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Assessment of Glucose-6-Phosphate Dehydrogenase Deficiency and Anaemia in Apparently Healthy Students in Gombe State University

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) is a key regulatory enzyme in the pentose phosphate pathway (PPP) which produces nicotiamide adenine dinucleotide phosphate (NADPH) to maintain an adequate reducing environment in cells and is especially important in red blood cells (RBC). Given its central role in the regulation of redox state, it is well known that deficiency of this enzyme causes haemolytic crisis and could affect both males and females. This study aimed at determining the packed cell volume (PCV) and finding out the prevalence of G6PD in both genders. Samples were collected randomly from apparently healthy students in Gombe state university and results were analysed according to age, gender and PCV and presented in percentages. The prevalence of G6PD in the total population screened indicates that the deficiency of G6PD in male subjects was 20% and 7.5% in female subjects which indicates that the male subjects of Gombe state University had higher number of deficient subjects. PCV (35.93±15%) of both male and female subjects who reacted positively to G6PD deficiency was significantly ($p<0.05$) lower when compared to the PCV (40.6 6±78%) of students without deficiency (normal group) and those with intermediate status of G6PD deficiency (39.32±7%). The study therefore suggests the need for routine G6PD screening test on anaemic patients to avoid factors which could further precipitate hemolytic crisis.

Keywords:
Glucose-6-phosphate dehydrogenase (G6PD), PCV, NADPH, Red blood cells (RBC).

INTRODUCTION

Red blood cell metabolism provides the cell with energy in the form of adenosine triphosphate (ATP) to pump ions against electrochemical gradients, maintain its shape, keep iron from haemoglobin in the reduced form and maintain enzyme and haemoglobin sulfhydryl groups (Nader *et al.*, 2012). The main source of metabolic enzyme comes from glucose. Glucose is metabolized through the Embden-Meyerhof glycolytic pathway and through the Hexose Monophosphate Shunt (HMP) to produce adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). 2, 3,-bisphosphoglycerate is an important regulator of the oxygen affinity of haemoglobin (Nader *et al.*, 2012). In the glycolytic pathway, glucose-6-phosphate dehydrogenase (G6PD) enzyme plays an active role in the survival of erythrocytes In the hexose monophosphate (HMP) pathway, also known as the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD) is critical for producing NADPH. NADPH maintains the redox balance within erythrocytes, protecting them from oxidative stress and ensuring their survival. It is known that in the pentose phosphate pathway of erythrocytes, glucose-6 phosphate dehydrogenase (G6PD) enzyme provides the NADPH and GSH. GSH produced by pentose phosphate pathway can react with H_2O_2 and reduce it to H_2O. This prevents the generation of oxidative stress within red blood cells, oxidative stress can be induced in erythrocytes whose G6PD enzymes are deficient. In this situation, GSH is not produced and H_2O_2 is not reduced to H_2O, leading to oxidative stress and haemolysis (Beutler, 2013; Luzzatto, 2015). This is the only mechanism available for the erythrocyte to generate reducing equivalents, therefore making it essential for the survival of erythrocytes. In individuals whose G6PD enzyme is deficient, different kinds of haemolysis from mild to severe are seen bound to differences in variants of the disease (Beutler, 2013; Luzzatto, 2015). Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathological disease in humans. This disease is described as a widespread, inheritable, X-chromosome linked, abnormality (Reclos *et al.*, 2014). It is estimated that it affects approximately 400 million people worldwide (Noori-Daloii *et al.*, 2014). This disease is seen most frequently in approximately all of Africa, Asia, and the countries near the Mediterranean Sea (Frank, 2015). Haemolytic anaemia is a type of anaemia that leads to the destruction of red blood cells (Qaseem *et al.*, 2018). It is caused by the inability of red blood cells to function properly leading to spherocytosis, elliptocytosis, fauvism, sickle cell disease, and thalassemia. It can also be induced externally with chemicals like phenyl-hydrazine, dapsone, and hydroxylamine, divicine and aluminium chloride (Tata *et al.*, 2016: Telford *et al.*, 2017). The most common signs of haemolytic anaemia are early fatigue and exhaustion, others include weakness, pale mucosal membrane and pale skin and nail bed, irregular or fast heartbeat, short nail, chest pain, light headedness or mild vertigo, numbness or coldness of the extremities, and headache (Lippi *et al.*, 2016). It could be so mild at early stage that its symptoms are not noticed, but as the anaemia continued to progress, its symptoms become severe.

