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Sickle cell anemia and the impact of G6PD activity on the Hematological parameters

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ABSTRACT
Objectives: Sickle cell anemia (SCA) and glucose-6-phosphate dehydrogenase (G6PD) deficiency are the most common inherited red blood cell (RBC) disorders. The aim of this study was to determine, compare, and correlate G6PD activities with hematological parameters in SCD patients with deficient and non-deficient G6PD and healthy controls in Sana'a, Yemen.
Materials and Methods: This cross-sectional study included 150 SCD patients (SCA = 84; SCT = 66) and 150 controls who attended some hospitals in Sana'a from April to June 2022. Five milliliters of venous blood were used for the estimation of CBC and G6PD activity. Data were analyzed using SPSS version 26 software.
Results: The SCD patients had significantly lower Hb, PCV, and RBC and higher reticulocytes than controls ($P < 0.020$). The SCA patients were significantly lower in Hb, PCV, and MCHC and higher in reticulocytes than SCT. G6PD deficiency was found in 26% of the SCD patients, and it was more prevalent in SCA than in SCT and in males than in females. The deficient patients had significantly lower Hb and PCV and higher reticulocytes than non-deficient patients. G6PD activity was found to be positively related to Hb and PCV.
Conclusion: G6PD deficiency was more common in SCA patients and had an impact on hematological parameters, which could lead to increased RBC hemolysis. As a result, screening SCA patients for G6PD levels during diagnosis and treatment is advised.

CONTENTS

Introduction:

Sickle cell anemia (SCA) and glucose-6-phosphate dehydrogenase (G6PD) deficiency are the most common inherited red blood cell (RBC) disorders. It causes chronic hemolytic anemia by increasing RBC hemolysis [1]. Sickle cell disease (SCD) is a genetic blood disorder inherited as an autosomal recessive disorder. It is caused by a point mutation in hemoglobin caused by the substitution of valine for glutamic

acid at position 6 of the beta-globin chain found on chromosome 11 [2], resulting in the formation of hemoglobin S (HbS). Sickle cell disease (SCD) or sickle cell anemia (SCA) occurs when the individual inherits two abnormal copies of the hemoglobin (Hb) genes, one from each parent. Sickle cell traits or carriers occur when the individual inherits a single abnormal copy and does not experience any symptoms [3]. Numerous studies have documented that the polymerization of the HbS molecule is the cornerstone in the pathogenesis of the disease and its complications in subjects with the HbS variant disorder. Consequently, the higher the erythrocyte HbS content, the more severe the clinical picture [4].

G6PD is the rate-limiting enzyme that is present in the pentose phosphate pathway, which converts glucose-6-phosphate into 6-phosphogluconate. G6PD is a vital enzyme that protects red blood cells (RBCs) from oxidative stress. G6PD prevents hemolysis of RBCs by supplying reduced energy to them. The levels of the reduced co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) are kept stable by G6PD, which also keeps the RBCs' supply of reduced glutathione (GSH) constant [5]. GSH is a powerful antioxidant that acts as an oxidant scavenger, scavenging any oxidants (free radicals) that may cause RBC damage [6-7].

G6PD deficiency is an X-linked recessive genetic disorder and the most common human enzyme deficiency, affecting an estimated 400 million people worldwide [8]. It affects both males and females and can be more common in males than females. It occurs most often in hemizygous males and homozygous females, but it can be a partial deficiency in a heterozygous female [9]. In affected individuals, G6PD deficiency causes RBCs to break down prematurely, resulting in chronic hemolytic anemia, which is mostly triggered by bacterial or viral infections, by certain antimalarial drugs, or after eating fava beans [10]. Hemolytic anemia leads to paleness, jaundice, dark urine, fatigue, shortness of breath, and a rapid heart rate [11]. A high incidence of G6PD deficiency has been reported in some areas of the world where the sickle cell gene is most prevalent [12]. There were variations in the effects of G6PD deficiency on the hematological parameters in SCD patients and the possible relationship between them. The aim of this study was to determine, compare, and correlate G6PD activities with hematological parameters in SCD patients with deficient and non-deficient G6PD and healthy controls in Sana'a, Yemen.

