**Prevalence of glucose-6-phosphate dehydrogenase deficiency in neonates in Egypt**

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Ann Saudi Med 2017; 37(5): 362-365

DOI: 10.5144/0256-4947.2017.362

**BACKGROUND:** Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked disorder which causes neonatal jaundice in most cases, and under certain conditions, can cause a spectrum of hemolytic manifestations.

**OBJECTIVE:** To determine the local prevalence of G6PD deficiency in newborns.

**DESIGN:** Cross-sectional.

**SETTING:** University hospital.

**METHODS:** Infants born during 2015 were prospectively screened for G6PD deficiency. Dried blood spot samples on filter paper were collected in collaboration with the central laboratories of the Ministry of Health. Quantitative measurement of G6PD enzyme activity was measured from the blood samples using fluorometric analysis. A value ≤1.3 U/g hemoglobin (Hb) was considered G6PD deficient.

**MAIN OUTCOME MEASURE(S):** G6PD enzyme activity (U/g Hb).

**RESULTS:** Of 2782 screened newborns (1453 males and 1329 females), 2646 (95.1%) newborns were normal, 17 (0.6%) exhibited intermediate deficiency; 119 newborns (91 male newborns; 28 female newborns) were deficient for G6PD for an overall prevalence of G6PD deficiency of 4.3% with a male:female ratio of 3.2:1. Enzyme activity was significantly higher in males than females ($P<.014$).

**CONCLUSION:** The overall prevalence of G6PD deficiency emphasizes the importance of neonatal screening for early detection and prevention together with proper intervention and genetic counseling.

**LIMITATION:** Lacked authority to collect the samples for testing directly from the local centers.
Menoufia Governorate in collaboration with central laboratories of the Ministry Of Health. Screening specimens were collected and preserved according to the conventional method of specimen acquisition for newborn dried blood spot screening. The screening specimens (n=118,829) were collected within the first week of life (maximally between 3-7 days of life), which corresponded to the number of births in Menoufia Governorate during the year 2015 (Directorate of Health Affairs, Ministry of Health Menoufia Governorate). Ethical approval was given by the Menoufia University Ethical Committee.

The sample size for a community-based random sample of newborn babies (in 1:1 male-to-female ratio) representative of the number of births in each of the ten centers of Menoufia governorate was calculated using the following formula:

\[ N = \frac{z^2 p (1-p)}{Me^2} \]

\[ z = 1.96 \text{ (constant).} \]

\[ p = \text{Estimated proportion of an attribute that is present in a studied population, based on reports on the prevalence of G6PD deficiency in Egypt.}^{7,2} \]

\[ Me = \text{the marginal error}^9 \]

All specimens were analyzed in our neonatal screening laboratory (Genetics, and Endocrinology Unit, Pediatric Department, Faculty of Medicine, Menoufia University Hospitals). After collection, packing and transfer, the samples were stored in a deep freeze at -20°C. They were then separated into groups by the 10 centers of the governorate as recommended by the International Society for Newborn Screening.10 The dried blood spot samples spotted on SS 903 filter paper were analyzed by fluorometry (WALLAC System, Perkin Elmer). G6PD enzyme activity was determined using the Neonatal Screening Kit for G6PD Deficiency (Neonatal G6PD Kit, ND-1000, Wallac OY, Mustionkatu 6, FI-20750 Turku, Finland, Perkin Elmer). The assay involves the oxidation of glucose-6-phosphogluconate (G6P), by the G6PD present in the blood spot sample and the concomitant reduction of NADP to NADPH. The blood spot is allowed to react with the substrate reagent, which consists of G6P and NADP, for 30 minutes at ambient temperature. Copper sulfate was added to slow the reaction. The fluorescence was measured in a Victor 2 D 1420 Multilabel Counter Fluorometer and NADPH was estimated. Hemoglobin (Hb) content was measured and weighed against the standard values documented in the kits.

Samples were considered normal if the hemoglobin content was >6U/g Hb and borderline between 1.3-6 U/g Hb. G6PD deficiency was considered present if the hemoglobin content was ≤1.3 U/g Hb. Statistical analysis was done using SPSS version 16. A P value below .05 was considered statistically significant.

RESULTS
Of 2782 randomly selected samples (1453 males and 1339 females), 2646 newborns (95.1%) were normal for G6PD enzymatic activity (Table 1). One hundred nineteen newborns had total deficiency, including (91 males and 28 females), and 17 newborns (0.6%) showed an intermediate deficiency, as compared to the standard reference supplied with kits. G6PD deficiency was present in 119 (4.3%) newborns. The prevalence of G6PD deficiency was 6.2% (91/1453) in males and 2.1% (28/1329) in females with a male:female ratio of 3.2:1. Enzyme activity for male newborns was significantly higher than for female newborns (Mann-Whitney test statistic=5.44, \( \chi^2=29.7 \), \( P<.014 \).

