SciMedCentral

Annals of Pediatrics & Child Health

Short Communication

Transportation is Pivotal to Newborn Screening of G6PD Deficiency

Yvanna L. D. C. Oliveira[1], Djane A. Oliveira[2], Lúcio H. S. Pinhero[1], Nathanielly L. Silva[1], Flávia O. Costa[2], Rosana Cipolotti[1], and Dulce M. Schimieguel[1]*

[1]Department of Pharmacy, Federal University of Sergipe, Brazil

[2]Unit of Laboratories of the Hospital of the Federal University of Sergipe, Brazil

*Corresponding author

Dulce Marta Schimieguel, Department of Pharmacy, Laboratory of Hematology, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil. CEP: 49100-000, Tel: 55 79 3194-6319; Email: hematox_ufs@outlook.com

Submitted: 09 April 2020

Accepted: 24 April 2020

Published: 27 April 2020

ISSN: 2373-9312

Copyright

© 2020 Oliveira YLDC, et al.

OPEN ACCESS

Keywords

• Newborn screening; Glucose-6-phosphate dehydrogenase deficiency; Standardization enzyme activity

Abstract

This study aimed to evaluate the interferents in a quantitative technique for glucose-6-phosphate dehydrogenase (G6PD), deficiency diagnosis. Newborn blood samples were collected in filter paper from May 2016 to January 2017 in Sergipe state, Brazil. Samples were divided into three groups with different pre-analytical and analytical conditions: in the pilot group was evaluated sample quality and time between collection and examination; in the standard group was analyzed the samples under regular conditions of newborn screening; and in the control group samples were transported with low temperature. We evaluated 11,677 samples, of which 8,213 (70.33%), were considered valid: 2,347 from Pilot Group, 5,766 from Standard Group, and 100 from Control Group. The prevalence obtained was 9.35% from the Standard Group. There was significant statistical difference among positive results of most groups. Prevalence decreased from 13.66% in Pilot Group to 1% in Standard Group, and the mean enzyme activity of samples from Pilot Group, Standard Group, and Control Group was 4.4, 4.3 and 7.5 U/g Hb, respectively. The optimal technical conditions observed were: completely filled filter paper (Pilot Group), three weeks between collection and examination (Standard Group), and refrigerated transport (Control Group). The confirmatory test was performed in 50 newborns from Standard Group, and the deficiency was confirmed in 20 patients. Among the established criteria, refrigerated transportation had the greatest impact in reducing prevalence. We suggest that quantitative measures of enzyme activity technique cannot be used to diagnose G6PD deficiency in regions with high temperature without refrigeration during transportation.

ABBREVIATIONS

G6PD: Glucose-6-phosphate Dehydrogenase; Hb: Hemoglobin

INTRODUCTION

The metabolic pathways involved in erythrocyte function and survival include non- oxidative anaerobic glycolysis, hexose monophosphate shunt, and glutathione metabolism pathway [1,2]. In a deviation from the glycolytic pathway, glucose-6-phosphate dehydrogenase (G6PD), is the rate-limiting enzyme whose main function is cellular protection against oxidative damages that decreases erythrocyte half-life [3].

G6PD deficiency is a recessive disorder encoded on the X chromosome, which is expressed on all body tissues. This genetic condition makes patients susceptible to hemolytic anemia with possible neonatal jaundice usually secondary to oxidative stress triggered by foods such as fava beans and food's artificial blue dyes as well as medications including sulfas, non-steroidal anti-inflammatories, and analgesics as acetaminophen and dipyrone [4].

This is the most incident erythroenzymopathy in humans affecting more than 400 million people worldwide – especially sub-Saharan and North Africa and the Middle East [5]. Latin America has a smaller incidence pattern due to miscegenation with African population, with frequency ranging from 0.39-4.09% [6-8]. In the Brazilian population the current prevalence is estimated between 3% and 6.9% [8]. Nonetheless, there are few studies conducted in Brazil with a large number of participants.

Most tests for G6PD deficiency consider the biochemical aspects: qualitative or quantitative measures of enzyme activity. Brazil is a tropical country where temperatures can reach from 30 to 40°C, and frequently it is accompanied by high humidity [9]. These environmental conditions can cause reduction of the enzyme activity while at low temperatures the integrity of the enzyme is preserved, with greater stability and reduction of activity. According to the cited limitations, this study aimed to evaluate the interferents of the quantitative technique for the diagnosis of glucose-6-phosphate dehydrogenase (G6PD), deficiency.

