Scientific Research Publishing

Open Journal of Blood Diseases, 2023, 13, 93-101
https://www.scirp.org/journal/ojbd
ISSN Online: 2164-3199
ISSN Print: 2164-3180

Prevalence of Association of Glucose-6-Phosphate Dehydrogenase Deficiency and Sickle Cell Disease at the National Teaching Hospital of Cotonou in Benin

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How to cite this paper: Zohoun, A., Baglo-Agbodande, T., Adje, T., Houssou, B., Massi, R., Guiwa, G.G.O., Dèhoumon, J., Mehou, J., Anani, L., Vovor, A. and Kinde-Gazard, D. (2023) Prevalence of Association of Glucose-6-Phosphate Dehydrogenase Deficiency and Sickle Cell Disease at the National Teaching Hospital of Cotonou in Benin. *Open Journal of Blood Diseases*, **13**, 93-101.
https://doi.org/10.4236/ojbd.2023.133012

Received: June 24, 2023
Accepted: September 8, 2023
Published: September 11, 2023

Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency and sickle cell disease are common genetic defects of red blood cells that lead to hemolytic anemia. The prevalence of G6PD deficiency in sickle cell patients is unknown in Benin. **Objective:** This study aimed to determine the prevalence of G6PD deficiency in sickle cell patients at the CNHU-HKM of Cotonou. **Methods:** This prospective study was conducted from April to November 2022 at the blood-related diseases teaching clinic and included sickle cell patients in the stationary phase. G6PD determination was performed using the enzymatic method on a Mindray BS 200 machine following the Herz method. Hematological parameters were determined using the XT 4000i analyzer and supplemented by a blood smear stained with May Grunwald Giemsa. Data were analyzed using Epi Info 3.5.4 software. **Results:** One hundred and sixty-four sickle cell patients (80 SS homozygotes and 84 SC heterozygotes) in the intercritical phase, with a mean age of 26.30 ± 10.76 years, were included. The prevalence of G6PD deficiency was 9.1% (15 cases found in 7 SS patients and 8 SC patients). In G6PD-deficient patients, the mean concentration of the enzyme was lower in Hb SC heterozygotes than in Hb SS homozygotes: 3.56 IU/g Hb versus 4.98 IU/g Hb. The mean reticulocyte count was 231.43 G/L in the deficient group, compared to 216.32 G/L in the non-deficient group. **Conclusion:** The preliminary results of our study reveal a high prevalence of G6PD deficiency in sickle cell patients. The impact of this association on he-

matologic and biological parameters should be evaluated for better management of sickle cell disease.

Keywords

Sickle Cell Disease, G6PD Deficiency, Prevalence, Hemogram

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme crucial for protecting red blood cells against oxidative stress. It plays a vital role in the pentose-phosphate pathway, facilitating the production of nicotinamide adenine dinucleotide phosphate (NADPH) from the oxidation of glucose-6-phosphate. NADPH, a co-enzyme of glutathione reductase, contributes to the synthesis of reduced glutathione, a key molecule in countering oxidation [1]. G6PD deficiency affects over 400 million individuals and closely correlates with regions endemic for malaria and sickle cell disease [2]. Both G6PD deficiency and sickle cell disease are common genetic defects of red blood cells associated with hemolytic anemia. Despite being carried on different chromosomes, these abnormalities can coexist in the same individual, potentially leading to increased hemolysis [2]. The impact of G6PD deficiency on sickle cell disease, as well as the disease's epidemiological, clinical, and evolutionary profiles, has been extensively studied and debated [3] [4] [5]. It is evident, however, that recognizing this association is vital for preventing hemolytic episodes in sickle cell patients, as it guides the avoidance of oxidizing drugs and foods. Yet, in Benin, there is a lack of data on the co-occurrence of these two erythrocytic pathologies. The current study aimed to determine the prevalence of G6PD deficiency among sickle cell subjects at the Clinique Universitaire des Maladies du Sang (CUMAS) of the Centre National Hospitalier Universitaire Hubert Koutoukou Maga in Cotonou (CNHU-HKM, National Teaching Hospital).