In Nigeria, G6PD deficiency occurs in 24% of boys and 5% of girls (Sodeinde *et al.*, 2015). It is also known to be a significant cause of anaemia in children, especially neonates (WHO, 2017). The resultant effect of the deficiency is the destruction of the red blood cells (RBCs) thereby giving rise to the episode of haemolytic anaemia, characterized by the physical signs and symptoms of jaundice with haemolysis of red cells seen in susceptible individuals (Ramnic, 2012).

Glucose-6-phosphate dehydrogenase deficiency is the most common enzymopathy in humans. It is triggered by predisposing biological agents, such as bacteria and virus infections and with drugs used for the treatment of malaria infection (e.g. quinine) and exposure to these drugs, bacteria and virus in association with the deficiency of the enzyme increases the breakdown of RBCs in vulnerable individuals, thus leading to haemolysis (Poole *et al.*, 2009).

Hereditary deficiency of red blood cell glucose-6-phosphate dehydrogenase (G6PD) is associated with clinical manifestations like drug-induced haemolytic anaemia, neonatal jaundice, favism, non-spherocytic congenital haemolytic anaemia (Ramnic, 2012). The drug-induced haemolytic anaemia associated with G6PD deficiency has an increased frequency of occurrence in some population due to high prevalence of malaria in those areas e.g. Asia, Mediterranean and Africa,

non-spherocytic congenital haemolytic anaemia occur mainly by involving the Embden-Meyerhof or hexose monophosphate pathway (Renu and Manoranjan 2013).

Reactive oxygen species (R0S) such as super oxide, hydrogen peroxide, hydroxyl radical, cause oxidative stress and damage of cells membrane (Savitha *et al.,* 2015). Increase production of ROS or decrease antioxidative defense enzymes play a major role in oxidative injuries in different organs, tissues, and cells including brain, heart, vascular cells (Renu and Manoranjan 2013), and causes brain disease like Alzheimer and Parkinson disease. They are considered to contribute to the aging process (Tsun-Yee *et al.,* 2017). Glucose-6-phosphate dehydrogenase, superoxide dismutases, catalase, glutathione peroxidases and glutathione reductase are antioxidant defense enzymes. In the human body, antioxidant defense system G6PD is an essential modulator enzyme with significant role in cells (red blood cells) (Leopold *et al.,* 2015). The G6PD functions in maintenance of redox potential in cell producing NADPH in pentose phosphate pathway (Leopold *et al.,* 2015), and in controlling of cell death.

Red blood cells exposure to certain drugs leads to the formation of hydrogen peroxide as the drug interacts with haemoglobin. The formation of free radicals from GSH, either through the action of peroxides or by direct action of drugs, may lead to the oxidation of GSH to its disulfide form. This process can result in the formation of a mixed disulfide with hemoglobin, which undergoes conformational changes that expose interior sulfhydryl groups to further oxidation and mixed disulfide formation. Normal red cell can defend themselves to a considerable extent against such changes by reducing the mixed disulphides of GSH and haemoglobin through glutathione to reductase reaction. The mechanism of anaemia is many, one of such could be deficiency of G6PD. Apparently healthy individuals therefore require the knowledge of the status of their G6PD which is lacking. G6PD is not routinely assess in clinical diagnosis. Therefore, there is need to determine the incidence to avoid encountering the agent that precipitates the crisis because of deficiency.

There are studies in the literature emphasizing the typical causes of haemolytic anaemia to be due to infections like plasmodium, hepatitis, concomitant administration of drugs and ingestion of fava beans. However, there is dearth of literature on the determination of status of Glucose-6-phosphate dehydrogenase G6PD and anaemia in an apparently healthy individual. Therefore, it is imperative to assess the status of Glucose-6-phosphate dehydrogenase G6PD in both normal and Anaemic individual. Understanding G6PD deficiency is crucial for mitigating public health risks, especially in environments where exposure to oxidative stressors, such as malaria medications or pollutants, is high. This study contributes to environmental science by highlighting the need for G6PD screening in regions prone to such stressors, potentially reducing adverse health impacts associated with environmental conditions and pharmacological treatments.