Materials and Methods

Subjects

This cross-sectional study included 150 SCD patients (81 males and 69 females) and 150 healthy controls aged 5 to 45 years. The SCD patients attended Al-Gumhori and Al-Thawrah hospitals and other government hospitals, as well as private clinics and hospitals in Sana'a, Yemen, from April to June 2022. The majority of SCD patients were referred to specialized medical laboratories such as Al-Aulaqi Specialized Laboratories, New Lab, and others for investigations. Hb electrophoresis and/or HPLC methods were used to diagnose them as having sickle cell anemia (SCA; n = 84; 56%) and sickle cell trait (SCT; n = 66; 44%).

Sample Collection

Five milliliters of venous blood was collected from each participant and divided into two potassium ethylene diamine tetra-acetate (EDTA) tubes. The first EDTA tube was used for the estimation of complete blood count (CBC) using automated Cell-Dyn 3700, while the second tube was used for the G6PD activity assay. Hemolysate from washed red blood cells was used for the G6PD assay. The G6PD activity was measured by spectrophotometry.

Methods

Cellulose acetate electrophoresis (CAE)

This technique is based on the principle of electrophoresis that mainly separates HbA, HbS, HbA2, and other forms of hemoglobin variants used in screening SCD and thalassemia. Cellulose acetate electrophoresis was performed at an alkaline pH of 8.6 on the prepared hemolysate from the blood sample to assess the spectrum of hemoglobinopathy [13].

High-performance liquid chromatography (HPLC)

The SCD patients were diagnosed by high-performance liquid chromatography (HPLC). These patients were referred to Al-Aulaqi Specialized Laboratories for quantitative assessment of hemoglobin variants (using the D-10 Hemoglobin Testing System (Bio-Rad Laboratories, USA) to detect different types of hemoglobin: Hb F, A, A2, and S). The different Hb variants were identified by using retention time windows that were specific for these variants. The normal reference ranges for these variants were 96–98.5% for Hb A, 1.5–3.2% for Hb A2, 0–0.8% for Hb F, and 0% for Hb S. SCT was diagnosed when a patient's RBC contained 30–40% Hb S and 60–70% Hb A [14].

Analysis of hematological parameters

A complete blood count (CBC) was carried out using an automated cell counter (Cell-Dyn 3700; Abbott Diagnostic, Dallas, USA). According to the manufacturer's instructions, the reference ranges for normal CBC in adults were as follows: white blood cells (WBCs, 4 - 11 × 103/µL); RBCs (males 4.5 - 6 × 106/µL, females 3.8 - 4.8 × 106/µL); hemoglobin (males 13 17 g/dL, females 12 - 16 g/dL); hematocrit (Hct, males 39-51%, females 36-48%); mean corpuscular volume (MCV, 76 - 96 fL); mean corpuscular hemoglobin (MCH, 26 - 32 pg); mean corpuscular hemoglobin concentration (MCHC, 32 - 37 g/dL); red cell distribution width (RDW, 12-15%), platelets (150 - 450 × 103/µL); reticulocytes (0.5-2.5%). MCV and MCHC were used as indicators of red cell size and chromaticity, respectively.

Analysis of G6PD activity

The G6PD assay was carried out on hemolysate prepared by mixing 9 volumes of lysing solution (2.7 mmol/L EDTA, pH 7.0, and 0.7 mmol/L 2-mercaptoethanol) with 1 volume of washed red cell suspension. The G6PD activity was calculated from the change in absorbance at 340 nm over 5 min by following the rate of production of NADPH, which has a peak of ultraviolet light absorption at 340 nm [15]. The absorbance of the blank was also measured. The test samples and the control samples were both analyzed at the same time. The ICSH recommendation was followed for expressing the G6PD activity results in terms of gHb. The enzyme activity was expressed in international units, with one unit of G6PD being the quantity of enzyme that reduces 1 µmol of NADP per minute. The normal range for G6PD activity in this study is 4.6–10 IUg/Hb at 30 °C. [15].

