

Evaluation of neonatal indirect hyperbilirubinaemia at Zanjan Province of Iran in 2001–2003: prevalence of glucose-6-phosphate dehydrogenase deficiency

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ABSTRACT

Introduction: Neonatal hyperbilirubinaemia, defined as a total serum bilirubin level above 5 mg/dL, is a frequent problem. This condition accounts for up to 75 percent of hospital readmissions in the first week of life. The purpose of this study was to evaluate the aetiology of indirect hyperbilirubinaemia and the prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in newborns who were admitted to Vali-e-Asr Hospital in Zanjan City during the period 2001-2003.

Methods: Medical records of 376 newborns who had been admitted for management of indirect hyperbilirubinaemia were reviewed. All necessary information, including the results of G6PD activity test (expressed as unit per gramme haemoglobin), were recorded on standardised questionnaires.

Results: The subject group included 159 (42.3 percent) boys and 217 (57.7 percent) girls. The prevalence of sepsis, ABO incompatibility, Rhesus incompatibility, and cephalhaematoma, G6PD deficiency was 15.7 percent (59 neonates), 3.7 percent (14 neonates), 2.1 percent (eight neonates), 0.5 percent (two neonates), and 2.1 percent (eight neonates), respectively. The median (interquartile range) of the highest total bilirubin level was 18 (15.8-20) mg/dL and 18.4 (16.3-19.5) mg/dL in normal G6PD and G6PD-deficient newborns, respectively (p-value equals 0.7).

Conclusion: We recommend performing G6PD testing in all Iranian and Mediterranean newborns with indirect hyperbilirubinaemia,

unless other investigators ascertain and document that this is unnecessary as a routine test.

Keywords: glucose-6-phosphate dehydrogenase, indirect hyperbilirubinaemia, neonatal hyperbilirubinaemia, neonatal jaundice

Singapore Med J 2007; 48(5):424–428

INTRODUCTION

Neonatal hyperbilirubinaemia, defined as a total serum bilirubin level exceeding 5 mg/dL, is a frequent problem.⁽¹⁾ Neonatal jaundice affects 60% of full-term infants and 80% of preterm infants in the first three days of life. Although transient, the condition accounts for up to 75% of hospital readmissions in the first week after birth.⁽²⁾ The mechanisms of neonatal hyperbilirubinaemia can be classified as:

- Bilirubin overproduction which occurs in haemolytic diseases with either positive Coombs test (ABO incompatibility, Rhesus incompatibility, and minor blood group antigens) or negative Coombs test (red blood cell membrane defects, e.g. spherocytosis, elliptocytosis, and/or red blood cell enzyme defects, such as glucose-6-phosphate dehydrogenase [G6PD] and pyruvate kinase deficiencies). Sepsis and some drugs are other examples of haemolytic diseases. Bilirubin overproduction may occur in non-haemolytic diseases, like cephalhaematoma, bruising, central nervous system (CNS) haemorrhage, swallowed blood, polycythaemia, ileal atresia, and pyloric stenosis.^(1,3) Breast milk jaundice has two forms: early and late onsets. The early onset form is due to low calorie intake and the late onset form is usually due to decreased bilirubin conjugation.⁽⁴⁾
- Decreased bilirubin conjugation that occurs in physiological jaundice, Crigler-Najjar syndrome, hypothyroidism, sepsis and premature newborns.

The above-mentioned mechanisms cause indirect hyperbilirubinaemia, but impaired bilirubin excretion,

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which is the third mechanism of neonatal jaundice, causes direct hyperbilirubinaemia.^(1,3)

Common risk factors for neonatal jaundice include prematurity, foetal-maternal blood group incompatibility, and a previously affected sibling. Cephalhaematoma, bruising, and trauma from instrumented delivery may increase the risk for serum bilirubin elevation. Delayed meconium passage also increases the risk. Infants with risk factors should be monitored closely during the first few days and up to two weeks of their lives.^(1,5) The initial laboratory evaluation of jaundice depends on the age of the newborn and the kind of hyperbilirubinaemia. Haemoglobin, haematocrit, reticulocyte count, peripheral blood smear, ABO blood group typing, Rhesus typing, and nonagglutinating antibody level (Coombs test) should be determined in indirect hyperbilirubinaemia.^(2,4) Evaluation of G6PD deficiency and pyruvate kinase deficiency should be considered, especially for infants who are older than four days, have a positive family history, or are of East Asian, Greek, Mediterranean, or African descent.⁽²⁾ The goal of this study was to evaluate the aetiology of indirect hyperbilirubinaemia and the prevalence of G6PD in newborns who had been admitted to Vali-e-Asr Hospital in Zanjan City from 2001 through 2003.

