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Original Article

SCREENING FOR G6PD DEFICIENCY IN BLOOD DONORS

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ABSTRACT

AIMS AND OBJECTIVES: 1. To screen blood donors [n=100] for G6PD deficiency, particularly in males. 2. To determine the frequency of G6PD deficiency in the population tested. **MATERIALS AND METHODS:** 50 samples of donors' blood from at KIMS Blood Bank. Method of collection of data: The Methaemoglobin reduction test: Principle: Sodium Nitrate oxidises MethHb to Hb and the subsequent enzymatic reconversion to Hb in the presence of Methylene blue. This occurs by activation of MethHb reductase in Pentose phosphate pathway. Interpretations: (a) Non sensitive individuals: sample is clear red like normal standard. (b) Full expression: sample is dark grey or brown, like positive standard. (c) Intermediate expression: Colour varies between the positive and negative controls in accordance with the degree of expression of the trait. **OBSERVATIONS AND RESULTS:** All the samples collected tested negative for Glucose-6-phosphate dehydrogenase (G6PD) deficiency. The percentage of male donors (93%) was greater than that of female donors (7%). the highest no. of donors, both male and female were of the blood group O positive. The age of the donors ranged from 18 to 49 years, with a mean of 33.5 years. Their Hemoglobin values ranged from 12.5 to 16 g/dl with a mean of 14.25 g/dl. **CONCLUSIONS:** This study was conducted to determine the prevalence of G6PD deficiency in blood donors, particularly male, and therefore to conclude whether screening for it should be included as a routine in blood banks prior to transfusion. A continued study for longer periods, involving a larger number of donors from different geographical areas, castes and communities may be required to ascertain the prevalence of the enzyme disorder and the necessity of routinely screening for it in healthy blood donors. Different study methods may also be used to determine the same.

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1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common known inherited human enzyme disease, affecting 10% of the world population, which amounts to 200 – 400 million affected people worldwide. It is an X-linked disorder genetically transmitted by a gene of intermediate dominance¹. According to world health organization 7.5% of world population are carriers of glucose-6-phosphate dehydrogenase deficiency and 2.9% were G6PD deficient. This enzyme deficiency is very prevalent in individuals of Africa, America, Mediterranean, and East Asia. In India the incidence of G6PD has been variably reported as 0–37% in different castes and communities. Higher incidence of G6PD deficiency is seen in north and west India (15%) as compared to south India (1–2%)².

Glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate: NADP 1-oxidoreductase, E.C.1.1.1.49) catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate cycle and reduces nicotinamide adenine dinucleotide phosphate (NADP) to NADPH^{1,6}.

G6PD is also described as a house keeping enzyme essential for basic cellular functions. As an antioxidant enzyme, G6PD is present in all human cells but it is particularly essential to red blood cells because these cells have no alternative source for producing NADPH. Oxidant damage to Hb leads to precipitation of Hb, which maybe morphologically recognized as Heinz bodies. NADPH generated by G6PD activity is utilized for glutathione (GSH) reductions, which restores Hb to the soluble form by protecting the sulfhydryl (-SH) groups from oxidation. This represents the major defence against oxidative damage of Hb1.

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G6PD deficiency represents a circumstance in which abnormalities both of the erythrocyte and of the environment contribute to rupture of the cell wall of the erythrocyte with resultant hemolytic anemia^{1, 14}. The likelihood of developing hemolysis and its severity is determined by the magnitude of the enzyme deficiency, which is consistent with the biochemical characteristics of each G6PD variant. G6PD deficiency is also known to be associated with neonatal jaundice, kernicterus and even death^{1,7}.

The hemolysis so induced is self-limiting. It is induced by a sudden destruction of the older and more deficient erythrocytes due to particular triggers in G6PD deficient patients, leading to oxidative damage to RBCs and hemolysis. Triggers can be exposure to certain drugs like primaquine or nitrofurantoin, viral or bacterial infections, ingestion of favabeans or metabolic abnormalities^{1, 14}. Since it is X-linked, males are usually affected while females mostly become carriers¹⁵.

Donor red blood cells from such donors, therefore, have shortened life span and will not benefit the recipients¹. A study, which used autologous G6PD-deficient A- RBC of healthy volunteers, found them inferior to normal cells¹¹. Susceptibility to hemolysis resides in the red cell enzyme. Consequently, were a patient to receive G-6-PD-deficient blood by transfusion and then be given a hemolytic drug or get infections, the donor cells might lyse¹². Exchange transfusion of G6PD-deficient blood has been found to cause hemolysis in infants and exaggeration of neonatal hyperbilirubinemia³.