2. Material and Methods

2.1. Study Design and Participants

This prospective descriptive study was conducted from April to November 2022 in the clinical hematology department of CNHU-HKM in Cotonou. The study included sickle cell patients who were either homozygous SS or double heterozygous SC, regularly followed up (with at least 3 consultations per year), aged 18 years and above, in a stationary phase for at least 3 months, and seen on an outpatient basis. Participants were excluded if they had received transfusions within the last 3 months or were under hydroxyurea treatment. The sample size was determined using the Schwartz formula, and eligible patients, no familial relationship, were consecutively enrolled. Socio-demographic variables including age and sex were recorded.

2.2. Sampling and Biological Analysis

For each participant, a sample was collected in a tube containing tripotassium ethylene diamine tetraacetic acid (EDTA-K3) and promptly sent to the Hematology and Biochemistry laboratory at the same center.

2.3. Biochemical Analysis of Blood Samples

G6PD activity was measured spectrophotometrically using a Mindray BS 200 automated system. Chronic hemolysis and continuous regeneration in sickle cell patients result in a high number of reticulocytes and young red blood cells, potentially leading to elevated G6PD activity that could mask a deficiency. To address this, Herz's micro-centrifugation technique was employed [6]. Reference interval of G6PD activity values in our laboratory range from 7 - 12 IU/gHb. Deficiency is considered partial when G6PD activity falls between 2.1 and 7 IU/g Hb and total when activity is below 2.1 IU/g Hb.

2.4. Hematological Analysis of Samples

Hematological analyses were conducted on the Sysmex XT 4000i automated system, utilizing hydro-focusing and flow cytometry. Various methods were applied to determine blood count parameters, such as spectrophotometry for hemoglobin, impedance-based hematologic analyzers for red cell, leukocyte, and platelet counts, and flow cytometry for leukocyte, reticulocyte, and erythroblast counts. Blood smears were routinely examined after fixation and staining with May-Grünwald Giemsa. Internal quality controls for blood counts and patient results were systematically validated.

2.5. Data Processing and Statistical Analysis

Data were entered and processed in Excel, and statistical analysis was performed using R. Means and standard deviations were presented for each parameter with a 95% confidence interval. Student's t-test was used for mean comparisons.

2.6. Ethical Considerations

This study was approved by the Institutional Committee of Ethics and Research of the University of Parakou (Authorization 0397/CLERB-UP/P/SP/R/SA). Participants provided informed consent after receiving information about the study's objectives, benefits, and risks. Research result confidentiality was maintained through unique patient codes.

3. Results

A total of 164 sickle cell patients in the intercritical phase (80 SS homozygotes and 84 SC heterozygotes) were enrolled, with 83 male (50.6%) and 81 female (49.4%) participants, yielding a male-to-female ratio of 1.1. The mean age of the study population was 26.3 ± 10.8 years. Female patients had a mean age of 30.2 ±

11.9 years, compared to 24.3 ± 9.7 years for male patients (p = 0.01) (Table 1).

SS homozygotes exhibited a mean hemoglobin level of 8.1 g/dL. The anemia was normocytic, characterized by a mean corpuscular volume of 85.7 fL, consistent with literature. Hyperleukocytosis was observed consistently, with an average of 15.5 G/L. Sickle cell SC patients displayed hemogram values approaching normal levels in healthy subjects, with microcytosis being a constant feature. Hematological parameter differences based on hemoglobin profile were statistically significant, except for MCHC (p < 0.001). Similar trends were observed in white blood cell and platelet counts, both of which were statistically elevated in SS homozygous patients (p < 0.001). Blood count data by hemoglobin profile can be seen in Table 2.

Table 1. Distribution of study population by hemoglobin profile, mean age, and gender.

Parameters	Male	Female	Total (%)
Hb SS	48	32	80 (48.8)
Hb SC	35	49	84 (51.2)
Mean age (years)	24.3 ± 9.7	30.2 ± 11.9	26.3 ± 10.8

Table 2. Mean hematology parameters by hemoglobin type.

