CASE REPORT

Prolonged indirect hyperbilirubinemia in a moderately preterm boy with Mediterranean glucose-6-phosphate dehydrogenase and glutathione S-transferase Mu 1 null mutations

Sameer Yaseen Al-Abdi

Department of Pediatrics, King Abdulaziz Hospital, MNGHA, Al-Ahsa, Saudi Arabia

Correspondence to

Dr Sameer Yaseen Al-Abdi,
abdis@ngha.med.sa

Accepted 23 December 2016

SUMMARY

A 33-week gestation boy with Mediterranean glucose-6-phosphate dehydrogenase (G6PD) and a glutathione S-transferase Mu 1 null mutations (GSTM1*0/*0) developed prolonged indirect hyperbilirubinemia (PIH). He had no laboratory evidence of haemolysis or infection, and no exposure to oxidising agents. He has two full-term older brothers who have no history of neonatal hyperbilirubinemia. One brother, who was exclusively breast fed, has only Mediterranean G6PD and the other has only GSTM1*0/*0. The three boys have no mutation in the uridine diphosphate glucuronosyltransferase 1A1 gene. This suggests that a combination of all or any two of prematurity, G6PD deficiency and GSTM1*0/*0 is a possible risk factor for PIH. However, this remains to be confirmed.

BACKGROUND

Neonatal indirect hyperbilirubinemia (total serum bilirubin (TSB) >85 µmol/L) that continues beyond 21 days of age in preterm infants is very common and termed prolonged indirect hyperbilirubinemia (PIH).^{1 2} Glucose-6-phosphate dehydrogenase (G6PD) deficiency is known to be a risk factor for the PIH.^{1 3 4} The impetus for this report is the recent suggestion that decreased hepatic glutathione S-transferase (GST) is a plausible mechanism of G6PD deficiency-associated hyperbilirubinemia.⁵ Thus, a case of PIH in a moderately preterm boy with Mediterranean G6PD and homozygous GST Mu 1 null mutation (GSTM1*0/*0) is presented here.

CASE PRESENTATION

A healthy, appropriate-for-gestational-age (AGA), preterm baby boy was born to Saudi parents at a 33^{3/7}-week gestation and 2095 g birth weight. The boy was admitted to level IIA nursery for 9 days. The mother is a 35-year-old healthy G3P2. Her blood group is A Rh+, and her indirect Coombs test and erythrocyte antibody screening were negative. Her pregnancy was complicated by preterm premature rupture membranes at 32^{5/7} weeks (5 days before delivery). The mother received ampicillin and erythromycin intravenously for 48 hours, followed by oral administration for 5 days, two doses of 12 mg intramuscular dexamethasone and no oxytocin. Despite the mother having no signs of chorioamnionitis, the placental histopathology showed severe acute suppurative chorioamnionitis

and funisitis. Besides, C reactive protein for the baby was 16.7 mg/L, thus, the baby was treated with 7 days intravenous ampicillin and gentamicin despite his blood culture being negative.

Cord blood was sent for measurement of G6PD activity, as universal Cord blood for G6PD deficiency is a standard care in our hospital. The baby's cord G6PD activity was 0.1 U/g of haemoglobin (U/g Hb) which is extremely low; our lower reference limit is 11.0 U/g Hb. The first TSB assessment was performed at 12 hours of age (HOA), which was 99.2 µmol/L and more than the phototherapy threshold of the National Institute for Health and Clinical Excellence (NICE), as shown in [figure 1](#). The feeding started in the second day of life and then increased gradually as per our unit protocol until full feeding was reached at day 7 of age. The baby received intravenous fluid during the first 8 days of life and he was discharged home at day 10 of life with a discharge weight of 2021 g. He was mostly being fed formula milk and occasionally breastmilk during hospitalisation. The baby was discharged home at day 10 of life with a bilingual (English and Arabic) leaflet listing food and herbal remedy advice, and was scheduled to repeat the TSB assessment as an outpatient.

He continued to have hyperbilirubinemia until his final clinic visit at day 72 of life with a TSB of 92 µmol/L ([figure 1](#)). He was exclusively feeding breastmilk after discharge (day 10) and until day 40 of age when the parents reintroduced milk formula. He was gaining weight adequately. The baby and his mother were never exposed to an oxidising agent that is known to cause haemolysis in patients with G6PD deficiency. He had no laboratory evidence of haemolysis ([table 1](#)).

