International Journal of Advanced Research in Engineering Technology & Science ISSN: 2349-2819
Email: editor@ijarets.org Volume-7, Issue-6 June- 2020 www.ijarets.org

ASSESSING ACTIVITY LEVELS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) IN DIFFERENT GROUPS

Pritima Sharma
Sri Satya Sai University of Technology & Medical Sciences, Sehore
Subject-Microbiology

ABSTRACT

Each and every tissue in the body has the enzyme G6PD, which is involved in the first step of the pentose phosphate pathway. Tropical and subtropical regions around the world have high rates of G6PD deficiency, in part because of the protection it provides against malaria infection. It is estimated that more than 200 million people worldwide suffer from a lack of the enzyme glucose-6-phosphate dehydrogenase (G6PD). G6PD deficiency with persistent non-spherocytic hemolytic anemia is a rare but severe condition observed worldwide. For the purpose of this study, 100 respondents were selected to assess the activity levels of G6PD in the control and normal group. In order to learn more about the extent of G6PD deficiency and the consequences for human health, the current investigation was carried out.

Keywords: Malaria, Activity, Disease, Glucose, Deficiency.

I. INTRODUCTION

G6PDd (glucose-6-phosphate dehydrogenase deficiency) is a critical concern in the treatment, management, and elimination of malaria. It affects roughly 400 million people and is the most common genetic condition in humans. The deficiency is prevalent in populations where malaria is common, with an average incidence of about 8%, most likely because G6PDd gives a survival benefit against infection, as seen in Fig. 1. G6PDd has a wide range of residual enzyme activity phenotypes (ranging from 1% to 150%) and underlying genotypes (many dozens of clinically significant mutants are known). There are mutations all throughout the length of this incredibly lengthy gene (20 K bases with 13 exons). G6PDd expression in females is frequently heterozygous (less frequently homozygous) as an X chromosome-linked trait, and the random process of lyonization of the trait during embryonic development determines the proportion of G6PD-normal to G6PD-deficient red blood cells (RBCs). Net G6PD enzyme activity, which is normally assessed from RBC lysate, can thus vary greatly depending on both the G6PDd variation and the proportion of RBC expressing it in females.

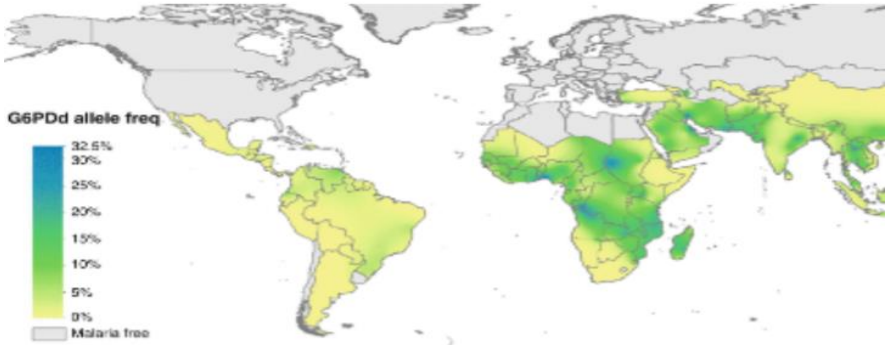

Fig. 1 Global distribution and prevalence of G6PDd

G6PD catalyses the hexose monophosphate shunt (HMS) rate-limiting process, which in RBC maintains a healthy reduction-oxidation (redox) equilibrium in the cytosol. When confronted with certain drugs, diets, or diseases, diminished G6PD activity limits the ability to maintain that equilibrium, resulting in an oxidizing cytosol. The risk of serious injury is determined by the extent to which G6PD activity is affected, as well as the dose and hemolytic potency of the challenging drug.

