WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 5, 60-71. Research Article ISSN 2277– 7105

THE PREVALENCE OF GLUCOSE-SIX-PHOSPHATE DEHYDROGENASE DEFICIENCY IN PATIENTS WITH SICKLE CELL ANEMIA IN SUDAN

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Article Received on
01 March 2021,

Revised on 22 March 2021,
Accepted on 11 April 2021

DOI: 10.20959/wjpr20215-20319

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ABSTRACT

Background: Sickle cell disease (SCD) and glucose-6-phosphate dehydrogenase (G6PD) deficiency are inherited disorders associated with chronic haemolysis. Therefore, coinheritance of both disorders could worsen haemolysis in the former and compound a haemolytic crisis. **Aim:** This study aimed to determine the co-incidence of glucose-6-phosphatedehydrogenase deficiency and sickle cell anaemia in Sudan. **Method:** A hundred samples of patients with SCD of both sexes with ages ranged between 6 months and 16 years and 50 samples from healthy individuals between the same ages as control group were collected from these patients and investigated for Hb concentration, RBCs count, and G6PD assay. **Results:** The study revealed that 45 patients were homozygous (SS), 55 patients were heterozygous (AS). And we found that out of the 100 SCD patients, 81 patients (81%) were within the normal range which lies between 245-299 mU/erythrocytes per ml blood and 19 patients (19%) were deficient less than 245 mU/erythrocytes per ml blood by G6PD assay method also there were 2 of the control group were deficient. The study showed that 77% of SCD patients had RBCs count less than 3.00×109 /L and the rest 23% had count between 3.00-4.00×109 /L, the Hemoglobin (Hb) concentration level between 3.6 - 10.0 g/dl with mean of 6.4 g/dl. **Conclusion:** The co-incidence of G6PD deficiency with SCD affect the red blood cells count and hemoglobin concentration.

G6PD deficiency may have slightly effect on the severity of hemolysis and the degree of anemia in deficient SCD patients.

KEYWORDS: Cell Disease, Glucose-6-phosphate Dehydrogenase, Coinheritance, Haemolysis.

1. INTRODUCTION

1.1. Sickle Cell Disease in Sudan

In Sudan, sickle cell anemia is the one of the major types of anemia especially in western Sudan where the sickle cell gene is frequent.[1] A rate of 11.2 – 30% was detected among the Baggara tribal group that include Hawazma and Masuria, and 1-18% was found in southern Nilotes and Nilo-Hamitic tribes.[2] Among tribes immigrated from west Africa and settled around the northern part of the blue nile, a prevalence of 16% was reported compared to 0 – 5% among the indigenous population. The aboriginal tribes of Biga and Nuba characteristically have zero frequency of HbS. And there was a studies on aboriginal Northern Nilotes (Dinka, Nuer and Shilluk) have shown variable low frequencies of the sickle cell gene, ranging from 0 – 4%.[3] However, the tribal groups residing along the northern part of the Nile (Nubians including Danagla, shaigia and Gaalyeen) also have a very low frequency (0.5 – 1.1%). It is noteworthy that the Nuba mountains where the Nuba tribe lives have been reported to be endemic for malaria infections,[4] where as Shaigia and Gaalyeen how are of Arab descend, live in a non-endemic area.[5] One previous study done on this suggests that the sickle cell gene may have been preferentially introduced through males of migrating from West African tribes to Sudan particularly Hosa, Folani and Bargo tribs.[6]

Another study about hemoglobinopathies in Sudan showed that hemoglobin "S" is the most common abnormal hemoglobin in western Sudanese ancestry.[3] In a study done in Sudan established by Omer et al,[7] to detect the incidence of abnormal hemoglobin among two groups, indigenous groups which include: [Nubians Northern Sudan), Kalakla (Central), Dinka (Southern), Ingassena (South Eastern), and Beja (Eastern)] and immigrant groups which include: [Nigerians (Western bank of the Blue Nile) and Tchiendians (Central Sudan)]. In the study they found that the highest sickle cell trait incidence was found in the immigrant tribes, the Nigerians (27%) and Tchiendians (20%). Igassena tribe showed no evidence of abnormal hemoglobin and thought to be a pure line of the original population of Sudan. Similarly, Beja tribe had no abnormal hemoglobin, which referred to its close community.