METHODS

The research team reviewed the medical records of all the newborns who had been admitted to the neonatal ward or neonatal intensive care unit (NICU) in Vali-e-Asr Hospital, with the diagnosis of neonatal hyperbilirubinaemia, from May 2001 through February 2003. The diagnosis was based on the International Classification of Diseases,⁽⁶⁾ according to the final diagnosis written by the treating paediatrician. This hospital is the only paediatric residency teaching hospital of Zanjan University of Medical Sciences in Zanjan City, with a neonatal ward and NICU. Zanjan City is in the centre of Zanjan Province, which is located in the northwest of Islamic Republic of Iran and has nearly one million inhabitants. Newborns (less than 30 days old) with pathological hyperbilirubinaemia were admitted to this hospital for evaluation (clinical and laboratory) and treatment (phototherapy and/or exchange transfusion), according to the criteria mentioned in Nelson Textbook of Pediatrics.⁽⁴⁾ Some of the criteria for pathological hyperbilirubinaemia were (a) jaundice appearing in the first 24–36 hours of life, (b) the total serum bilirubin level rising at a rate faster than 5 mg/dL/day, (c) jaundice persisting after 14 days of life, (d) jaundice associated with pallor, hepatomegaly, splenomegaly, symptoms and signs of sepsis, abnormal vital signs, and signs of kernicterus, and (e) serum bilirubin level greater than 12 mg/dL in full-term (especially in the absence of risk factors) or 14 mg/dL in premature infants. The main

inclusion criterion was the presence of the G6PD activity test (normal or deficient) in the medical records.

Medical records were reviewed precisely and deliberately for pallor, cephalhaematoma, skin bruising, hepatomegaly, splenomegaly, signs and symptoms of sepsis, hypothyroidism, CNS haemorrhage, type of treatment, maternal and neonatal blood groups, direct antibody test (Coombs test), serum levels of total and direct bilirubin concentration, haemoglobin and haematocrit levels, reticulocyte count, peripheral blood smear G6PD test, and results of blood, cerebrospinal fluid, urine and faecal cultures, as well as other tests and X-rays. It is important to mention that maternal and neonatal blood groups, Coombs test, haemoglobin and haematocrit levels, reticulocyte count, peripheral blood smear, and G6PD activity test were performed routinely for all the newborns admitted for the management of neonatal hyperbilirubinaemia, but if there were clinical manifestations of hypothyroidism (or jaundice persisting beyond 14 days) or sepsis, clinicians attending to the infants would request the measurement of thyroxine and thyroid stimulating hormones, and order sepsis work-up, respectively. All necessary information had been recorded on standardised questionnaires.

In the absence of other causes, aetiology of sepsis was ascribed to newborns with positive blood cultures and/or features of infection necessitate antibiotics therapy for seven or more days. In this study, an undetermined aetiology included:

1. Exaggerated physiological jaundice (jaundice occurring after the third day of life in healthy, full-term newborns),
2. Prematurity-associated jaundice (exaggerated physiological jaundice occurring in newborns less than 37 weeks of gestational age),
3. Breastfeeding jaundice (breastfeeding newborns who had no identifiable risk factor for jaundice and which settled by 10–14 days of age),
4. Unknown causes which included:
 - a. Haemolysis with positive Coombs test, and/or abnormal peripheral smear, and/or elevated reticulocyte count that were neither ABO/Rhesus incompatible nor G6PD deficient,
 - b. Maternal blood swallowed during labour without bloody vomiting,
 - c. CNS haemorrhage with normal clinical manifestations (so imaging studies were not done), and
 - d. No identifiable aetiology was found.