II. REVIEW OF RELATED STUDIES

Luzzatto et al., (2020) One of the most frequent human enzymopathies is glucose 6-phosphate dehydrogenase (G6PD) impairment, which is caused by hereditary mutations in the X-linked gene G6PD. Red blood cells with a G6PD deficiency are more prone to oxidative damage and thus hemolysis. There are around 200 G6PD mutations identified, nearly half of which are polymorphic and thus widespread in different populations. Approximately 500 million people with any of these mutations are mostly asymptomatic throughout their lives; however, when triggered by the ingestion of fava beans, any of a number of drugs (e.g. primaquine, rasburicase), or, more rarely, infection, any of them can develop acute and sometimes very severe hemolytic anaemia. Approximately half of G6PD mutations are spontaneous, resulting in persistent non-spherocytic hemolytic anemia in a small number of patients. Almost all G6PD mutations are missense mutations, resulting in amino acid substitutions that result in G6PD enzyme deficiency, either because the protein's stability is compromised, or because the catalytic activity is reduced, or a combination of both mechanisms: thus, genotype-phenotype correlations have been fairly well clarified in many cases. G6PD deficiency correlates strongly with past/present malaria endemicity in terms of geographic distribution: it is a rare example of an X-linked human polymorphism balanced by the protection of heterozygotes from malaria mortality. If acute hemolytic anaemia is detected early enough, it can be adequately treated. Where primaquine and its counterpart tafenoquine, which was recently introduced, are required for the elimination of malaria, reliable diagnostic tools are available, with point-of-care diagnostics becoming increasingly relevant.

Wong et al., (2017) G6PD deficiency is a known cause of severe newborn hyperbilirubinemia, and determining which infants are at risk could help to better allocate care and resources. We wanted to see if G6PD enzyme activity (EA) and particular gene variations were linked to neonatal hyperbilirubinemia that need phototherapy in the first week after delivery. Methods: Newborn infants with G6PD deficiency and a control group with normal fluorescent spot test results were chosen for analyses of G6PD EA and the 10 most common G6PD mutations in this region, with the goal of determining whether the infant's required phototherapy before being discharged from the hospital in the first week. A total of 222 newborns were enrolled, with an average gestation and birth weight of 38.3±1.8 weeks and 3.02± 0.48≤kg, respectively. Of these, n=121 had EA≤6.76 U/g Hb deficiency, and nearly half (43 percent) underwent phototherapy within the first week after delivery. The average EA concentration was 3.7 U/g Hb. With an area under the receiver-operating characteristic curve of 0.81 ± 0.05, the EA was quite accurate in predicting phototherapy use. Infants using phototherapy were more likely to have World Health Organization Class II mutations (remaining EA of 10%). Deficiency in EA and mutation at c.1388G>A (adjusted odds ratios of 1.5 and 5.7; 95 percent confidence intervals: 1.31-1.76 and 1.30-25.0, respectively) were found to be independent risk factors for phototherapy in a logistic regression study. Low G6PD EA (<6.76 U/g Hb) and the G6PD gene variation c.1388G>A are risk factors for newborn infants needing phototherapy in the first week after delivery.

Faruky et al., (2010) One of the most prevalent enzymopathies is glucose-6-phosphate dehydrogenase (G6PD) impairment, which can be a risk factor for a problematic pregnancy. Measure Hb, TC of RBC, serum bilirubin, and reticulocyte count in pregnant women with preeclampsia to monitor this enzyme

status, as well as Hb, TC of RBC, serum bilirubin, and reticulocyte count to monitor hemolytic state. In addition, to see whether there are any correlations between this enzyme level and all of these haematological markers. From January to December 2008, this cross-sectional study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka. Thirty pregnant women with preeclampsia, ranging in age from 20 to 34 years, were evaluated during their third trimester (>24 weeks) (group B). They were chosen from BSMMU's Obstetrics and Gynecology Out Patient Department (OPD) and Dhaka Medical College Hospital (BMCH). A control group of 30 seemingly normal pregnant women of the same gestational age (group A) was also studied for comparison. They were chosen through a personal connection. The level of G6PD in erythrocytes was determined using a Spectrophotometric technique and a Randox kit. Standard laboratory techniques were used to measure serum bilirubin, haemoglobin concentration, total RBC count, and reticulocyte count. ANOVA, independent sample t test, Chi-square test, and Pearson's correlation coefficient test were done using SPSS for Windows version 12 as needed for statistical analysis. The level of erythrocyte G6PD in preeclampsia was much lower than in controls in this investigation, however the percentages of participation were not statistically significant. Furthermore, the study group's haemoglobin concentration and RBC count were significantly lower, while serum bilirubin and reticulicyte count were significantly greater than the control groups. The amount of erythrocyte G6PD, on the other hand, was positively connected with hemoglobin concentration and total RBC count, but negatively correlated with serum bilirubin and reticulocyte count in the study group, and all of these correlations were statistically significant. The existence of G6PD deficit linked with hemolysis in preeclampsia may operate as a contributory element in the development of this problematic pregnancy, according to the findings of this study.

