# Exploring the Coexistence of Glucose-6-Phosphate Dehydrogenase Deficiency in Sickle Cell Anemia Patients: Insights from Maiduguri, Nigeria's North-East Region


## Abstract

**Background:** Sickle cell anaemia (SCA) and Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency are inherited red cell disorders associated with chronic haemolysis that have a similar pattern of occurrence in malaria-endemic areas, including Nigeria.

**Objectives:** This study aims to determine the effect of co-inheritance of red blood cell G-6-PD deficiency and SCA regarding some clinical parameters.

**Methods:** This cross-sectional study was conducted over 13 months involving 235 SCA patients and 235 voluntary HbAA controls. Patients were HbSS as confirmed by Hb electrophoresis in an alkaline medium (pH 8.4-8.6) in a steady state. Quantitative G-6-PD enzyme activity among the study population was assayed using the spectrophotometric method.

**Results:** The prevalence of G-6-PD deficiency was similar in patients (29.3%)and controls (25.5%). Only 3.8% of patients had total G-6-PD deficiency, and 25.5% had partial deficiency. The mean (SD) G-6-PD activity of patients was totally deficient; 1.49(0.43), partially deficient; 4.95(1.45), and normal; 10.39(2.66). Similarly, G-6-PD activity in controls was totally deficient; 1.62(0.36), partially deficient; 4.93(1.54) and normal 9.00(1.89). The mean age at first transfusion (±SD) was lower in patients with total G-6-PD deficiency (4.89 years ±3.96) when compared with patients with normal G-6-PD activity (10.73 years ±2.27).

**Conclusion:** The prevalence of G-6-PD deficiency is high in both SCA patients and normal controls. Sickle cell anaemia patients with co-existing G-6-PD deficiency commence transfusion at a younger age than those without G-6-PD deficiency.

**Keywords:** Co-existing; deficiency; disorder; G-6-PD; Sickle cell; hereditary.

## Introduction

Sickle cell disease (SCD) and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency are inherited haemoglobin and red cell enzyme disorders of red blood cells, respectively, are associated with prevalent chronic haemolysis in Nigeria.[1] There is the possibility that an individual could inherit both SCD and G-6-PD deficiency since the two disorders have a typical relationship of having a similar geographic and ethnic distribution.[2] The commonest form of SCD is sickle cell anaemia (SCA), in which the sickle beta-globin gene is inherited in a homozygous state (HbSS) following the Mendelian autosomal recessive fashion of genetic disease inheritance.[3] Other forms of SCD include compound heterozygous states for the sickle β-globin gene and HbC (HbSC) disease, β-thalassaemia (HbSβ-thal), HbD (HbSD), HbO (HbSO) and Hb Lepore (HbSLepore).[3,4] The sickle haemoglobin mutation is a single nucleotide substitution in the β-globin gene, which results in the synthesis of a β-globin protein with a hydrophobic valine instead of the hydrophilic glutamic acid at the sixth position of the amino acid codon. This gene is widely distributed throughout Africa, the Middle East, the Mediterranean, and Southeast Asia, and by population migration to the Caribbean, North America and Northern Europe.[3] The frequency of the carrier state (HbAS) is 20-25% in West Africa, including Nigeria, and about 10% in Afro-Caribbeans.[5] This frequency has reached high levels in the population of West Africa because the carrier state protects against severe forms of Malaria that are endemic in the region, the phenomenon of balanced polymorphism.[6] Sickle cell

disease is associated with a highly pro-oxidant environment due to increased production of reactive oxygen species (ROS) that is generated as a result of elevated levels of pathological free heme and iron and a reduction in anti-oxidant systems such as reduced glutathione.[7]

The clinical presentation of SCA is primarily due to the vaso-occlusive episodes resulting from the polymerisation of the deoxygenated sickled haemoglobin (HbS), which characteristically leads to a change in the shape of the erythrocytes to a sickle or crescent shape.[3]These sickled erythrocytes have poor deformability with a high tendency to adhere to the vascular endothelial surfaces, ultimately leading to its damage and subsequent exposure of the sub-endothelial structures and collagen with resultant platelet activation and aggregation within the microvasculature.[8,9] Furthermore, a recent study suggests that sickled erythrocytes increase vascular endothelial production of adhesion molecules, which creates the favourite condition for intravascular cellular adhesions, stasis, and prolongation of blood flow transit time, thereby increasing the chances of erythrocytes sickling.[10] These cascades of events finally lead to the blockade of the small blood vessels, resulting in tissue infarctions of structures supplied by the blocked vasculature, which present clinically as the characteristic painful vaso-occlusive crises commonly affecting the bones.[3] The effect of vascular occlusion in SCA is not only restricted to the bones; virtually all body organs, including the central nervous system, the lungs and the kidneys, are particularly affected and may result in multiple system organ damage.[8] In addition, sickling drastically shortens the life span of erythrocytes, leading to chronic haemolytic anaemia and jaundice with the possibility of the formation of bilirubin stones within the gall bladder in the long run.[8]

