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RESEARCH ARTICLE

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G6PD Deficiency in Indonesian Newborn

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Introduction: In many countries screening for Glucose-6-Phosphate Dehydrogenase (G6PD) is a part of the newborn screening program. In Indonesia the National Newborn screening program started in 2012 exclusively for Congenital Hypothyroidism and screening for G6PD has not been included in the National Screening Program. Deficiency of G6PD can cause many manifestations with high morbidity rate. Different areas in Indonesia might have different proportion of G6PD deficiency. In Indonesia, there is no report on screening for G6PD deficiencies in newborn babies. *Objective*: To estimate the prevalence of G6PD deficiencies in Indonesian newborn. *Methodology*: Dried blood spot (DBS) samples were collected from many provinces all over Indonesia as part of the National Screening Program for Hypothyroid, and 676 DBS samples received in May–June 2017 were analyzed for G6PD deficiency using LabSystem Neonatal G6PD kit and Fluorometer Thermo Scientific instrument. *Results*: From 676 newborn this study it was found that the G6PD deficiency was observed in 7.39 male and 6.95% female babies. The cutoff value for G6PD deficiency was less than 4,5 IU/gHb. The proportion was higher in males babies compared to females. Based on this finding, the G6PD screening should be performed in all newborn because the total prevalence was more than 3–5% as stated by the WHO recommendation.

Keywords: G6PD, Newborn Screening.

1. INTRODUCTION

G6PD is one of the enzymes within the erythrocyte which allows the cell to perform its function. During its 120-day lifespan, erythrocyte is mainly essential for oxygen delivery to body tissues. To function effectively, enzymes within erythrocyte play roles in maintaining glycolysis, maintaining the shape and deformability of the erythrocyte membrane, and protecting the hemoglobin from oxidative denaturation.[1]

G6PD will catalyze the first reaction in the detoxification of oxygen radicals, which is the conversion of hydrogen peroxide to H_2O. Erythrocyte with a G6PD deficiency is not capable of producing sufficient NADPH to reduce glutathione. As a result, hydrogen peroxide, a product of oxidative stress, fails to be detoxified into H_2O. Oxidative stress would instead cause hemoglobin to undergo precipitation, forming Heinz bodies. These Heinz bodies will then attach to the inside of the erythrocyte membrane and cause permanent damage to the membrane. Erythrocyte with Heinz bodies is hence removed more quickly from the circulation compared to normal erythrocyte, due to the membrane damage and loss of membrane elasticity.[1]

Glucose-6-phosphate dehydrogenase deficiency is the most common enzyme deficiency worldwide, which affects approximately 400 million individuals, or 7% of the world population. It is spread along Africa (7.5%), Middle East (6%), Asia (4.7%), Europe (3.9%), and America (3.4%). The distribution showed a correlation with the malaria distribution, including in Indonesia. Most affected individuals are asymptomatic, exposure to oxidative agents such as drugs, traditional medicines, certain foods, and infection, can cause acute or chronic hemolysis, neonatal jaundice and anemia.[1,2]

G6PD deficiency is an X-linked hereditary genetic defect caused by mutations in the G6PD gene; it has many variants with different biochemical and clinical outcomes. G6PD gene is located on the X chromosome, on Xq28. Since it is located on the X chromosome, G6PD deficiency is more common in males than in females.[10,11] Males can inherit normal gene (normal hemizygote/normal allele) or hemizygote deficiency (mutant allele), while females can inherit normal homozygote (both normal allele), homozygote deficiency (both allele

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with the same mutation), heterozygote combination (both allele with different mutation), or heterozygote (one normal allele and one mutated allele). Males with mutant allele will clinically manifest G6PD deficiency and pass the abnormality to their daughters. In females, the clinical manifestation will depend on random X-chromosome gene inactivation process (lyonization). If the normal allele randomly inactivated in heterozygote carrier, then G6PD deficiency will manifest in the affected females. About 180 mutations have been described, mostly with amino acid substitutions. The effective management of G6PD deficiency is to prevent hemolysis by avoiding the oxidative stress substances. Screening programs for the disorder are undertaken, depending on the prevalence of G6PD deficiency in a particular community.[1, 3, 4]

In many countries, the G6PD screening is performed as part of their national newborn screening program.[5–7] In Indonesia the National Newborn screening program started in 2012 exclusively for Congenital Hypothyroidism and screening for G6PD has not been included in the National Screening Program. Deficiency of G6PD can cause many manifestations with high morbidity rate. Indonesia consists of many islands with multi-ethnicity. Different areas in Indonesia might have different proportion of G6PD deficiency. In Indonesia, there is no national report on screening for G6PD deficiencies in newborn babies.[8]

There are multiple techniques that can be used in G6PD deficiency examination. This research aims to know the proportion of G6PD deficiency in healthy neonates from several provinces in Indonesia.

