ORIGINAL ARTICLE

Prevalence and Clinical Significance of Glucose-6-Phosphate Dehydrogenase Deficiency among Apparently Healthy Blood Donors in Kano, North-West Nigeria

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Received: March 31st, 2018
Accepted: May 16th, 2018

DISCLOSURE
This publication was not funded by any organization and none of the authors has any conflict of interest in this publication.

ABSTRACT

Background: Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency is high in the tropics because it protects heterozygous carrier females from severe malaria. Donors who are G6PD-deficient may not be symptomatic at time of donation. Nonetheless, G6PD-deficient donor red cells may undergo haemolysis when they encounter drugs or infections in recipient patients. Hence, World Health Organization (WHO) recommends routine screening of donors for G6PD deficiency in populations with high prevalence of G6PD deficiency. Unfortunately, donors are not routinely screened for G6PD deficiency in Nigeria. Hence, prevalence of G6PD among blood donors in North-West Nigeria has not been determined.

Objective: To determine the prevalence and significance of G6PD deficiency among apparently healthy blood donors in Kano, North-West Nigeria.

Methodology: Cross-sectional prospective study in which 4mls of ethylene-diamine tetra-acetate anti-coagulated blood were collected from 500 healthy consenting blood donors were screened for G6PD deficiency by UV-light fluorescent screening technique conducted between January and June 2014 at Aminu Kano Teaching Hospital Kano, North-West Nigeria.

Result: Glucose-6-phospahte dehydrogenase deficiency was found in 102(20.4%) of donors studied. All of the G6PD-deficient donors were males and had normal haematological parameters at the time of donation.

Conclusion: Prevalence of G6PD deficiency in blood donors in Kano, North-West Nigeria is high. Transfusion of G6PD-deficient red cells is potentially deleterious especially to neonates and young children, and all G6PD-deficient patients irrespective of age. Hence, Nigeria and other tropical countries must upgrade their transfusion safety by ensuring that donors are routinely screened for G6PD deficiency as recommended by WHO.

Key Words: *Transfusion, Red Cell, Enzymopathy, Screening*

INTRODUCTION

The World Health Organization (WHO) recommends that blood donation should in all cases be absolutely altruistic.[1] Medical staff in charge of blood donation and transfusion must ensure that donors are protected from any possible harm that may arise from blood donation, while the recipients must equally be protected from any possible harm that may

arise from the transfusion of any donated blood product.[1] In developed countries, centralized blood transfusion services are responsible for donor selection and recruitment, blood collection and fractionation, and storage of blood products.

However, in Nigeria the national blood transfusion service is still at a formative stage and cannot satisfy the national requirement for donor blood. Hence, the responsibility of donor recruitment and blood collection are virtually relegated to individual hospital blood banks where the suitability of prospective donors is determined by a pre-donation assessment of general health, medical and social history, weight and blood pressure, as well as infectivity markers of HIV 1 and 2, hepatitis B and C, and syphilis.[2] Healthy persons who are between the ages of 18 and 65 years with haemoglobin (Hb) levels of not less than 13.5 g/dl for males and 12.5 g/dl for females are acceptable as donors if they test negative for transfusion transmissible infections.[1]

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked recessive genetic disorder with a geographical distribution that corresponds with the endemicity of malaria within Africa and other tropical regions of the world.[3] G6PD is an important enzyme in the pentose mono-phosphate pathway wherein it is involved in the production of reduced nicotinamide dinucleotide phosphate (NADPH) which combats oxidative stress and injury to haemoglobin and red cell membrane, thereby protecting the red cells from haemolysis.[4]

There are two abnormal G6PD variants in Africa: G6PD-A and G6PD-A(minus).[4] G6PD-A is enzymatically non-deficient and is thus clinically insignificant, whereas G6PD-A(minus) is enzymatically deficient and is thus clinically significant in the aetiology of haemolytic anaemia.[4] In tropical countries such as Nigeria, the prevalence of G6PD deficiency is as high as 20% in males (symptomatic hemizygotes), while among the females up to 32% are asymptomatic heterozygotes (carriers) and 4% are symptomatic homozygotes.[4] The high prevalence of G6PD deficiency in the tropics is due to the fact that the heterozygous state, through the process of balanced polymorphism, offers protection against severe *falciparum* malaria.[5,6]

