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Original Research Article

Liver function assessment in Glucose-6-Phosphate Dehydrogenase deficient neonates in Benin

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the major underlying causes of jaundice in neonates. However, the exact cause of the hyperbilirubinemia is still unknown. This study was aimed to assess liver function in G6PD deficient neonates.

Methods: This study was carried out as a pre-posttest design study with a control group. A total of 410 neonates aged ≤7 days were included in the study. Intra-erythrocyte G6PD activity was determined by quantitative enzymatic method. Plasma glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, direct and total bilirubin, calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels were measured using ELITech reagents. Hematological analyses were performed using an automatic hematological analyzer.

Results: Of the 410 neonates, 101 (24.63%) were G6PD-deficient with no significant difference between gender (male, 49 (11.95%); female, 52 (12.68%). G6PD activity level was significantly ($p < 0.001$) decreased in G6PD-deficient male (2.06 ± 0.82 U/g Hb vs 16.63 ± 5.13 U/g Hb) and in female (5.42 ± 1.28 vs 19.73 ± 2.71 U/g Hb) compared to G6PD-normal controls. Blood glucose showed no variation, but conjugated bilirubin ($p = 0.03$) and total bilirubin ($p = 0.01$) levels were significantly increased in G6PD-deficient than in G6PD-normal controls. Calcium level was significantly ($p = 0.03$) higher in G6PD-deficient while Magnesium level did not vary. Serum AST ($p = 0.01$) and ALT ($p = 0.03$) levels were significantly increased in G6PD-deficient neonates compared to G6PD-normal controls. AST and ALT levels in erythrocytes showed no changes.

Conclusion: Results suggest that hyperbilirubinemia in G6PD deficient neonates may be caused by liver malfunction.

Keywords: G6PD deficiency, neonates, hyperbilirubinemia, liver function, Benin.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the major underlying causes of jaundice in neonates. In tropical African countries, the overall prevalence of G6PD deficiency was ranged from 15 to 26% [1]. Neonatal screening for G6PD deficiency is recommended by the WHO in regions where the prevalence of G6PD deficiency is ≥ 3% [1]; unfortunately, this practice are not yet applied in West Africa. In Benin, neonates are not routinely screened for G6PD deficiency and many neonates without any jaundice baseline risk assessment are so discharged. G6PD-deficient neonates are at risk of severe acute hemolytic anemia after exposure to oxidant stresses [2,3]. Severe neonatal hyperbilirubinemia resulting in kernicterus, the most serious complication of G6PD deficiency in neonates; which leads to permanent neurological damages and death [4]. G6PD deficiency was reported to be associated with kernicterus in up to 62% of infants in Nigeria [5]. Hyperbilirubinaemia in neonates with G6PD deficiency is thought to be secondary due to reduced

hepatic conjugation and excretion of bilirubin [6,7] rather than increased bilirubin production from haemolysis[8, 9]. Anaemia is often not reported in G6PD deficient neonates who develop hyperbilirubinaemia or even kernicterus mainly because massive acute haemolysis is thought not to occur in the absence of trigger factors [7] in neonates. It was reported that the plasma bilirubin increase is largely the result of an impairment of liver function caused by G6PD deficiency [10] suggesting that frequent and severe hemolytic episodes can have adverse effects on liver in new born as one of the main organs involved in the hemolytic processes. In this study, we aimed to determine the influence of G6PD deficiency on glycaemia, bilirubin and transaminases levels in order to assess liver function in absence of exposure to known icterogenic agents on the first week after birth.

2. Material and methods

2.1 Design and study participants

This study was carried out as a pre-posttest design study with a control group. From March to October 2017, a total of 486 neonates aged ≤ 7 days ,birthed in 6 different maternity hospitals across Benin and referred for G6PD deficit screening were enrolled in the study. The study was approved by the National Research Ethics Review Boards of Benin and mothers provided informed consent.

2.2 Measurement of Biochemical of Hematological Parameters

A 2 mL venous blood sample was collected from each newborn by trained nurses, from the cubital vein, in ethylene diamine tetra acetic acid (EDTA) tubes and carried immediately to the laboratory where biomedical parameters were determined within 12 hours. The red cell G6PD activity was determined by an enzymatic kinetic assay for the quantitative determination of G6PD enzymatic activity using a commercial kit (Cypress Diagnostics, Belgium). The principle of the test is that, in the process of conversion of glucose-6-phosphate to 6-phosphogluconate, a reaction catalyzed by G6PD, NADP+ is reduced to NADPH. The amount of NADPH produced is an index of G6PD activity. Formation of NADPH is measured over a set period of time.