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Inc., New York, USA). The frequency of tables and descriptive statistics were used to summarize the data. Quantitative data were compared between cases and controls. and between G6PD-deficient and non-deficient participants using two samples-test. The correlation of G6PD activity with the hematological parameters was computed using Pearson's correlation coefficient. The P values of less than 0.05 were considered statistically significant.

Results

Table 1 shows the characteristics of SCD patients (group 1) and healthy controls (group 2), aged 5 to 45 years, with male predominance in both groups. The SCD Group 1 included 150 patients, of whom 81 (54%) were males and 69 (46%) were females, with a mean age of 15.5 ±8.5. Control group 2 consisted of 150 healthy individuals with a mean age of 15.0 ±8.0, including 87 (58%) males and 63 (42% females). The SCD patients in Group 1 were divided into Group 1a (SCA) and Group 1b (SCT). The SCA group 1a included 84 (56%) patients, of whom 47 (56%) were males and 37 (44%) were females, with a mean age of 14.5 ±8.5. The SCT group 1b included 66 (44%) patients, of whom 34 (52%) were males and 32 (48%) were females, with a mean age of 16.5 ±8.4. Hematological parameters (Hb, PCV, and RBC) were significantly lower and reticulocytes were higher in SCD patients compared to controls (P =.0020). There were no significant differences in age, gender, WBCs, platelets, MCV, MCH, MCHC, and G6PD activity between patients and controls (P > 0.05). Similar results were obtained

when comparing SCA patients (group 1a) with SCT patients (group 1b), the Hb, PCV, and MCHC were significantly lower (P = 0.020) and the reticulocytes were higher (P = 0.040) in SCA patients (Table 1).

Table 1: Laboratory characteristics of sickle cell disease (SCD) patients and control group.

Parameter	Total SCD (group 1) (n=150)	Control (group 2) (n=150)	P value	SCA (group 1a) (n=84)	SCT (group 1b) (n=66)	P value
	Mean ±SD	Mean ±SD		Mean ±SD	Mean ±SD	
Age (years) Mean ±SD Range	15.5 ±8.5 5-45	15.0 ±8.0 5-43	0.754	14.5 ±8.5 5-41	16.5 ±8.4 5-45	0.486
Gender	**n (%)**	**n (%)**		**n (%)**	**n (%)**	
Male	81 (54)	87 (58)	NS	47 (56)	34 (52)	NS
Female	69 (46)	63 (42)		37 (44)	32 (48)	
Hematological parameters						
Hb (g/dL)	10.53 ± 2.70	14.35 ± 1.61	0.010	9.10 ± 2.31	11.50 ± 3.62	0.010
PCV (%)	33.10 ± 3.42	43.10 ± 3.14	0.020	27.33 ± 3.81	40.34 ± 4.12	0.020
RBCs (× 10^{12}/L)	4.65 ± 1.35	5.90 ± 1.75	0.020	3.90 ± 1.25	5.00 ± 1.54	0.058
WBCs (× 10^{9}/L)	7.68 ± 4.50	7.37 ± 1.90	0.185	8.65 ± 5.53	6.68 ± 3.46	0.512
Platelets (× 10^{9}/L)	318.50 ± 98.84	325.63 ± 57.47	0.154	324.17 ± 78.53	315.87 ± 99.75	0.253
MCV (fL)	75.50 ± 13.50	81.42 ± 4.40	0.235	77.40 ± 9.79	74.64 ± 14.10	0.743
MCH (pg)	25.85 ± 4.35	29.89 ± 1.13	0.368	27.50 ± 3.73	25.44 ± 4.96	0.267
MCHC (g/dL)	35.60 ± 9.10	32.62 ± 1.24	0.485	30.27 ± 2.10	39.54 ± 2.23	0.020
Reticulocytes (%)	2.60 ± 2,6	0.9 ± 0,4	0.020	3.50 ± 3,4	2.80 ± 1,6	0.040
G6PD activity (IU/gHb)	6.8 ± 3,0	7.0 ± 2.4	0.614	6.6 ± 2.9	6.8 ± 3.1	0.537

P value was calculated with unpaired sample *t*-test. P value < 0.05 was considered significant. SCD: sickle cell disease; SCT: sickle cell trait; SD: standard deviation; CBC: complete blood cell count; WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PCV: hematocrit or packed cell volume; NS: non-significant.