2. METHODS

The study subjects were all neonates who had undergone TSH examination in Cipto Mangunkusumo National Referral Hospital (RSUPNCM) Clinical Pathology Laboratory in the period of April–May 2017. The inclusion criteria included the age 0–28 days, weight >2500 grams, gestation age >37 weeks. The exclusion criteria were if the dried blood sample in filter paper that did not meet the requirements.

The sample originated from the remains of blood sample in filter paper for neonates TSH examination in RSCM hematology laboratory that came from 25 provinces and districts in Indonesia. The examination sample is the blood dried blood spot (DBS) from babies heels put on filter paper. G6PD activity examination was performed by using Ani Labsystems Neonatal G6PD Deficiency Screening Assay reagent and measured with Fluorometer Thermo Scientific.

Ani Labsystems Neonatal G6PD Deficiency Screening Assay examination principle was based on enzymatic method, used to determine G6PD activity quantitatively with DBS sample. Nicotinamide adenine dinucleotide phosphate (NADP+) was reduced by G6PD with the presence of glucose-6-phosphate (G6P), and the formed NADPH levels were equal to G6PD activity, and also determined by fluorometry. Stop solution containing was added to stop the ongoing reaction and stabilize fluorescence complex. Fluorescence was measured on the wavelength of excitation 355 nm and emission wavelength 460 nm.[9]

To determine precision and accuracy of the test, a preliminary evaluation was performed. Within-run precision is tested using control 1, control 2, and normal sample. Each sample were tested five times consecutively. Mean, standard deviation, and coefficient of variations calculated for each sample. For between day precision test, each sample was tested once in 5 consecutive days. Mean, standard deviation, and coefficient of variations also calculated for this test. Precision and accuracy of laboratory method and data analysis for sample results will be calculated using Microsoft Excel 2016.

Table I. Neonates's characteristic.

Parameter	
Gender	♂ 51,47%
Age	1,8 ± 2 days
Birth weight	3100 (2500–4200) gram
Gestational age	38,7 ± 1,2 weeks
Source of sample	Jakarta : 84,16%
	Out of Jakarta: 15,8%

3. RESULT

The result of within-run precision using control 1 was an average of 3.9 IU/gHb, standard deviation (SD) 0.05, and coefficient of variation (CV) 1.4%. Within-run precision tested using control 2 ingredient resulted in 7.1 IU/gHb average, SD 0,1, and CV (0.8%). Within-run precision test with normal samples resulted in 10.8 IU/gHb average, SD 0.2 and CV 2.1%.

Between day precision test using control one result were average of 3.6 IU/gHb, 0.04 SD, and 1.2% CV. Between day precision test with control two resulted in 7.2 IU/gHb average, 0.13 SD, and 1.8% CV. Between day precision test with normal sample resulted in 10.5 IU/gHb average, 0.2 SD, and 1.9% CV.

With precision test using control 1, we obtained +2.6% deviation (d). With control two ingredients in precision test, the deviation is +1.4%. The total of errors from control 1 and two ingredient examination was 4.9% and 2.7%, consecutively.

From 676 examined neonates, the proportion of G6PD deficiency is 7.39% in healthy males and 6.95% in healthy females. The characteristics of research subjects are shown in Table I.

4. DISCUSSION

Precision tests which were performed in this research include both within-run and between-day precision tests, using control and normal sample population. In the within-run precision test, the resulting coefficient of variation (CV) for control 1 was 1.4%, while the CV for control 2 was 2.1%. The between-day precision test for control 1 resulted in a CV of 1.2%, while control 2 and normal sample resulted in a CV of 1.8% and 1.9% respectively. In the precision tests evaluating the activity of the G6PD in control 1 and control 2, the respective deviations (d) were +2.6% and +1.4%. The respective values of total error for control 1 and control 2 were 4.9% and 2.7%. The Westgard desirable bias value for the test on G6PD activity was 3.2%, while the Westgard allowable total error was 9.2%. Hence, the result of the precision tests performed in this research is indeed acceptable.[12]

The tests subjects to investigate the proportion of G6PD deficiency in neonates which consisted of 676 neonates: 348 males and 328 females. From these subjects, the proportion of G6PD deficiency was 14.3%, which consisted of 50 male neonates (7.39%) and 47 female neonates (6.95%). The proportion of G6PD deficiency in neonates varies widely according to

countries. In 2013, Molou E et al. performed a study in Greece, and it was discovered that the proportion of G6PD deficiency among neonates there was 7.74%. Similarly, a study by Al-Naama et al. in 1994 found that the proportion of G6PD deficiency in male and female neonates in Iraq were 7.9% and 9.7% accordingly.[13, 14]

