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ORIGINAL ARTICLE

Hematological Effects of the Co-Inheritance of Glucose-6-Phosphate Dehydrogenase Deficiency and Sickle Cell Disease

Michael H. Mezgebe, Nazeer Alli, Aamina Moosa, Susan Louw

University of the Witwatersrand, Faculty of Health Sciences, Department of Molecular Medicine and Haematology, Johannesburg, South Africa

ABSTRACT

Background: **Glucose-6-phosphate dehydrogenase deficiency (G6PDD) and sickle cell disease (SCD) both result in hemolysis associated with anemia. Studies on the hematological effects of SCD and G6PDD co-inheritance are limited, with conflicting results. The objective of the study was to compare the hematological parameters and biochemical markers of hemolysis viz., lactate dehydrogenase (LDH) and unconjugated bilirubin (UBR), in patients with SCD to patients with SCD/G6PDD co-inheritance.**

Methods: **A total of 214 patients with SCD including both homozygous (HbSS) and heterozygous (HbAS) variants, with or without concomitant G6PDD were identified over a 14-year period at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in South Africa. SCD was diagnosed with high-performance liquid chromatography (HPLC), while G6PD status was determined with G6PD spot testing. The full blood count (FBC) parameters, along with the UBR and LDH levels, were recorded.**

Results: **The median ages at diagnosis were 4.5 and 20 years for HBSS (with and without G6PDD) and HBAS (with and without G6PDD), respectively. The FBC parameters viz., red blood cell count (RCC), hematocrit (Hct), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean cell volume (MCV), as well as UBR levels, showed no significant differences between patients with HbSS alone and those with HbSS/G6PDD. However, patients with HbAS/G6PDD had significantly higher mean UBR levels ($p < 0.05$) in comparison to those with HbAS alone, although the FBC parameters showed no statistically significant differences between these groups.**

Conclusions: **Co-inheritance of G6PDD with HbAS does not result in anemia, but elevated biochemical markers of hemolysis suggest ongoing hemolysis. This raises concern about potential complications associated with chronic hemolysis, beyond anemia.**

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Correspondence:
Dr. Susan Louw
Room 12, Charlotte Maxeke
Johannesburg Academic Hospital
York Road
Johannesburg, Gauteng
South Africa
Phone: +27 11 4898822/ +27 82 6063487
Fax: +27 11 4898513
Email: Susan.louw@nhls.ac.za

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INTRODUCTION

Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is the commonest congenital red blood cell (RBC) enzyme deficiency, with a global prevalence of ~ 400 million people globally [1]. G6PDD is inherited as an X-linked condition and therefore males are predominantly affected. Homozygous females with G6PDD are far less common. Heterozygous females are obligate

carriers potentially affecting male offspring. Approximately 11% of affected patients are of Black African ancestry [2,3]. The G6PD enzyme drives the hexose monophosphate shunt, which is a branch of glycolysis that is responsible for NADPH (nicotinamide adenine dinucleotide phosphate) generation from NADP. NADPH reduces glutathione (GSH) which plays a critical role in neutralising reactive oxidant species, an inherent physiological by-product of red cell metabolism [4]. Red cells are also exposed to exogenous oxidants such as those released by endothelial and immune cells during inflammation or infection, as well as exogenous agents such as drugs and toxins. A decrease or absence of reduced GSH results in oxidative damage to proteins and lipids with destabilisation of the red cell membrane and eventual hemolysis [4]. The degree of hemolysis in patients with G6PDD depends on the severity of the enzyme deficiency [4-7].

Sickle cell disease (SCD) encompasses a group of sickling disorders that include homozygous (HbSS) and heterozygous SCD (HbAS) and compound heterozygous states such as HbS/HbC, HbS/beta thalassemia, HbS/HbO, etc. SCD affects 300 million people worldwide, with over 200,000 annual births in Africa [8,9]. The SCD genetic lesion is a single nucleotide substitution in the 6th codon of the beta globin gene that results in substitution of glutamic acid by valine at this codon position. The mutant hemoglobin (hemoglobin S (HbS)) is insoluble in the deoxygenated state due to an exposed hydrophobic domain of valine. Binding of valine to other amino acids from adjacent hemoglobin (Hb) tetramers renders the bound tetramers soluble and hence stable. This results in the formation of a continuous chain of HbS molecules, called sickle tactoids, that ultimately force the red cell into a sickle shape. Sickle cells are rigid and often become trapped in the microcirculation with resultant tissue hypoxia and severe pain localised to that region, referred to as a sickle crisis. Vasoocclusive episodes are triggered by hypoxia, infection, decrease in pH, trauma, etc. Repeated sickling and unsickling of red cells also causes damage to the red cell membrane and ultimately hemolysis.

