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Original Research Article

Glucose-6-phosphate Dehydrogenase Deficiency and Hemoglobinopathy among Patients of the Yemeni Society of Thalassemia and Blood Genetic Disorders in Sana'a, Yemen

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency and hemoglobinopathies such as sickle cell anemia (SCA), sickle cell trait (SCT), and thalassemia are the most common congenital causes of hemolysis. The study aimed to determine the prevalence of G6PD deficiency in hemoglobinopathy patients and its effect on RBC indices.

Materials and methods: This case-descriptive study was carried out on a total of 100 hemoglobinopathy patients (51 males and 49 females) aged between 3 and 38 years. They attended the Yemeni Society for Thalassemia and Genetic Blood Disorders (YSTGBD) in Sana'a, Yemen, during the period between January and February 2021. Hb electrophoresis and/or HPLC methods were used to categorize them as sickle cell anemia (HbSS; n = 70), sickle cell trait (HbAS; n = 12), HbS/thalassemia major (n = 12), and HbS/thalassemia minor (n = 6). Five milliliters of venous-EDTA blood were collected from each patient and used to determine G6PD activity and the complete blood count (CBC). Data were analyzed using SPSS version 26 software.

Results: G6PD deficiency was detected in 29 (29.0%) of patients with hemoglobinopathy, of whom 16 (16.0%) were males and 13 (13.0%) were females. G6PD deficiency was discovered in 20 (20%), 3 (3%), 5%, and 1% of patients with HbSS, AS, S/β-thalassemia major, and minor hemoglobinopathies, respectively. In G6PD-deficient patients, G6PD activity significantly correlated positively with RBC (p= 0.048), MCH (p= 0.040), and MCHC (p= 0.002).

Conclusion: The prevalence of G6PD deficiency was high among hemoglobinopathy patients, particularly those with sickle cell anemia (SCA), which may contribute to a further increase in the hemolysis of RBCs. Therefore, screening hemoglobinopathy patients for G6PD levels is recommended during diagnosis and treatment.

Keywords: Glucose-6-phosphate Dehydrogenase (G6PD), Hemoglobinopathy, Sickle cell anemia (SCA), Sickle cell trait (SCT) and β-thalassem

Introduction

The most common congenital causes of hemolysis include glucose-6-phosphate dehydrogenase (G6PD) deficiency and hemoglobinopathy such as sickle cell anemia (SCA), sickle cell trait (SCT) and β-thalassemia. G6PD is the rate-limiting enzyme that presents in the pentose phosphate pathway which converts glucose-6-phosphate into 6-phosphogluconate [1].

G6PD is a vital enzyme which protects red blood cells (RBCs) from oxidative stresses and preventing hemolysis by supplying reducing energy to them by maintaining the level of reduced co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH), which in turn, maintains the supply of reduced glutathione (GSH) in the RBCs [2].

The GSH is an important antioxidant which acts like oxidant scavenger that is used to mop up any oxidants (free radicals) that will cause damage to the RBCs [3.4].

G6PD deficiency is X-linked recessive genetic disorder and the most common human enzyme deficiency affecting an estimate of 400 million people worldwide [5]. 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 partial deficiency in a heterozygous female [6]. 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 [7]. Hemolytic anemia leads to paleness, jaundice, dark urine, fatigue, shortness of breath, and a rapid heart rate [8]. The high incidence of G6PD deficiency has been reported in some areas of the world where sickle cell gene is most prevalent [9].

Sickle cell disease (SCD) is a genetic blood disorder inherited as an autosomal recessive disorder. It is caused by a point mutation in hemoglobin by the substitution of valine for glutamic acid at position 6 of the beta (β)-globin chain found on chromosome 11 [10] 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 [11]. There were variations in previous studies about the prevalence of G6PD deficiency in hemoglobinopathy patients and the possible relationship between them. The aim of this study is to determine the prevalence of G6PD deficiency in hemoglobinopathy patients and its effect on RBC indices.

Materials and Methods

Subjects

This case-descriptive study was carried out on total of 100 hemoglobinopathy patients (51 Males and 49 females) aged between 3 to 38 years who attended the Yemeni Society for Thalassemia and Genetic Blood Disorders (YSTGBD) in Sana'a city, Yemen, during the period between January and February 2021. These patients were diagnosed by Hb electrophoresis and/or high- performance liquid chromatography (HPLC) methods into sickle cell anemia (HbSS; n=70), sickle cell trait (HbAS; n=12), HbS/β thalassemia HbS/β thalassemia major (n=12), and HbS/β thalassemia minor (n=6).