The lifespan of G6PD deficient red cells is subnormal. Therefore, the earliest clinical manifestation of G6PD deficiency is often neonatal jaundice and anaemia, and later in life it may manifest as chronic haemolytic anaemia or acute intermittent haemolytic anaemia triggered by the oxidant effects of drugs, chemicals or infections.[4] The possibility of encountering the oxidant effects of drugs, chemicals or infections among hospitalized patients requiring blood transfusion is substantial. Therefore, it is important to screen blood donors for G6PD deficiency in countries where the deficiency is prevalent as recommended by WHO.[1] Unfortunately, in Nigeria and most other tropical countries, donors are not routinely screened for G6PD deficiency.[7]

Published data on the prevalence of G6PD deficiency among blood donors in North-West Nigeria is lacking. Hence, in this study we evaluated the prevalence of G6PD deficiency among blood donors in Aminu Kano Teaching Hospital Kano, North-West Nigeria and discussed the implication of this on blood transfusion safety.

METHODOLOGY

This is a cross-sectional prospective study of prevalence of G6PD deficiency among 500 blood donors carried out between January and June, 2014 at the Aminu Kano Teaching Hospital Kano, North-West Nigeria. The minimum sample size (n) was determined using the formula $n=Z^2Pq/d^2$. Where: z=1.96 (confidence interval), P = 20% prevalence rate from a previous national study[4], q=1−P=complimentary probability, d=precision (margin of error) at 95% confidence limit=0.05. The minimum sample size was calculated as 239. However, because the subjects (blood donors) were easy to get,

the sample size was augmented to 500, which would in turn increase the accuracy of data generated from the study.

Inclusion Criteria and Ethics

Apparently healthy donors who scaled through the pre-donation clinical evaluation and Hb estimation, and tested negative for transfusion transmissible diseases were consecutively recruited at the donation bay. Their demographic details were documented and their haematological parameters were determined and collated.

A written informed consent which was approved by the Institutions Ethics Committee on human research was obtained from all subjects enrolled in this research. All research procedures were fully compliant with ethical principles and provisions of the World Medical Association Declaration of Helsinki as amended in 2008 at Seoul.

Exclusion Criteria

Donors who failed the pre-donation clinical evaluation, Hb estimation, and/or tested positive for transfusion transmissible diseases were excluded from this study.

Determination of Haematological Parameters and G6PD Status of Blood Donors

Venous blood (4ml) was collected from each donor into potassium ethylenediamine tetra-acetic acid specimen containers and analyzed for haematological parameters and G6PD status immediately after collection. Haematological parameters (haematocrit, haemoglobin, red cell indices, leucocyte cell count and differentials, and platelet counts) were determined using automatic blood analyzer (Celltac Alpha MEK 6400: Nihon Kohden Corporation, Tokyo, Japan). G6PD status was determined by UV light fluorescent screening technique. The enzyme G6PD catalyzes the oxidation of G6P in the pentose monophosphate pathway; it then reduces NADP to NADPH, which fluoresces under long wave-length UV light.

Based on these principles, all eligible donors were screened for G6PD deficiency by the fluorescent spot test as described by Dacie and Lewis, using commercial test reagents made by Randox Laboratories Crumlin, UK.[8] The tests were executed by addition of G6P and NADP to donor red cell haemolysate and incubated at room temperature for 10 minutes in compliance to the reagent manufacturer's guidelines. The mixture was subsequently spotted on filter paper, dried and examined under long wave-length UV light.

Fluorescence indicates the presence of NADPH, which has been generated by normal G6PD activity in the donor haemolysate. Lack of fluorescence indicates G6PD deficiency with less than 20% of normal G6PD activity.[8] All tests were carried out in parallel with normal G6PD-replete and G6PD-deficient controls as provided by reagents manufacturers.