Data entry, statistical analysis and calculations were performed with the use of the Statistical Package for Social Sciences software for Windows version 10.0 (SPSS Inc, Chicago, IL, USA). Results were shown as frequency, percent, median, interquartile range, range, minimum and maximum. For continuous variables, the

Mann-Whitney U-test was used to compare data that did not have a normal distribution. Significance was defined as $p < 0.05$. The G6PD activity of red blood cells was determined by measurement of the rate of increase in nicotinamide adenine dinucleotide phosphate absorbance at 340 nm⁽⁷⁾ using a photometer (Eppendorf ECOM-E 6125, Germany), and the results were expressed as unit per gramme haemoglobin (U/g Hb). The normal range of G6PD activity was 7.5–14.5 U/g Hb. Serum total and conjugated bilirubin levels were determined with a modified diazo method,⁽⁸⁾ using an automated clinical analyser (Vitalab selectra 2, Netherlands). The study was approved by the research committee of Zanzan University of Medical Sciences.

RESULTS

The study included 376 newborns, 159 (42.3%) of them were boys and 217 (57.7%) were girls. 375 newborns were breast-fed. The prevalence of ABO incompatibility, Rhesus incompatibility, cephalohaematoma, sepsis, and G6PD deficiency were 3.7% (14 neonates), 2.1% (eight neonates), 0.5% (two neonates), 15.7% (59 neonates), and 2.1% (eight neonates) in the total study population, respectively (Table I). The aetiology was not determined in 75.8% (285) of newborns. We did not find any cases of CNS haemorrhage, polycythaemia, intestinal atresia or stenosis, delayed meconium passage, hypothyroidism, and trauma from instrumented delivery. Among G6PD-deficient newborns, seven (1.9%) were boys and one

Table I. Causes of indirect neonatal hyperbilirubinaemia.

Causes	Number (%)
ABO incompatibility	14 (3.7)
Rhesus incompatibility	8 (2.1)
Cephalohaematoma	2 (0.5)
Sepsis	59 (15.7)
G6PD deficiency	8 (2.1)
Undetermined (Exaggerated physiological jaundice, prematurity, breastfeeding jaundice, unknown)	285 (75.8)
Total	376 (100)

(0.3%) was a girl. The median, range, interquartile range, minimum and maximum of the highest total bilirubin concentration (mg/dL), reticulocyte count (percent), and the lowest haemoglobin level (g/dL) of normal G6PD and G6PD-deficient newborns are shown in Table II.

Direct Coombs test was positive in six (1.6%) newborns. All of them had ABO incompatibility with spherocytosis in their peripheral blood smears. In Coombs-positive newborns, the median (and interquartile range) of the highest total bilirubin concentration, reticulocyte count and the lowest haemoglobin level were 21.3 (19.3–31.5) mg/dL, 11.5% (5.9%–18.6%), and 11.4 (9.4–13.4) g/dL, respectively. Direct Coombs test was negative in 369 newborns (the result for one girl was missing). In

Table II. The highest total bilirubin concentration, lowest haemoglobin level and the reticulocyte count in normal G6PD and G6PD-deficient newborns.

Variable	Normal G6PD (n = 368)	G6PD-deficient (n = 8)
Highest bilirubin concentration (mg/dL)		
Median	18	18.4
Interquartile range	15.8–20	16.3–19.5
Range	23.9	10
Minimum	9.1	14.9
Maximum	33	24.9
Lowest haemoglobin level (g/dL)		
Median	15.4	15.5
Interquartile range	13.6–17.1	13.5–18.4
Range	16.5	7.8
Minimum	6.5	12
Maximum	23	19.8
Reticulocyte count (percent)		
Median	1.8	1.7
Interquartile range	1.2–3.5	1.2–3.8
Range	34.5	4.3
Minimum	0.5	1.2
Maximum	35	5.5