DISCUSSION
Glucose 6 phosphate dehydrogenase enzyme is involved in the pentose phosphate pathway, generating NADPH, which maintains reduced glutathione that defends against oxidative damage in red blood cells.11 A lack of G6PD makes the red corpuscles fragile to oxidative stresses causing hemolysis. G6PD deficiency is characterized by clinical, biochemical and molecular heterogeneity,11 and the prevalence varies widely.12-14 By 1988, approximately 400 types of G6PD were recognized with the most prevalent mutations in people from the Mediterranean, the west of Africa and Southeast Asia.8 Black Africans often have a mild deficiency; Asians are more deficient than Africans, and peoples of the Mediterranean have the most severe form.11

Reports from throughout the world indicate that G6PD deficiency is a common cause for neonatal hyperbilirubinemia with the frequency of G6PD deficiency among jaundiced neonates reported in different studies.15-19 The frequency of enzyme deficiencies in jaundiced newborns in Egypt varies by province, possibly because of the varied ethnic origins in coastal and seaside districts from the Egyptian Delta.2,20,21 Recently, Abo El Fotoh and Risk reported a frequency of 8.9% in neonates with indirect hyperbilirubinemia in newborns admitted to NICU, Menoufia University Hospitals, Egypt.22 Enzyme deficiency was more frequent among icteric newborns compared to the population samples, which signifies the importance of neonatal screening programs and the necessity for G6PD screening for all neonates, especially those with high or prolonged jaundice.21 In the current study, the frequency of enzymatic deficiency in females was 2.25% (28 of 1298 female neonates).

Determination of the frequency of enzyme deficiency in female subjects has gone unstudied with heterozygotes for enzyme deficiency assumed not to be at...
risk for many years. Heterozygotes may suffer severe neonatal jaundice with a risk equal to that observed in heterozygous male subjects. G6PD deficiency is not infrequent among females even with this particular inheritance pattern. Different clinical phenotypes may result from non-random inactivation of the X chromosome. Enzyme activity in red blood cells may vary, ranging from normal to absolute enzyme deficiency in heterozygous female individuals in relation to the X chromosome inactivation pattern. Of the newborns with borderline G6PD activity in our study, 14 were males. A possible explanation for this finding may be erroneous measurement or different mutations with a resultant low enzyme activity. Borderline activity in males warrants confirmation by molecular genetic analysis to identify common mutations among Egyptian neonates.

Quantitative measurement of enzymatic activity using freshly collected blood is the most widely used method for diagnosis of enzymatic deficiency. The main rationale for using a quantitative measurement is based on the fact that G6PD activity in newborns is higher than in elderly children or adults. In our study, we used relatively low cut-off reference values for determining predominantly deficient males and mostly heterozygous females, as provided with the manufacturer’s kit. As in our study, Riskin et al favored use of low cut-off reference values for deficiency (<2 U/g Hb). However, other studies considered newborn males and females with G6PD activity of <7 and 9.5-10 U/g Hb, respectively, as a high-risk group for neonatal hyperbilirubinemia. Nevertheless, all these studies emphasized the imperative issue that quantitative screening occur before discharge from hospital nurseries to offer appropriate care and increase parent awareness of possibly unfavorable sequelae with serious consequences that might befall the newborn. For this reason, WHO recommends routine screening in countries with a prevalence of ≥3-5% in males. G6PD deficiency is one of the disorders that should be screened in all newborns so that major health problems and/or hazards of blood transfusion can be eliminated.

Due to a lack of authority, we were unable to collect samples directly from each local center and instead obtained them from the central laboratory of the Ministry of Health. Besides the easier transfer and preservation of samples, we might have obtained more detailed information from medical records if had been able to go directly to the local centers.

**Conflict of interest**
No conflict of interest to be declared.

**Acknowledgments**
Many thanks to all medical staff of Community Medicine and Public Health Department, Menoufia University for providing help and assistance and also for central laboratories of the Ministry of Health for their collaboration, during both sample design and collection.
REFERENCES

1. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. Blood 2008; 111:16-24.
2. LaRue N, Kann M, Murray M, Leader BT, Bansil P, McGary S, et al. Comparison of quantitative and qualitative tests for glucose-6-phosphate dehydrogenase deficiency. J Trop Med Hyg 2014;91:854-61.
3. Glader B. Hereditary hemolytic anemias due to red blood cell enzyme disorders. In: Wintrobe’s Clinical Haematology, 12th ed, Greer, JP, Foerster, J, Rodgers, GM, et al. (Eds), Lippincott, Williams & Wilkins, Philadelphia 2009, pp:933.
4. WHO Working Group. Glucose-6-phosphate dehydrogenase deficiency. Bull World Health Organization 1989; 67: 601-11.
5. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet 2008; 371:64-74.
6. Kaplan M, Hammerman C. Neonatal screening for glucose-6-phosphate dehydrogenase deficiency: biochemical versus genetic technologies. SEMIN Perinatol 2011 Jun;35(3):155-61.
7. Seifin A, Ali-Haggar M, Al-Raz R, Youssif H, Osarn N. Screening of G6PD Mediterranean mutation among Egyptian neonates with high or prolonged jaundice. Haematologica 2006; 91(1):83-90.
8. Mohareb S, El-Tahan H, El-Sallab S, Sayed M, El-Tonbary Y. Some aspects of G6PD deficiency anemia in the North East Delta. J Egypt Pediatr Assoc 1982;1:34-38.
9. Lachou S, Louini N, Sahli CA, Daoubi R, Becher M, Jouini L, et al. Molecular identification of Gd A-and Gd B-G6PD deficient variant by ARMIS-PCR in a Tunisian population. Ann Biol Clin 2016;74:219-226.
10. Satyagraha AW, Sadchewa A, Elvira R, Elazer I, Feriandika D, Antonyjaya U, et al. Assessment of point-of-care diagnostics for G6PD deficiency in malaria endemic rural Eastern Indonesia. PLoS Neglected Tropical Diseases 2016;10:e0004457.
11. Hammad SE, Bad El-Din AA, Abdel-Aal AA and El-Kenany HA. The erythrocyte enzyme G6PD deficiency and its relation to neonatal jaundice. MD Thesis, Alexandria University, Egypt, 1986.
12. Shebli S SH. The common problems among the Egyptian newborn. Neonatal Scientific Conference, 1992 Jan;6:10-1-17.
13. Abo El Fotoh WM, Risk MS. Prevalence of glucose-6-phosphate dehydrogenase deficiency in jaundiced Egyptian neonates. J Matern Fetal Neonatal Med. 2016 Dec;29(9):3834-7.
14. Hameed NN, Na’Ma AM, Vilms R, Bhutan VK. Severe neonatal hereditary hyperbilirubinemia and adverse short-term consequences in Baghdad, Iraq. Neonatology. 2011;100(1):57-63.
15. Iranpour R, Hashimpor M, Talaei S M, Soroshnia M, Amini A. Newborn screening for glucose-6-phosphate dehydrogenase deficiency in Isfahan Iran: a quantitative assay. J Med Screen 2008;15:62-64.
16. Olusanya BO, Emokpae AA, Zamora TG, Slusher TM. Addressing the burden of neonatal hyperbilirubinemia in countries with significant glucose-6-phosphate dehydrogenase deficiency. Acta Pediatr. 2014; 103(11):1102-1109.
17. Nasserrullah Z, Alshammary A, Al Abbas M et al. Regional experience with newborn screening for sickle cell disease, other hemoglobinopathies, and G6PD deficiency. Ann Saudi Med 2003;23:354-357.
18. Altay C, Gurnuk F Red cell glucose-6-phosphate dehydrogenase deficiency in Turkey. Turk J Hematol 2008; 25:1-7.
19. Alay C, Gurnuk F Red cell glucose-6-phosphate dehydrogenase deficiency in Turkey. Turk J Hematol 2008; 25:1-7.
20. Anggur N, Avraham I, Hammerman C, Kaplan M. Quantitative Neonatal Glucose-6-Phosphate Dehydrogenase Screening: Distribution, Reference Values, and Classification by Phenotype. J Pediatr 2012 Apr 10.
21. Riskin A, Grey N, Kugelman A, Spevak I, Bader D. Glucose-6-Phosphate Dehydrogenase Deficiency and Borderline Line Deficiency: Association with Neonatal Hyperbilirubinemia. J Pediatr 2012 Aug;161(2):191-6.e1.
22. Kaplan M, Algar N, Hammerman C. Intermediate values of glucose-6-phosphate dehydrogenase deficiency. J Pediatr 2012 Sep;161(3):571.
23. Riskin A, Kugelman A, Bader D. Intermediate values of glucose-6-phosphate dehydrogenase deficiency. J Pediatr 2012 Sep;161(3):571-2.
24. Kilicdag H, Gokmen Z, Ozkira S, Guican H, Tarcan A. Is it accurate to separate glucose-6-phosphate dehydrogenase activity in neonatal hyperbilirubinemia as deficient and normal? Pediatr Neonatol 2014 Jun; 55(5):202-7.