Hemoglobin (Hb) was measured by spectrophotometry on the same sample. G6PD activity was recorded as U/gHb. On the basis of frequency distribution of activity levels, the critical levels for diagnosis of G6PD deficiency in neonates aged ≤ 7 days were7.00 U/gHb (male neonates) and 9.50U/gHb(female neonates)[11]. Any neonate with an activity below this value was diagnosed as G6PD deficient. Plasma glucose was measured by Glucose Oxidase and Peroxidase (GOD-POD) method (ELITech Group, Puteaux, France) according to manufacturer instructions.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured on plasma and hemolysate using an automated blood analyzer Hitachi 705 (Hitachi, Japan) with DiaSys reagents (Diagnostic System Gmbh, Germany). Serum levels of direct and total bilirubin, calcium (Ca^{2+}) and magnesium (Mg^{2+}) were measured using ELITech reagents (ELITech Group, Mizy, France). Hematological analyses were performed using an automatic hematological analyzer (cell Dyn 3500, Abbott) from blood samples collected in the EDTA tubes.

2.3 Statistical analyses

Data were analyzed by Sigma Plot statistical analysis software 2010 (Systat Software, Inc. San Jose, CA, USA). Means and standard errors of the mean (SEM) of blood parameters were calculated. Student's t-test was used to ascertain any difference between the group characteristics. A p value of < 0.05 was deemed significant.

3. Results

3.1Glucose-6-phosphate dehydrogenase activity in neonate

Of 486 neonates enrolled in the study, 76 (47 males and 29 females) were excluded, either because of clotted or insufficient blood samples, or because the parents did not give consent for sampling. Of the 410 newborns screened over a period of eight months, 220(53.66%) were males and 190 (46.34%) were females. A total of101 (24.63%) neonates wereG6PD deficient, of which 49 (11.95%) were male and 52 (12.68%) female (Table 1). Normal neonates were 309 (75.37%) with 171 (41.71%) male and 138 (33.66%) females) (Table 1). Frequency in male sample was 22.27% (49 neonates of 220 male neonates) and in female sample was27.36% (52 neonates of 190 female neonates). The sex ratio for G6PD deficiency was 1.06. A total of 227 neonates (67.56%) were 3 days old and under and 133 (32.44%) aged between 3 and 7 days (Table 1).

Table 1: Demographic characteristics of study participants, 2017

Parameters	G6PD Status		p-Value
	G6PD Deficient	G6PD Normal	
N (%)	101 (24.63%)	309 (75.37%)	<0.001
Sex			
Male	49 (11.95%)	171 (41.71%)	<0.001
Female	52 (12.68%)	138 (33.66%)	<0,001
Age			
≤ 3 days	64 (15.61%)	213 (51.95%)	<0.001
> 3 days	39 (09.51%)	94 (22.93%)	<0.001

G6PD activity level was significantly decreased in G6PD-deficient male neonates (2.06 ± 0.82 vs. 16.63 ± 5.13 U/g Hb; p <0.001) and in G6PD-deficient female ones (5.42 ± 1.28 vs. 19.73 ± 2.71 U/g Hb; p <0.001) compared to G6PD-normal controls (Table 2).

Table 2: Glucose-6-phosphate dehydrogenase activity in neonates, 2017

	G6PD Activity (U/g Hb)				Reference value [11]
	Range	Mean	SEM	p-Value	
G6PD Deficient					
Male	0.35 - 5.27	2.06	0.82	-	< 7.0
Female	0.69 - 8.41	5.42	1.28	<0.001	< 9.0
G6PD Normal					
Male	9.19 - 35.43	16.63	5.13	-	≥ 9.0
Female	9.54 - 39.88	19.73	2.71	0.68	≥ 9.5

3.2 Biochemical parameters in neonate

Blood glucose level did not vary significantly in G6PD-deficient neonates compared to G6PD-normal controls. Conjugated bilirubin (p =0.03), total bilirubin (p =0.01) and Calcium (p =0.03) levels were significantly higher in G6PD-deficient neonates compared to G6PD-normal controls while Magnesium level was similar in both groups. Serum AST (p =0.01) and ALT (p =0.03) levels were significantly increased in G6PD-deficient neonates compared to controls. AST and ALT levels measured in erythrocytes showed no significant changes between the two groups (Table 3).