Calculations and Statistical Analysis

Prevalence of G6PD deficiency was calculated as percentage. Mean values of demographic and haematological parameters were compared between G6PD replete and deficient donors by using the t-test with p-values of less than 0.05 taken as significant. Statistical analysis was performed using computer software SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 500 donors consisting of 474(94.8%) males and 26(5.2%) females were studied. Out of the 500 donors studied, 102(20.4%) were G6PD deficient and 398(79.6%) were G6PD replete. All of the G6PD deficient donors were males. The demographic and haematological parameters of the blood donors with respect to G6PD status are shown in Table 1. There were no statistically significant differences between G6PD replete and G6PD deficient donors with respect to age and haematological parameters at the time of blood donation.

DISCUSSION

Donors in Nigeria are predominantly young as shown by their mean ages of about 30 years in this study, which is a reflection of the

demographic structure of Nigeria as a developing nation with a relatively young population. In addition, the younger people are relatively more educated and more amenable to donor recruitment campaign. Despite the fact that blood donation is acceptable from healthy females that are not pregnant or breast-feeding, there is a general misconception in Nigeria that women are not eligible to donate blood.[9] This may have significantly contributed to the scarcity of female donors as revealed by the highly skewed predominance of male donors in this study.

The prevalence rate of G6PD deficiency among blood donors in Kano (North-West Nigeria) was 20.4%, which is similar to the prevalence rates of 20% found in North-Central Nigerian blood donors and 19.5% found in South-West Nigerian blood donors, but is higher than the prevalence rate of 13% found among blood donors in south east Nigeria.[10,11,12] This discrepancy may be related to differences in the study procedures.

Table 1: Demographic and haematological parameters among 500 blood donors with respect to G6PD status

Parameters	G6PD deficient donors(N=102)	G6PD replete donors(N=398)	*P*-values
Age (years) Mean ± SD	32.4± 5.6	31.8± 5.2	P>0.05
Hb (g/dl) Mean ± SD	15.2 ± 1.0	15.4 ± 1.1	P>0.05
Haematocrit (l/l) Mean ± SD	0.45 ± 0.02	0.46 ± 0.03	P>0.05
MCV (fl) Mean ± SD	86.2 ± 4	85.3 ± 3	P>0.05
MCH (pg) Mean ± SD	30.3 ± 1.2	30.5 ± 1.1	P>0.05
MCHC (g/dl Mean ± SD	34.1 ± 1.1	33.4 ± 1.2	P>0.05
Total leucocyte count ($x10^9$/l) Mean ± SD	8.2 ± 1.5	7.8 ± 1.3	P>0.05
Neutrophil count ($x10^9$/l) Mean ± SD	5.44 ± 0.7	5.16 ± 0.33	P>0.05
Lymphocyte count ($x10^9$/l) Mean ± SD	2.2 ± 0.26	2.1 ± 0.25	P>0.05
Monocyte count ($x10^9$/l) Mean ± SD	0.35 ± 0.11	0.3 ± 0.12	P>0.05
Eosinophil count ($x10^9$/l) Mean ± SD	0.18 ± 0.06	0.2 ± 0.06	P>0.05
Basophil count ($x10^9$/l) Mean ± SD	0.03 ± 0.01	0.04 ± 0.01	P>0.05
Platelet count ($x10^9$/l) Mean ± SD	225 ± 28	236 ± 31	P>0.05

There were no significant differences (p>0.05) between corresponding mean values of age and haematological parameters of G6PD deficient and G6PD replete donors.

In this study G6PD deficient and G6PD replete donors were not significantly different with respect to age and haematological parameters. Lack of significant difference between G6PD replete and deficient donors with respect to haematological parameters in this study indicated the absence of active haemolysis among our G6PD deficient donors at the time of donation. However, G6PD deficiency in blood donors has enormous potential clinical significance and implications on the recipients.