The clinical course of SCA is typically characterised by variable periods of steady state during which the patient remains reasonably healthy.[11] This state of relative well-being is

periodically interrupted by crises, which may be mild and manageable at home or severe and warranting hospital admissions.[11] However, the clinical status of patients with SCA could be affected by co-inheritance of additional genetic red cell disorders. For example, the co-inheritance of the gene for hereditary persistence of foetal haemoglobin (HPFH) leads to higher intracellular levels of HbF, which interacts less effectively with HbS in the process of sickling. In contrast, the co-inheritance of alpha thalassaemia gene reduces the rate of polymerisation of HbS by decreasing the intracellular concentration of HbS in the red cells thereby inhibiting sickling.[12] Therefore, the co-inheritance of HPFH genes or alpha thalassaemia reduces the rate of red cell sickling, thereby reducing disease severity and improving the overall prognosis of SCA.[12] While the favourable effect of co-inheritance of HPFH and alpha thalassaemia genes on the prognosis of SCA is clear and well established, the significance of co-inheritance of G-6-PD deficiency remains inconclusive. While some researchers had earlier reported that G-6-PD deficiency could benefit the clinical course of SCA,[13] this correlation was not confirmed in other studies carried out in Nigeria by Ahmed et al.,[14] Jamaica[15] and the United States.[16]On the other hand, inheritance of G-6-PD deficiency is sex-linked as the enzyme is controlled by one gene locus on the X chromosome.[17] However, only variants with significant deficient activity are associated with clinical disease in the form of haemolytic anaemia like SCA.[18] The two most common deficient variants are the Mediterranean type and the A-type found in the black populations of West Africa, including Nigeria.[19] Co-inheritance of the disorder of SCA and G-6-PD deficiency in a patient may, therefore, result in worsening of haemolysis in SCA patients having co-inherited G-6-PD deficiency and, therefore, leading to hyperhaemolytic anaemic crisis.[20]

In this paper, we present a report on determining the effect of G-6-PD deficiency in patients with SCA concerning complete blood count, reticulocyte count and reticulocyte index in Nigerian patients with SCA as seen in Maiduguri, North-Eastern Nigeria.

## Materials and Methods

### Study design

This study was a hospital-based prospective cross-sectional study conducted in October 2020 to November 2021 at the haematology department of the University of Maiduguri Teaching Hospital.

### Study participants

The study population was adult sickle cell anaemia patients 18 years and above in steady state and normal voluntary HbAA subjects. Patients with HbSS confirmed by Hb electrophoresis in an alkaline medium (pH 8.4-8.6) in steady state as defined by Akinola *et al.*,[21] as the period free of crisis extending from at least three weeks since the last clinical event and three months or more since the previous blood transfusion to at least one week before the start of a new clinical event. The controls were healthy adult volunteers who were aware of their HbAA status and enrolled from willing staff, medical students and voluntary blood donors at National Blood Transfusion Services, UMTH.

**Study procedure:** In the reagent kit, glucose-6-phosphate dehydrogenase in the RBCs is released after lysis with digitonin. The G-6-PD released catalyses the oxidation of G-6-P in the pentose phosphate pathway with the reduction of $NADP^+$ to NADPH.[22] The reduction rate of $NADP^+$ to NADPH is measured as an increase in absorbance at 340nm proportional to the G-6-

PD activity in the sample.[23] Based on this principle, all patients were screened for G-6-PD deficiency by the Spectrophotometric quantitative assay as recommended by W.H.O.[24] using commercial test reagent kit, cat No PD410, manufactured by Randox Laboratories, U.K.[23] The tests were executed by addition of two and a half millilitres of blood into Ethylene diamine tetra acetic acid (EDTA) bottle, a volume of 0.2ml of blood was washed with 2mls aliquots of normal saline and centrifuged after each wash for 10 minutes at around 2000rpm. The procedure was repeated three times, and a red cell suspension in 0.5mls of the solution was made. The suspension was allowed to stand for 15 minutes in a refrigerator at $4^0$C and then centrifuged again. The supernatant was assayed within 2 hours.