Table 2 shows the G6PD activity in deficient and non-deficient SCD patients. G6PD deficiency was found in 39 (26%) patients with SCD; 21 (14%) were males and 18 (12%) were females, with a mean age of 15.0 SCD; 21 (14%) were males and 18 (12%) were females, with a mean age of 15.0 ± 8.5; 24 (16%) of them had SCA while 15 (10%) had SCT. Partial deficiencies of G6PD activity were found in 8 (38.1%) of males and 14 (77.8%) of females, while full deficiencies were found in 13 (61.9%) of males and 4 (22.2%) of females. G6PD non-deficiency was found in 111 (74%) patients with SCD, 60 (40%) of whom were males and 51 (34%) were females, with a mean age of 16.0 ±9.0, and 60 (40%) of them had SCA while 51 (34%) had SCT (Table 2).

Table 2: G6PD activity in deficient and non-deficient SCD patients (n=150)

Parameter	SCD patients (n=150), n (%)		
	G6PD-deficient (n=39)	G6PD non-deficient (n=111)	Total SCD patients (n=150)
Age (years)			

Mean ±SD	14.0 ± 8.0	16.0 ±9.0	15.0 ±8.5
Gender:	**n (%)**	**n (%)**	**n (%)**
Male	12 (8)	69 (46)	81 (54)
Female	24 (16)	45 (30)	69 (46)
Total	36 (24)	114 (76)	150 (100)
SCD	**n (%)**	**n (%)**	**n (%)**
SCA	16 (10.7)	68 (45.3)	84 (56)
SCT	20 (13.3)	46 (30.7)	66 (44)
Total	36 (24)	114 (76)	150 (100)

Table 3 shows the hematological parameters in G6PD-deficient and non-deficient SCD patients (n = 150). There were statistically significant differences among the two groups in Hb and PCV, which were lower, and reticulocytes were higher in G6PD-deficient than non-deficient SCA patients (P < 0.020). There were no significant differences among the two groups in RBCs, WBCs, platelets, MCV, MCH, and MCHC (P >0.05) (Table 3).

Table 3: Hematological characteristics in SCD patients (n=150)

Parameter	**G6PD-deficient patients (n=39)**	**G6PD non-deficient patients (n=111)**	**P value**
	(Mean ±SD)	**(Mean ±SD)**	
Hb (g/dL)	8.55 ± 2.60	1145 ± 2.80	0.012
PCV (%)	23.10 ± 3.40	43.10 ± 3.44	0.018
RBCs (× 10^{12}/L)	3.75 ± 1.30	4.65 ± 1.40	0.063
WBCs (× 10^{9}/L)	6.15 ± 4.0	8.10 ± 5.0	0.612
Platelets (× 10^{9}/L)	310 ± 96	330 ± 100	0.357
MCV (fL)	72.80 ± 9.0	76.80 ± 10.0	0.723
MCH (pg)	25.55 ± 4.3	27.50 ± 4.4	0.274
MCHC (g/dL)	32.5 ± 8.5	39.5 ± 9.4	0.178
Reticulocytes (%)	4.5 ± 3.3	3.60 ± 3.5	0.015

P value < 0.05 was considered significant.

Table 4 shows the correlations between G6PD activity and hematological parameters in deficient and non-deficient patients. Among deficient patients (n = 39), there were significant positive correlations between G6PD levels and Hb and PCV (P < 0.05) and non-significant correlations with age, RBCs, WBCs, platelets, MCV, MCH, and MCHC (P >0.05). Among non-deficient patients (n = 111), there were non-significant correlations between G6PD activity and all hematological parameters (P > 0.05) (Table 4).