Coombs-negative newborns, the median (interquartile range) of the highest total bilirubin concentration, reticulocyte count, and the lowest haemoglobin level were 18 (15.8–20) mg/dL, 1.8% (1.2%–3.5%), and 15.4 (13.8–17.2) g/dL, respectively. Exchange transfusions were done in 14 (3.7%) newborns, of which seven were male and seven were female. Two had positive Coombs test, and nine had sepsis. Pre-exchange serum bilirubin levels for these babies were above 20 mg/dL (except in one septic newborn girl who exchanged with bilirubin level of 17.6 mg/dL, reticulocyte count of 5.6 and negative Coombs test), and post-exchange serum bilirubin levels were approximately less than 50% of pre-exchange levels. Phototherapy was considered for all of these babies before exchange transfusion. No exchange transfusion was done in G6PD-deficient newborns.

DISCUSSION

In this study, the cause of jaundice could not be determined in 75.8% of the newborns. Undetermined aetiology was higher in our study in relation to other investigations.^(2,9) It may be due to:

1. Lack of symptoms, signs and laboratory evidences of CNS haemorrhage, polycythaemia, intestinal atresia or stenosis, delayed meconium passage and hypothyroidism.
2. Different classifications of the causes of neonatal hyperbilirubinaemia. We categorised exaggerated physiological jaundice, prematurity and breastfeeding jaundice under the title of undetermined aetiology. The aetiology of extreme hyperbilirubinaemia in newborns admitted to an NICU in southern Turkey has been idiopathic in 65.6% of cases. The Turkish classification included isoimmunisation (presumed ABO incompatibility), increased haemolysis (no sepsis or G6PD deficiency), G6PD deficiency, sepsis, hypothyroidism, and idiopathic.⁽¹⁰⁾ Some investigators agree that in up to 50% of infants with severe jaundice, breastfeeding and prematurity are the main causes identified despite extensive work-ups. In such cases, laboratory evaluation is suggested to be fairly minimal because test results are often not revealing and helpful, even in the presence of haemolysis.⁽²⁾ There is no data available on the sensitivity and specificity of routine testing for hyperbilirubinemia.⁽²⁾
3. Incapability to confirm decreased bilirubin conjugation or to measure the activity of uridine diphosphoglucuronic acid glucuronosyl transferase (UGT). Physiological and breast milk jaundice are usually due to decreased bilirubin conjugation.⁽⁴⁾ Incomplete maturation of UGT 1A1 enzyme in premature infants may result in diminished bilirubin conjugation, placing these neonates at especial risk of hyperbilirubinaemia.⁽¹¹⁾ Some investigators believe that diminished bilirubin conjugation is another mechanism

of jaundice in the G6PD-deficient newborns.^(11–13)

4. In patients with acute haemolysis, the result of G6PD deficiency test may be falsely negative because immediately after haemolytic episode, reticulocytes and young erythrocytes predominate. Reticulocytes and young erythrocytes have normal or near-normal enzyme activity.⁽¹²⁾

Statistical analyses of our results showed that there were no differences in the highest total bilirubin concentration ($p = 0.7$), reticulocyte count ($p = 0.7$), and the lowest haemoglobin level ($p = 0.8$) between normal G6PD and G6PD-deficient newborns. Comparison of Coombs-negative with Coombs-positive newborns did not reveal a significant difference in the highest bilirubin concentration ($p = 0.08$), but reticulocyte count ($p = 0.0005$) and the lowest haemoglobin level ($p = 0.02$) reveal significant differences. It means that in Coombs-positive newborns, the reticulocyte count was higher and haemoglobin level was lower, in comparison to Coombs-negative newborns. In the present study, one girl was G6PD-deficient, and her G6PD activity was 4 U/g Hb. She may be a G6PD-deficient homozygote or heterozygote. The gene for G6PD deficiency is transmitted as a sex-linked trait with severe enzyme deficiency, occurring only in hemizygote males and homozygote females. Heterozygous females often have normal G6PD activity, but some may have intermediate activity, and others may have low activity.⁽¹⁴⁾ Random X-chromosome inactivation results in two RBC populations in female heterozygotes. One population consists of RBCs with normal G6PD activity, and the other consists of G6PD-deficient cells. X-inactivation may be non-random or one or the other clone may be selected preferentially. There may be varying phenotypes, and the RBCs of heterozygotes may exhibit normal, intermediate, or grossly deficient G6PD activity.⁽¹⁵⁾