At the start of the procedure, the spectrophotometer was set at a wavelength of 340nm with a temperature of $37^0$C, and the light path of the cuvette was set at 1cm. Next, double distilled water was used as blank, and its absorbance was measured and recorded; subsequently, the absorbance of the test samples was measured as follows:

A test tube was set on a rack, and 1ml of Triethanolamine buffer EDTA solution was added to the tube, followed by 0.03ml of NADP solution and 0.015ml of the prepared haemolysate. This test tube content was mixed and then incubated at $37^0$C for 5 minutes, after which 0.015ml of substrate solution was added and mixed gently, ready to read the absorbance. For the reading, the test tube content was emptied into a cuvette placed in the spectrophotometer, and the initial absorbance was read while starting a timer simultaneously, followed by further readings of the absorbance at 1, 2 and 3 minutes. Finally, the change in absorbance per minute (-A340nm/min) was calculated by using absorbance at 3 minutes minus initial absorbance divided by 3.[25]

**Calculation of G-6-PD activity:**

The following formula was used to calculate the G6PD activity[25]

mU/ erythrocytes per ml blood =33650 x -A340nm/min.

The G-6-PD activity was then converted to mU/g haemoglobin using the equation

G-6-PD $mU/gHb = mU\text{x}\ \frac{Eryt\,hrocytesperml\ \text{x}\ 100}{Hb\ (g/dl)}$

Normal Reference range; 6.97 - 20.5 U/g Hb[25]

**Statistical Methods:** Data obtained were analysed using a statistical package for social science (IBM SPSS Statistics version 23.0 software), SPSS Inc., Chicago, Illinois, USA. The sex, marital status, educational level, and transfusion history were summarised and presented using frequency, percentages, and tables as appropriate. The ages, age at first transfusion, and total units of blood transfused were presented in either mean ± SD when normality is not violated or median (Interquartile range) when normality is violated. Factorial (Two-way) ANOVA was used to compare the level of G-6-PD deficiency; multivariate analysis of variance (MANOVA) was used to correlate the age at first transfusion and total units of blood received in patients with varying degrees of G-6-PD activity. Statistical significance was set at $\boldsymbol{P} < 0.05$.

**Ethical considerations:** Ethical approval was obtained from the Health Research Ethics Committee of the University of Maiduguri Teaching Hospital (UMTH/REC/22/618). Participants were counselled individually, after which informed consent was obtained from them.

## Results

### Socio-demographic profile of participants

The study population consisted of 470 participants, 235 of whom were SCA and the other 235 were HBAA controls. The patients consisted of 128(54.5%) females and 107(45.4%) males,

while the controls consisted of 82(34.9%) females and 153(65.1%) males. The study participants' ages ranged from 18 to 52 years. Sickle cell anaemia patients had a range of 18-45 and Controls 18-52. The median age in years (interquartile range) among SCA patients was 22.0(8.0) and control was 25.0(10.0). The majority of the participants were single, with 206(87.7%) of patients and 149(63.4%) of controls. The remainder were either married 26(11.1%) of patients and 84(35.7%) of controls; or divorced 3(1.3%) of patients and 2(0.9%) of controls. More than half of the participants were educated, of which 128(54.5%) SCA and 182(77.4%) had attained a tertiary level of education (Table I).



**Table I:** Socio-demographic profile of participants

| | SCA = 235 | Control = 235 |
|---|---|---|
| | Frequency (%) | Frequency (%) |
| **Age (years)** | 22.0(8.0)[α] | 25.0(10.0)[α] |
| **Sex** | | |
| Male | 107(45.5) | 153(65.1) |
| Female | 128(54.5) | 82(34.9) |
| **Marital status** | | |
| Married | 26(11.1) | 84(35.7) |
| Single | 206(87.7) | 149(63.4) |

| | | |
|---|---|---|
| Divorced | 3(1.3) | 2(0.9) |
| **Educational level** | | |
| None | 11(4.7) | 6(2.6) |
| Primary | 14(6.0) | 6(2.6) |
| Secondary | 82(34.9) | 41(17.4) |
| Tertiary | 128(54.5) | 182(77.4) |

The participants were mostly students 167(71.1%) SCA and 130(55.3%) controls (Figure 1).

