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Glucose-6-Phosphate Dehydrogenase Deficiency in Individuals Infected with Human Immunodeficiency Virus at the Aminu Kano Teaching Hospital Kano, North Western Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author HA designed the study and wrote the first draft of the manuscript. Authors UM and AMD managed sample collection and laboratory analyses. All authors shared in data analysis and interpretation of results. Author HA was responsible for manuscript writing and final editing. All authors read and approved the final revised manuscript.

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ABSTRACT

Background: Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency is the most important disorder of the pentose phosphate pathway in erythrocyte metabolism resulting in decreased activity of the enzyme. In individuals infected with Human Immunodeficiency Virus (HIV), G-6-PD deficiency could induce hematological complications.
Aim: Given the large number of people living with HIV in Nigeria, this study was carried out to determine G-6-PD activity and the prevalence of its deficiency in HIV infected individuals. Also its possible role in inducing hematological complications in the infected individuals on treatment with Antiretroviral Therapy (ART) and prophylactics was evaluated.

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Method of Study: Blood samples collected from 150 HIV infected individuals and 50 apparently healthy individuals (controls) aged 21-60 years were subjected to CD4 count, complete blood count analysis and a quantitative G-6-PD activity assay.
Results: A 22.5% prevalence of G-6-PD deficiency was found in the study population. We found no significant correlation (*P*=0.32) between G6PD activity and CD4 count. Although, hemolytic anemia was absent in all G-6-PD deficient individuals in all study groups, hemoglobin and packed cell volume concentrations were significantly lower (P=.05) in the G-6-PD deficient individuals in the HIV group with opportunistic infections who were on ART and antimicrobial medication compared with the control group and the HIV ART naïve group. We also found a significant (P=.001) correlation between hemoglobin and packed cell volume with G-6-PD deficiency in the HIV group on ARTs and prophylactics
Conclusion: The high prevalence of G-6-PD deficiency in the study indicates the need for more attention to be given to this enzymopathy. The absence of hemolytic anemia found in this study should not deter clinicians from thorough G-6-PD screening of patients before prescription of medications for HIV infected individuals.

Keywords: Glucose 6 phosphate dehydrogenase; HIV; hemoglobin; packed cell volume; hemolytic anemia; deficiency.

1. INTRODUCTION

Glucose-6-phosphate dehydrogenase [G-6-PD, EC 1:1:1:49; D–Glucose–6–phosphate: NADP Oxidoreductase] is a key enzyme in the pentose phosphate pathway (PPP) that is essential for adequate supply of phosphorylated nicotinamide-adenine dinucleotide (NADPH), which protects red blood cells (RBCs) from oxidative stress [1]. Reduced form of NADPH is needed to maintain glutathione (GSH), which in turn keeps the sulfhydryl groups of hemoglobin and other RBC proteins in a reduced active form. This activity enables the RBCs to withstand lysis from oxidant damage, instituted particularly during viral/bacterial or protozoa infections, or following exposure to oxidant drugs with high redox potential such as antimalarials (primaquine and pamaquine), sulfonamide, sulfamethoxazole and other drugs and chemicals and consumption of certain food stuff (fava beans) [2,3,4]. G-6-PD deficiency increases the vulnerability of erythrocytes to oxidative stress and, thus, increases the risk of hemolytic anemia [5]. [6] reported that G-6-PD deficiency is the most common enzymopathy known worldwide with about 400 million people affected. In Nigeria, the prevalence of G-6-PD deficiency ranges from 4 – 26% with the male population having about 20 – 26% [7,8]. This prevalence rate varies from one community to another [9]. Lack of severe clinical effects in most G-6-PD deficient individuals has led to low attention and insufficient and unclear data on this enzymopathy. This has led to the high use of drug regimens (sulfonamides, antimalarials, nonsteroidal anti inflammatory drugs) that could possibly induce some hematological complications in G-6-PD deficient individuals. Nigeria is among the top ranked countries with high HIV burden in the world. There are many factors that contribute to increasing rates of HIV in Nigeria, such as poverty which prevails in northern Nigeria. In Nigeria as in other parts of the world, the corner stone for HIV treatment is antiretroviral therapy (ART) and prophylaxis for the prevention of opportunistic infections (OIs). Hematologic complications like anemia have been found to lead to disease progression and eventually morbidity and mortality. Anemia is the most commonly encountered hematologic abnormality in HIV patients, occurring with increasing frequency and is a significant predictor of progression to AIDS or death, with more than 70% of patients developing anemia and requiring transfusion [10]. Certain drugs that are used in the treatment of HIV and other conditions, such as malaria, are known to induce hemolysis in G-6-PD deficient individuals. Among G-6-PD deficient patients, sulfa drugs may cause hemolytic anemia. Some workers have reported that although HIV itself does not seem to be an oxidative stressor in patients with G-6-PD deficiency, HIV-infected individuals often receive oxidant drugs (particularly dapsone, primaquine and sulfonamides) as prophylactics. [11] reported that theoretically, even mild hemolytic events due to G-6-PD deficiency may be significant in patients with HIV infection because of depressed bone marrow reserve and concomitant anemia from other causes. Despite this, few studies have evaluated the clinical and laboratory presentations of ART and OI prophylactic - related drug toxicities including hemolytic anemia