The incidence of abnormal hemoglobin in Dinka was about 8% that was higher than the previously reported studies. Different results in Dinka were attributed to the test method variations. Kalakla displayed zero percent of abnormal hemoglobin, however, it is considered to be of mixed origin.[8]

Sickle cell trait: This is a heterozygous type benign condition with no anemia and normal appearance of red cells on a blood film. Hematuria is the most common symptom and is thought to be caused by minor infarcts of the renal papillae. Hb S varies from 25 to 45% of the total hemoglobin. Care must be taken with an aesthesia, pregnancy and at high altitude.[9] Sickling rare unless O2 saturation falls < 40%. Crises have been reported with severe hypoxia. Hb, MCV, MCH and MCHC are normal. Sickling test is positive. Detection of the carrier state relies on hemoglobin electrophoresis. The amount of HbS always is less than the concentration of Hb A.[10]

1.2. Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency

Glucose-6-phosphate dehydrogenase (G6PD) which is the rate limiting enzyme in the pentose phosphate pathway converts glucose-6-phosphate into 6-phosphogluconate.[11] It protects red blood cells (RBCs) from oxidative damage by supplying reducing energy to them by maintaining the level of reduced co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH).[12] Te NADPH in turn maintains the supply of reduced glutathione (GSH) in the RBCs, which acts like oxidant scavenger that is used to mop up any oxidants (free radicals) that will cause damage to the RBCs.[13]

Glucose-6-phosphate dehydrogenase deficiency is an X-linked recessive hereditary disease characterized by abnormally low levels of glucose-6-phosphate dehydrogenase (abbreviated G6PD), a metabolic enzyme involved in the pentose phosphate pathway, especially important in red blood cell metabolism.[14] G6PD deficiency is includes a large number of allelic variants, such that variants may be classified as severely deficient (<10% enzyme activity) or moderately deficient (10 – 60% activity).[15] G6PD deficiency is closely linked to *favism*, a disorder characterized by a hemolytic reaction to consumption of broad beans. The name favism sometimes used to refer to the enzyme deficiency as a whole, although this is misleading, as not all people with G6PD deficiency will manifest a physically observable reaction to consumption of broad beans.[16]

1.3. Sickle Cell Disease and Glucose-6-phosphate Dehydrogenase Deficiency

The possible interaction of sickle cell anemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency has been the subject of a number of studies in different populations.[17, 18] Hemoglobin S (HbS) and G6PD deficiency (A– type) are common in Central Africa. Their high frequency related to a selective advantage against malaria.[19-21] These two disorders are genetically independent, their loci being located on chromosome 11 for sickle cell disease and chromosome X for G6PD deficiency. They are thus expected to assort independently.[22] Nevertheless, different studies have suggested that the incidence of G6PD deficiency is higher in HbSS patients than in the general population, but others do not confirm this association.[23, 24] Some of these reports may be difficult to interpret since the methods used to determine G6PD deficiency were only based on measurement of enzyme activity.[15, 25] Sickle cell anemia is one of the major types of anemia found in Sudan, especially in western Sudan in which the sickle cell gene is frequent. Sickle cell disease leads to serious crises and complication, which considered life-threatening and fatal. The co-incidence of glucose-6-phosphate dehydrogenase deficiency and sickle cell disease may increase the severity of the disease. In addition, there is no study published on the co-incidence of glucose-6-phosphate dehydrogenase deficiency and sickle cell disease in Sudan. Therefore, this study is an attempt to find out the co-incidence of G6PD deficiency and sickle cell disease patients in Sudan and these will provide evidence for clinician to manage their patients probably and thus reduce the risk of serious complication and improve the health care services for sickle cell disease patients in the future.

2. OBJECTIVES

2.1 General objectives

- To determine the prevalence of the co-incidence of glucose-6-phosphate dehydrogenase (G6PD) deficiency and sickle cell disease in Sudan.

2.2 Specific objectives.

• To estimate the HB% concentration in sicklecell diseasepatients with G6PD deficiency and control.

• To determine the red blood cells count in sickle celldisease patients with G6PD deficiency and control.

• To measure theenzyme G6PD activity in sickle cell disease patients with G6PD deficiency.

• To comparebetween G6PD deficient and normal G6PD patients.

3. MATERIALS AND METHOD

3.1 Study design

A case–control study conducted to determine the Co-incidence of G6PD deficiency and sickle cell anaemia in Khartoum state from December 2018 to August 2019.

3.2 Study Area

This study was conducted at Ahmed Gassim pediatric Hospital, Omdurman hospital and Al-buluk pediatric Hospital in Khartoum state.

3.3 Inclusion criteria

A hundred subjects who were known SCD patients diagnosed by hemoglobin electrophoresis and sickling test, aged between 6 and 16 years who neither have infection, nor any crisis in the past one month and they hadn`t blood transfusion at least three months prior to the study. The controls were 50 healthy volunteers with no history of SCD.

