly caused by excessive bilirubin production during hemolysis [11-13]. Nevertheless, some studies have found minimal evidence of hemolysis in jaundiced neonates with G6PD deficiency [14-16]. In our cohort of infants with hyperbilirubinemia, the hemoglobin levels in the G6PD-deficient group were significantly lower than those in the normal G6PD group (p<0.001), and slightly higher than those in the ABO hemolysis group (134.33±24.18 g/L) (P = 0.014). Since fetal erythropoiesis in infants with G6PD deficiency was the same as that in controls, and G6PD was dispensable for human erythroid cell differentiation [18, 19]. Our findings suggest that decreased hemoglobin levels may be due to hemolytic factors in jaundiced infants with G6PD deficiency. Another possible explanation is the disruption of the oxidant-antioxidant balance and impaired recycling of peroxiredoxin 2, which can impact bilirubin clearance (4). Moreover, co-inheritance of a uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) gene variant is an additional risk factor for neonatal jaundice in G6PD-deficient infants [5]. G6PD is caused by loss-of-function mutations in the G6PD gene and follows an X-linked recessive in- heritance pattern. The distribution of G6PD deficiency is predominantly found in the south of the Yangtze River in China, with Guangdong province exhibiting a high incidence of G6PD deficiency (8). Interestingly, common variants among G6PD-deficient individuals in southern China are unique to these populations [19]. In our study, Canton (c.1376 G > T) and Kaiping (c.1388 G > A) were the most frequent variants, accounting for over 78% of G6PD-deficient infants with jaundice. This distribution pattern was consistent throughout Guangdong province and the entire country [20].

Conclusion

In newborns with G6PD deficiency, infection, and neonatal hemolytic disease were identified as the main causes of hyperbilirubinemia and acute bilirubin encephalopathy. Specifically, Hemolytic factors in infants with G6PD deficiency may lead to reduced hemoglobin and increased bilirubin levels in jaundiced infants.

Conflict of Interest

Not available

Financial Support

Not available

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