Patients with HbAS are generally asymptomatic [10]. HbAS provides partial protection against Plasmodium falciparum malaria, significantly influencing the geographical distribution of the mutant allele [11]. Similarly, the high prevalence of G6PDD in malaria-endemic regions suggests a selective advantage against malaria, offering a survival advantage to these individuals [12]. Expectedly, there is a significant rate of co-inheritance of SCD and G6PDD in malaria endemic regions [13-15]. The combined effect of SCD and G6PDD on the rate of hemolysis and the severity of anemia remains unclear, with studies reporting conflicting results [13, 15,16].

In this study, we aimed to investigate the severity of anemia and hemolysis in patients with HbSS/G6PDD and HbAS/G6PDD) co-inheritance, compared to those with SCD alone.

MATERIALS AND METHODS

The results of 214 patients were retrospectively analysed (Table 1).

The study was conducted at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and included results of patients between January 2008 and April 2022 at diagnosis of SCD with or without associated G6PDD. Blood samples were collected in ethylenediaminetetraacetic acid (ETDA) containing BD (Becton Dickinson®, Plymouth, UK) vacutainers. Full blood counts were performed on the Sysmex XN9000® haematology blood counting analyser (Sysmex® Corporation, Kobe, Japan) at the National Health Laboratory Service (NHLS) laboratory, CMJAH. Results were obtained within 4 hours post collection. Hemoglobin subtypes were identified using 2 methods viz., 1) High-performance liquid chromatography (HPLC) (Bio-Rad® D-10 analyser®, CA, USA), and 2) Hb electrophoresis using the semi-automated Hydrasys® analyser (SEBIA® Hydrasys, Evry Cedex, France). G6PD activity was qualitatively measured with the fluorescent spot test [17,18]. Bilirubin and lactate dehydrogenase (LDH) levels were measured with Roche cobas® 8000 analysers (Roche®, Basel, Switzerland).

The data were captured on a Microsoft Excel spreadsheet (Microsoft®, Redmond, WA, USA). Quantitative data was tabulated and summarised with standard statistical analysis using SPSS® software (Statistical Package for the Social Sciences 11.0, Chicago, IL, USA). The normal distribution of the quantitative data in each group was tested using the Kolmogorov-Smirnov test. Chi-squared cross tabulation was used to categorise the data based on age, gender, and severity of anemia. The Student's *t*-test was utilised to compare the median values of Hb, red cell indices (viz., RCC, HCT, MCV, MCH, MCHC), UBR, TBR and LDH in all patients. A p-value of < 0.05 was considered statistically significant.

RESULTS

The results of 214 patients were analysed. Breakdown of patient numbers and conditions is summarised in Table 1.

Table 1. Number of study patients and hemoglobinopathy.

The median age at diagnosis was 4.5 and 20 years for HbSS and HbAS respectively (with or without coexisting G6PDD). The male: female ratio of the SCD/G6PDD group was 2:1 and 1:1 in the SCD patients without G6PDD.

In both the HbSS and HbAS groups, individuals with co-inherited G6PDD had lower RCC, Hb, HCT and MCV values compared to those without G6PDD, though these differences were not statistically significant ($p > 0.05$).

In assessing the degree of hemolysis, the HbAS/G6PDD

Table 1. Number of study patients and hemoglobinopathy.

Number	Diagnosis
HbSS	83
HbSS + G6PDD	21
HbAS	69
HbAS + G6PDD	41

HbSS: homozygous sickle cell disease, HbAS: heterozygous sickle cell disease, G6PDD: glucose-6-phosphate dehydrogenase deficiency.

Table 2. Median red blood cell indices, bilirubin and lactate dehydrogenase (LDH) of cohorts.