3.4 Exclusion criteria

Healthy individuals, patients on crisis and patients who receive recent transfusion of fresh blood.

3.5 Ethical consideration

The study has been grantee ethical approval by Alzaeem ALazhari University research ethics committee and the parent of each participant gave informed consent.

3.6 Sample Collection and Analysis

Three milliliters of venous blood was collected from each participants using standard procedure into a specimen container containing potasium ethylenediamine tetra-acetic acid (K2EDTA) and analysed within 24 h at room temperature for the quantitative invitro determination of glucose –six- phosphate dehydrogenase in serum and erythrocytes by spectrophotometer, Shimadzu UV-1800 (Shimadzu, Japan). G6PD activity was calculated from the change in absorbance at 340 nm over a period of 5 min, as per the manufacturer’s instructions, and Hb (U/gHb) normalized the derived G6PD activity (mU/ml).

G6PD activity was expressed as units of enzyme activity per gram of hemoglobin (U/g Hb). G6PD deficiency was defined as activity < 60% of the normal mean (<3.9 U/g Hb).

3.6.1 Reagents

G6PDassaykits.Distilledwater.

3.6.2 Test performed

Glucose-6-phosphate dehydrogenase (G6PD) assay

For the quantitative *in vitro determination of Glucose-6-Phosphate Dehydrogenase in serum and erythrocytes.*

3.6.3 Laboratory procedure

G6PD assay was carried out on hemolysate prepared by mixing 9 volume of lysing solution (2.7 mmol/L EDTA, pH 7.0 and 0.7 mmol/L 2-mercaptoethanol) to 1 volume of washed red cell suspension. The assay was conducted within 1 h of the freshly prepared haemolysate. The G6PD activity was calculated from the change in the absorbance at 340 nm over a period of 5 min by following the rate of production of NADPH, which has a peak of ultraviolet light absorption at 340 nm. The absorbance of the blank was also measured.

Other investigations carried out were red blood count and hemoglobin estimation by using hematology analyzer (Horiba ABX - India).

3.7 Data analysis

The data was collected in master sheet paper, subjected to statistical analysis using Microsoft excel 2007and statistical professional for social science (SPSS) computed program, version 20. Descriptive statistics, proportion and percentages, were determined. The statistical significance of the difference in the proportion of the studied groups was obtained using T test. Correlation was evaluated by Pearson's correlations. $P<0.05$ was significant throughout the study.

4. RESULTS

The total number of hundred SCD patients, 45 patients were homozygous (SS), 55 patients were heterozygous (AS) and 50 control group (AA). Of the total 100 patients, 68 patients were males (68%) and 32 patients were females (32%) and the study reveal that the prevalence of glucose-6-phosphate dehydrogenase deficiency in SCD patients was 19 deficient (10 male and 9 female) whereas 81 was normal (58 males and 23 females), the statically analysis showed insignificant differences *P* value = 0.442 (Table 1). The prevalence of G6PD deficiency was higher in SCD patients than controls with *P* value = 0.026 (Table 2).

Also we found that the red blood cells count range between 1.30 - 4.00 ×109 /L with mean of 2.37×109 /L and in the in deficient SCD patients there were 15 less than 3.00×10^9 /L and 4 deficient patients were between $3.00\text{-}4.00\times10^9$ /L. However, 77 % of SCD patients had RBCs count less than 3.00×10^9 /L and the rest 23 % had count between $3.00\text{-}4.00\times10^9$ /L, with statistically significant difference *P* value = 0.000 (Table 3). In this study we found that the Hemoglobin (Hb) concentration level between 3.6 - 10.0 g/dl with mean of 6.4 g/dl and all the SCD patients were anemic. And 74% of patients had Hb level between 5-10 g/dl; the rest 26% had level less than 5 g/dl however, there was significantly differences was found between G6PD assay and hemoglobin concentration *P* = 0.000 (Table 4). In addition, we found that in the major types of the SCD there was 45 homozygous patients (SS) and 55 heterozygous patients (AS) and G6PD deficiency among them were 8 (17.8%) and 11 (20%), respectively which showed no statistically significances with P value = 0.620 as shown in (Table 5). The severity of G6PD deficiency among SCD patients was determined by G6PD assay in mU/erythrocyte per ml blood. And the study revealed that out of the total 100 patients, there was 81 patients (81%) were within the normal range which lies between 245-299 mU/erythrocytes per ml blood and 19 patients (19%) were deficient less than 245 mU/erythrocytes per ml blood with significant differences *P* value = 0.000 (Table 6).