MATERIALS AND METHODS

Collection and sample transportation

Blood samples were collected from newborns by heel puncture and stored on filter paper disks, preferably between three and seven days after birth, from May 2016 to January 2017. The sample collection was performed in Sergipe/Brazil as part

of the newborn screening routine, and the samples were sent to the Neonatal Screening Laboratory at the University Hospital of Federal University of Sergipe by truck without refrigeration and stored in paper envelops.

Glucose-6-phosphate dehydrogenase dosing technique

The glucose-6-phosphate dehydrogenase assay was performed using the Neonatal G6PD kit (Perkin Elmer, Turku, Finland), which adopts quantitative methodology with time-resolved fluorescence measurement. Filter paper disks with blood sample were cut directly to a white microplate with an automatic chopper. Then, it was incubated at room temperature with G6PD substrate reagent with slow stirring on the agitator. The floating blood spots were removed. At the end of the reaction copper reagent was added and the fluorescence was measured in up to 15 minutes with 355nm excitation filter and 460nm emission. Patients were defined as **deficient** when the test value was below the cut-off value (2.6U/g Hb).

Methodology standardization

Samples were analyzed and divided into five groups (Pilot Group – subdivided in Subgroup 1, 2 and 3 – Standard Group, and Control Group) according to the following flowchart (Figure 1). Samples were considered with satisfactory quality if presented complete filling of the filter paper with blood and showed no signs of degradation (dilution, fungal contamination, color change).

Confirmatory test of G6PD deficiency

Positive patients from Standard Group and their parents were invited to confirmatory test, and blood samples in filter paper were collected in the laboratory where the test was carried out and refrigerated afterwards.

Additional tests and clinical evaluation

A 1mL aliquot of peripheral blood collected with EDTA was used for the quantitative and qualitative analysis of blood cells and reticulocyte counts. Another aliquot of peripheral blood collected without anticoagulant was used for the bilirubin dosage and performed according to the methodology of the dichlorophenyldiazonium salt (DPD). Patients who had the deficiency of the enzyme confirmed were included in a clinical evaluation to elucidate the disease.

Statistical analysis

The results were analyzed by Microsoft Excel 2010 and R version 3.3.1 by descriptive statistics using the Shapiro-Wilk Normality Test and the G-Test. The significance level was 5%.

RESULTS AND DISCUSSION

A total of **11,677 samples** were analyzed and the results of the screening were demonstrated in the following flowchart (Figure 2). Samples were excluded if showed poor quality, if exceeded the time limit established between collection and analysis, if there was flotation of the filter papers even after being sunk in the microwell, or no result within the linear dynamic range.

The **prevalence** of deficient population of this study was **1.0%.** This value was obtained from 101 samples of the Control Group, considering this group met all pre- analytical and analytical criteria established in this study for an ideal analysis.

There was statistically significant difference among positive results of all groups ($p<0.05$), except between Subgroup 1 and 2 ($p = 0.7396$), and Subgroup 3 and Standard Group ($p = 0.2196$). Prevalence decreased from 13.66% to 1%, and the mean enzyme activity of samples from Pilot Group, Standard Group, and Control Group was 4.4, 4.3 and 7.5 U/g Hb, respectively. These results demonstrated that 1) the quality sample evaluation; and 2)