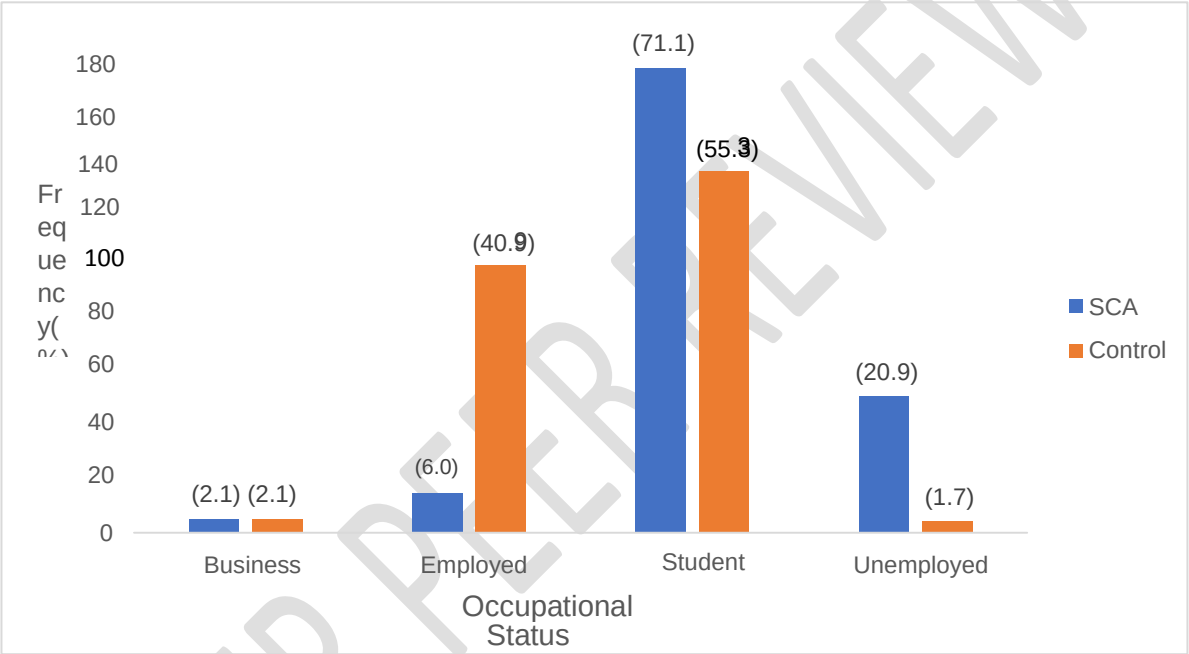


**Figure 1:** Occupational status of participants

**Red cell G-6-PD activity of the study participants**

The mean (SD) G-6-PD activity was totally deficient 1.49(0.43) in patients and 1.62(0.36) in controls; partially deficient 4.95(1.45) in patients and 4.93(1.54) in controls; normal activity 10.39(2.66) in patients and 9.0(1.89) in controls (Table II). The G-6-PD activity was similar in patients and controls except in normal G-6-PD, which was slightly higher in patients than in controls, with a mean difference of 1.39 (Table II).

**Table II:** Comparison of G-6-PD activity at different levels among SCA and controls

| G6PD | SCA[a] Mean ±SD | Controls Mean ±SD | Estimated Mean |
|---|---|---|---|
| Totally deficient | 1.49 ±0.43 | 1.62 ±0.36 | 1.552 |
| Partially deficient | 4.95 ±1.45 | 4.93 ±1.54 | 4.941 |
| Normal[c] | 10.39 ±2.66 | 9.00 ±1.89 | 9.696 |
| Groups (SCA & Control): F (df) = 1.512 (1), *P*= 0.219 | | | |
| G6PD: F (df) = 314.989(2), *P*<0.0001 | | | |
| Groups * G6PD (interaction): F (df) = 5.061(2), *P*= 0.007[α] | | | |

Factorial ANOVA (Two-way ANOVA)
Multiple Comparison
G-6-PD:a & b = *P*<0.0001, a & c = *P*<0.0001, b& c = *P*<0.0001,
Interaction: Normal (a & b = *P*<0.0001)

Patients were found to have a G-6-PD deficiency prevalence of 29.3%, with 3.8% having total deficiency and 25.5% having partial deficiency. Controls had a prevalence of 24.3%, with 5.1% having total deficiency and 19.2% having partial deficiency (Figure 2).