MATERIALS AND METHODS

This study was conducted with a total of 100 samples. The relatively small sample size may limit the generalizability of the findings. A larger, more diverse sample across different regions or universities would provide more robust and representative data (Rueangweerayut *et al.,* 2017).

Chemicals

All chemicals used for this screening test were of analytical grade and include sodium nitrite, D-glucose, methylene blue chloride.

Preparation of Reagents

Methylene Blue0.4mmol/L

0.15g of methylene blue chloride was weighed and transferred into a volumetric flask (1L). Some distilled water was added to completely dissolve the dye. The final volume was made up to 1 Litre (1L) and transferred into a dark brown bottle. It was then stored in the dark at room temperature.

Sodium Nitrite-Glucose Reagent

This was prepared by dissolving 0.5g sodium nitrite ($NaNO_2$) and 2.0 D-glucose in 40ml of distilled water.

Study Subjects

This study was carried out on a total of 100 apparently healthy subjects who are undergraduate students in Gombe State University, out of which 50 subjects were males while the other 50 were females and were randomly selected within the age

range of 19 to 30+ years.

Sample Collection

The blood samples used for this study were collected through venipuncture from the antecubital vein of the forearm with cotton wool soaked in 70% alcohol.

4ml of the blood was collected from each subject into Dipotassium Ethylene Diaminetetracetic acid (K2EDTA) containers and swirled to avoid clotting. The collected samples were screened immediately.

Methaemoglobin Reduction Test

The Methaemoglobin Reduction Test (see Table 1) was chosen for its simplicity and reliability in detecting G6PD deficiency (Brewer *et al.*, 1962**)**. Although enzymatic assays provide more quantitative results, the reduction test is cost-effective and widely accepted in field studies. However, it has limitations in detecting partial deficiencies, particularly in females with heterozygous mutations (Kalnoky *et al*., 2018; Beutler, 2013)

Principle of the test

Hemoglobin is oxidized to methaemoglobin by sodium nitrite. Methylene blue stimulates the hexose monophosphate pathway, which if intact, supplied NADPH, which in turn reduces brown methaemoglobin to red oxyhaemoglobin in subjects with normal G6PD activity.

Table 1: Procedure - Pipette into each test tube as follows:

Test Tube	Test	Normal	Deficient
Fresh Sodium-nitrate	0.1ml	-	0.1ml
Glucose	-	-	-
Methylene blue reagent	0.1ml	-	-
Subjects blood	1ml	1ml	1ml

i. The test tubes were sealed and mixed well (gently) and incubated at 37^0C for 3hrs.
ii. Three large tubes of 15ml capacity were taken and labeled “Test”, “Normal”, and “Deficient”. 10ml of distilled water was pipetted into each tube.
iii. 0.1ml of the well mixed sample from the test, normal and deficient tubes were transferred to the large tubes and mixed.
iv. The color of the solution in each tube was examined.

Test for packed Cell Volume

Heparinized capillary with well-mixed EDTA anticoagulated blood was filled with freshly collected blood sample to about three quarters of the capillary tube. The capillary tube was sealed at the unfilled end using sealant material. The capillary tube was centrifuged in a micro haematocrit centrifuge at RCF 12000-15000xg for 10 minutes to obtain constant packing of the red cells. The PCV value was read from the scale of a micro-haematocrit reader and expressed as percentage (see Table 2).

Table 2: Interpretation of Test Results

Observation	Inference
The color of the test solution is like the red color of the normal tube	Normal G6PD Activity
The color of the test solution is like the Brown Color of the deficient tube	Reduced G6PD Activity
The color of the test solution is neither like the red color of the normal tube nor the brown color of the deficient tube	Deficient G6PD Activity

Data Analysis

Data generated were subjected to statistical analysis using Microsoft Excel. Results were presented as mean + SEM and in percentages. The level of significance was obtained at 95% confidence limit.