as a result of G-6-PD deficiency among HIV patients in Nigeria. [12] reported the existence of scarce and conflicting reports on the adverse events that occur following the use of ARV drugs and prophylactic medications in HIV patients. Most studies have focused on Zidovudine use as one of the major causes of anemia in HIV infected individuals and also on G-6-PD deficiency in neonates as neonatal jaundice is perhaps one of the common clinical manifestations of this deficiency. As G-6-PD deficiency is very common in many parts of the world [13], it is surprising that acute hemolysis has not been described as a complication in primary HIV infection more often. [11] reported that it remains unclear whether this is a rarity or whether a systematic review of cohorts that show a high prevalence of G-6-DP deficiency (e.g. Sub-Saharan Africa) will reveal that hemolysis is a more common, yet easily overlooked complication of primary HIV infection. This study was carried out to determine the prevalence of G-6-PD deficiency in HIV infected individuals and determine its possible role in causing hemolytic anemia in HIV infected individuals receiving treatment (on ARTs and prophylactics).

2. MATERIALS AND METHODS

2.1 Study Area and Ethical Approval

The study was carried out at the Aminu Kano Teaching Hospital (AKTH), Kano, North Western Nigeria. Ethical approval for this study was obtained from the ethical committee of the Aminu Kano Teaching Hospital, Kano. Approval was granted subject to patient anonymity being maintained, good laboratory practice/quality control being ensured within the PEPFAR laboratory, chemical pathology and hematology laboratories along with the blood donor clinic. Also every finding being treated with utmost confidentiality and for the purpose of the research only.

2.2 Study Subjects

A total of 200 individuals were enrolled for the study comprising 150 HIV infected individuals aged 18 to 55 years who sought treatment in the S.S Wali Center for HIV, Aminu Kano Teaching Hospital (AKTH) Kano, and 50 apparently healthy individuals (controls) were recruited from the population of blood donors from the blood donor unit, staff of AKTH and students of Bayero University, Kano.

2.2.1 Inclusion criteria

Individuals with established HIV infection who agreed to participate were included in the study. Apparently healthy (HIV negative, hepatitis B and C negative, and anemia free) individuals were included in the study as controls.

2.2.2 Exclusion criteria

Individuals that were HIV negative as well as HIV positive individuals that declined to give consent were excluded as test subjects and for the control group individuals that were HIV positive, hepatitis B or C positive, and who were anemic were excluded from the study.

Informed consent was sought and obtained from the subjects and baseline clinical details obtained from hospital records including basic demographic information and for the control subjects from personal interviews. Subjects were divided into four groups. Group 1 (n= 50): ART Naive HIV positive individuals- (have not commenced any treatment); Group 2 (n= 50): Stable HIV individuals – (on ARTs and prophylactics for at least 3 months without any clinical episodes); Group 3 (n= 50): HIV infected individuals with opportunistic infections specifically pulmonary tuberculosis and pneumonia (also on ARTs) and Group 4 (n= 50): Control group (Apparently Healthy individuals). The diagnosis of OI (co- infection with tuberculosis) was based entirely on clinical grounds, where physicians involved in HIV care had made diagnosis of pulmonary tuberculosis (TB) based on assessment of required compatible clinical features and suggestive chest X-ray (CXR) findings or positive sputum acid fast bacilli.