Although it has been reported that G6PD deficient red cells remain stable during storage, nonetheless it has been suggested that storage-related biochemical changes and depletion of antioxidant mechanisms may predispose stored G6PD deficient donor red cells to exacerbated storage-induced alterations, which increases their susceptibility to post-transfusion haemolysis in the recipients. [13,14]

In addition, G6PD replete patients requiring red cell transfusions may also receive oxidant medications or have acute infections, both of which can induce haemolysis of transfused G6PD deficient red cells.[14] This is consistent with previously reported cases of rising levels of serum bilirubin in patients of all ages transfused with G6PD deficient donor blood.[7]

Moreover, the haemolytic effects of transfused G6PD deficient red cells on young children and neonates appear to be more deleterious than the effects reported in adult patients.[7] The neonates are particularly vulnerable to haemolytic risk associated with transfusion of G6PD deficient donor red cells because the newborns, especially premature babies, have low antioxidant reserves and therefore cannot effectively combat the effect of oxidative stress on transfused G6PD deficient red cells.[15] It has been reported that exchange transfusion with G6PD deficient red cells was associated with exacerbated post-transfusion haemolysis and less effective clearance of serum bilirubin in babies with neonatal jaundice.[16] In addition, studies have shown that stored G6PD deficient red cells are more susceptible to potassium leakage, which would increase the risks of hyperkalaemia and arrhythmia in neonates and other susceptible recipients.[13,17]

The haemolytic risk and complications associated with transfusing G6PD deficient donor red cells will be even higher if the recipient patient is also G6PD deficient. In such circumstances, the transfusion of G6PD-deficient donor red cells into a G6PD deficient patient may predictably lead to aggravated intravascular hyper-haemolysis, which may cause a number of adverse events including worsening jaundice with increased risk of kernicterus, as well as elevated risks of thrombosis and/or acute renal failure.[18,19,20,21]

In order to mitigate the potential adverse effects of G6PD deficient donor red cells, the WHO recommends that individuals with G6PD deficiency with history of haemolysis should be not be accepted as donors, while individuals with G6PD deficiency without history of haemolysis can be accepted as donors but their red cells should not be used for intrauterine and neonatal transfusion or for transfusing any patient with G6PD deficiency irrespective of age.[1]

Thus, there is need for Nigeria and other tropical African countries in which G6PD deficiency is prevalent to upgrade the safety level of their transfusion services by making sure that all prospective donors are routinely screened for G6PD deficiency as recommended by the WHO and other relevant studies in the literature. [1,7,14]

CONCLUSION

The prevalence of G6PD deficiency among apparently healthy blood donors in Kano, North-West Nigeria is 20.4%, which though high, is consistent with the national prevalence rate as reported in the Nigerian general population.

