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Glucose-6-phosphate dehydrogenase deficiency in Northeastern Thailand: prevalence and relationship to neonatal jaundice

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Background : *Glucose 6-phosphate dehydrogenase (G6PD) deficiency is a common cause of neonatal hyperbilirubinemia and hemolytic anemia in Thai population. The prevalence of G6PD deficiency in Northeastern Thailand is high.*

Objective : *To determine the prevalence of G6PD deficiency in Burirum province and compare bilirubin levels between the G6PD-deficient and normal neonates. To evaluate whether methemoglobin reduction test (MRT) of cord blood can be used as a screening test for G6PD deficiency.*

Setting : *Burirum Provincial Hospital*

Research design : *A prospective study*

Patients : *Normal full-term neonates born by Caesarian section between April - May, 2003 at the Burirum Provincial Hospital.*

Methods : *185 cord blood samples (97 males, 88 females) were assayed for G6PD enzyme activity by the WHO-recommended standard test and MRT. Bilirubin level was prospectively transcutaneously measured in the neonates once daily for 3-4 days and the results were compared between the G6PD-deficient and that of the normal.*

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Results : *The prevalence of G6PD deficiency is 21.7 % in male and 8.0 % in female. In 156 neonates with normal G6PD, their average bilirubin levels in the first 4 days of life were: 5.1, 7.9, 9.3, and 9.8 (SD = 2.8-3.3) mg/dl. The bilirubin levels during the first 4 days of life of the 28 neonates with G6PD-deficiency were slightly higher, but the difference was not of statistical significance than that of normal infants (6.1, 8.3, 9.9, and 10.9 mg/dl, respectively). The overall sensitivity of MRT of cord blood screening is high (92.9 %) but its specificity is low (73.9 %).*

Conclusions : *G6PD deficiency in Burirum is high, both in the male and the female. Neonates with G6PD-deficiency had slightly higher bilirubin levels than normal neonates in their early days of life. Methemoglobin reduction test is a useful cord blood screening test for G6PD deficiency.*

Keywords : *G6PD deficiency, Northeastern, Thai, Thailand, Population, Prevalence.*

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เพชรรัตน์ กิตติวัฒนาสาร, ชาลิสา หลุยเจริญ, ภัทธดนย์ สุขพันธ์, อิศรางค์ นุชประยูร. ภาวะพร่องเอนไซม์กลูโคส-6-ฟอสเฟต ดีไฮโดรจิเนส ในชาวภาคตะวันออกเฉียงเหนือ: ความชุกและผลต่อระดับบิลิรูบินในทารกแรกเกิด. จุฬาลงกรณ์เวชสาร 2546 ส.ค; 47(8): 471 - 9

วัตถุประสงค์ : *เพื่อศึกษาความชุกของภาวะภาวะพร่องเอนไซม์กลูโคส-6-ฟอสเฟต ดีไฮโดรจิเนส (G6PD) ในชาวอีสาน และผลภาวะพร่องเอนไซม์ต่อระดับบิลิรูบินในทารกแรกเกิด รวมทั้งบทบาทของการตรวจกรองเมทฮีโมโกลบิน รีดัคชั่น (MRT) ใน การวินิจฉัยภาวะพร่อง G6PD*

สถานที่ที่ทำการศึกษา : *โรงพยาบาลบุรีรัมย์*

รูปแบบการวิจัย : *การศึกษาแบบไปข้างหน้า*

ผู้ป่วยที่ได้ทำการศึกษา : *เด็กทารกที่คลอดโดยการผ่าตัดทางหน้าท้อง และตรวจวัดระดับบิลิรูบินในเด็กทารกเหล่านี้*

วิธีการศึกษา : *ผู้วิจัยทำการตรวจหาภาวะพร่อง G6PD ในเลือดจากรก 185 ตัวอย่าง ด้วยวิธี MRT และวิธีมาตรฐานขององค์การอนามัยโลก และติดตามวัดระดับบิลิรูบินในเด็กทารกแรกเกิดเหล่านี้ทางผิวหนังโดยไม่ต้องเจาะเลือด วันละครั้งเป็นเวลา 3-4 วันหลังคลอด และวิเคราะห์เปรียบเทียบระดับบิลิรูบินในเด็กที่พร่อง G6PD กับเด็กที่ระดับเอนไซม์ปกติ*