INVESTIGATIONS

In this preterm infant, a PCR amplification and pyrosequencing of chromosome Xq28 for the following six G6PD mutations were performed: c.202G>A, c.376A>G, c.563C>T, c.1003G>A, c.1376G>C and c.1376G>T. It revealed that he has c.563 C>T mutation (Mediterranean G6PD mutation). His PCR-restriction fragment length polymorphism of glutathione S-transferase Mu1 (GSTM1) and glutathione S-transferase Theta 1 (GSTT1) genes revealed that he has GSTM1*0/*0 and at least one active allele of GSTT1 gene. His PCR amplification and direct sequencing of the

CrossMark

To cite: Al-Abdi SY. *BMJ Case Rep* Published online: [please include Day Month Year] doi:10.1136/bcr-2016-218404

Figure 1 Total serum bilirubin levels and phototherapy duration plotted on phototherapy chart for 33-week gestation. The figure is adapted and modified with permission from the 2010 National Institute for Health and Clinical Excellence phototherapy chart (NICE Clinical guideline 98) updated on October 2016: available from <https://www.nice.org.uk/guidance/cg98>.

uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) gene (chromosome 2q37.1) revealed that he has a homozygous normal sequence promoter (A(TA)₆TAA/A(TA)₆TAA) and no mutation in exons 1–5 and respective exon–intron boundaries. His maximum direct bilirubin was 8.7 µmol/L. His electrolytes and blood urea nitrogen were normal, albumin was 3.4–3.9 g/dL and cord blood thyroid-stimulating hormone was 1.89 mIU/L. His other relevant blood investigations are outlined in [table 1](#).

This preterm infant has two older brothers, 6 years and 10 years old, who were born at full term (≥37 weeks gestation) and were AGA. Both older brothers have no history of neonatal

hyperbilirubinemia. The 6-year-old brother is known to have G6PD deficiency. His cord G6PD activity was 0.3 U/g Hb and his TSB was 96 µmol/L at 25 HOA. The 10-year-old brother is G6PD normal. His G6PD activity was 12.4 U/g Hb at 10 years of age and his TSB was 209 µmol/L at 75 HOA. Genetic analyses of these two brothers were performed based on the parents' request. Their molecular testing revealed that the 6-year-old brother has Mediterranean G6PD mutation and at least one active GSTM1 allele. The 10-year-old brother has GSTM1*0/*0 and none of the six tested G6PD mutations were detected. Their results of GSTT1 and UGT1A1 gene testing were similar to their younger brother (proband case).

Table 1 Basic laboratory characteristics of the preterm infant

Laboratory test	Age of the preterm infant			
	At birth	At Day 4	At Day 26	At Day 72
Haemoglobin level (g/L)	145	126	113	5
Haematocrit (%)	46	37	32	30
Mean corpuscular volume (fL)	95.4	93	85	80
Mean corpuscular haemoglobin (pg)	30	30	30	25
Mean corpuscular haemoglobin concentration	315	327	350	315
Red blood cell (RBC) distribution width (%)	18.6	18.7	17.6	16.8
Nucleated RBC/100 white cell counts (%)	2.0 (manual)	7.0 (automated)	Zero (automated and manual)	2.0 (automated)
Automated reticulocytes (%)	Not performed (NP)			2.05
Peripheral blood smear				
Anisocyte	1+	NP	1+	1+
Polychrome	1+		1+	None
Ovalocytes	1+		None	None
Target cells	Occasional		Occasional	Occasional
Tear drop	none		1+	None
Spherocyte	none		Occasional	None
Schistocyte	none		Occasional	None
Hypochrome	1+		None	1+
Toxic granules	1+		None	None
Toxic vacuoles	1+		None	None

DIFFERENTIAL DIAGNOSIS

A combination of all or any two of prematurity, G6PD deficiency and GSTM1*0/*0 is a possible risk factor for PIH.

TREATMENT

The baby's hyperbilirubinemia was treated by 96 hours of phototherapy on two separate occasions, of which 36 hours was double phototherapy (figure 1).

OUTCOME AND FOLLOW-UP

He continued to have hyperbilirubinemia until his final clinic visit at day 72 of life with a TSB of 92 $\mu\text{mol/L}$ (figure 1).

DISCUSSION

This moderate preterm infant developed PIH and is deficient in G6PD and GSTM1, whereas neither of his two full-term brothers, who are deficient in only one of these (G6PD or GSTM1), developed hypalbuminemia. In addition, his G6PD-deficient brother was exclusively breast fed during his first 6 months of life. This preterm infant had no exposure to oxidising or haemolysing trigger agents,^{6,7} no laboratory evidence of haemolysis or infection,⁸ and no mutation in UGT1A1 gene. Thus, it can be inferred that a combination of all or any two of prematurity, G6PD deficiency and GSTM1*0/*0 mutation is a possible risk factor for PIH. However, prematurity is less like the cause of PIH here as discussed later.

The G6PD Mediterranean is known to be a risk factor for indirect neonatal hyperbilirubinemia.⁴ Identifying G6PD Mediterranean in the proband case and one of his brothers is expected for two reasons. First, their cord G6PD levels were extremely low.⁹ Second, the G6PD Mediterranean is the most common type of G6PD deficiency among people from AL-Ahsa area, Eastern Province of Saudi Arabia.¹⁰

The GSTM1 is the most expressed GST in the liver, followed by GST-Theta 1 (GSTT1).⁵ These GSTs bind cytosolic bilirubin and transfer it to the smooth endoplasmic reticulum for conjugation.⁵ Supposedly, bilirubin will return to the circulation in case of GSTM1 or GSTT1 null allele mutation (zero activity).⁵ Results from the studies on the relationship between null allele mutation of GSTM1 (GSTM1*0/*0) or GSTT1 (GSTT1*0/*0) and hyperbilirubinemia are inconsistent.⁵ One study found that mean TSB levels were significantly higher in infants with ABO incompatibility and with no GSTM1 activity (GSTM1*0/*0 mutation) compared to infants with ABO incompatibility and with some GSTM1 activity (GSTM1 gene).¹¹