Ethical Considerations

Ethical consideration was taken and all participants were informed about the objectives and protocol of this study before their informed consent was obtained.

Sample Collection

Five milliliters (5ml) of venous blood sample was collected from each patient and divided into two ethylene diamine tetra-acetate (EDTA) tubes. The first EDTA tube sample was used for G6PD activity assay, while the second tube sample was used for complete blood count (CBC). The samples were transported in an ice chest to the hematology laboratory for processing and analysis.

Methods

Cellulose acetate electrophoresis

This technique was based on the principal 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 alkaline pH (8.6) on the prepared hemolysate from the blood sample to assess the spectrum of hemoglobinopathy [12].

High-performance liquid chromatography (HPLC)

The majority of SCD patients were diagnosed by high-performance liquid chromatography (HPLC). These patients attended Al-Aulaqi Specialized Laboratories for determination of Hb type using D-10 Hemoglobin Testing System (Bio-Rad, USA).

G6PD assay

Assay for G6PD activity was carried out using the quantitative in-vitro test kit by BIOTEC© Laboratories Limited (Cloud Hill, Temple Cloud, Bristol, Uk). Its principle was based on the reduction of NADP+ by G6PD present in red blood cells. The NADPH generated fluoresces under ultraviolet light at a wave length of 340 nm (NADP to NADPH giving increase) (G6P + NADP GS HD → 6-Phosphogluconate + NADPH + H').

Enzyme activity was determined by the rate of absorbance change. A measured G6PD activity of < 202 U/g RBCs defined G6PD deficiency while values ≥202 U/g RBCs were regarded as normal. All samples were refrigerated immediately after collection at 4˚C - 8˚C and analysed within 24 hours.

Complete blood count (CBC)

Complete blood count (CBC) was carried out by using the Sysmex Automated Haematology Analyzer (Sysmex Co, Japan) on samples in the EDTA tube, according to the manufacturer's instructions that automatically generated values for Hb, PCV, RBCs count, RBC indices, WBC and platelet counts.

Statistical analysis

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Inc., New York, USA). Frequency of tables and descriptive statistics were used to summarize the data. Descriptive data were given as mean ± standard deviation (SD). Pearson correlation coefficients (R) were calculated to quantify the relationship between parametric parameters and $P<0.05$ were considered statistically significant.

Results

Table (1) shows that G6PD deficiency was detected in 29 (29.0%) of patients with hemoglobinopathy of which 16 (16.0%) were males and 13 (13.0%) were females. G6PD deficiency was detected in 20 (20.0%), 3 (3.0%), 5 (5.0%) and 1 (1.0%) of hemoglobinopathy patients with HbSS, HbAS, HbS/β thalassemia major and HbS/β thalassemia minor, respectively. On the other hand, G6PD non-deficient was detected in 71 (71.0%) of patients with hemoglobinopathy of which 35 (35.0%) were males and 36 (36.0%) were females. G6PD non-deficient was detected in 50 (50.0%), 9 (9.0%), 7 (7.0%), and 5 (5.0%) of hemoglobinopathy patients with HbSS, HbAS, HbS/β thalassemia major and HbS/β thalassemia minor, respectively.

Table 1: Prevalence of glucose6phosphate dehydrogenase deficiency in hemoglobinopathies patients by gender and types of Hb (n=100)

Parameter	Hemoglobinopathies patients (n=100), n (%)		
Variables	**G6PD-deficient n (%)**	**G6PD non-deficient n (%)**	**Total patients n (%)**
Sex			
- Male	16 (55)	35 (49)	51 (51)
- Female	13 (45)	36 (51)	49 (49)
- Total	**29 (100)**	**71 (100)**	**100 (100)**
Types of Hb			
- HbSS	20 (69)	50 (70)	70 (70)
- HbAS	3 (10)	9 (13)	12 (12)
-HbS/βthalassemia major	5 (17)	7 (10)	12 (12)
-HbS/βthalassemia minor	1 (4)	5 (7)	6 (6)
- Total	**29 (100)**	**71 (100)**	**100 (100)**

Table (2) shows the comparison of G6PD level among the different types of hemoglobin in G6PD-deficient hemoglobinopathy patients (n=29). The majority of the G6PD deficiency was found in the SCD patients with genotype HbSS with a mean deficiency of (124.20 ± 45.58), HbAS with a mean deficiency of (127.00 ± 30.95), HbS/β-thalassemia major with a mean deficiency of (117.00 ± 55.19) and HbS/β-thalassemia minor with a mean deficiency of (147.20).