ผลการศึกษา : *เด็กทารก 185 รายโดยเป็นของทารกชาย 97 รายและหญิง 88 ราย ได้รับการตรวจ G6PD พบว่าเด็กชายพร่อง G6PD 21.7 % และเด็กหญิงพร่อง G6PD 8.0 % เด็กแรกเกิดปกติคือไม่พร่อง G6PD 156 ราย มีระดับบิลิรูบินเฉลี่ยในวันแรกถึงวันที่ 4 ของชีวิต คือ 5.1, 7.9, 9.3, และ 9.8 มก./ดล. ตามลำดับ (ค่าเบี่ยงเบนมาตรฐาน 2.8-3.3 มก./ดล.) ส่วนเด็กแรกเกิดที่พร่อง G6PD 28 ราย มีระดับบิลิรูบินสูงกว่าเด็กปกติเพียงเล็กน้อย (6.1, 8.3, 9.9, และ 10.9 มก./ดล.) แต่ไม่มีนัยสำคัญทางสถิติ การตรวจกรอง MRT มีความไวสูง (92.9 %) แต่ความจำเพาะค่อนข้างต่ำ (73.9 %)*

วิจารณ์และสรุป : *ภาวะพร่อง G6PD พบได้บ่อยในชาวอีสานทั้งชายและหญิง และทำให้เด็กแรกคลอดตัวเหลืองกว่าปกติเพียงเล็กน้อย สามารถตรวจกรองได้ด้วย MRT*

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common enzyme deficiency in Thailand and many parts of the world.[1] G6PD deficiency is an important cause of neonatal hyperbilirubinemia as well as episodic hemolysis in childhood and young adults.[1, 2] G6PD deficiency is highly prevalent in Northeastern Thai population,[3] but its relationship to neonatal jaundice has never been reported prospectively.

Methemoglobin reductase test (MRT)[4] has been widely used as a screening test to detect the prevalence of G6PD deficiency in various regions of Thailand because of its low cost. However, there were few published studies on the sensitivity of the test. Using G6PD activity assay as the gold standard, we examined the value of MRT in cord blood sample as a screening test for G6PD deficiency.

Patients and methods

All newborns who were delivered by Caesarian section at the Burirum Provincial Hospital between April 2 - May 25, 2003, were recruited in the study. Umbilical cord blood samples were obtained from the placenta after delivery of the newborns in operating rooms. Five milliliters of cord blood were mixed with acid-citrate-dextrose (ACD) and stored at 4°C until assay. All samples were tested by methemoglobin reduction test in Burirum, and shipped to King Chulalongkorn Memorial Hospital in ice for standard G6PD activity assay within 3 days after collection.

The methemoglobin reduction test (MRT) was performed according to a modified method of Brewer.[4] Forty μl ACD-preserved blood samples (packed cell volume 35-45 %) were mixed with 20 μl of 0.18 M Sodium nitrite ($NaNO_2$) solution and 20 μl methylene blue chloride (15 mg/dl in 0.9 % NaCl) and incubated at 37°C for 3 hours. For positive control, only $NaNO_2$ was added. For negative control, no reagent was added to the blood samples. Color change of the blood was assessed qualitatively. It was interpreted as G6PD deficiency when the sample was dark brown and similar to the positive control, G6PD was normal when the color was red and similar to negative control, and intermediate if the color was between red and brown.

G6PD activity assay was performed according to the WHO-recommended standard test [5] with a minor modification. Two ml of citrated blood were washed with 5 volumes of cold normal saline 3 times, then buffy coat was removed. The washed red cells were assayed for hemoglobin concentration, then 50 μl were mixed with 950 μl ddH_20, mixed and frozen at -20°C for 40 minutes. Lysed red cells were centrifuged at 3000 rpm (5000 g) for 20 minutes, the supernatant was used for the G6PD activity assay. In a final volume of 1 ml, 50 μl of the red cell lysate was incubated with 200 μM nicotinamide adenine dinucleotide phosphate (NADP), 100 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, and 600 mM glucose-6-phosphate at 22°C. The NADPH was assayed spectrophotometrically at 340 nm over 5 minutes with Spectronic 401 (Milton Roy). The G6PD activity (IU/g Hb) was calculated with an equation (OD change per minute x 2138.08 / Hb). The G6PD activity that was less than 1.5 IU/ g Hb was considered deficient. [6]