Two physiological characteristics of preterm infants may be accounted for their higher risk of developing hyperbilirubinemia than full-term infants. First, life span of red blood cells (RBCs) is a gestational age dependent.⁸ It is expected that preterm infants have shorter RBC life span than term infants.⁸ Lacking laboratory evidence of haemolysis despite coexistence of severe G6PD deficiency indicates that reduced RBC life span plays a minor role here, if any. Having said that, it should be kept in mind that carboxyhaemoglobin and end-tidal carbon monoxide, which are more sensitive and specific tests for diagnosis of haemolysis, were not performed.⁸ Secondly, at birth, UGT1A1 activity in infants born between 32 and 34 weeks gestation

(moderate preterm) on average is about 50% of those born at full-term.¹² Activity of UGT1A1 in preterm infants markedly rises after 7 days of life regardless of gestational age and became equivalent to that of full-term infants aged 8–28 days.¹² Thus, UGT1A1 activity after 7 days of life of proband case supposed to be equivalent to UGT1A1 activity of his two brothers at birth.

Nevertheless, the assumption that the combination of G6PD deficiency and GSTM1*0/*0 mutation is the possible risk factor for PIH needs to be confirmed in future studies, including re-analysing the stored DNA of previous studies on G6PD deficiency-associated neonatal hyperbilirubinemia.

Learning points

- A combination of G6PD deficiency and GSTM1*0/*0 mutation is a possible risk factor for prolonged indirect hyperbilirubinemia-GSTM1*0/*0 mutation should be considered in the future studies addressing mechanisms of G6PD deficiency-associated neonatal hyperbilirubinemia or at least re-analysing the stored DNA of previous studies.

Competing interests None declared.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- Gundur NM, Kumar P, Sundaram V, *et al.* Natural history and predictive risk factors of prolonged unconjugated jaundice in the newborn. *Pediatr Int* 2010;52:769–72.
- Maisels MJ, Clune S, Coleman K, *et al.* The natural history of jaundice in predominantly breastfed infants. *Pediatrics* 2014;134:e340–5.
- Turbendian HK, Perlman JM. Glucose-6-phosphate dehydrogenase deficiency in triplets of African-American descent. *J Perinatol* 2006;26:201–3.
- Minucci A, Concolino P, De Luca D, *et al.* A prolonged neonatal jaundice associated with a rare G6PD mutation. *Pediatric blood Cancer* 2009;53:475–8.
- Al-Abdi SY. Decreased glutathione S-transferase level and neonatal hyperbilirubinemia associated with glucose-6-phosphate dehydrogenase deficiency: a perspective review. *Am J Perinatol* 2016.
- Lee SW, Lai NM, Chaiyakunapruk N, *et al.* Adverse effects of herbal or dietary supplements in G6PD deficiency: a systematic review. *Br J Clin Pharmacol* 2017;83:172–9.
- Youngster I, Arcavi L, Schechmaster R, *et al.* Medications and glucose-6-phosphate dehydrogenase deficiency: an evidence-based review. *Drug Saf* 2010;33:713–26.
- Christensen RD, Yaish HM. Hemolysis in preterm neonates. *Clin Perinatol* 2016;43:233–40.
- Al-Abdi SY, Alsaigh AS, Aldawoud FL, *et al.* Lower reference limits of quantitative cord glucose-6-phosphate dehydrogenase estimated from healthy term neonates according to the clinical and laboratory standards institute guidelines: a cross sectional retrospective study. *BMC Pediatr* 2013;13:137.
- Al-Abdi SY, Mousa TA, Al-Aamri MA, *et al.* Hyperbilirubinemia in glucose-6-phosphate dehydrogenase-deficient Male newborns in Al-Ahsa, Saudi Arabia. *Saudi Med J* 2010;31:175–9.
- Abdel Ghany EAG, Hussain NF, Botros SKA. Glutathione S-transferase gene polymorphisms in neonatal hyperbilirubinemia. *J Invest Med* 2012;60:18–22.
- Kawade N, Onishi S. The prenatal and postnatal development of UDP-glucuronyltransferase activity towards bilirubin and the effect of premature birth on this activity in the human liver. *Biochem J* 1981;196:257–60.

Copyright 2017 BMJ Publishing Group. All rights reserved. For permission to reuse any of this content visit <http://group.bmj.com/group/rights-licensing/permissions>.
BMJ Case Report Fellows may re-use this article for personal use and teaching without any further permission.

Become a Fellow of BMJ Case Reports today and you can:

- ▶ Submit as many cases as you like
- ▶ Enjoy fast sympathetic peer review and rapid publication of accepted articles
- ▶ Access all the published articles
- ▶ Re-use any of the published material for personal use and teaching without further permission

For information on Institutional Fellowships contact consortiasales@bmjgroup.com

Visit casereports.bmj.com for more articles like this and to become a Fellow