Parameter (RI)	HBSS (n = 83)	HBSS + G6PDD (n = 21)	p-value	HBAS (n = 69)	HBAS + G6PDD (n = 41)	p-value
Median age at diagnosis in years	4.5	4.5	-	20	20	-
M:F	1:1	2:1	-	1:1	2:1	-
HB (11.6 - 16.4 g/dL)	11.8	9.7	0.10	12.7	11.8	0.10
HCT (0.340 - 0.480%)	0.35	0.28	0.06	0.39	0.36	0.05
RCC ($3.93 - 5.40 \times 10^9$/L)	4.39	3.57	0.09	4.9	4.6	0.15
MCV (78.9 - 98.5 fL)	89.2	79.3	0.25	81.9	79.6	0.22
RCC ($3.93 - 5.40 \times 10^9$/L)	4.39	3.57	0.09	4.9	4.6	0.15
MCH (26.1 - 33.5 pg)	29.9	27.4	0.31	26.3	25.6	0.32
MCHC (32.7 - 34.9 g/dL)	34.0	34.5	0.65	31.9	32.1	0.57
Tbil (5 - 21 µmol/L)	70.48	54.18	0.35	9.38	57.27	0.04
Inbil (0 - 3 µmol/L)	53.90	32.83	0.53	6.15	39.74	0.02
LDH (100 - 190 U/L)	771.04	654.54	0.12	-	-	-

RI: reference index, HBSS: homozygous sickle cell disease, G6PDD: Glucose-6-phosphate dehydrogenase deficiency, HBAS: heterozygous sickle cell disease, HB: hemoglobin, HCT: hematocrit, RCC: red cell count, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration, Tbil: total bilirubin, Inbil: indirect bilirubin, LDH: lactate dehydrogenase.

group had significantly higher unconjugated bilirubin levels with a median value of 39.74 µmol/L compared to 6.15 µmol/L in the HbAS group without G6PDD (p = 0.02). The HbSS/G6PDD group also had higher median indirect bilirubin and LDH levels (53.90 and 771.04 U/L, respectively), compared to HbSS without G6PDD (32.83 and 654.54 U/L, respectively). These differences, however, were not statistically significant (p = 0.53 and 0.12, respectively). A summary of the results of the study population is provided in Table 2.

Table 2. Median red blood cell indices, bilirubin and lactate dehydrogenase (LDH) results of the study cohorts.

DISCUSSION

This study aimed to investigate the impact of G6PDD on HbSS and HbAS with respect to anemia and degree of hemolysis. The findings provide valuable insights into how G6PDD influences hematological parameters in the two genotypic groups.

The most notable finding was a significant increase in UBR levels in patients with co-inherited HbAS/G6PDD compared to HbAS alone, suggesting a higher rate of hemolysis in the former group. In contrast, there was no statistically significant difference in the mean hemoglobin levels between the two cohorts, indicating that

these patients can adequately compensate for the hemolysis. This finding is of clinical relevance, given the well-documented adverse effects of chronic hemolysis including iron overload, cholelithiasis, bony abnormalities, thromboembolic phenomena, etc. Chronic hemolysis contributes to a hypercoagulable state through several mechanisms, including nitric oxide (NO) scavenging by free hemoglobin, inflammation and platelet activation [19]. Further evaluation is needed to assess the clinical impact of co-inheritance and explore the need for more intensive long-term monitoring of HbAS patients with G6PDD.

Within the HbSS group, the results showed no statistically significant difference in the severity of anemia or the rate of hemolysis between HbSS patients with and without G6PDD. However, the severity of G6PDD was not assessed in this study, and the likely inclusion of individuals with mild enzyme deficiency could have influenced this result. Future studies specifically examining the impact of severe G6PDD in HbSS patients may yield more meaningful insights.

Unconjugated bilirubin and LDH levels were used as markers of hemolysis in this study. Even though haptoglobin is known to be a more sensitive and specific quality marker of hemolysis [19], it was not consistently measured in this cohort and could therefore not be included in the analysis. Haptoglobin is also not useful in assessing the degree of hemolysis [19].

Limitations of the study were as follows: 1) This was a retrospective record review, without the flexibility of performing additional tests such as quantitative G6PD assay (the inclusion of individuals with mild deficiency may have skewed the results); 2) The study was conducted at a single centre, limiting the generalisability of the findings; 3) Nutritional assessments were not included (deficiencies may have contributed to the anemia); 4) As a laboratory-based study, clinical information such as transfusion history and hydroxyurea therapy was not available.

CONCLUSION

While the degree of anemia in patients with HbSS and HbAS co-inheriting G6PDD does not appear to be worse, the observation of increased hemolysis in patients with co-existing HbAS and G6PDD is a notable finding. This warrants further investigation with prospective controlled studies.

Ethics Approval:
Ethics approval for this retrospective record review was obtained from The Human Research Ethics Committee of the University of the Witwatersrand, waiving the need for individual informed consent.