RESULTS AND DISCUSSION

Results

The results for G6PD and anaemia screening of apparently healthy students in Gombe state university were presented in the tables below. Results were grouped, compared and analyzed according to PCV, gender and age. As shown in Table 3, the prevalence of G6PD in the total population screened i.e. both anaemic subjects and apparently healthy subjects were grouped in tables each showing the percentages and results deduced from the research.

Prevalence of Glucose 6 Phosphate Dehydrogenase (G-6-P-D) in the total population screened

The prevalence of Glucose 6 Phosphate Dehydrogenase (G6PD) in both anaemic (sickle cell) and apparently healthy students in Gombe State University is shown in Table 1. Out of the total 100 subjects tested, 15 were anaemic; only 3 had glucose 6 phosphate dehydrogenase deficiency, 7 show reduced G6PD activity while 5 were normal. Among the apparently healthy subjects, 12 had G6PD deficiency, 19 display reduced G6PD activity while 54 were normal.

Table 3: G6PD prevalence in the total population screened

G6PD Status	Anaemic No.(%) of subjects	Apparently Healthy No. (%) of Subjects
Normal	5(33.3)	56(65.8)
Reduced	7(48.6)	17(20)
Deficient	3(20)	12(14.1)
Total	15(100)	85(100)

Gender Distribution of Glucose 6 Phosphate Dehydrogenase (G-6-P-D)

Table 4 shows the Gender distribution of G6PD deficiency frequency in apparently healthy students and Anaemic students in Gombe State University Out of the apparently healthy male, 23(%) were normal, 9(20%) showed G6PD deficiency and 5(%) showed reduced G6PD activity while 3 out of the 5 anaemic patients tested had normal G6PD activity, 1 was G6PD deficient and 1 showed reduced G6PD activity.

Table 4: Gender Distribution of G6PD in Anaemic and Apparently Healthy Students in Gombe state university based on Gender

Gender	Groups	Apparently healthy	Gender (%)	Anaemic subjects
Male	Normal	36	80	2
	Reduced	-	-	-
	Deficient	9	20	1
	Total male	45	100	5
Female	Normal	30	75	3
	Reduced	7	15	5
	Deficient	3	7.5	2
	Total female	40	100	10
Overall	Male + Female	100	200	100

Relationship between Glucose 6 Phosphate Dehydrogenase Deficiency (G6PD) and Gender

Table 5 shows the relationship between Glucose 6 Phosphate Dehydrogenase (G6PD) deficiency and gender in apparently healthy and Anaemic Students. Out of the anaemic patients tested, 5 were males and 2 showed normal G6PD activities while 10 were females, out of which 3 had normal G6PD, 2 were G6PD deficient; 1showed homozygote deficiency while the other was heterozygote deficient. However, out of the apparently healthy students tested, 45 were males and 24 showed normal G6PD activity and 9 were G6PD deficient. Out of the 40 females tested, 30 were normal while 3 were deficient (2 heterozygotes and 1 homozygote).

The gender distribution of G6PD deficiency highlights a significant disparity, with males being more frequently affected. Socio-demographic

Table 5: Relationship between G6PD and gender

G6PD Status	Anaemic No.(%) of subjects		Apparently Healthy No. (%) of Subjects	
Gender n (%)	Male	Female	Male	Female
Normal	3	2	33	23
G-6-P-D deficient	2	1	2	3

factors, such as age and environmental exposure to oxidative stressors (e.g., malaria-endemic areas), may influence the prevalence of G6PD deficiency. Additional studies are needed to investigate the role of these factors (Reclos *et al.*, 2014).

Relationship between Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency and Age of Subjects

Table 6 shows the relationship between G6PD and age of subjects in sickle cell (Anaemic) subjects as well as apparently healthy subjects. The selected age group ranges from 18-21, 22-25, 26-29, 37 and above. Among the 5 sickle cell subjects within the age group 18-21, 1 showed G6PD deficiency while out of the 8 subjects within age group 22-25, only 2 showed deficiency in G6PD. However out of the 43 apparently healthy subjects within age group 18-21, 6 showed G6PD deficiency and among the 32 apparently healthy subjects ranging from 22-25, 6 were G-6-P-D deficient.