Mandas et al., (2009) In the pentose phosphate pathway (PPP), which produces reducing power (NADPH) and pentose phosphates, glucose-6-phosphate dehydrogenase (G6PD) is an essential metabolic regulatory site. The former is primarily responsible for the detoxification of chemical reactive substances, whereas the latter is responsible for cell proliferation regulation. G6PD insufficiency is the most frequent enzymopathy in humans, defined by a reduction in G6PD activity, primarily in red blood cells but also in nucleated cells. This drop in activity is not related to a problem with enzyme synthesis, but rather to a decrease in enzyme stability, which results in a shorter half-life. As a result, understanding the underlying processes linking G6PD deficiency to oxidative stress and cell proliferation is a huge challenge. In order to address this issue, we used fibroblasts isolated from pterygium, an eye proliferative lesion, from G6PD normal and deficient (PFs+ and PFs-, respectively) patients as an experimental paradigm. The fact that pterygium is thought to be produced by persistent oxidative stress induced by UV exposure, and that pterygium fibroblasts have a tumorigenic phenotype, influenced our decision. We used fibroblasts obtained from the conjunctiva of G6PD-normal and G6PD-deficient patients (NCFs+ and NCFs-, respectively) who had cataract surgery as controls. PFs grow quicker than NCFs, yet PFs- and PFs+ increase at the same pace, despite the fact that NCFs- grow more slowly than NCFs+. This was linked to considerably lower G6PD activity in NCFs+ compared to NCFs-, while there were no significant differences in G6PD activity between PFs+ and PFs-. The fact that G6PD mRNA levels were substantially higher in PFs- than in PFs+ backed up this conclusion. Increased green autofluorescence in both NCFs- and PFs- compared to equivalent positive cells was another intriguing discovery of this study, indicating substantial oxidative stress in deficient cells. Finally, in comparison to NCFs- and NCFs+, aberrant buildup of neutral lipids, primarily cholesterol esters, was found in both PFs- and PFs+. Though more research is needed to fully understand the mechanism linking G6PD to oxidative stress and cell proliferation, our findings allow us to speculate on the role of G6PD in tumorigenesis and to place G6PD-deficient people at a higher risk of developing common and dreaded proliferative disorders like atherosclerosis and cancer.

Bonilla et al., (2007) Glucose-6-phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway and the main intracellular source of reduced nicotidamineadenine nucleotidephosphate (NADPH), which is involved in a variety of physiological processes including antioxidant defence, endothelial growth modulation, erithropoyesis, vascularization, and phagocitosis (for example in the erythrocyte The most frequent X-chromosome-linked enzymopathy in humans is G6PDH deficiency. Despite the fact that it can be found in any cell type, its absence is fatal to life. G6PD insufficiency affects 400 million individuals worldwide, according to the WHO, while in Colombia, the severe type affects around 3% to 7% of the population. There are no data on minor and moderate changes that have therapeutic implications. This research examines various biomolecular components of G6PD, as well as its classification based on activity and electrophoretic mobility, as well as several key clinical issues associated to activity changes.

III. MATERIALS AND METHODS

Sample

Identification and other essential details of the patient and their family members were recorded in structured schedules for family studies in order to study familial aggregation andgenetic basis as well as for future reference. A total of 100 individuals were chosen for the present study.

Procedure

The assays were carried out at 30° C. The procedure was performed by UV-Kinetic Method using Commercial kits manufactured and suppliedby *Enzopak*. The assay was performed according to the instructions includedin the kit. On the basis of frequency distribution of activity levels, the critical level for diagnosing G6PD deficiency was considered 5.8 U/gHb. Any subject with an activity below this value was diagnosed as G6PD deficient.

Preparation of Red Cell Hemolysate

0.1 ml of whole blood was washed with 2 ml. aliquots of physiological saline (0.9%) 3 times, and then washed, packed and centrifugederythrocytes were suspended in precooled 0.5 ml of 3G6PDH (Lysing reagent). This was Mixed well and kept in the refrigerator (2-4° C) for at least 15 minutes and maximum for 2 hours. Lysate was centrifuged at 3000 rpm. for 5 minutes prior to use.