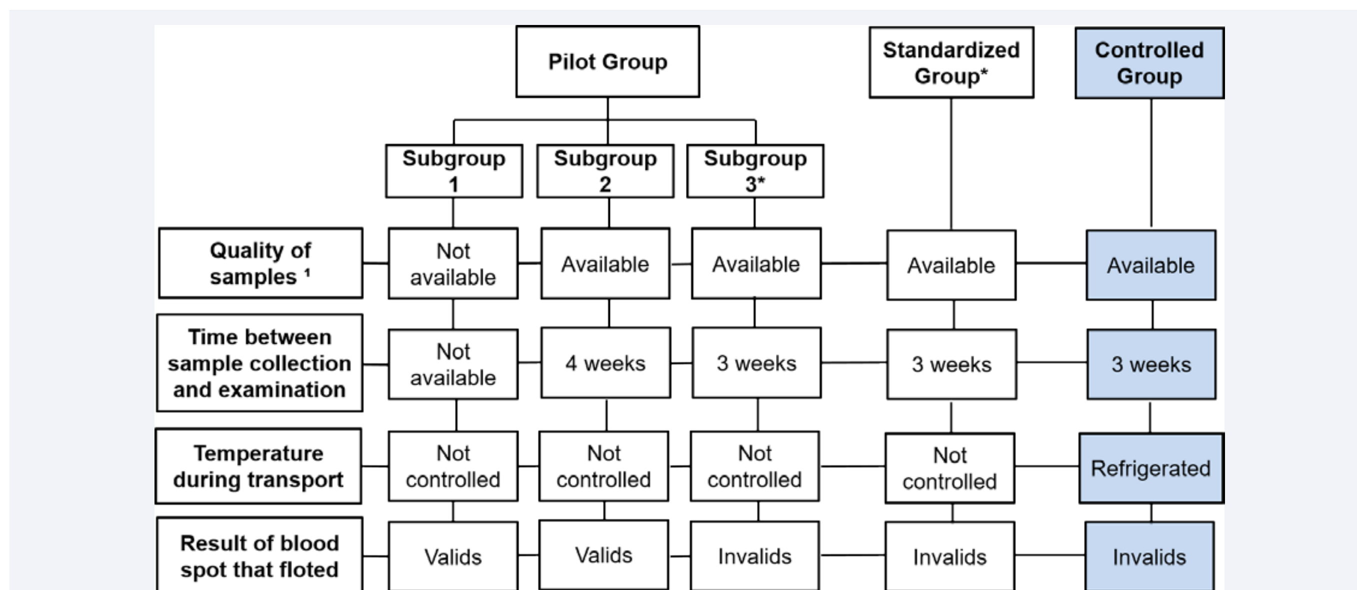

Figure 1 Samples grouped by methodological standards. Blood samples that completely fill the filter paper disc diameter.
* The methodological difference between the groups occurred in the sinking of the perforated filter paper: after 30 minutes of reaction in Subgroup 3 and after 15 and 30 minutes of reaction in Standardized Group. The figure was created in Power Point 2013

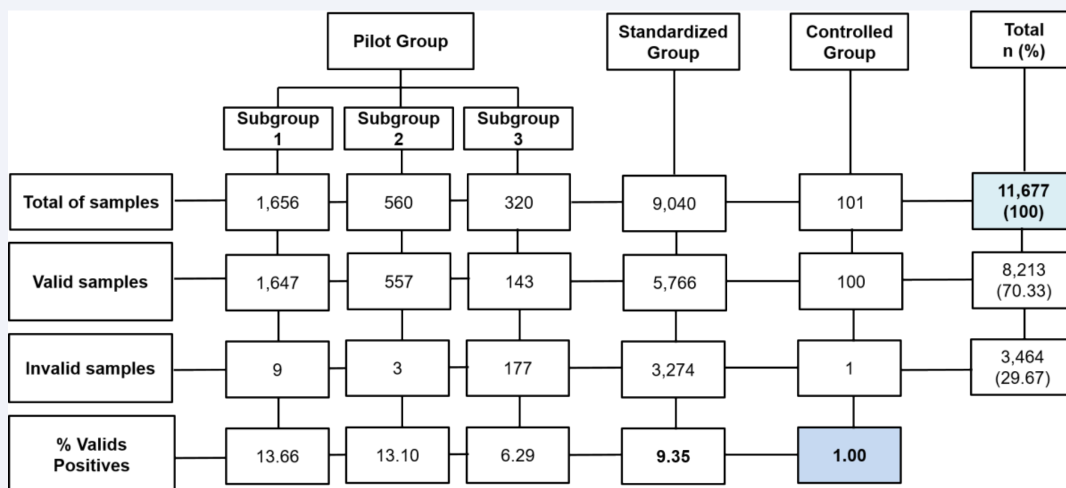

Figure 2 Total of samples analyzed in each of the five groups and mean of the enzyme activity. The figure was created in Power Point 2013.

adoption of the limit time of three weeks between the collection and examination, adopted in Subgroup 3; and 3), refrigerated transport, adopted in Control Group, are the optimal conditions for carrying out the test.

One hundred positive patients from Standard Group were invited to repeat the test. From these, 50 patients were attended and the mean enzyme activity for confirmatory test was 3.5 U/g Hb, greater than the initial test that presented a mean enzyme activity of 1.7 U/g Hb.

Among the 50 newborns, glucose-6-phosphate dehydrogenase deficiency was confirmed in 20 patients. In the family assessment, the deficiency was observed in four mothers and one father. Four patients presented results below the reference value for hemoglobin (8.94, 8.99, 9.01, and 9.94 g/dL), and reticulocyte count (1.62, 2.00, 2.26, and 2.43%), and 2 patients presented results above the reference value for bilirubin dosing (1.7 and 3.5 g/dL). The 20 patients, who had the deficiency of the enzyme confirmed, participated in a clinical evaluation to elucidate the disease, prophylaxis and care with food and use of medicines that can trigger a hemolytic crisis.