Table 2: The comparison of G6PD level among G6PD-deficient hemoglobinopathy patients (n=29)

G6PD-deficient hemoglobinopathy patients (n=29)		G6PD level
	No.	**Mean ± SD**
HbSS	20	(124.20 ± 45.58)
HbAS	3	(127.00 ± 30.95)
HbS/β-thalassemia major	5	(117.00 ± 55.19)
HbS/β-thalassemia minor	1	147.20
Total	**29**	-

Table (3) shows the laboratory parameters in hemoglobinopathy patients (n=100) and G6PD-deficient patients (n=29). The value is expressed as Mean ±SD. Among all hemoglobinopathy patients (n=100), the mean age was 15.07 ±7.26 years, the mean G6PD level was 281.21 ± 138.30 U/gRBC, the mean Hb level was 9.44 ± 1.55 g/dl, the mean haematocrit (PCV) was 26.10 ± 4.54 %, the mean RBC count was 5.55 ± 25.41 /l, the mean WBC count was 11.89 ± 6.37 /l, the mean platelet count was 317.50 ± 122.52 /l, the mean MCV was 89.18 ± 12.65 fl, the mean MCH was 32.18 ± 5.42 pg, and the mean MCHC was 35.79 ± 1.59 g/dl. On the other hand, among G6PD-deficient patients (n=29), the mean age

was 17.41 ± 8.47 years, the mean G6PD level was 123.82 ± 43.97 U/gRBC, the mean Hb level was 9.61 ± 1.74 g/dl, the mean haematocrit (PCV) was 26.62 ± 4.90 %, the mean RBC count was 3.03 ± 0.69 /l, the mean WBC count was 10.63 ± 4.92 /l, the mean platelet count was 331.86 ± 103.27 /l, the mean MCV was 89.42 ± 12.54 fl, the mean MCH was 32.37 ± 5.27 pg, and the mean MCHC was 36.10 ± 0.96 g/dl.

Table 3: Laboratory parameters in hemoglobinopathy patients (n=100) and G6PD-deficient patients (n=29)

Parameter	Hemoglobinopathy patients (n=100)	G6PD-deficient patients (n=29)
	Mean ±SD	Mean ±SD
Age (years)	15.07 ±7.26	17.41 ± 8.47
G6PD (U/g RBC)	281.21 ± 138.30	123.82 ± 43.97
Hb (g/dl)	9.44 ± 1.55	9.61 ± 1.74
PCV (%)	26.10 ± 4.54	26.62 ± 4.90
RBC (X1012/l)	5.55 ± 25.41	3.03 ± 0.69
WBC (X109/l)	11.89 ± 6.37	10.63 ± 4.92
Platelets (X109/l)	317.50 ± 122.52	331.86 ± 103.27
MCV (fl)	89.18 ± 12.65	89.42 ± 12.54
MCH (pg)	32.18 ± 5.42	32.37 ± 5.27
MCHC (g/dl)	35.79 ± 1.59	36.10 ± 0.96

Table (4) shows the correlation between G6PD activity with other parameters in emoglobinopathy patients (n=100) and G6PD-deficient patients (n=29). Among hemoglobinopathy patients (n=100), there was non-significant correlation between G6PD level with age, Hb, PCV,RBC, WBC, platelets counts, MCV, MCH and MCHC. On the other hand, among G6PD-deficient patients (n=29), G6PD activity was significantly correlated positively with RBC (p= 0.048), MCH (p= 0.040), MCHC (P= 0.002) and platelets (p= 0.034) and non-significantly correlated with age, Hb, PCV, WBC and MCV.

Table 4: Correlation between G6PD activity and other parameters in hemoglobinopathy patients (n=100) and G6PD-deficient patients (n=29)

Parameter	Hemoglobinopathy patients (n=100)	G6PD-deficient patients (n=29)
	***P* value**	***P* value**
Age (years)	0.238	0.118
Hb (g/dl)	0.655	0.609
PCV (%)	0.588	0.323
RBC (X1012/l)	0.600	0.048*
WBC (X109/l)	0.710	0.804
Platelets (X109/l)	0.371	0.034*
MCV (fl)	0.452	0.100
MCH (pg)	0.624	0.040*
MCHC (g/dl)	0.146	0.002*

* statistically significant

Discussion

The present study examined 100 patients that have been recently diagnosed with hemoglobinopathy, including sickle cell anemia (SCA), sickle cell trait (SCT) and β-thalassemia. Fifty-one (51.0%) out of the total patients were males and forty-nine (49.0%) were females. From these 100 hemoglobinopathy patients, 70 (70.0%) of them were had sickle cell anemia (HbSS), 12 (12.0%) had sickle cell trait (HbAS), 12 (12.0%) had HbS/β thalassemia major, and 6 (6.0%) had HbS/β thalassemia minor.

