

Lessons from the Molecular Biology of Neonatal Hyperbilirubinaemia

H. Van Rostenberghe¹, ~~N. Yusoff~~ H. Nishio, M. Matsuo, R. Noraida and W.I. Wan Pauzi

¹Medical School, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Summary

Compared to Caucasians, South East Asian babies have more frequently jaundice, requiring phototherapy. This is partly due to the high prevalence of G6PD deficiency and other genetically determined haematological abnormalities. If no underlying cause is found, these babies tend to be labelled as having 'excessive' physiological jaundice or alternatively idiopathic pathological jaundice.

It is likely however that jaundice in newborns is a complex interplay between multiple genetic and environmental risk factors. Often more than one risk factor may be present such as in G6PD deficiency where recently it was discovered that neonatal jaundice is often only not accompanied by haemolysis. An association was found with Gilbert syndrome

One relatively recently described risk factor in the South East Asian population is the presence of point mutations in the coding regions for the gene of the uridine diphosphate glucuronyl transferase enzyme. These point mutations may decrease the activity of the enzyme responsible for glucuronidation of the indirect bilirubin just slightly. This can cause jaundice that is really excessive in babies with other risk factors.

Neonatal jaundice is most likely a real multifactorial pathological event based on many genetic and environmental factors. In future we will look more at a combination of risk factors for jaundice, rather than just attributing the jaundice to one risk factor alone. The number of babies labelled as 'excessive physiologic jaundice' or idiopathic jaundice might decrease significantly in the near future.

Introduction

Neonatal jaundice is the most common condition requiring medical attention in the newborn period. It has been classically attributed to a number of more or less common causes. The causes are classically divided in two main groups. The first group is comprising of conditions associated with an increase in break down of red blood cells such as Rhesus incompatibility, ABO blood group incompatibility, G6PD deficiency, birth trauma and polycythaemia. A second group consists of conditions in which the excretion of bilirubin is diminished such as breast feeding jaundice, breast milk jaundice, Gilbert syndrome or the more severe Crigler Najjar syndrome.

Many babies however get jaundice without any of the above causes being identified. In these babies the term physiological jaundice is often applied. Even if bilirubin levels go up in the phototherapy range it has been called 'excessive physiological jaundice' and if it approaches exchange transfusion level it tends to be called idiopathic pathological jaundice.

In the latest decade and especially since the advent of genomics there have been some remarkable discoveries that may change our perception of the pathophysiology of neonatal jaundice thoroughly.¹ Instead of attributing neonatal jaundice to a single condition as is classically done, we may realise in the near future that the presence of one risk factor for NNJ is often not enough to cause severe or even moderately severe NNJ. New risk factors have been identified and an association of several risk factors may be often present^{2,3} so that it may become more appropriate to count the number of risk factors for the development of neonatal jaundice in a particular child rather than attributing the jaundice to one or another risk factor.

G6PD Deficiency

G6PD deficiency is among the most common causes of neonatal jaundice in many populations. G6PD deficiency can lead to severe haemolytic crises in older children and adults after exposure to oxidant stresses (favism). That is why it was classically believed that the neonatal jaundice, associated with G6PD deficiency, is due to haemolysis as well. There are indeed very occasionally babies with G6PD deficiency having real favism-like presentations.⁴ In these babies a known oxidant stress or infection can often be identified. Most babies however with G6PD deficiency and neonatal jaundice present quite differently and have a gradual onset of jaundice, often only becoming visible on day 3 of life. In this latest kind of presentation, usually no obvious signs of haemolysis can be found.

Multiple studies have been undertaken looking at the presence of haemolysis in G6PD deficient neonates with jaundice. Measurement of

CO-haemoglobin (COHb) is a good way to assess bilirubin production and haemolysis since in the production of bilirubin, protoheme is split, releasing CO which is produced in equimolar quantities with bilirubin.⁵ Except for one study⁶ done in a Nigerian population, most studies measuring CO production in G6PD deficient neonates^{7,8,9} showed only a very slight increase in CO production compared to G6PD normal controls but CO production was found to be completely similar in those G6PD deficient neonates with and those without jaundice.