Table 4: Correlations between G6PD activity and hematological parameters in SCD (n=150)

Parameter	G6PD-deficient patients (n=39)	G6PD non-deficient patients (n=111)
	P value	P value
Age (years)	0.121	0.238
Hb (g/dL)	**0.011**	0.543
PCV (%)	**0.022**	0.538
RBCs ($\times 10^{12}$/L)	0.062	0.436
WBCs ($\times 10^{9}$/L)	0.612	0.748
Platelets ($\times 10^{9}$/L)	0.357	0.396
MCV (fL)	0.135	0.468
MCH (pg)	0.065	0634
MCHC (g/dL)	0.245	0.146
Reticulocytes (%)	0.182	0.257

Pearson correlation; P value < 0.05 was considered significant.

Discussion

Sickle cell anemia and G6PD deficiency are both inheritable diseases of the red blood cells that can co-exist in an individual. Numerous studies have reported on the effects of comorbidity, although their conclusions vary. The present study found the prevalence of 26.0% G6PD deficiency in SCD patients to be within the range found in various national and international studies. From previous studies, the prevalence of G6PD deficiency in SCA patients ranged from 12% to 27.3% [16]. Lower rates of 13.6% were observed in international research among SCA patients in the United States [17]. The burden of illness, including SCA and G6PD deficiency, was substantially lower in the United States because of the use of genetic assays, which are more precise than biochemical assays. An incidence of 35.8% was noted in a more recent study conducted in Ghana [17]. Studies that define deficiency states using a cut-off higher than the WHO cut-off of 3 IU per gram of hemoglobin tend to report relatively lower prevalence rates [18].

In the present study, we found that SCD patients had hematological abnormalities compared to controls. The hematological parameters (Hb, PCV, and RBC) were significantly lower and reticulocytes were higher in SCD patients when compared to controls (P < 0.020). Also, non-significant differences were obtained in age, gender, leukocytes, platelets, MCV, MCH, MCHC, and G6PD between SCD patients and controls (P > 0.05). Similar results were obtained in other studies by comparing SCA patients with controls [19]. Khaled et al., (2022) found significantly lower levels of Hb, PCV, RBC, MCV, and MCH and higher levels of reticulocytes in SCD patients than controls, as well as non-significant differences in age, gender, WBC, platelets, and MCHC [19].

In the current study, we found significantly lower Hb, PCV, and MCHC and higher reticulocytes in SCA patients than in SCT patients. Our findings were in agreement with other studies [19]. Also, we found higher MCHC in SCT patients compared to SCD patients, while MCHC was low in SCD patients. These findings could be explained by a possible concomitant iron deficiency anemia, which is the most common form of anemia. These results were consistent with the findings of Akodu et al., who found decreased MCHC in their SCD patients. They used the prevalence of malarial infection in Nigeria to explain their findings. However, they concluded that SCD is a disorder with higher MCV and MCH than controls [20]. Chronic hemolysis, according to Mouele et al., explains macrocytosis in SCD patients with a high MCV and a high number of young RBCs [21]. Another