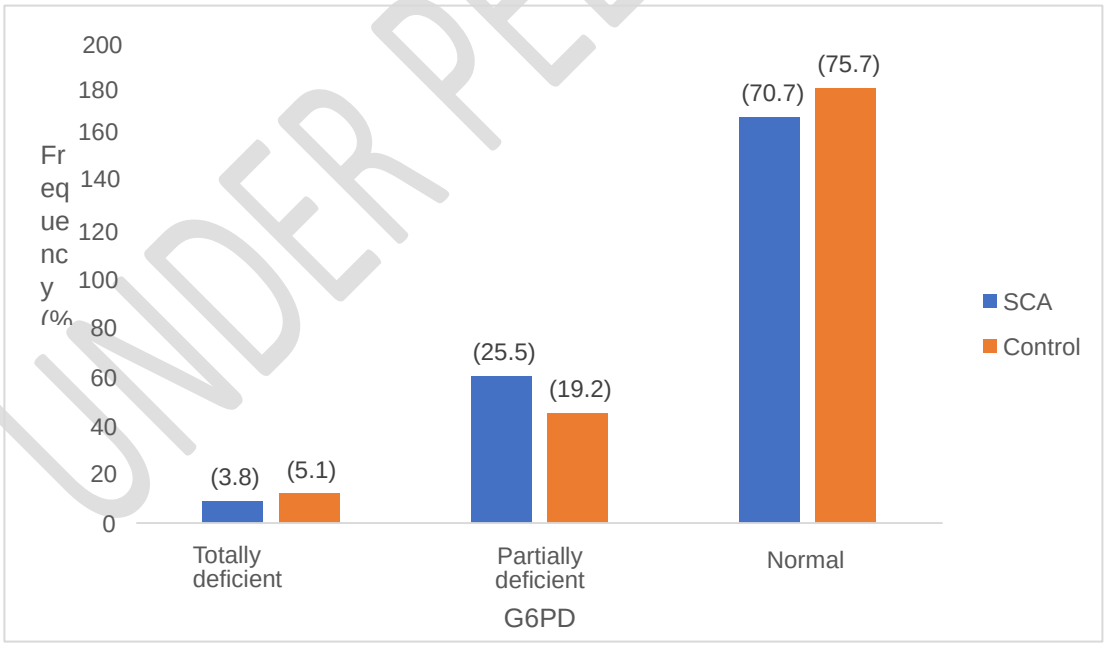


**Figure 2:** Distribution of G6PD deficiency in SCA and controls

The prevalence of G-6-PD deficiency in patients was higher in females 42(60.9%) compared to males 27(39.1%), though the difference was insignificant. However, prevalence in controls was higher and statistically significant in males 48(84.2%) compared to females 9(15.8%) (Table III).



**Table III:** Comparison of G-6-PD deficiency and gender of SCA and Controls

| | G-6-PD | | | | |
|---|---|---|---|---|---|
| Sex | Deficient | Normal | Total | Chi-square (df) | *P** |
| **SCA** | | | | | |
| Male | 27(11.5) | 80(34.0) | 107(45.5) | 1.614(1) | 0.204 |
| Female | 42(17.9) | 86(36.6) | 128(54.5) | | |
| Total | 69(29.4) | 166(70.6) | 235(100) | | |
| **Control** | | | | | |
| Male | 48(20.4) | 105(44.6) | 153(65.0) | 12.089(1) | 0.001 |
| Female | 9(3.8) | 73(31.0) | 82(34.8) | | |
| Total | 57(24.3) | 178(75.7) | 235(100) | | |

*Chi-square independent

The prevalence of total deficiency was 5(4.7%) in males and 4(3.1%) in females' patients, while the prevalence was 12(7.8%) in males and 0(0%) in females controls.

**G-6-PD deficiency and transfusion requirements in SCA patients**

The mean age (SD) at first transfusion of patients was 10.84 years (8.93). Patients that had total G-6-PD deficiency had their first transfusion at a younger age, 4.89 years (3.96), when compared with patients with partial deficiency at 12.60 years (2.17) and normal G-6-PD activity 10.73 years (2.27). However, they had a similar moderate number of blood units transfused to date across all three levels. Therefore, patients with total deficiency are likely to have higher transfusion requirements, but it was not statistically significant ***P*** = 0.098 (Table IV).