The most devastating clinical consequence of G6PD deficiency is neonatal hyperbilirubinaemia which can be severe, and result in kernicterus or even death.^(13,15) Brown and Johnson reported 23 cases of kernicterus occurring since 1989, 16 in term newborns and seven in near-term newborns.⁽¹⁶⁾ In these newborns, peak unconjugated bilirubin concentrations measured 22–50 mg/dL. Kernicterus was associated with G6PD deficiency (five infants), ABO incompatibility (one infant), haemolysis of unknown cause (five infants), dehydration (seven infants), familial aetiology (one infant) and unexplained jaundice in the first 24 hours of age (six infants). Following the introduction of neonatal population screening programmes and major health awareness campaigns by the government, there was a drastic decrease in the incidence of neonatal hyperbilirubinaemia and acute haemolytic anaemia in G6PD-deficient patients in Taiwan and Singapore.⁽¹⁵⁾

According to the report of World Health Organisation (WHO), 2.9% of the world's population are G6PD-deficient and Iran is in a moderately high incidence area (10%–15%) for G6PD deficiency.⁽¹⁷⁾ The WHO recommends neonatal screening on cord blood samples in populations where G6PD deficiency is common.⁽¹⁷⁾ Cord blood screening of newborns for G6PD deficiency in 2,000 neonates (50.3% were boys) in two hospitals in Tehran (capital of Iran) was done from April to December 1999.⁽¹⁷⁾ Their results showed that 2.1% of the total population (3.6% of males and 0.6% of females) were G6PD-deficient. Their research revealed a relatively lower incidence of G6PD deficiency, similar to our study. There are reports of G6PD deficiency prevalence in other parts of Iran that differ from the Tehran study.⁽¹⁶⁾ These reports indicate that the northern and southeastern provinces of Iran have higher rates of G6PD deficiency (8.65%–16.4% in the northern part [Mazandaran and Guilan Provinces], 12% in the southern part [Shiraz] and 19.3% in the southeastern part of Iran). High rates of G6PD deficiency in certain areas of Iran may represent the higher rate of G6PD deficiency throughout the country, compared to our results and those from the Tehran report.⁽¹⁷⁾ These differences are mainly due to geographical and population variations, but there are also differences in study population. The population of Mazandaran and Guilan Provinces differ from the Shiraz and Tehran populations in ethnicity. The Tehran population was mostly from the central provinces of Iran and included some immigrants from other provinces, and did not represent the population of all parts of Iran.

While studies from the northern Pakistan (predominantly of Path ethnicity) reported a 7%–8% prevalence of G6PD deficiency, the results from Karachi (a multiethnic city in southern Pakistan) revealed only a 2% prevalence.⁽¹⁸⁾ This is comparable to reports from Singapore and Malaysia, although the prevalence is less than reports from the Middle East and India.⁽¹⁸⁾

The major limitation of this study is that it has an analytical retrospective design. In our residency teaching hospitals, nearly all patients were examined thoroughly and deliberately by the attending clinicians and residents, as well as by medical students. Moreover, all medical records were written correctly and completely every day, and were reviewed carefully.

In conclusion, neonatal hyperbilirubinaemia is one of the most common problems and requires hospital admission for investigation and treatment. Despite a low prevalence of G6PD deficiency in our study, we recommend that G6PD deficiency tests be performed in all Iranian and Mediterranean icteric newborns, unless

other investigators ascertain and document that G6PD deficiency tests are not necessary to be done routinely. In addition, we recommend that measurement of the enzyme UGT be made available for the clinical use in the evaluation of neonatal hyperbilirubinaemia.

ACKNOWLEDGEMENTS

This study was supported by grants from the Research Committee of Zanjan University of Medical Sciences. We thank Dr Nouraddin Mousavinasab, Assistant Professor of Medical Statistics, Zanjan University of Medical Sciences, for analysing the data used in the study. We are grateful to Miss Elham Hosseinejad, Ms Parvin Soleymanloo and Mr Siamak Moraveji for their help with editing.

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