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

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**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.

Glucose 6 phosphate dehydrogenase (G6PD) deficiency is a genetic disease frequently complicated with neonatal jaundice.[1] First reported in India, the disease occurs in persons of Mediterranean, African and/ or Asian descent, reaching a prevalence of 25% in some countries.[2] The gene encoding G6PD is located on the X-chromosome, band Xq28. Because the disease has an X-linked recessive pattern of inheritance, males are frequently affected.[3] An inherited G6PD-deficient genotype can manifest as many syndromes: as a distinctive response to drugs and chemicals with acute hemolysis, as a complication of illnesses or as neonatal jaundice causing kernicterus.[3] Children with G6PD deficiency are commonly asymptomatic, but certain agents such as specific foods, medications, and infectious agents may result in a hemolytic reaction.[3] Red blood cell transfusion is required in severe enzymatic deficiency (G6PD activity <10% below normal values).[2] Parents of deficient newborns should be counseled on the risks of jaundice and the importance of avoiding offending agents.[2] Treatment for G6PD deficiency is simple and inexpensive,[4] and can be started before symptoms appear.[5,6] We aimed to determine the prevalence of G6PD deficiency among newborns in the Menoufia governorate, located north of Cairo.

## PATIENTS AND METHODS

Infants born during the year 2015 were prospectively screened for G6PD deficiency in 10 centers of the

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=118829) 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 1.1:1 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:

$N=z^2p\ (1-p)/Me^2$.

z =1.96 (constant).

p= Estimated proportion of an attribute that is present in a studied population, based on reports on the prevalence of G6PD deficiency in Egypt.[7,8]

Me=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 defend 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.[4] 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.[7,20,21] 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.[23] 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

**Table 1.** Distribution of G6PD deficiency in dried blood spot samples (n=2782).

| Classification | n (%) | Median | IQR | Min-Max | Phenotype |
|---|---|---|---|---|---|
| **Male (n=1453)** | | | | | |
| ≤1.3 U/g Hb | 91 (6.3) | 0.26 | 0.19-0.37 | 0.01-1.13 | Deficient |
| 1.3-6 U/g Hb | 14 (0.9% | 3.41 | 2.08- 4.72 | 1.07-5.22 | Intermediate[a] |
| >6 U/g Hb | 1348 (92.8%) | 17.24 | 15.21-22.41 | 6.02-31.43 | Normal |
| **Female (n=1339)** | | | | | |
| ≤1.3 U/g Hb | 28 (2.1%) | 0.48 | 0.23-1.2 | 0.05-1.23 | Deficient |
| 1.3-6 U/g Hb | 3 (0.2%) | 5.62 | 3.42-5.82 | 1.24-5.46 | Intermediate |
| >6 U/g Hb | 1298 (96.7%) | 18.25 | 16.24-4.04 | 6.04-32.54 | Normal |

IQR: Interquartile range; [a]Intermediate males with borderline enzyme activity may be due to mutations that require further study to identify.

risk for many years.[24,25] 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.[24] 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.[26,27] 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.[28] 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).[29] 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.[30,31] 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.[32] For this reason, WHO recommends routine screening in countries with a prevalence of ≥3-5% in males.[4] 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

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