Due to the heterogeneity of clinical signs in glucose-6-phosphate dehydrogenase deficiency, laboratory diagnosis is essential—both for the confirmation of this erythroenzimopathy and to distinguish it from other hemolytic anemia subtypes with similar signs and symptoms. Early diagnosis of glucose-6-phosphate dehydrogenase deficiency in newborns helps pediatricians to manage the neonatal jaundice and to avoid hemolytic crisis triggered by specific foods or medications.

In Brazil, there are few recent studies that have investigated this deficiency, however they have been performed evaluating a limited number of participants [10-13]. Our results were the most judicious regarding to samples screening and standardization of the pre- analytical and analytical steps. In addition, it was analyzed a large number of random samples received from all over the state, which corresponds to almost entirely all newborns in Sergipe during the analyzed period.

Another found in this study that should be highlighted is the large number of samples that were excluded (approximately 30%), in the pre-analytical step due to poor quality that could hide positive and negative results related to errors during sample collection and processing. Regard to sample quality, two aspects were evaluated: full filling of the filter paper disc diameter and time between sample collection and examination. The filter paper discs have from 1-2% lower blood volume at the edges than at the center [14], which could imply variations in enzyme levels in each sample. Furthermore, according to Flores and collaborators (2013), after three weeks of collection, samples stored under high temperature and humidity had enzymatic activity reduced below the cut-off point [15]. Therefore, it is very important that periodic training be conducted with health professionals, and samples be sent to the laboratory as soon as they be dried to avoid errors in the tests performed during newborns screening to ensure the reliability of results.

The Standard Group met the necessary methodological modifications for the reduction of interferences excepting temperature control. Our outcomes from this Standard Group present a prevalence of 9.35%, which is consistent with the results presented by Monteiro et al. (2016), that conducted a systematic review performed in Latin American countries, showing prevalence between 0-13.6% in Brazil. However, prevalence obtained in our study was still higher than some studies performed in Brazil indicating prevalence between 1.76 and 7.9% [10-13,16].

This difference may have occurred due to transport conditions. Flores and collaborators [15], conducted a study with 144 newborns to evaluated G6PD enzyme stability in dried blood spots under various temperature and humidity environments, and they observed that humidity must be controlled and storage temperature should not exceed 25°C if analyzed 5 days after collection. These criteria were also recommended by the Haematology Guideline of British Society [17]. In Sergipe, during the sample collection and transportation, the temperature can suffer great variations because room temperature frequently exceeds 30ºC, and it can cause enzyme denaturation. Temperature effect can be observed at confirmatory test phase performed by

random sampling, which only 40% revealed G6PD deficiency, showing a great number of false positive results at first collection.

To reaffirm this hypothesis, we conducted a trial on Control Group with samples transported under refrigeration, and a low percentage of positive results (1%), was observed. The enzymatic activity values were higher than the other groups, and there was a statistically significant difference between positive results on this and other groups. Kuwahata and collaborators (2010), demonstrated a 5% decrease in G6PD activity when stored at 4°C, 15% when stored at 25°C, and 40% at 37°C [18].

Among the established criteria, the one that had the greatest influence in reducing prevalence, with high statistical significance ($p = 0.00004$), was refrigeration during transportation: from 9.35% in the Standardized Group, whereas other methodological criteria were adopted, to 1% in the Control Group. For these reasons, despite the importance and need in performing the early diagnosis in areas with high prevalence of this deficiency in order to avoid future damages [19], we suggest that this technique cannot be adopted in tropical and subtropical regions with high temperature without refrigerated transport.

CONCLUSION

Prevalence of G6PD deficiency in this study decreased from 13.66% to 1% after adoption of methodological criteria. Among these factors, the least taken into account in diagnosis routine is refrigeration during transportation. However, it was the one that had the greatest impact in reducing prevalence, which shows that even with the adoption of other criteria, the possibility of having false-positive results is still very high. Considering the limitations of the public health service regarding to samples' collection and transport, this technique cannot be used to diagnose G6PD deficiency in regions with high temperature. Nonetheless, we suggest that new studies may be made in different geographic regions of Brazil with climatic and ethnical variation to confirm our hypothesis.

ACKNOWLEDGEMENTS

We are grateful to the Laboratory of Clinical Analyzes of the University Hospital of Sergipe for physical and material support given to accomplish the tests, to Farmac (Sergipe, Brazil), and Perkin Elmer (Turku, Finland) who donated the kits and supported us with research and scientific advice.