Parameters	Reference interval adult AA in our laboratory [7]		Obtained values by hemoglobin type		p
	Male	Female	SS	SC	
Hb (g/dL)	12 - 16	11.5 - 14	8.1 ± 1.9	10.9 ± 2.0	<0.001
RBC (T/L)	4 - 6	3.5 - 5	3.2 ± 1.5	4.3 ± 0.8	<0.001
WBC (G/L)	3 - 8		15.5 ± 34.2	6.9 ± 2.5	<0.001
HTE (%)	36 - 49	30 - 40	25.1 ± 6.2	32 ± 5.4	<0.001
MCV (fL)	80 - 90		85.7 ± 11.6	75.4 ± 7.3	<0.001
MCH (pg)	25 - 32		28.5 ± 4.2	25.7 ± 2.7	<0.001
MCHC (%)	30 - 36		33.3 ± 1.5	34.2 ± 1.6	0.002
NEUT (G/L)	1.5 - 6		7.2 ± 24.6	2.9 ± 1.7	0.268
EO (G/L)	0.15 à 0.4		0.6 ± 1.4	0.2 ± 0.3	0.117
BASO (G/L)	0.05 à 0.15		0.02 ± 0.1	0.02 ± 0.1	0.628
MONO (G/L)	0.2 à 0.8		0.3 ± 0.5	0.3 ± 0.5	0.609
LY (G/L)	1.5 - 4		4.3 ± 8.4	2.7 ± 1.8	0.23
PLT (G/L)	150 - 400		423.6 ± 187.7	292.3 ± 162.1	<0.001

Hb: Hemoglobin; RBC: Red Blood Cell, WBC: White Blood Cell; HTE: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; NEUT: Neutrophils; EO: Eosinophils; BASO: Basophils; MONO: Monocytes; LY: Lymphocytes; PLT: Platelets.

Fifteen patients exhibited decreased G6PD activity, resulting in a G6PD deficiency prevalence of 9.1%. Among these patients, 14 were classified in class III (moderate to mild), and 1 in class II (severe) according to current World Health Organization (WHO) classification and guidance [8]. The mean G6PD activity was 4.2 ± 1.5 IU/g Hb for patients of class III and G6PD activity was 0.49 IU/g Hb for the only class II patient. Out of the 15 G6PD-deficient patients, 10 were male, and 5 were female. Regarding the distribution of G6PD-deficient patients by hemoglobin profile, 7 had the SS phenotype, and 8 had the SC phenotype. For these G6PD-deficient patients, the mean enzyme concentration was lower in SC heterozygotes compared to SS homozygotes, measuring 3.6 IU/g Hb and 4.9 IU/g Hb, respectively. G6PD activity classification of deficient patients by phenotype and sex was reported in **Table 3**. The mean reticulocyte count was 231.4 G/L in G6PD-deficient patients, compared to 216.3 G/L in non-deficient patients. Mean values for key hemogram parameters (hemoglobin, white blood cell, and platelets) between G6PD-deficient and non-deficient patients exhibited no significant association.

4. Discussion

In our study, we observed a discreet male predominance with a sex ratio of 1.1. This trend is consistent with the findings of Antwi-Baffour *et al.* in Ghana [4], who reported a male-to-female sex ratio of 1.3. However, contrasting results have been reported by other researchers, such as Fasola *et al.* in Nigeria [3], Bouanga *et al.* in Congo [9], and Diop *et al.* in Senegal [10], where a predominance of females was observed, with sex ratios of 0.8, 0.7, and 0.8, respectively.

Sickle cell patients in a stable phase exhibit different mean hemogram values compared to healthy subjects. In our study, SS homozygotes had an average hemoglobin level of 8.1 g/dL. This mean level is close to those reported by Dahmani *et al.* [11] and Doupa *et al.* [12], with mean hemoglobin levels of 7.6 and 8.2 g/dL, respectively. The observed anemia was normocytic, with a mean corpuscular volume of 81.6 fL, which is in line with the literature. The normocytic, occasionally macrocytic anemia can be attributed to the chronic hemolysis observed in SS sickle cell patients. We consistently noted hyperleukocytosis, with an average value of 11.9 G/L, across all SS patients. This hyperleukocytosis has been reported by several authors, including Dahmani *et al.* [11] and Nacoulma *et al.* [13]. It can be explained by a high level of bone marrow regeneration, combined

Table 3. G6PD activity classification of deficient patients by phenotype and sex.