Table 6: Relationships between G6PD and Age of Subjects

Age group (years)	Anaemic		Apparently Healthy	
	Total subjects	Deficient subjects	Total subjects	Deficient subjects
18-21	5	1	43	-
22-25	8	2	32	6
26-29	2	-	6	6
>30	-	-	4	-

Please indicate if values shown are frequencies or percentages. Provide this information under each table as appropriate.

Relationship between Glucose 6 Phosphate Dehydrogenase Deficiency (G6PD) and Packed Cell Volume (PCV)

Table 7 shows the relationship between PCV and G6PD deficiency in both anaemic and apparently healthy individuals (Figure 1). The selected PCV was between 29-34, 35-40, 41-46, and 47-53. The PCV between 22-28 had 1 anemic subject that showed deficiency in G6PD, PCV between 29-34 had 2 Anemic subject and they showed deficiency in G6PD, PCV between 35-40 had 4 apparently healthy subjects deficient in G6PD, PCV between 41-46 also had 2 apparently healthy subjects deficient in G6PD while PCV between 47-53 also had 2 apparently healthy subjects deficient in G6PD.

Table 7: Relationship between PCV and G-6-P-D deficiency

Groups PCV	Sickle cell	Apparently healthy
	No of deficient subjects	No of deficient subjects
29-34	2	3
35-40	0	4
41-46	0	2
47-53	0	0

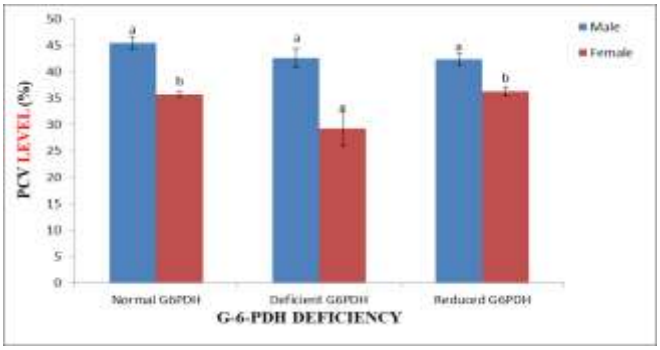

Figure 1: Relationship between G6PD and PCV

Effect of Glucose 6 Phosphate Dehydrogenase (G6PD) Deficiency on Packed Cell Volume (*PCV*)

Effects of G6PD Deficiency on PCV on apparently healthy Students in Gombe State University is presented in Table 8 The PCV (35.93±6.68%) of students who reacted positively to G6PD deficiency was significantly ($p<0.05$) lower when compared with the PCV (40.66±4.85%) of students without G6PD deficiency (normal group) and those with intermediate status of G6PD deficiency (39.32±3.01%).

Table 8: Effect of G6PD on packed cell volume

Group	N	Status (%)	PCV (%)
Normal G6PD activity	73	73	40.66
Reduced G6PD activity	12	12	39.32
Deficient G6PD activity	15	15	35.93

DISCUSSION

The higher prevalence of G6PD deficiency in males (20%) compared to females (7.5%) is expected due to the X-linked inheritance pattern of the gene. Males, having only one X chromosome, are more likely to express the deficiency if the gene is mutated, while females require mutations on both X chromosomes to be fully affected, hence the lower prevalence (Luzzatto *et al.*, 2015; Wang *et al.*, 2009). Due to the X-linked inheritance of G6PD deficiency, males are more likely to be affected, while females with heterozygous mutations may present intermediate activity. This highlights the need for gender-specific health interventions to manage G6PD deficiency, especially among women with intermediate enzyme activity who may be at risk of haemolytic episodes under oxidative stress (Luzzatto *et al.*, 2016; Willkins *et al.*, 2018). G6PD deficiency is the most common red blood cell enzyme deficiency worldwide, affecting over 400 million individuals across the globe. The clinical expression of G6PD deficiency involves a wide range of disease severity related to the ability of red blood cells to generate NADPH. Likewise, the observed prevalence of G6PD deficiency in both male and female subjects of Gombe State University shows that the male subjects within the region were much more affected than the female subjects. Screening was carried out as described previously by (Sharma *et al.*, 2020).