2.2.3 Sample collection and analyses

Exactly 6 ml of blood sample was collected from peripheral vein (antecubital vein puncture) using a 10 ml syringe. Aliquots of 2 ml of whole blood was then dispensed into Disodium Ethylene Diaminotetra-Acetate anticoagulant (EDTA) sample tubes, 2 ml into tubes containing Acid Citrate Dextrose (ACD) and 1 ml into plain sample tubes from all subjects. Samples collected in EDTA tubes were processed immediately for determination of CD4 T lymphocyte counts, Complete Blood Count and the Direct Antiglobulin test while samples collected in ACD tubes were kept at 4℃ for the determination of Glucose-6-Phosphate

Dehydrogenase (G-6-PD) activity. The remaining 1 ml collected in the plain sample tubes without anticoagulant was allowed to clot and retract. Serum was extracted and then stored at -20℃ until needed for analyses (lactate dehydrogenase assay).

2.3 Biochemical Analyses

2.3.1 Glucose-6-Phosphate Dehydrogenase activity determination

G-6-PD activity was measured using a quantitative G-6-PD assay kit (BioVision Inc.). In the assay, glucose-6-phosphate is oxidized with the generation of a product which is utilized to convert a nearly colorless probe to an intensely colored product with an absorbance at 450 nm. Also one unit defines as the amount of enzyme that catalyzes the conversion of 1.0 µlmol of glucose-6-phosphate into 6-phosphoglucono-δ-lactone and generates 1.0 µmol of NAD+ to NADH per minute at 37℃). Based on the manufacturer's instruction/cut off value decreased activity or G-6-PD deficiency was taken as any level of enzyme activity less than 118 mU/mL (Biovision Diagnostic Procedure).

2.3.2 Lactate dehydrogenase assay

Lactate dehydrogenase activity was determined using LDH-L Reagent set –kinetic procedure (TECO diagnostics, USA) to test for presence of hemolytic anemia. The test is based on the principle where LDH catalyzes the oxidation of lactate to Pyruvate in the presence of NAD, which is subsequently reduced to NADH. The rate of NADH formation measured at 340 nm is directly proportional to serum LDH-L activity. LDH activity level above 460U/l was considered to indicate possible presence of hemolytic anemia in combination with levels of other parameters measured.

2.3.3 CD4 T lymphocyte count determination

CD4 T lymphocyte count test was determined by CD4 easy count kit (Partec GmbH, Germany) using flow cytometer.

2.4 Hematological Analyses

2.4.1 Complete blood count analysis

Complete Blood Count (CBC) analysis of each sample was carried out using the fully automated analyzer (Sysmex KX 21 N hematology analyser).

2.4.2 Direct antiglobulin test

Direct Antiglobulin test (Coombs test) was carried out using ATLAS Medical Anti-human globulin (AHG) test kit (ATLAS Medical, Cambridge UK). This test was carried out to detect the presence of hemolytic anemia, any red cell disorders and also differentiate between acquired and congenital hemolytic anemia if anemia was detected. This method demonstrates *in vivo* sensitization of cells. The test procedure is based on agglutinin principle where human immune globulins and/or complements attached to the red cell surface agglutinates in the presence of polyspecific AHG indicating a positive result.

2.5 Statistical Analysis

Data recording was done on Microsoft excel before being exported to Statistical Package for Social Sciences (SPSS) program version 16 (Chicago, IL, USA). Analyses were carried out using both inferential and descriptive statistics with mean and standard deviations (SD) range and percentages, for continuous or categorical variables, respectively. Microsoft Excel and Word in Windows 2007 were used for graphics and tables. The student t test and ANOVA were used to test for significant differences in means of various groups. All reported p-values <0.05 were considered statistically significant. Pearson's correlation was used to ascertain relationship between groups' parameters for each dependent variable.