Table-1: Prevalence of glucose-6-phosphate dehydrogenase deficiency in sickle cell disease patients by gender.

		Gender		Total
		Males	Females	
G6PD assay	Deficient	10	9	19
	Normal	58	23	81
Total		68(68%)	32(32%)	100
P value	0.442			

Table 2: Prevalence of glucose-6-phosphate dehydrogenase deficiency between SCD patients and controls.

		glucose-6-phosphate dehydrogenase deficiency		Total
		Deficient	Normal	
G6PD assay	patients	19	81	100
	control	2	48	50
Total		21	129	150
P value	0.026			

Table 3: Comparison of red blood cells count in deficient/normal G6PD among the SCD patients.

		RBCs count×10^9/L		Total
		< 3.00	> 3.00	
G6PD assay	Deficient	15	4	19
	Normal	62	19	81
Total		77	23	100
P value	0.000			

Table 4: Comparison between G6PD deficiency and hemoglobin concentration among patients.

		Hemoglobin conc.		
		< 5.0 g/dl	5.0-10.0 g/dl	Total
G6PD assay	Deficient	11	8	19
	Normal	15	66	81
Total		26(26%)	74(74%)	100
P value	0.000			

Table 5: Comparison between G6PD assay in the homozygous and heterozygous patients.

				Total
		Homozygous	Heterozygous	
G6PD assay	Deficient	8 (17.8%)	11(20%)	19
	Normal	37	44	81
Total		45	55	100
P value	0.620			

Table 6: Comparison between G6PD enzyme activities in mU/erythrocytes per ml blood among SCD patients.

		G6PD assay in mU/erythrocytes per ml blood		Total
		< 245	245-299	
G6PD assay	Deficient	19	0	19
	Normal	0	81	81
Total		19	81	100
P value	0.000			

5. DISCUSSION

A case control study was conducted in Khartoum state during the period from December 2018 to August 2019 to find out the prevalence G6PD deficiency among hundred sickle cell disease patients that have been clinically diagnosed with sickle cell disease (SCD), of them 45 patients were homozygous (SS), 55 patients were heterozygous (AS). This study showed that the prevalence of G6PD deficiency was higher in SCD patients than apparently healthy

controls, this findings was similar with many studies done by Samuel AP, Saha N. et al.[24] , a study done by Diop et al. in Dakar,[26] and the other study in Burkina Faso done by Simpore J, Ilboudo D. et al.[22] On the other hand, our prevalence is lower than that study done by R. A. Lewis et al in Ghana in 1966, which was 43% among 95 sickle cell anemia patients.[27] Also the study revealed that there were significant differences in the hemoglobin concentration and RBCs count among G6PD deficient patients and none G6PD deficient with sickle cell diseases, this result similar the results obtained by Samuel Antwi-Baffour et al.[28] and was in line with the study of Lionnet et al.[29] In addition, the prevalence of G6PD deficiency in the SCD patients were 19 patients (19%) and 81 patients (81%) and the study found that the severity of G6PD deficiency which determined by G6PD assay among the deficient patients was less 245 mU/erythrocytes per ml and among the none deficient patients was lies between 245 and 299 mU/erythrocytes per ml, so the severe form of the deficiency was more obvious in the SCD patient's blood, this findings was similar to a study done by Steinberg, MH. et al.[18] and Bienzle, U. et al.[30] Comparing the clinical and laboratory profile (RBCs count, Hemoglobin concentration and G6PD assay in mU/erythrocytes per ml blood) of SCD patients with G6PD deficiency and SCD patients with normal G6PD activity showed statistical difference between the two groups. So the present data would suggest the likelihood of G6PD deficiency increased the hemolysis in SCD patients.

6. CONCLUSIONS

In this study, the evaluation of clinical status in sickle cell disease patients with G6PD showed slight harmful effect of this enzyme abnormality upon the sickle cell disease. Hematological, the coincidence of G6PD deficiency with SCD slightly affected the red blood cells count, hemoglobin concentration and G6PD assay in mU/erythrocytes per ml blood. However, the G6PD deficiency should be looked for in all subjects with sickle cell anemia. Since the homozygous individual with SS hemoglobin is uniquely unfit to tolerate increased hemolysis and when the two problems coexist. Therefore, selective decision should be taken in patients with two conditions coexist in order to deduce preventive and therapeutic measures.

Conflicts of Interest

All the authors have no any possible conflicts of interest.

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