Usually the affected individuals are asymptomatic unless they are provoked by oxidative stress. Currently, the donor's blood is not routinely screened for G6PD deficiency in blood banks¹. Severe manifestations of G6PD deficiency can be prevented if necessary precautions are taken, by screening for the enzyme deficiency in the asymptomatic population represented by healthy blood donors before transfusing their blood into recipients².

Hence the need for screening.

This was a short observational study done on 100 voluntary blood donors in the Blood bank attached to Department of Pathology, Kempegowda Institute of Medical Sciences, Hospital and Research centre, Bangalore.

MATERIALS AND METHODS:

This was a short observational study done on 100 voluntary blood donors in the Blood bank attached to Department of Pathology, Kempegowda Institute of Medical Sciences, Hospital and Research centre, Bangalore. Originally proposed as n=50, it was increased during the study period to 100 when the first 50 samples yielded no positives.

Any donor whose blood is rejected for storage or transfusion because of limitations with respect to age, weight, Hb levels and other physiological conditions like pregnancy or presence and/or increased risk of transfusible infections or malignancies were not included in the study.

Method of collection of data:

(A): The Methaemoglobin (methylene blue dye) reduction test:

Principle: The test consists of the oxidation of Hb to MethHb by Sodium nitrite and the subsequent enzymatic reconversion to Hb in the presence of Methylene blue. This occurs by stimulation of the Pentose phosphate pathway and activation of Methaemoglobin reductase.

Reagents required:

Normal saline,

0.28M glucose solution,

Acid Citrate Dextrose Inosine (ACDI) solution,

0.18M Sodium nitrite solution,

0.0004M Methylene blue chloride solution,

0.02M Phosphate buffer,

0.4M Sodium cyanide solution,

0.6M Potassium ferricyanide solution.

Preparation & storage of blood samples: 2ml of venous blood mixed with ACDI (0.15ml per ml of whole blood) is taken. If it is not used immediately, it can be kept refrigerated and stored till one week for reliable test results.

Procedure: Place 0.5ml of the blood sample in a test tube. Add 0.025ml of the sodium nitrite solution. Then add 0.025ml of the methylene blue chloride solution. Mix the sample by gentle inversion 12 times.

Place it in a water bath at 37°C. At 180 minutes, the incubation is finished.

Pipette 0.1ml of the incubated mixture into 10ml of phosphate buffer (pH 6.6). Between 2.5 and 5 minutes after adding the blood, compare the unknown samples with the standard by visual inspection.

Interpretations:(Table 1)

(a) Non sensitive individuals: Colour of diluted sample is clear red like the normal standard.

(b) Intermediate expression: Color varies between the positive and negative controls in accordance with the degree of expression of the trait.

(c) Full expression: Color of diluted sample is dark grey or brown, like the positive standard.

(B): G6PD enzyme assay was done for confirmation of deficiency wherever possible.

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All the data collected in this study were analysed by computing descriptive statistics like mean, standard deviation and percentages.

TABLES: 1. Interpretation of the test result

SAMPLE	TEST	POSITIVE CONTROL	NEGATIVE CONTROL
BLOOD	0.5 ml	0.5 ml	0.5 ml
DEXTROSE + SODIUM NITRITE SOLUTION	0.025 ml (25 µl)	0.025 ml (25 µl)	----
METHYLENE BLUE	0.025 ml (25 µl)	----	----

Table 1: Interpretation of the methylene blue reduction test.

OBSERVATIONS AND RESULTS: (Table 2 and 3)

All the samples collected tested negative for Glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Total no. of donors, N = 100 (Males = 93, Females = 7)

Age range: Males: 18 – 49 years

Females: 26 – 45 years

Hemoglobin range: Males: 12.8 – 16 g/dl

Females: 12.5 – 14 g/dl

As stated before, all the samples collected during the time of study tested negative for G6PD deficiency.

As shown by Table 2, the percentage of male donors (93%) was greater than that of female donors (7%). As shown in Table 1, the highest no. of donors, both male and female were of the blood group O positive. The age of the donors ranged from 18 to 49 years, with a mean of 33.5 years. Their Hemoglobin values ranged from 12.5 to 16 g/dl with a mean of 14.25 g/dl.