For neonates whose cord blood was obtained and their mothers had consented for the study, their bilirubin level was monitored transcutaneously by Bilicheck®, a multi-wavelength spectral reflectance bilirubinometer device (SpectRx, Norcross USA)[7]

according to manufacturer's instruction, beginning from the first 24 hours of life (day 1), and then once daily until discharged. Briefly, the device is calibrated before each measurement by a reference standard (called Bilical). The transcutaneous bilirubin measurement was performed on the forehead, where the probe was positioned on the neonate's skin. In each measurement, the bilirubinometer device scan for the reflection of light spectrum 5 times, calculate the an average value of bilirubin level, and display in mg/dl. If an erroneous measurement was found, an error message was displayed and the scan needed to be repeated.

The serial transcutaneous bilirubin results were compared between G6PD-deficient and non-deficient (G6PD normal) group and statistically tested using a unpaired T-tests using Excel® (Microsoft, USA) 6.0 program.

Results

Prevalence of G6PD deficiency

During the 2-month study period, there were 197 full-term neonates born by Caesarian section at the Burirum Provincial Hospital. One hundred and eighty-five cord blood samples were successfully collected and were subsequently assayed for red cell G6PD activity. The average gestational age (± 1 Standard deviation; SD) was 38.5 ± 1.7 weeks. The cut-off level for the diagnosis of G6PD deficiency was below 1.5 IU/g Hb.[5] Twenty-one (21.7 %) of 97 male newborns and 7 (8.0 %) of 88 female newborns were G6PD deficient. Some of the G6PD deficient male newborns (4 of 21) had undetected G6PD activity.

Neonatal hyperbilirubinemia in G6PD deficiency

G6PD is a common cause of neonatal hyperbilirubinemia.[2, 8] However, it was not known whether all G6PD-deficient neonates will develop jaundice. The bilirubin level of each newborn whose cord blood G6PD activity was known was prospectively measured transcutaneously once everyday form the first day of life until discharged. Of 180 newborns, most of them (167, 92.8 %) remained in the nursery for 72 hours, and 114 (63.3 %) after 96 hours.

The transcutaneous bilirubin levels of 28 G6PD-deficient newborns and 152 G6PD-normal newborns were compared. In both groups, their bilirubin levels rose during the first 72 hours and reached a plateau between 72-96 hours. The bilirubin levels of G6PD-deficient newborns were slightly higher, but without significance, during their first 4 days of life (Table 1).

Table 1. Transcutaneous bilirubin levels (mg/dl) in neonates with or without G6PD deficiency.

G6PD activity	Hr <24		Hr 24-48		Hr 48-72		Hr 72-96	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
normal	140	5.1 ± 3.5	152	7.9 ± 2.3	144	9.3 ± 2.8	95	9.8 ± 3.6
deficient	28	6.1 ± 2.5	26	8.3 ± 2.8	23	9.9 ± 2.8	19	10.9 ± 3.3
T-test	p = 0.15		p = 0.26		p = 0.14		p = 0.09	

Table 2. The methemoglobin reduction test (MRT) compared to the standard G6PD activity in cord blood screening. Each cord blood sample was assayed for both tests. Number of samples with each result is shown.

G6PD Activity / MRT	<1.5 IU/g Hb (Deficient)		>1.5 IU/g Hb (normal)		Total		Grand Total
	Male	Female	Male	Female	Male	Female	
Deficient	20	6	14	27	34	33	67
Intermediate	0	0	12	12	12	12	24
Normal	1	1	50	42	51	43	94
Total	21	7	76	81	350	88	185
Grand Total	28		157		185		

The role of the methemoglobin reduction test (MRT) as screening test for G6PD deficiency

To assess the role of MRT as a screening test, the result of MRT was compared to the gold standard G6PD assay (Table 2). Of 28 G6PD-deficient cord blood samples, MRT was able to diagnose correctly, 26 cases (92.0 %). Of 157 non-G6PD-deficient samples, MRT correctly identified only 92 (60 %), representing rather a non-specific test. If the intermediate results were also considered non-deficient, the specificity of MRT rose to 73.9 %. The specificity was higher in males (62 of 76; 81.9 %) than in females (54 of 81; 66.7 %). The positive predictive value of MRT for G6PD deficiency was rather low (38.8 %), but the negative predictive value was high (98.3 %).