reason for these inconsistent findings is that, in contrast to the current investigation, the majority of these studies involved pediatric patients, whose hemolysis rates are higher than those of adults. Consistent with other previous studies, the prevalence of G6PD deficiency in the present study was 26.0% in SCD patients, which is comparable to other previous studies in different countries in SCD patients. G6PD deficiency was more prevalent in SCA (6%) than in SCT (10%), in males (14%) than in females (12%), and had a higher proportion of partial deficiency (n = 22; 14.7%) than full deficiency (n = 17; 11.3%). Igwilo et al. (2021) found that the prevalence of G6PD deficiency in SCA patients was 23.9% [22]. Fasola et al. (2019) found the prevalence of G6PD deficiency was higher in SCD patients than in controls (28.6% vs. 22.3%, *P* = 0.18), and SCD patients were twice as likely to have enzyme activities below 3.0 IU/gHb [18]. Gautam et al. (2019) discovered G6PD deficiency in 40% of SCA, 18.4% of SCT, 4.8% of TT, and 2.8% of normal cases. He discovered that only SCT (5.3%) and β-TT (4.8%) developed increased G6PD. According to him, the two most common hemoglobinopathies that coexist with G6PD deficiency are sickle cell disease (SCD) and β-TT [23]. Also, Antwi-Baffour et al. (2019) found the prevalence of G6PD deficiency in SCD patients to be 35.83% in SCD patients [17]. Simpore et al. (2007) in Burkina Faso found G6PD deficiency in 27.03% of patients with severe sickle cell disease [24]. A previous study in Yemen was done by Al-Nood (2011), who found G6PD deficiency in 22.6% of patients with SCD in Taiz, Yemen [25]. In contrast, Igwilo et al. (2021) found that G6PD deficiency was significantly higher in females [22].

In our study, we found that the prevalence of G6PD deficiency was higher in men than in women, which could be attributed to the fact that G6PD deficiency is an X-linked disease and more men have full enzyme abnormalities than women since men are hemizygous for the X chromosome while women are dizygous. As a result, there is a lesser chance of discovering the G6PD mutation's genes on the two X chromosomes [26].

In the present study, we found significant differences in hematological parameters in G6PD-deficient patients compared to non-deficient SCD patients. In G6PD-deficient SCA patients, Hb and PCV were significantly lower, while reticulocytes were higher (P <0.020). Also, we found non-significant differences in age, gender, RBCs, WBCs, platelets, MCV, MCH, and MCHC between the two groups (P > 0.05). This is consistent with other studies [22, 24, 27]. The relatively lower hematocrit may be attributed to the slightly accentuated hemolysis in G6PD-deficient SCA patients [17, 18, 24]. We found that there was no difference in age between SCA patients with G6PD deficiency and those with normal G6PD activity. Both disorders have hereditary etiologies, so aging may not have a big impact on the burden. The impact of the comorbidity, if severe, could have a negative impact on the life expectancy of those affected; however, this has not been proven. On the other hand, Abubakar et al. (2015) reported a relatively higher rate of G6PD deficiency among females than males in a study conducted in northern Nigeria [16]. Some other studies have found no difference in the incidence of G6PD deficiency between males and females.[16].

There were variations in other studies on the hematological parameters in deficient and non-deficient patients. Fasola et al. (2019) found that deficient patients were more likely to have a lower hematocrit (22.8 ± 3.9% vs. 24.5 ± 5%, *P* = 0.04) and non-significantly higher reticulocyte counts. Also, he concludes that the coinheritance of SCA and G6PD deficiency could worsen hemolysis in SCD patients, and care should, therefore, be taken in the choice of drugs in deficient SCD patients [18]. On the other hand, Igwilo et al. (2021) found similar results in the hematological parameters between G6PD-deficient and G6PD normal SCA persons except that the G6PD-deficient SCA persons have a significantly higher reticulocyte response (P = 0.001) [22].

In our study, we found significant correlations between G6PD activity and

hematological parameters in G6PD-deficient SCA patients. There were significant positive correlations between G6PD levels and Hb and PCV ($P < 0.02$) and non-significant correlations with age, gender, RBC, WBCs, platelets, MCV, MCH, MCHC, and reticulocytes ($P > 0.05$). On the other hand, among G6PD non-deficient patients (n = 114), G6PD levels were non-significantly correlated with age, gender, and hematological parameters ($P > 0.05$). Our observations were in line with other studies. Fasola et al. (2019) found that G6PD activity correlated positively with hematocrit ($r = 0.91$, $P = 0.01$) and mean corpuscular hemoglobin concentration ($r = 0.17$, $P = 0.02$) [18].

Conclusion

G6PD deficiency was more common in SCA patients and had an impact on hematological parameters. which could lead to increased RBC hemolysis. As a result, screening SCA patients for G6PD levels during diagnosis and treatment is advised.

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