2. Blood groups and sex distribution of blood donors

SEX	BLOOD GROUP							
	O+ve	O-ve	A+ve	A-ve	B+ve	B-ve	AB+ve	AB-ve
Male	38	1	19	2	22	1	9	1
Female	5	1	-	-	1	-	-	-

Table 2: Sex distribution of the different blood groups

3. Hemoglobin levels of blood donors

SEX	AGE (yrs)	Hb (g/dL)	No. of donors
Male	33.5	14.4	93
Female	35.5	13.25	7

Table 3: HB levels of blood donors with sex distribution

DISCUSSION:

This study was attempted to screen for the presence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the asymptomatic population represented by the healthy blood donors and to infer whether the screening test should be added as a routine test before accepting blood donations. But the data collected did not achieve any statistical significance with respect to G6PD deficiency.

Our results differ from a few previous studies which have shown a higher prevalence of the disorder among the blood donors, indicating the need for screening as a routine test in blood banks.

In the study by A M Shanthala Devi et al, sixteen blood donors out of 2005 screened by methaemoglobin reduction test were found to be G6PD deficient. Among these, two blood donors were from West Bengal, one from Kerala and the rest of the 13 subjects were from Karnataka. This makes the incidence of the disorder to be 0.6% in the sampled population from Karnataka. But the study concluded that the prevalence of G6PD being very low in the study and as many people remain asymptomatic unless challenged with oxidative drugs or infection, screening the whole population for G6PD deficiency was not warranted, but screening of blood to be transfused for select population like neonates was advisable².

In another study by Ching-Shan Huang et al, most (88, 90.7%) of the 97 donors tested were confirmed to be G6PD deficient at the DNA level. But since the technique used was molecular biology, our test fails to match its specificity and sensitivity. But again, the study concluded that it was unnecessary to screen G6PD activity for donors of adult recipients in Taiwan, since no significant hemolytic reactions in the recipients were found post-transfusion³.

But in a study done by H. Amoozegar et al among blood donors in Shiraz, Iran, a prevalence of 6% was noted (27 out of 450 blood donors were G6PD deficient). The study concluded the prevalence to be noteworthy and recommended screening the blood bags for this enzyme prior to use for simple or exchange transfusion in premature infants⁷.

Similarly, in a study conducted by E. O. Akanni et al among the blood donors in Osogbo, Nigeria, a prevalence of 19.5% (39 out of 200 donors) was obtained for the disorder. This study therefore concluded the necessity of including G6PD testing in the blood donors screening criteria in the study area¹.

Our study design differs from that of these studies and many more with respect to geography, ethnicity, the number of samples screened, the study period and some other criteria. Even the tests used in some of these studies are different from ours (methemoglobin reductase test).

Our test results coincide with the data by WHO which states the prevalence of the disorder in various parts of the world. According to this, the disorder is very prevalent in individuals of Africa, America, Mediterranean, and East Asia. In India the incidence of G6PD has been variably reported as 0–37% in different castes and communities. Higher incidence of G6PD deficiency is seen in north and west India (15%) as compared to south India where it is only 1–2 percent².

The very low prevalence of the disorder in South India and also the short period of study (2 months) accounts for a portion of the cause behind the negative results. All the samples collected were from donors hailing from the same geographical area and of the same ethnicity. Also, the test itself fails to match the sensitivities and specificities of some of the other tests available for detecting the enzyme disorder.

One study never answers all the questions conclusively and definitively. Thus, more studies done for longer intervals, with regard to determining the prevalence of this enzyme disorder involving a larger number of samples from donors coming from various geographical areas and if possible various castes and communities are required to arrive at a conclusive answer. Also, studies that determine the consequences of transfusing G6PD deficient blood into adult as well as neonatal recipients under various conditions would substantiate the answers obtained from similar studies.

CONCLUSIONS:

This study was conducted to determine the prevalence of G6PD deficiency in blood donors, particularly male, and therefore to conclude whether screening for it should be included as a routine in blood banks prior to transfusion. In our study consisting of 100 blood donors, 93 of them male and 7 female, none of the samples tested were G6PD deficient, as determined by the Methemoglobin reductase (methylene blue) method. Thus according to this study, screening for the enzyme deficiency need not be included under the routine tests in blood banks. A continued study for longer periods, involving a larger number of donors from different geographical areas, castes and communities may be required to ascertain the prevalence of the enzyme disorder and the necessity of routinely screening for it in healthy blood donors. Different study methods may also be used to determine the same.