Moustafa et al. performed a study as well in Egypt in 2015 and found that the proportion of G6PD deficiency in neonates with jaundice was 8.9%. In 2015, Kumar et al. performed similar research in Pakistan, and the resulting proportion was 9.3%. Kaban et al. did a study in 2009–2010 using test subjects from RSIA Bunda Jakarta, and it was revealed that the proportion of G6PD deficiency among neonates at the hospital was 5.2%. Moreover, a study performed in 145 male neonates in Yogyakarta by Suryantoro in 1998 revealed that the proportion of G6PD deficiency among the test subjects was 1.6%.[15–18]

In many studies on G6PD, it was found that the proportion of G6PD deficiency in males is higher than in females, owing to the location of the gene on X chromosome. Galates et al. carried out a study in 2016 in Mozambique and discovered that G6PD deficiency was indeed more common in males. Similar result was also discovered by Ntalos et al. who performed a study in Northern Greece in 2008; the total proportion of G6PD deficiency was 3.7%, with males' proportion twice higher than female's.[19, 20]

5. CONCLUSION

A study on the proportion of G6PD deficiency in neonates using fluorometric method was performed. The proportion of G6PD deficiency in male was 7.39%, while the proportion in female was 6.95%. A screening to evaluate the activity of the G6PD enzyme in neonates in Indonesia hence must be done regularly, because as recommended by WHO, in a population in which the proportion of G6PD deficiency in males exceeds 3–5%, screening on the activity of G6PD in neonates must be performed.

References and Notes

1. K. Doig, Introduction to increased destruction of erythrocytes, Rodak's Hematology Clinical Principles and Applications, edited by B. Rodak, G. Fritsma, E. Keohane, Elsevier, Missouri **(2016)**.
2. A. Riskin, N. Gery, A. Kugelman, M. Hemo, I. Spevak, and D. Bader, *J. Pediatr.* 161, 191 **(2012)**.
3. B. Branchi and Mohandas, Hereditary disorder of the read cell membrane and disorders of red cell metabolism, Postgraduate Haematology, edited by A. V. Hoffbrand, D. Higgs, D. Keeling, A. Metita, New Delhi **(2016)**, pp. 132–133.
4. B. O. Olusya, A. A. Emokpae, T. G. Zamora, and T. M. Slusher, *Acta Paediatr.* 103, 1002 **(2014)**.
5. J. Jiang, B. Li, W. Cao, X. Jiang, X. Jia, Q. Chen, and Wu., *Genet. Mol. Res.* 13, 4272 **(2014)**.
6. WHO Working Group, Glucose-6-Phosphate Dehydrogenase Deficiency, Bull World Health Organ. **(1989)**, Vol. 103, pp. 6–14.
7. R. Lam, H. Li, and M. Nock, *J. Perinatol.* 35, 1027 **(2015)**.
8. Aditarahman and I. S. Timan, Nilai rujukan aktivitas glucose-6-phosphate dehydrogenase (G6PD) neonatus di Jakarta menggunakan kertas saring, Fakultas Kedokteran Universitas Indonesia, Jakarta **(2007)**.
9. Ani Labsystems, Insert kit G6PD.
10. Q. Li, F. Yang, R. Liu, L. Luo, Y. Yang, L. Zhang, H. Liu, W. Zhang, Z. Fan, Z. Yang, L. Cul, and Y. He, *PLos One* 30 **(2015)**.
11. B. Glader, Hereditary hemolytic anemias due to red blood cell enzyme disorders, Wintrobe's Clinical Hematology, Wolters Kluwer, Philadelphia **(2014)**.
12. Westgard, Desirable Biological Variation Database Specification, Available from: https://www.westgard.com/biodatabase1.htm.
13. M. Al-Naama, L. Al-Naama, and T. Al-Sadoon, *Ann. Trop. Paediatr.* 14, 195 **(1994)**.
14. E. Molou, K. Schulpis, G. Thodi, V. Georgiou, Y. Dotsikas, K. Papadopoulos, and S. Biti, *Scand. J. Clin. Lab. Invest.* 74, 259 **(2014)**.
15. K. Kumar, A. Sohaila, S. Tikmani, I. Khan, and A. Zafar, Screening for G6PD deficiency among neonates with neonatal jaundice admitted to tertiary care center: A need in disguise, Available from: [cited 2017 March].
16. W. Moustafa, A. Fotoh, and M. Rizk, *J. Matern. Fetal. Neonatal. Med.* 29, 3834 **(2016)**.
17. R. Kaban and V. Wijaya, Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in neonates in Bunda women's and children's hospital, Jakarta, Indonesia, Available from.
18. P. Suryantoro, *Southeast Asian J. Trop. Med. Public Health* 3, 138 **(2003)**.
19. B. Galatas, L. Mabote, L. Simone, G. Matambisso, L. Nhamussua, Q. Bassat, P. Alonso, and P. Aide, *Malar. J.* 16, 36 **(2017)**.
20. G. Ntaos, A. Chatzinikolaou, C. Tomos, C. Manolopolos, P. Karalazou, A. Nikolaidou, and S. Alexiou-Daniel, *Intern. Med. J.* 38, 204 **(2008)**.

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