This study found that the prevalence of G6PD deficiency was high among hemoglobinopathy patients. G6PD deficiency was detected in 29.0% of hemoglobinopathy patients of which 16 (16.0%) were males and 13 (13.0%) were females, while 71% of hemoglobinopathy patients were G6PD non-deficient (Table 1).

The present study also found that the majority of G6PD deficiencies were found in patients with sickle cell anemia (HbSS) (Table 1 and 2). The G6PD deficiencies were detected in 20.0%, 3.0%, 5.0% and 1.0% of hemoglobinopathy patients with sickle cell anemia (HbSS), sickle cell trait (HbAS), HbS/β thalassemia major and HbS/β thalassemia minor, respectively. The mean deficiency of G6PD with HbSS, HbAS, HbS/ β-thalassemia major and HbS/ β-thalassemia minor was 124.20 ± 45.58, 127.00 ± 30.95, 117.00 ± 55.19, and 147.20, respectively. The mean G6PD deficiency among hemoglobinopathy patients (n=100) and G6PD-deficient patients (n=29) was 281.21 ± 138.30 and 123.82 ± 43.97, respectively (Table 3).

In line with other previous studies, the prevalence of G6PD deficiency obtained in the present study was 29.0% among hemoglobinopathy patients of which 23.0% was among patients with sickle cell disease (SCA and SCT), which is similar to other previous studies in different countries on SCD patients. Fasola et al. (2019) have found that the prevalence of G6PD deficiency in SCD patients was 28.6% [13]. Similarly, Simpore et al. in Burkina Faso, have detected G6PD deficiency in 27.03% of patients with major sickle cell disease [14]. In Yemen, there was a previous study done by Al-Nood who has detected G6PD deficiency in 22.6% of patients with SCD in Taiz, Yemen [15]. On the other hand, the prevalence G6PD deficiency in this study is lower than that detected in other studies. Gautam et al. have observed G6PD deficiency in 40.0% SCA, 18.4% SCT and 4.8% β-thalassemia [16] and 35.83% in SCD patients [17].

In the current study, in terms of gender, it is found that the prevalence of G6PD deficiency was higher among the male patients (16%) than in female patients (13%) (Table 1). This finding is similar to that of Fasola et al. (2019), which was higher in males (28.7%) than females (24.50%) [13], and that of Jacques et al. (2007) which was 20.5% among males and 12.3% among females [14].

The prevalence of G6PD deficiency was higher among males than females, and this could be explained by the presence of full enzyme defects in males more than females, which is due to the fact that males are hemizygous whilst females are dizygous for the X chromosome. Therefore, the probability

of finding the genes for the G6PD mutation on the two X chromosome is lower [18].

Furthermore, it is observed in the present study that, among hemoglobinopathy patients (n=100), G6PD activity was non-significantly correlated with age, Hb, PCV,RBC, WBC, platelets counts, MCV, MCH and MCHC. Among G6PD-deficient patients (n=29) it is found that G6PD activity was significantly correlated positively with RBCs (p= 0.048), MCH (p= 0.040), MCHC(P= 0.002), and platelets (p= 0.034), and non-significantly correlated with other parameters. Also, among G6PD-deficient males (n=16), it is observed that the significant correlation was more pronounced with RBC indices. G6PD activity was significantly correlated positively with RBCs (p= 0.003), MCV (p= 0.030), MCH (p= 0.016), and MCHC (P= 0.001), but non-significantly correlated with age, Hb, PCV, WBC, and platelets count. On the other hand, among G6PD-deficient females (n=13), it is found that there was non-significant correlation between G6PD level and other parameters.

Conclusion

The prevalence of G6PD deficiency was high among hemoglobinopathy patients, particularly those with sickle cell anemia (SCA), which may contribute to a further increase in the hemolysis of RBCs. Therefore, screening hemoglobinopathy patients for G6PD levels is recommended during diagnosis and treatment.

Competing interest

The authors declare no competing interests to disclose.

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