Furthermore Kaplan¹⁰ has shown quite conclusively that the levels of direct bilirubin were lower in G6PD deficient neonates with jaundice than in G6PD normal neonates with jaundice, indirectly indicating that there may be also a decrease in glucuronidation of bilirubin in those G6PD deficient neonates with jaundice. Further genetic analysis² of the same study population showed indeed a high prevalence of polymorphism of the promotor region of the UGT1A1 gene. (Gilbert Syndrome).

The currently available data suggest that the majority of babies with G6PD deficiency and neonatal jaundice do not have severe haemolysis. They have rather a very mild haemolysis that by itself may be not severe enough to cause jaundice but a combination of G6PD deficiency and other risk factors may be necessary to make the infants jaundiced.

The molecular basis of G6PD deficiency has been studied in the Malaysian population and it was discovered that the genotype is heterogeneous in Malays as well as in the Chinese race¹¹⁻¹³.

ABO Incompatibility

ABO incompatibility is another major cause of jaundice in the neonatal period. Often the babies get jaundice on day one of life. The direct Coombs test is 'falsely' negative in up to 70 % of the cases. This means that in up to 70 % of cases of ABO incompatibility with early onset jaundice we fail to show the presence of maternal antibodies against the blood group of the baby. This may be due to low titers of antibodies or to the immature expression on the red blood cells of the babies. Often we fail to show a drop in haemoglobin levels and if the expression of the blood group is so immature in many red blood cells, other factors than haemolysis alone may play a role in the pathophysiology of ABO incompatibility associated neonatal jaundice. Kaplan found that in a Jewish population, Gilbert syndrome was a determining factor for jaundice related to ABO incompatibility³.

Other Well Known Causes of Jaundice

A similar reasoning may be made for most of the other well known causes of jaundice. Some children with mild birth trauma develop severe jaundice while others with severe subaponeurotic haemorrhage do

not develop jaundice. Similar observations can be made for polycythaemia, breastfeeding jaundice and others. The subsequent discussion will cover some of such additional risk factors.

Gilbert Disease

This is an autosomal dominant condition in which a polymorphism of the promotor region of the UGT1A1 gene is observed. This disease occurs with a high frequency in some Caucasian populations. It can present in the neonatal period with jaundice and patients may get mild jaundice in episodes of stress. The babies with only this genetic variant and no other risk factors for jaundice may not get jaundice but those with other risk factors may be the ones who get jaundice.¹⁴

Other Mutations in the UGT1A1 Gene

A large variety of other mutations have been described in populations of different ethnic origin. However in the Southeast Asian population where jaundice has a very high incidence, only recently some attention has been given to the molecular analysis of the UGT1A1 gene. A first mutation has been described in the Thai population¹⁵ and has subsequently been found in a relatively high proportion of Malay babies with jaundice¹⁶.

Two other novel mutations in the same gene have been recently described in Malaysian babies¹⁷. These could be either real mutations or polymorphisms. In either case, it could be predisposing the neonates to develop jaundice if other risk factors are present. By itself it might disturb the bilirubin metabolism rather mildly and not cause severe jaundice

Other Potential Molecular Explanations of the NNJ

There are numerous other enzymes or transport proteins involved in the metabolism of bilirubin. There is the transport protein of bilirubin across the liver cells. There are also the heme oxidase and the biliverdin reductase. In each of these, there may be a genetic or environmental factor interfering enough with the metabolism of bilirubin to cause jaundice or be at least a significant risk factor worsening jaundice in the presence of other risk factors. Examples of these include the fact that some stress hormones such as corticoids or some endotoxins could increase the activity of biliverdin reductase and stimulate as such the bilirubin production¹⁸.

There is a vast field for future research in order to elucidate fully the pathophysiology of neonatal jaundice

Conclusion

Results of recent studies and developments in molecular biology are leading to newer insights in the pathophysiology of neonatal jaundice. Often significant jaundice may not be attributable to just one cause but neonatal jaundice has most likely a truly multifactorial causality. As our knowledge in the bilirubin metabolism on the molecular level advances, we may meet less and less 'excessive physiological jaundice' or idiopathic pathological jaundice. A better knowledge of all risk factors involved will lead to a more effective prevention of severe neonatal jaundice through identification and early treatment of high-risk cases.