REFERENCES

1. Da Costa J, Galimand J, Fenneteau O, Mohandas N. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev. 2013; 27: 167-178.

2. Koralkova P, van Solinge WW, van Wijk R. Rare hereditary red blood cell enzymopathies associated with hemolytic anemia – pathophysiology, clinical aspects, and laboratory diagnosis. Int J Lab Hematol. 2014; 36: 388-397.

3. Stanton RC. Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. IUBMB Life. 2012; 64: 362-369.

4. Shah SS, Macharia A, Makale J, Uyoga S, Kivinen K, Craik R, et al. Genetic determinants of glucose-6-phosphate dehydrogenase activity in Kenya. BMC Med Genet. 2014; 15: 93.

5. Renzaho AMN, Husser E, Polonsky M. Should blood donors be routinely screened for glucose-6-phosphate dehydrogenase deficiency? A systematic review of clinical studies focusing on patients transfused with glucose-6-phosphate dehydrogenase– deficient red cells. Transfus Med Rev. 2014; 28: 7-17.

6. Zamorano-Jimenez CA, Baptista-González HA, Bouchán-Valencia P. Identificación Molecular de la Glucosa-6-Fosfato Deshidrogenasa detectada en el tamiz neonatal. Gac Med Mex. 2015; 151: 34-41.

7. Rezaei N, Naderimagham S, Ghasemian A, Saeedi Moghaddam S, Gohari K, Zareiy S, et al. Burden of hemoglobinopathies (thalassemia, sickle cell disorder, and G6PD deficiency) in Iran. Arch Iran Med. 2015; 18: 502-507.

8. Freitas PFVB, Segre CAM. Deficiência de glicose-6-fosfato desidrogenase. Grupo Editorial Moreira Júnior. 2013. 376-381.

9. World Weather and Climate Information. Climate and average weather in Brazil. 2019.

10. Castro S, Weber R, Dadalt V, Tavares V, Giugliani R. Prevalence of G6PD deficiency in newborns in the south of Brazil. J Med Screen. 2006; 13: 85-86.

11. Ferreira MFC. Neonatal screening for glucose-6-phosphate dehydrogenase deficiency and prevalence of G202A (G6PD A-) and C563T (G6PD Mediterranean) mutations in Mato Grosso / Brazil [thesis]. São Paulo, SP: Faculty of Medicine, University of São Paulo; 2014.

12. Iglessias MAC, Santos RMV, Amorim MST, Silva RT, Moreira SS, Barretto OCO, et al. Erythrocyte glucose-6-phosphate dehydrogenase deficiency in male newborn babies and its relationship with neonatal jaundice. Rev Bras Hematol Hemoter. 2010; 32: 434-438.

13. Belisário AR. Evaluation of the influence of genetic, laboratory and clinical markers on the occurrence of cerebrovascular disease in children with sickle cell anemia triaged by the neonatal screening program in Minas Gerais and monitored at the Belo Horizonte hemocenter of the hemominas foundation [thesis]. Belo Horizonte, MG: Faculty of Medicine, Federal University of Minas Gerais; 2015.

14. Mei JV, Alexander JR, Adam BW, Hannon WH. Use of filter paper for the collection and analysis of human whole blood specimens. J Nutr. 2001; 131: 1631S-6S.

15. Flores S, Hall EM, Jesus V. Stability of Glucose-6-Phosphate Dehydrogenase Proficiency Testing Dried Blood Spot Materials. CDC. 2013.

16. Roper D, Layton M, Rees D, Lambert C, Vulliamy T, De la Salle B, et al. Laboratory diagnosis of G6PD deficiency. A British Society for Haematology Guideline. Br J Haematol. 2020; 189: 24-38.

17. Deficiency G6PD [Internet]. Unisert: National Union of Neonatal Screening Services

18. Kuwahata M, Wijeshing R, Ho MF, Pelecanos A, Bobogare A, Landry L, et al. Population screening for glucose-6- phosphate dehydrogenase deficiencies in Isabel Province, Solomon Islands, using a modified enzyme assay on filter paper dried bloodspots. Malaria J. 2010; 9: 223.

19. Kumar RK, Nagar N, Ranieri E. Newborn Screening for G6PD Deficiency- Why is it Important for India? Indian J Pediatr. 2014; 81: 90-91.

Cite this article

Oliveira YLDC, Oliveira DA, Pinhero LHS, Silva NL, Costa FO, et al. (2020) Transportation is Pivotal to Newborn Screening of G6PD Deficiency. Ann Pediatr Child Health 8(3): 1176.