IV. RESULTS

On the basis of the frequency distribution of activity levels, the critical level for diagnosing G6PD deficiency was considered 5.8 U/g Hb. Any subject with an activity below this value was diagnosed as G6PD deficient. Quantitative G6PDH activities in G6PD deficient and normal subjects were evaluated and 89.13% G6PD deficient subjects exhibited the range below 4.6 U/g Hb (Table 1).

Table 1: Activity of glucose-6-phosphate dehydrogenase (G6PD) in G6PD deficient and normal control

Range (U/g Hb)	ZZG6PD deficient (n= 50)	Control (Normal in qualitative test) (n= 50)
<4.6	(88.15)	(0.00) **
4.7-13.5	(11.85)	(35.24) *
>13.5	(0.00)	(64.76) **

Figures in parentheses are the percentage

*Statistically significant difference ($p<0.05$).

**Statistically significant difference ($p<0.01$).

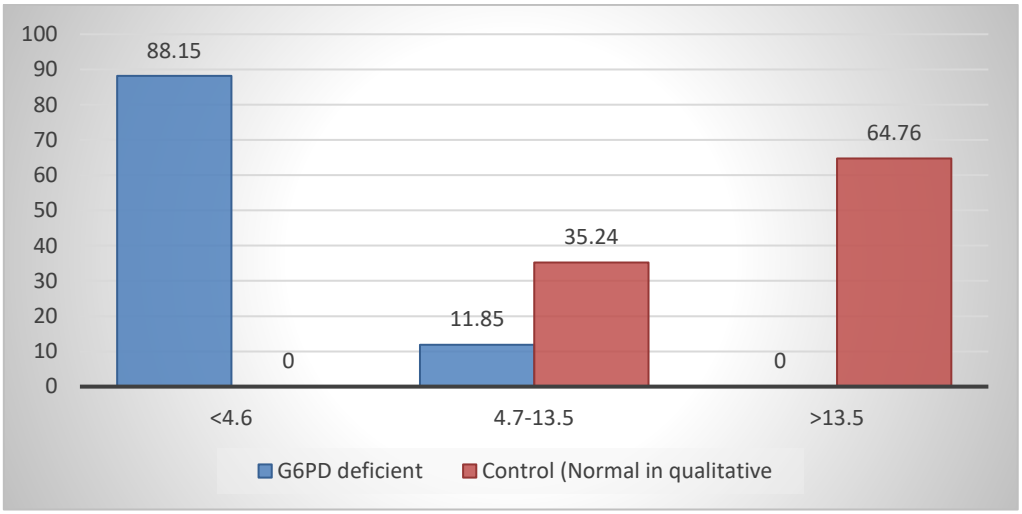

Fig. 4.4. Activity of G6PD in G6PD deficient and normal control

From the above table and graph it is clear that only 10.87% G6PD deficient subjects showed the activity in the range of 4.7-13.5 U/g Hb. The activity of G6PD in normal individuals was frequently noted in the range >13.5 U/g Hb (65.22%) and 4.7-13.5 U/g Hb (34.78%).

Table 2: Activity of G6PD enzyme in G6PD deficient infants, children and adults

Group	G6PD activity, U/g Hb			
	Range	Mean	SD	Variance
Infants (n=6)	3.9-13.5	9.62	4.07	16.39
Children (n=11)	2.4-12.8	4.41	2.79	8.11
Adults (n=33)	2.5-13.3	5.11*	3.31	10.59

Normal values: G6PDH activity (at 30° C): 4.6-13.5 U/g Hb

*Statistically significant difference with infants ($p<0.05$).