REFERENCES

1. World Health Organization. Blood donor selection: guidelines on assessing donor suitability for blood donation. Geneva, 2012. Available at: http://www.who.int/bloodsafety/publications/bts_guideline_donor_suitability/en/. [Accessed March 3, 2018].
2. Aneke JC, Okocha CE. Blood transfusion safety; current status and challenges in Nigeria. *Asian J Transfus Sci* 2017; 11:1-5. doi:10.4103/0973-6247.200781.
3. Howes RE, Piel FB, Patil AP. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS Med* 2012; 9:e1001339. doi:10.1371/journal.pmed.1001339.
4. Luzzatto L, Gordon-Smith EC. Inherited haemolytic anaemias. In: Hoffbrand AV, Lewis SM, Tuddenham EGD, editors. Postgraduate haematology. London: Arnold; 2001. p. 120-143.
5. Luzzatto L. G6PD deficiency: a polymorphism balanced by heterozygote advantage against malaria. *Lancet Haematol* 2015; 2:e400-1. Doi: 10.1016/ S2352-3026(15)00191-X.
6. Sirugo G, Predazzi IM, Bartlett J, Tacconelli A, Walther M, Williams SM. G6PD A- deficiency and severe malaria in the Gambia: heterozygote advantage and possible homozygote disadvantage. Am *J Trop Med Hyg* 2014; 90:856-9. doi:10.4269/ajtmh.13-0622.
7. 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. doi:10.1016/j.tmrv.2013.10.003.
8. Roper D, Layton M. Investigation of the hereditary haemolytic anaemias: membrane and enzyme abnormalities. In: Lewis SM, Bain BJ, Bates I. Editors. Practical Haematology. London: Churchill Livingstone; 2006. p.205-236.
9. Erhabor O, Isaac Z, Abdulrahaman Y, Ndakotsu M, Ikhuenbor DB, Aghedo F, *et al*. Female gender participation in the blood donation process in resource poor settings: case study of Sokoto in north western Nigeria. *J Blood Disord Transfus* 2013;5:176. doi:10.4172/2155-9864.1000176.
10. Egesie OJ, Egesie UG, Jatau ED, Isiguzoro I, Ntuhun DB. Prevalence of sickle cell trait and glucose-6-phosphate dehydrogenase deficiency among blood donors in a Nigerian tertiary hospital. *Afr J Biomed Res* 2013;16:143-147.
11. Akanni EO, Oseni BS, Agbona VO. Glucose-6-phosphate dehydrogenase deficiency in blood donors and jaundiced neonates in Osogbo, Nigeria. *J Med Lab Diagn* 2010;1:1-4.
12. Aneke JC, Ibeh NC, Okocha CE, Orakpor E. Evaluation of glucose-6-phosphate dehydrogenase enzyme deficiency and methemoglobin concentration in blood donors in a Nigerian tertiary hospital-based blood bank. *Glob J Transfus Med* 2017;2:137-142. doi:10.4103/GJTM.GJTM_21_17.
13. Tzounakas VL, Kriebardis AG, Georgatzakou HT. Glucose 6-phosphate dehydrogenase deficient subjects may be better "storers" than donors of red blood cells. *Free Radic Biol Med* 2016;96:152–165. doi:10.1016/j.freeradbiomed.2016.04.005.
14. Francis RO, Jhang JS, Pham HP, Hod EA, Zimring JC, Spitalnik SL. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. *Vox Sang* 2013;105:271–82. doi:10.1111/vox.12068.
15. Perrone S, Negro S, Tataranno ML, Buonocore G. Oxidative stress and antioxidant strategies in newborns. *J Matern Fetal Neonatal Med* 2010;23:63-65. doi:10.3109/14767058.2010.509940.
16. Samanta S, Kumar P, Kishore SS, Garewal G, Narang A. Donor blood glucose 6-phosphate dehydrogenase deficiency reduces the efficacy of exchange transfusion in neonatal hyperbilirubinemia. *Pediatrics* 2009; 123: e96-100. doi:10.1542/peds.2008-2021.
17. Lee AC, Reduque LL, Luban NL, Ness PM, Anton B, Heitmiller ES. Transfusion associated hyperkalaemic cardiac arrest in paediatric patients receiving massive transfusion. *Transfusion* 2014;54:244–254.
18. Farouk ZL, Ibrahim M, Ogala WN. Glucose-6-phosphate dehydrogenase deficiency; the single most important cause of neonatal hyperbilirubinaemia in Kano, Nigeria. *Nig J Paediatr* 2017;44:44-49.
19. Farouk ZL, Muhammed A, Gambo S, Mukhtar-Yola M, Umar Abdullahi S, Slusher TM. Follow-up of children with kernicterus in Kano, Nigeria. *J Trop Pediatr* 2018;64:176-182. doi:10.1093/tropej/fmx041.
20. Thompson PA, Chew E, Szer J. Deep vein thrombosis in association with acute intravascular haemolysis in glucose-6-

phosphate dehydrogenase deficiency: a unique case. *Intern Med J* 2013;43:1164–1165. doi:10.1111/imj.12268.

21. Abdel Hakeem GL, Abdel Naeem EA, Swelam SH. Detection of occult acute kidney injury in glucose-6-phosphate dehydrogenase deficiency anemia. *Mediterr J Hematol Infect Dis* 2016;8,e2016038. doi:10.4084/MJHID.2016.038.