References

1. Watchko JF, Daoood MJ, Biniwale M. Understanding neonatal hyperbilirubinaemia in the era of genomics. *Semin Neonatol*, 7(2):143-152,2002.
2. Kaplan M, Renbaum P, Levy-Lahad E, Hammerman C, et al. Gilbert syndrome and glucose-6-phosphate dehydrogenase deficiency: a dose-dependent genetic interaction crucial to neonatal hyperbilirubinemia. *Proc Natl Acad Sci U S A*, 94(22):12128-12132,1997.
3. Kaplan M, Hammerman C, Renbaum P, Klein G, et al. Gilbert's syndrome and hyperbilirubinaemia in ABO-incompatible neonates. *Lancet*, 356(9230):652-653,2000.
4. Kaplan M, Hammerman C. Glucose-6-phosphate dehydrogenase deficiency: a potential source of severe neonatal hyperbilirubinaemia and kernicterus. *Semin Neonatol*, 7(2):121-128,2002.
5. Engel RR, Rodkey FL, Krill CE, Jr. Carboxyhemoglobin levels as an index of hemolysis. *Pediatrics*, 47(4):723-730,1971.
6. Slusher TM, Vreman HJ, McLaren DW, Lewison LJ, et al. Glucose-6-phosphate dehydrogenase deficiency and carboxyhemoglobin concentrations associated with bilirubin-related morbidity and death in Nigerian infants. *J Pediatr*, 126(1):102-108,1995.
7. Kaplan M, Vreman HJ, Hammerman C, Leiter C, et al. Contribution of haemolysis to jaundice in Sephardic Jewish glucose-6-phosphate dehydrogenase deficient neonates. *Br J Haematol*, 93(4):822-827,1996.
8. Seidman DS, Shiloh M, Stevenson DK, Vreman HJ, et al. Role of hemolysis in neonatal jaundice associated with glucose-6 phosphate dehydrogenase deficiency. *J Pediatr*, 127(5):804-806,1995.
9. Van Rostenberghe H, Selamah G, Nik Zainal A, Nishio H. Poor correlation between hemolysis and neonatal jaundice in Malaysian G6PD-deficient babies. Submitted for publication to *Pediatr Int*.
10. Kaplan M, Muraca M, Hammerman C, Vilei MT, et al. Unconjugated and conjugated bilirubin pigments during perinatal development. V. Effect of phototherapy on serum conjugated bilirubin in hyperbilirubinemic neonates. *Biol Neonate*, 73(3):155-160,1998.
11. Yusoff NM, Shirakawa T, Nishiyama K, Ghazali S, et al. Molecular heterogeneity of glucose-6-phosphate dehydrogenase deficiency in Malays in Malaysia. *Int J Hematol*, 76(2):149-152,2002.

12. Ainoon O, Joyce J, Boo NY, Cheong SK, et al. Glucose-6-phosphate dehydrogenase (G6PD) variants in Malaysian Chinese. *Hum Mutat*, 14(4): 352,1999.
13. Ainoon O, Yu YH, Amir Muhriz AL, Boo NY, et al. Glucose-6-phosphate dehydrogenase (G6PD) variants in Malaysian Malays. *Hum Mutat*, 21(1):101,2003.
14. Ulgenalp A, Duman N, Schaefer FV, Whetsell L, et al. Analyses of polymorphism for UGT1*1 exon 1 promoter in neonates with pathologic and prolonged jaundice. *Biol Neonate*, 83(4):258-262,2003.
15. Sutomo R, Laosombat V, Sadewa AH, Yokoyama N, et al. Novel missense mutation of the UGT1A1 gene in Thai siblings with Gilbert's syndrome. *Pediatr Int*, 44(4):427-432,2002.
16. Sutomo R, Norlelawati A, Yusoff N, Van Rostenberghe H et al. Screening for G71R mutation of the UGT1A1 gene in the Javanese-Indonesian and Malay-Malaysian populations. *Pediatr Int*, 46(5), 2004
17. Norlelawati A, Van Rostenberghe H, Sutomo R, Matsuo M. et al. Novel Missense Mutations 964A→G and 1477G→C, in the UGT1A1 Gene in Malay Babies with Early Onset of Severe Neonatal Jaundice. Submitted for publication to *Annals Med Gen*
18. Dennery PA. The biology of heme oxygenase during development. *Neoreviews*, 2: e67-e73, 2001