Table 1 shows the prevalence of Glucose 6 Phosphate Dehydrogenase (G6PD) in both anaemic and apparently healthy Students in Gombe State University 15 were anaemic and only 3 had glucose 6 phosphate dehydrogenase deficiency. Among the apparently healthy subjects, 12 subjects had G6PD deficiency. Table 4 shows the Gender distribution of G6PD deficiency frequency in apparently healthy students and anaemic students in Gombe State University Out of the apparently healthy male 9(20%) showed G6PD deficiency while 3 out of the 5 anaemic patients only 1 patient was G6PD deficient. Among the female subjects 3(7.5%) had G6PD deficiency and 12(30%) had reduced G6PD activity. This pattern aligns with findings from studies in Nigeria and other African regions where G6PD deficiency is common (Sodeinde *et al.*, 2015; Noori-Daloii *et al.*, 2014). Early detection of G6PD deficiency is critical in regions where oxidative stressors, such as malaria treatments, are common. Routine screening will help identify at-risk individuals, particularly males and heterozygous females, and reduce the incidence of hemolyticepisodes (Bancone *et al.*, 2017). G6PD deficiency is most frequently observed in Africa, Asia, and Mediterranean regions where malaria prevalence is high (Frank, 2015). The correlation between G6PD deficiency and lower PCV observed in this study reflects findings from previous research linking G6PD deficiency to higher susceptibility to hemolysis. This leads to reduced red blood cell count and a lower PCV, particularly under oxidative stress conditions like infections or drug exposure (Luzzatto *et al.*, 2015; Beutler, 2013).

CONCLUSION

The prevalence of G6PD in the total population screened indicates that the deficiency of G6PD amongst male subjects of Gombe State University is higher than that of the female subjects. It was also concluded that the PCV for both male and female subjects who reacted positively to G6PD deficiency within the deficiency was lower when compared to the PCV of normal subjects with no G6PD deficiency and subjects with intermediate status of G6PD deficiency. The study therefore suggests the need for routine G6PD screening test on anaemic patients to avoid factors which could further precipitate hemolytic crisis. Although the study provides insights into the prevalence of G6PD deficiency, the sample size remains a limitation. Future research should include a larger, more diverse population to confirm these findings and explore further implications for public health interventions. The primary limitation of this study is the small sample size, which reduces the generalizability of the findings. Additionally, the lack of data on environmental factors (e.g dietary habits, exposure to oxidative stress) may have influenced G6PD activity. Future studies should

include a larger, more diverse sample and explore the role of these environmental factors in G6PD deficiency. Routine screening for G6PD deficiency should be integrated into public health initiatives, especially in malaria-endemic regions where oxidative stressors are prevalent. Early detection can prevent hemolytic crises and improve patient outcomes (WHO, 2012). Public health interventions should focus on education and awareness to help manage G6PD deficiency, particularly in susceptible populations.

Ethical Statement: This study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. Approval for the research protocol was obtained from the Institutional Review Board of Gombe State University, and written informed consent was obtained from all participants. The data collected were anonymized to ensure confidentiality and privacy. Participation was voluntary, and subjects had the right to withdraw from the study at any point without penalty

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Conflict of Interest: The authors declare that they have no competing interests.

Supplementary Material: Supplementary material related to this article, including (e.g., raw data, tables, or figures), is available upon request or in the online version of this manuscript.

REFERENCE

Bancone, G., Kalnoky, M., & Chu, C. S. (2017). The G6PD flow-cytometric assay is a reliable tool for diagnosis of G6PD deficiency in women and anaemic subjects. Scientific Reports, 7, 9822.

Beutler E. (2013) G6PD deficiency. International Journal of molecular science; glucose-6-phosphate dehydrogenase: update and analysis of new mutations around the world. Blood; 84-3613–36.