Table 3: Biochemical parameters in neonates, 2017

Parameters	G6PD Deficient		G6PD Normal		p-Value
	Mean	SEM	Mean	SEM	
Glucose (g/L)	0.48	0.06	0.56	0.04	0.70
Direct Bilirubin (mg/L)	16.79	1.63	11.42	1.53	0.03
Total Bilirubin (mg/L)	92.96	10.92	57.16	6.30	0.01
Ca^{2+} (mg/L)	96.74	2.21	90.13	1.24	0.03
Mg^{2+} (mg/L)	18.65	0.55	20.83	1.01	0.15
Serum AST (UI/L)	69.45	4.26	37.66	6.70	0.01
Serum ALT (UI/L)	28.18	2.38	13.88	3.57	0.02
Intra-erythrocyte AST (UI/L)	52.25	4.13	55.41	6.69	0.42
Intra-erythrocyte ALT (UI/L)	23.18	3.57	18.57	2.46	0.20

3.3 Hematological parameters in neonate

Hematological parameters were measured in collected venous blood samples in all neonates. There were no significant changes in red blood cell count (RBC), hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), reticulocyte and platelet levels in G6PD-deficient neonates compared to G6PD-normal controls. Only white blood cells number increased significantly (p <0.001) in G6PD-deficient neonates compared to control group (Table 4).

Table 4: Hematological parameters in neonates, 2017

Parameters	G6PD Deficient		G6PD Normal		p-Value
	Mean	SEM	Mean	SEM	
WBCs (10^3/mm^3)	10.18	0.56	7.52	0.54	<0.001
RBC (10^6/mm^3)	4.56	0.18	4.86	0.11	0,17
Hemoglobin (g/L)	14.45	0.56	15.01	0.40	0,42
Hematocrit (%)	45.19	1.72	47.13	1.19	0,34
MCV (fL)	99.70	1.75	97.76	1.45	0,41
MCHC (%)	31.95	0.16	31.82	0.14	0,58
Reticulocyte (10^5/mm^3)	1.17	0.20	1.25	0.22	0,39
Platelet (10^3/mm^3)	309.7	18.70	288.7	17.69	0,45

4. Discussion

Quantitative measurement of G6PD enzymatic activity is the most widely used method for diagnosis of the G6PD deficiency [13, 14]. In this study, we used reported cut-off reference values to determine G6PD-deficient neonates [13, 15]. The prevalence of G6PD deficiency in our study was 24.63% with no significant difference between genders. G6PD deficiency prevalence varies widely around the world. Our result is higher than those reported in European countries (1.57-2.10%) [16, 17] and among American Blacks (14%) [18], but similar to those reported in West Africa (15-30%) [19, 20].

Our results showed that G6PD activity level is much lower than cut-off values (2.06 U/g Hb vs. 7 U/g Hb in male and 5.42 U/g Hb vs. 9 U/g Hb in female). This observation suggests that the method used for G6PD activity measurement clearly differentiate deficient neonates. In several populations, systematic screening of G6PD activity suggested an increased prevalence of G6PD deficiency in individuals with diabetes, compared with the background rate of the general population [21]. The relationship between G6PD deficiency and diabetes has been a matter of debate. Both hypotheses that G6PD deficiency is associated with hyperglycemia and the occurrence of diabetes has been raised. In several populations, systematic screening of G6PD activity suggested an increased prevalence of G6PD deficiency in individuals with diabetes, compared with the background rate of the general population [22]. Results of glycaemia in our study showed no significant variation in G6PD-deficient compared to G6PD-normal controls, suggesting the absence of diabetes in the study participants. In G6PD-deficient neonates, both direct (p <0.03) and total serum bilirubin (p <0.01) levels and both serum AST (p <0.01) and ALT (p <0.02) levels were significantly higher than in G6PD-normal controls. Our results agree with the report that G6PD deficiency can cause severe neonatal hyperbilirubinemia, which may cause kernicterus. In a study from Oman, 71% of kernicterus patients were reported to suffer from G6PD deficiency [23]. Here, our results showed no significant difference in reticulocyte count, hematocrit level and intra-erythrocyte AST and ALT between G6PD-deficient and -normal controls. Our findings suggest that the hyperbilirubinemiain G6PD-deficient neonates may not due to massive hemolysis [24] but to liver malfunction [25].

The hypothesis of liver function impairment is in accordance with some other studies that reported elevated levels of AST in majority and ALT in some G6PD deficient patient [26]. Hyperbilirubinemia in G6PD deficient neonates is thought to be in part due to reduced hepatic conjugation and excretion of bilirubin, rather than increased bilirubin production resulting from hemolysis [27]. Our results showed no significant sign of anemia. However, the

fact that white blood cells count showed significant increased level in neonate with G6PD deficiency, suggests a presence of infections in this group. G6PD deficiency has been linked to neonatal sepsis [28, 29] and prevalence of some infections has been reported higher in G6PD-deficient neonates [30, 31].

In conclusion, the present study, the first in Benin, showed that G6PD deficiency in≤7 days old neonates is accompanied by hyperbilirubinemia without evidence of increased hemolysis. The results suggest liver malfunction in G6PD-deficient neonates in Benin.

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