Discussions

The prevalence of G6PD deficiency in the Northeastern region of Thailand is high.[3] According to previous reports,[3,9-12] the range of the prevalence was between 12.7 % to 20.1 %. These studies were done between 1962-1968 based on various screening methods, including MRT and dye-screening test. In our study, we provided an accurate and unbiased prevalence in a population survey using the gold standard assay for G6PD activity in cord blood samples and confirmed the high prevalence of G6PD deficiency in Northeastern region of Thailand.

The prevalence of G6PD deficiency in the Northeastern region of Thailand is higher than that of Bangkok population (11-12 % of male).[8,13] This may be explained by a higher proportion of assimilated Chinese, the population with much lower prevalence of G6PD deficiency (2.6 % in male), [14] in Bangkok. G6PD deficiency is inherited in an X-linked pattern, which explains that males who have defective genes are more commonly found to be G6PD-deficient than females. With the prevalence of 21.6 % mutant gene in the X-chromosomes, 4.7 % of the female are predicted to be homozygote for G6PD mutants according to Hardy-Weinberg principles. Therefore, by the standard G6PD assay, a significant number of G6PD-deficient females (3.3 % of females) are heterozygotes.

G6PD deficiency is a well recognized cause of neonatal jaundice. The prevalence of G6PD deficiency is almost twice higher in jaundiced infants (22 % in males) than in general Thai population (11 % in males).[8] However, there is no prospective study that measures bilirubin level serially in newborns with G6PD deficiency probably because serial measurements of serum bilirubin in asymptomatic neonates would be deemed unethical. In this study, we used a non-invasive transcutaneous bilirubin measurement device which has been proved accurate a measurement of serum bilirubin level (r=0.92).[7] This device has been extensively validated in Western and Thai populations.[15-16] Herein, we only recruited into the study newborns who were delivered by Caesarian-section because their bilirubin levels could be serially measured up to 4 days. A longer prospective bilirubin measurement beyond day 4 after birth was not feasible, since most neonates would be discharged.

We found that G6PD-deficient neonates had slightly higher bilirubin levels than those who had normal G6PD during their early days of life. However, the differences were not statistically significant. It is possible that G6PD deficiency contribute to jaundice later than 4 days as it had been observed that pathological jaundice late in the neonatal course were often due to G6PD deficiency.[17] It was not feasibly in our setting to measure bilirubin level in neonates beyond 4 days to prove such hypothesis. In addition, ABO incompatibility which was not excluded in this study may contribute to a high variation in the bilirubin levels, therefore it obscured the statistical difference. It is also possible that the type of *G6PD* mutation that is prevalent in the Thais in the Northeastern provinces may not contribute to neonatal jaundice. It is not yet known whether the common G6PD variant in Thais, *G6PD Viangchan*,[6] would contribute to jaundice like the common Chinese variant, *G6PD Canton*.[14] Further molecular analysis of mutations in Thais is needed to correlate the findings with hyperbilirubinemia.

MRT has been widely used as a screening test to study the prevalence of G6PD deficiency in various populations of Thailand,[9-12] probably because of its low cost and the simplicity of the technique. We estimated the material cost of MRT was approximately 1 Baht per test, compared to approximately 200 Baht per assay of the technique which measured G6PD activity. Our result in this and a previous study [8] confirmed that MRT is an acceptable screening test of G6PD deficiency from cord blood. However, because of somewhat high false positive rate, MRT had a low positive predictive value for G6PD deficiency.

The high prevalence of G6PD deficiency in our population and the low cost of MRT test should make routine cord blood screening for G6PD deficiency with MRT useful. Children who are tested deficient by MRT should be further monitored for neonatal jaundice. The costly G6PD activity may be done from cord blood to confirm the result of MRT test only when they developed neonatal jaundice.

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