REFERENCES:

1. E.O.Akanni, B.S.A.Oseni, V.O.Agbona, B.A.Tijani, E.Tosan, E.E.Fakunle and V.O.Mabayoje. Glucose-6-phosphate dehydrogenase deficiency in blood donors and jaundice neonates in Osogbo, Nigeria. *Journal of Medical Laboratory and Diagnosis* Vol. 1(1); 1-4; April 2010.
2. A.M.Shanthala Devi, Rose Helen, Alwar Vanamala, V.Chaitra and Rameshkumar Karuna. Screening for G6PD Deficiency in Blood Donor Population. *Indian Journal of Hematology and Blood Transfusion*, 2010 September; 26(3): 122-123.
3. Ching-Shan Huang, Yung Chan Sung, May-Jen Huang, Chung-Shi Yang, Wei-Shang Shei and Tang K. Tang. Content of Reduced Glutathione and Consequences in recipients of Glucose-6-Phosphate Dehydrogenase Deficient Red Blood Cells. *American Journal of Hematology*, 1998; 57: 187-192.
4. Sridevi Sukumar, Malay B. Mukherjee, Roshan B. Colah, Deepika Mohanty. Molecular Basis of G6PD Deficiency in India. *Blood cells, Molecules and Diseases* 2004; 33: 141-145.
5. WY Au, SK Ma, AKW Lie, R Liang, T Cheng and YL Kwong. Post-transplant Complications: Glucose-6-phosphate Dehydrogenase Deficiency and Hematopoietic Stem Cell Transplantation. *Bone Marrow Transplantation*, 2002; 29: 399-402.
6. Muayad M. Abboud, Wajdy Al-Awaida. Synchrony of G6PD Activity and RBC Fragility Under Oxidative Stress Exerted At Normal and G6PD Deficiency. *Clinical Biochemistry* 2010; 43: 455-460.
7. H. Amoozegar. M. Mirshakeri, N. Paishva. Prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency Among Male Donors in Shiraz, Southern Iran. *IJMS*, Vol. 30, No. 2, June 2005.
8. WY Au, JC So, SK Ma, Albert KW Lie. Glucose-6-Phosphate Deficiency and Hematopoietic Stem Cell Transplantation in Chinese Patients. *Hong Kong Medical Journal* Vol. 15, No. 3, Supplement 3, June 2009.
9. A. Morelli, U. Benatti, G. F. Gaetani and A. De Flora. Biochemical Mechanisms of Glucose-6-Phosphate Dehydrogenase Deficiency. *Proc. Natl. Academy of Sciences, USA*. 1978; Vol. 5, No. 4: 1979-1983.
10. Fatemeh Emamghorashi, Farhang Hooshmand, Abdolrahman R. Mohtashamifar. Screening for Glucose-6-phosphate Dehydrogenase Deficiency in Blood Donors. *Transfusion*; November 2009; Vol. 49 (2).
11. Oded Shalev, Noga Manny, Raphael Sharon. Posttransfusional Hemolysis in Recipients of Glucose-6-Phosphate Dehydrogenase Deficient Erythrocytes. *Vox Sang* 1993; 64: 94-98.
12. P. R. McCurdy and E. E. Morse. Glucose-6-Phosphate Dehydrogenase Deficiency and Blood Transfusion. *Vox Sang* 1975; 28: 230-237.
13. A. Tizianello, I. Pannacciulli, E. Salvidio and A. Gay. Erythrocytic Glucose-6-Phosphate Dehydrogenase Deficiency as a Problem in the Selection of Blood Donors. *Vox Sang* 1963; 8: 47-50.
14. Ernest Beutler. Study of Glucose-6-Phosphate Dehydrogenase: History and Molecular Biology. *American Journal of Hematology* 1993; 42: 53-58.
15. E. Beutler. G6PD: Population Genetics and Clinical Manifestations. *Blood Reviews* 1996; 10: 45-52.
16. Chun Hay Ko, Karen Li, Chung Leung Li, Pak Cheung Ng, Kwok Pui Fung, Anthony Edward James, Raymond Pui-On Wong, Goldie Jia-Shi Gu, Tai Fai Fok. Development of a Novel Mouse Model of Severe G6PD Deficiency for in vivo and in vitro Assessment of Hemolytic Toxicity to Red Blood Cells. *Blood Cells, Molecules and Diseases*; 2011; 47: 176-181.
17. Anna L. Peters and Cornelis J. F. Van Noorden. Glucose-6-Phosphate Dehydrogenase Deficiency and Malaria: Cytochemical Detection of Heterozygous G6PD Deficiency in Women. *Journal of Histochemistry and Cytochemistry* 2009; Vol. 57(11): 1003-1011.
18. Harish Nair. Neonatal Screening Program for G6PD Deficiency in India: Need and Feasibility. *Indian Pediatrics*; December 17, 2009; Vol. 46: 1045-1049.