Source of Funds:
No costs were incurred in this record review study.

Data Availability Statements:
Raw data were generated at University of the Witwatersrand. Derived data supporting the findings of this study are available from the corresponding author on request and subject to the ethics committee guidance.

Disclaimer:
The views expressed in the submitted article are the authors own and not an official position of the institution.

Declaration of Interest:
The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

References:

1. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis 2009;42(3):267-78. (PMID: 19233695)

2. Mohanty D, Mukherjee MB, Colah RB. Glucose-6-phosphate dehydrogenase deficiency in India. Indian J Pediatr 2004;71(6):525-9. (PMID: 15226563)

3. Belfield KD, Tichy EM. Review and drug therapy implications of glucose-6-phosphate dehydrogenase deficiency. Am J Health Syst Pharm 2018;75(3):97-104. (PMID: 29305344)

4. Luzzatto L, Nannelli C, Notaro R. Glucose-6-Phosphate Dehydrogenase Deficiency. Hematol Oncol Clin North Am 2016;30 (2):373-93. (PMID: 27040960)

5. Harcke SJ, Rizzolo D, Harcke HT. G6PD deficiency: An update. JAAPA 2019;32(11):21-6. (PMID: 31609781)

6. Howes RE, Battle KE, Satyagraha AW, Baird JK, Hay SI. G6PD deficiency: global distribution, genetic variants and primaquine therapy. Adv Parasitol 2013;81:133-201. (PMID: 23384623)

7. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. Blood 2008;111(1):16-24. (PMID: 18156501)

8. Tsaras G, Owusu-Ansah A, Boateng FO, Amoateng-Adjepong Y. Complications associated with sickle cell trait: a brief narrative review. Am J Med 2009;122(6):507-12. (PMID: 19393983)

9. Diallo D, Tchernia G. Sickle cell disease in Africa. Curr Opin Hematol 2002;9(2):111-6. (PMID: 11844993)

10. Alli NA, Patel M, Alli HD, et al. Recommendations for the management of sickle cell disease in South Africa. S Afr Med J 2014; 104(11):743-51. (PMID: 25909112)

11. Williams TN, Mwangi TW, Roberts DJ, et al. An immune basis for malaria protection by the sickle cell trait. PLoS Med 2005;2 (5):e128. (PMID: 15916466)

12. Mukherjee MB, Colah RB, Martin S, Ghosh K. Glucose-6-phosphate dehydrogenase (G6PD) deficiency among tribal populations of India - Country scenario. Indian J Med Res 2015;141(5): 516-20. (PMID: 26139767)

13. Fasola FA, Fowodu FO, Shokunbi WA, Kotila TR. The effect of the coinheritance of Glucose-6-phosphate dehydrogenase deficiency on the severity of sickle cell disease. Niger Postgrad Med J 2019;26(2):118-22. (PMID: 31187752)

14. Adu P, Simpong DL, Takyi G, Ephraim RK. Glucose-6-Phosphate Dehydrogenase Deficiency and Sickle Cell Trait among Prospective Blood Donors: A Cross-Sectional Study in Berekum, Ghana. Adv Hematol 2016;2016:7302912. (PMID: 27703480)

15. Nouraie M, Reading NS, Campbell A, et al. Association of G6PD with lower haemoglobin concentration but not increased haemolysis in patients with sickle cell anaemia. Br J Haematol 2010;150 (2):218-25. (PMID: 20507315)

16. Steinberg MH, West MS, Gallagher D, Mentzer W. Effects of glucose-6-phosphate dehydrogenase deficiency upon sickle cell anemia. Blood 1988;71(3):748-52. (PMID: 3345344)

17. Pienthai N, Pongpunyayuen P, Ruengdit C, Pornprasert S. Development of suitable external quality control material for G6PD deficiency screening with the fluorescent spot test. Clin Chem Lab Med 2019;58(1):e18-e2. (PMID: 31815380)

18. Henriques G, Phommasone K, Tripura R, et al. Comparison of glucose-6 phosphate dehydrogenase status by fluorescent spot test and rapid diagnostic test in Lao PDR and Cambodia. Malar J 2018;17(1):243. (PMID: 29929514)

19. Dimitrov JD, Roumenina LT, Perrella G, Rayes J. Basic Mechanisms of Hemolysis-Associated Thrombo-Inflammation and Immune Dysregulation. Arterioscler Thromb Vasc Biol 2023;43(8): 1349-61. (PMID: 37317847)