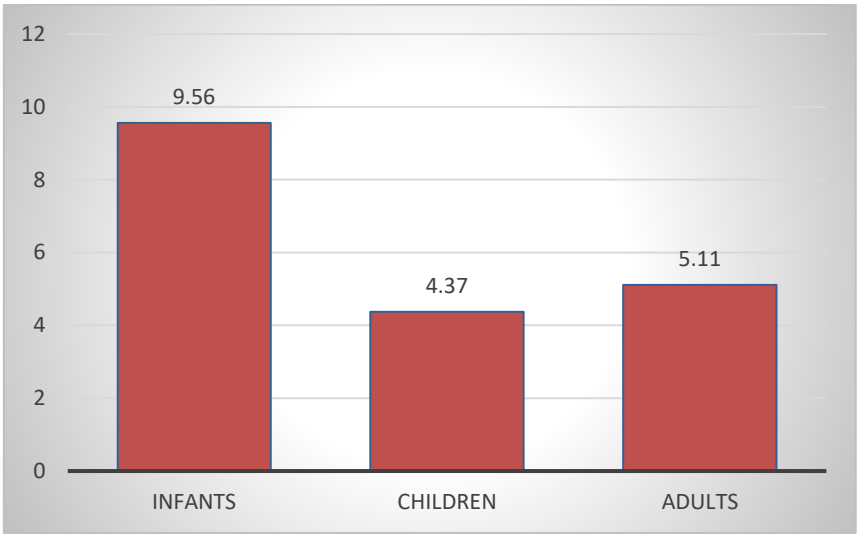

Figure 2: Mean Values of G6PD enzyme in G6PD deficient infants, children and adults

The mean G6PD activities in G6PD deficient infants, children and adults were 9.62 + 4.07, 4.41 + 2.79 and 5.11 + 3.31 respectively (Table 2).

V. CONCLUSION

Present study reveals that G6PD deficient people have greater risk of acquiring anaemia and jaundice. This study proposes preventing and minimising the clinical episodes of G6PD deficiency by avoiding fava beans, oxidative drugs and powerful infections that cause hemolysis. This calls for the deployment of low-cost, easy-to-use G6PD diagnostic devices that can be used even in remote areas without access to electricity. The challenge of reviewing and certifying such devices will involve many of the technologies mentioned in this article.

REFERENCES: -

[1] Beutler, E. (2008): Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood* **111** (1): 16–24.

[2] Bhasin M.K, Walter H. (2006): Genetics of castes and tribes in India. Kamla- Raj Enterprises: Delhi.

[3] Bonilla-Briceño, Javier & Sanchez-Corredor, Magda-Carolina & Chuaire-Noack, Lilian. (2007). Glucose-6-phosphate dehydrogenase (G6PD). Response of the human erythrocyte and another cells to the decrease in their activity. Revista Colombia Médica (ISSN: 0120-8322) Vol 38 Num 1. 38.

[4] Chalvam, R; Mukherjee, M.B;, Colah, R.B; Mohanty, D,; Ghosh, K. (2007): G6PD Namoru (208 T--> C) is the major polymorphic variant in the tribal populations in southern India. *Br J Haematol.*,**136**: 512-513.

[5] Dennery, P.A; Seidman, D.S; Stevenson, D.K. (2001): Neonatal Hyperbilirubinemia. *N Engl J Med*, **344:** 581-90.

[6] Faruky, Kazi & Begum, Noorzahan & Begum, Shelina & Ali, Taskina & Ferdousi, Sultana & Amin, Ahsanul. (2010). Study of Glucose6-phosphate Dehydrogenase (G6PD) Status in Preeclampsia. Bangabandhu Sheikh Mujib Medical University Journal. 2. 10.3329/bsmmuj.v2i2.4758.

[7] Luzzatto, Lucio & Ally, Mwashungi & Notaro, Rosario. (2020). Glucose-6-Phosphate Dehydrogenase Deficiency. Blood. 136. 10.1182/blood.2019000944.

[8] Mandas, Antonella & Peiretti, Enrico & Cocco, Pierluigi & Norfo, Claudia & Abete, Claudia & Angius, Fabrizio & Pani, Alessandra & Diaz, Giacomo & Dessì, Sandra & Fossarello, Maurizio. (2009). Glucose-6-phosphate-dehydrogenase deficiency as a risk factor in proliferative disorder development. Nature Precedings. 4. 10.1038/npre.2009.3001.1.

[9] Wong, Feiliang & Ithnin, Azlin & Othman, Ainoon & Cheah, Choe. (2017). Glucose-6-phosphate dehydrogenase (G6PD)-deficient infants: Enzyme activity and gene variants as risk factors for phototherapy in the first week of life. Journal of paediatrics and child health. 53. 10.1111/jpc.13509.