G6PD-deficient Status	Hb SS		Hb SC	
	Male	Female	Male	Female
Class II (severe)	1	0	0	0
Class III (moderate to mild)	5	1	4	4
Total	6	1	4	4

with permanent demargination, a chronic inflammatory state, and increased susceptibility to infection. In cases of acute hemolysis, the high level of bone marrow regeneration leads to erythroblastosis, resulting in a false hyperleukocytosis that must be corrected if the percentage of erythroblasts is greater than or equal to 10% [14]. The data obtained from the hemogram of heterozygous SC subjects in a stable phase align with what's described in the literature. Sickle-cell SC patients present values close to the normal values of healthy subjects, but with constant mi crocytosis. Hemoglobinopathy C is often associated with microcytosis, with or without anemia. In the case of chronic hemolysis, this microcytosis may transition to normocytosis [14].

Finally, the blood count parameters of G6PD-deficient patients did not differ statistically from those of non-deficient patients. Our study suggests that the association of G6PD deficiency and sickle cell disease does not have a direct impact on the hematobiological profile of carriers in the absence of a crisis.

Assaying G6PD activity and interpreting results require special attention, as the activity of Glucose-6-phosphate dehydrogenase depends on the condition of the red blood cell. Young red blood cells (reticulocytes) are rich in G6PD and therefore have high enzymatic activity. In addition to G6PD spectrophotometric assays, it's essential to include reticulocyte or as partate-Amino-Transferase (ASAT) counts. Relating the value found to that of another enzyme, as mentioned earlier, is crucial to avoid considering a value increased by a regenerative phenomenon as normal or a low value as an activity due to an a regenerative phenome non [14]. In our study, we chose to couple G6PD assay with reticulocyte count due to the ease of assay and availability of reagents. Additionally, Herz microcentrifugation is a method that allows G6PD levels to be determined even during crises while avoiding young red blood cells with high G6PD activity [6]. Reticulocyte count values obtained based on G6PD-deficient or non-deficient status in our study reveal that the measurement of the enzyme's activity was not impacted by this parameter. The mean G6PD activity in our study was 16.99 IU/g Hb, showing no significant difference based on patient gender.

The prevalence of G6PD deficiency in our study was 9.1%, with a higher occurrence in males. This data is consistent with the epidemiological trend of higher susceptibility in males. G6PD deficiency affects over 400 million individuals worldwide, with more than 90% being male [15]. The gene coding for G6PD is located on the X chromosome, explaining the predominance of male sufferers [15]. Notably, homozygous or composite heterozygous women may exhibit clinical and hematological profiles similar to those of men. Rarely, women may express G6PD deficiencies due to preferential inactivation of the normal X chromosome or other factors [15].

A prevalence range of 1% to 25% has been reported in various studies. For instance, Varret *et al.* in Congo Brazzaville [16] and Boutchi *et al.* in Niger [17] reported prevalences of 1.9% and 7.08%, respectively, while Saad *et al.* in Brazil [18] and Steinberg *et al.* in the USA [19] reported prevalence rates of 12.9% and 10.4%, respectively. Using molecular methods, Doupa *et al.* found a prevalence

of 13% in Senegal [20]. Higher prevalence rates have also been documented by other authors [21] [22].

The co-occurrence of G6PD deficiency and sickle cell disease presents significant risks due to the oxidative nature of drugs commonly used by sickle cell patients. Therefore, systematic screening for G6PD deficiency in sickle-cell patients is imperative.

The limitation of our study lies in the sample size, which affected the statistical power. Further research with a larger sample size will provide a more accurate estimation of the observed prevalence. The study of G6PD deficiency in sickle-cell subjects using molecular methods should be considered for a more precise prevalence estimate. Additionally, the cross-sectional nature of our study may be influenced by variations in erythrocyte constants due to various factors such as parasitosis, nutritional deficiencies, and iron deficiency. Also, study of the clinical impact of the deficiency represents a perspective for future studies.

Authors' Contributions

All authors contributed to the paper. All authors helped to conceptualise ideas, interpret the findings and contributed to the revision of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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