Beutler, E. (2013). G6PD deficiency. *International Journal of Molecular Sciences*, 14(3), 4619–4660.

Frank, J. E. (2015). Diagnosis and management of G6PD deficiency. *American Family Physician*, 72(7), 1277-1282.

Kalnoky M, Crow JF. Hardy, Bancone G, Kahn M Eur J Haematol (2018). Cytochemical flow analysis of intracellular G6PD and aggregate analysis of mosaic G6PD expression. Weinberg and language impediments. Genetics; 100294–303. 152:821–5.

Lippi, Renu M, Bancone G, Kahn M Eur J Haematol Kalnoky M, (2016). Cytochemical flow analysis of intracellular G6PD and aggregate analysis of mosaic G6PD expression. 100:294–303.

Luzzatto L, Nannelli C, Notaro R., North Am (2016) Glucose-6-phosphate dehydrogenase deficiency. Hematol Oncol Clin; 30:373–93.

Luzzatto, L., & Poggi, V. (2015). Glucose-6-phosphate dehydrogenase deficiency: from genetics to clinical practice. Blood, 126(24), 2751–2763.

Noori-Daloii, M. R., et al. (2014). Glucose-6-phosphate dehydrogenase deficiency: Molecular and clinical aspects. *Blood*, 124(21), 1950.

Poole C, Nkhoma ET, Vannappagari V et al (2009). The global prevalence of glucose-6 phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis; 42:267–78.

Qaseem M., Kalnoky M, Bancone G, Kahn M., (2018) Cytochemical flow analysis of intracellular G6PD and aggregate analysis of mosaic G6PD expression. 100:294–303.

Reclos GJ, Kotaka, Morelli, Moltulsky T, Campbell-Kraut, Hatzidakis CJ, Schulpis KH. (2011) Glucose-6-phosphate dehydrogenase deficiency neonatal screening: preliminary evidence that a high percentage of partially deficient female neonates are missed during routine screening. J Med Screen 7:46–51.

Reclos LaRue N, Kahn M, Murray M., (2014). Comparison of quantitative and qualitative tests for glucose-6-phosphate dehydrogenase deficiency. *Am J Trop Med Hyg;* 91:854–61.

Reclos, G. J., Kotaka, M., Schulpis, K. H., & Tsun-Yee, P. (2014). Glucose-6-phosphate dehydrogenase deficiency: Screening, genetic variants, and new therapeutic insights. *American Journal of Medical Genetics*, 164(3), 233-246.

Rueangweerayut R, Bancone G, Harrell EJ (2017) Hemolytic potential of tafenoquine in female volunteers heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PD mahidol variant) versus G6PD-normal volunteers. Am J Trop Med Hyg. 97:702.11.

Sodeinde, O., *et al*. (2015). Prevalence of glucose-6-phosphate dehydrogenase deficiency in Nigerian populations. *Journal of Molecular Biology*, 22(5), 212–218.

Wang FL, Boo NY, Ainoon O Singapore Med J (2009). Comparison of detection of glucose-6-phosphate dehydrogenase deficiency using fluorescent spot test, enzyme assay and molecular method for prediction of severe neonatal jaundice. 50, 62–7.

WHO Scientific Group. WHO. Geneva: WHO; (2012) Standardization of procedures for the study of glucose-6-phosphate dehydrogenase: *report of a Technical Report Series* No. 366.

WHO South East Asia J Publ Health (2017). Implementation of G6PD testing and primaquine for P. vivax radical cure: operational perspectives from Thailand and Cambodia. 6:60–8.

WHO. Geneva: WHO (2012) Standardization of procedures for the study of glucose-6- phosphate dehydrogenase: report of a WHO Scientific Group. WHO Technical Report Series No. 366.

Willkins, Adam, Fetman, Noshad, Anjum, Kenangalem E, Karyana M, Burdarm L Malar J (2018). Plasmodium vivax infection: a major determinant of severe anaemia in infancy. 15:321.

World Health Organization (2012). Glucose-6-phosphate dehydrogenase deficiency: public health implications. Bulletin of the World Health Organization, 67(6), 601–611.