**Table IV:** Comparison of transfusion requirements and G-6-PD deficiency in SCA

| | Totally deficient | Partially deficient | Normal | | |
|---|---|---|---|---|---|
| | Mean ±SD | Mean ±SD | Mean ±SD | F(value) | ***P*** |
| Age at first transfusion | 4.89 ±3.96 | 12.60 ±2.17 | 10.73 ±2.27 | 1.977(0.956) | 0.098[α] |
| Total units | 5.28 ±4.16 | 5.36 ±2.43 | 5.73 ±2.76 | | |

[α]MANOVA (Wilks' Lambda), Box's Test = ***P***=0.159,

**Discussion**

Glucose-6-phosphate dehydrogenase enzyme activity varies among individuals in a population; low enzyme activity or a deficient state of the enzyme is associated with episodes ofhaemolytic states following exposure to infection or some drugs, which is also a feature of SCA, a monogenic disorder that affects red blood cells. Determination of the enzyme activity in SCA is relevant because, in this study, the prevalence of G-6-PD deficiency was higher among SCA patients than among controls (29.3% and 24.3%, respectively), which is similar to the prevalence found in a study by Fasola *et al.*,[1] in Ibadan. The prevalence is higher than that found in a previous study in the same environment by Ahmad *et al*. 14, in which 15.6% of male patients were found to be G-6-PD deficient, and none of the female patients were deficient. The differences in prevalence could be a result of the use of the non-quantitative method (fluorescent spot test), which is less sensitive and has a drawback of substantially missing deficiency in female heterozygotes.[26]Our findings also demonstrate a higher prevalence than the studies by Egesie *et al*.,[27] in Jos, and Ogunkanbi *et al*.,[28] in Ilorin. The disparity in findings compared to thosetwo studies is probably due to differences in patient selection and the lower sample size used in their studies. However, the study by Ogunkanbi et al., conducted in children, found that G-6-PD deficiency was more frequently encountered in older age groups, even though the finding was not statistically significant.[28]In contrast, our findings have a lower prevalence when compared to the study by Antwi-Baffour *et al.*,in Ghana[29]whichfound a higher prevalence; this was probably due to differences in study participants selection, method of G6PD assay and geographic location of the study. It has also been reported that even within a given country, there can be a marked variation in the prevalence of G-6-PD deficiency.[30] Only 3.8% of our SCA patients were totally G-6-PD deficient, and 25.5% of them were partially deficient; this is similar to findings obtained by

Antwi-Baffour *et al*.,[29] The prevalence of total G-6-PD deficiency is similar between males and female patients, unlike the marked difference in male and female controls. This could be due to the higher number of male controls used in the study. Female patients had a higher prevalence of G-6-PD deficiency in our series; this is in contrast to the expectation of an X-linked disorder, which makes female offspring of a G-6-PD deficient father all to be carriers (heterozygotes) of the trait with a variable range of G-6-PD activity while each male offspring of a carrier or affected mother has a 50-100% chance of being deficient.[26] This could result from categorising acquired G-6-PD deficiency due to an increased oxidative state as inherited G-6-PD deficiency since a genetic study to confirm diagnosis was not done. The higher burden in females could be due to the relatively higher life expectancy. Similar findings were obtained by Igwilo*et al.*,[31] and Abubakar *et al.*,[32]

With transfusion support remaining an essential component of patient management in SCA and making a significant contribution to patients' morbidity and mortality, this study found that a high proportion of patients, 188 (80%), have been transfused before, which was also reported by Diop *et al*.,[33] and Tshilolo *et al*.,[34] When the disorder is co-inherited with G-6-PDdeficiency it is expected to have a higher transfusion requirement. In this study, SCA patients with total G-6-PD deficiency had a lower age at first transfusion and, therefore, a higher transfusion requirement, though not statistically significant, which was similar to the cases with those with G-6-PD deficiency ina study by Benkerrou *et al*., that found a high frequency of transfusion.[35] The relatively small population of patients with total deficiency in this study will require further study with a larger population to validate such deductions.

**Conclusions**

The prevalence of G-6-PD deficiency is high in both SCA patients and normal controls. Sickle cell anaemia patients with co-existing G-6-PD deficiency commence transfusion at a younger age than those without co-existence of the two haemolytic disorders. Long-term outcomes depend on optimal transfusion policies and require further investigations in larger cohorts.

**Limitation**

This study is limited by the fact that the assessment of G-6-PD enzyme activity was not done by a molecular study, which is more accurate and specific. Moreover, patients with acquired deficiency as a result of increased oxidative stress maybe wrongly be classified as G-6-PD deficient and thus overestimate the burden of the disease.

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