3. RESULTS

3.1 Variation in G-6-PD Activity

The mean value for G-6-PD activity across the study population was 236.6±133.2 mU/mL. Among the groups, mean values were 213.3±140.9, 228.2±138.8, 219±126.5 and 286±133.2 mU/mL for HIV ART naïve, HIV stable, HIV-OI and apparently healthy groups respectively. A significant difference (P=0.05) in G-6-PD activity was found between the apparently healthy group and the HIV groups with mean values of 220.1±134.8 mU/mL for the HIV groups and 286.0±115.9 mU/mL for the apparently healthy group. The HIV stable and the apparently healthy groups had lower enzyme activity than the HIV naïve and HIV-OI groups. Distribution of G-6-PD normal and G-6-PD deficient individuals enzyme activity in all study groups is shown in Fig. 1. The HIV stable

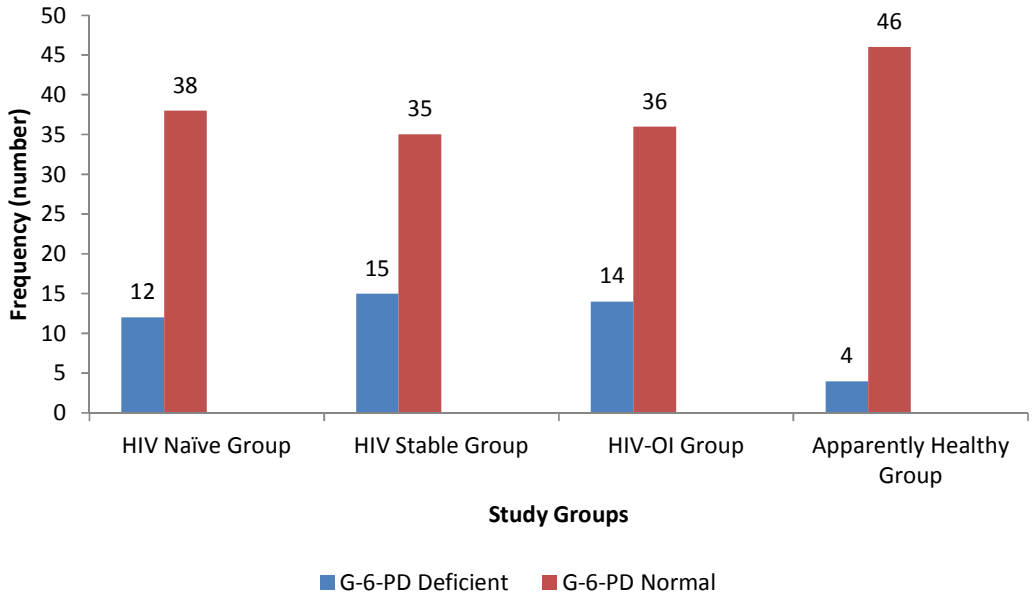

Fig. 1. Distribution of G-6-PD deficient and G-6-PD normal individuals in study groups

and HIV-OI groups had higher number of individuals with enzyme deficiency.

3.2 Prevalence of G-6-PD Deficiency in Study Population

Based on the manufacturer's cut off value of < 118 mU/mL, 22.5% (45/200) of subjects had deficient enzyme activity (Table 1). Of the 200 individuals in the study, 24.0% (12/50) were G-6-PD deficient in the HIVART naïve group, 30.0% (15/50) in the HIV stable group, 28.0% (14/50) in the HIV-OI group and 8.0% (4/50) were deficient in the apparently healthy group. Of the 22.5% G-6-PD deficient individuals, 91.0% (41/45) were from the HIV groups while 9.0% (4/45) were from the apparently healthy group.

3.3 Socio Demographic Characteristics of G-6-PD Deficient Subjects

Gender distribution in this study showed that 27 of the G-6-PD deficient individuals were females while 18 were males (Table 2). A higher number of G-6-PD deficient individuals was found in the 3rd decade age group (Table 3).

3.4 CD4 Count and Hematological Parameters of G-6-PD Deficient Individuals

The mean values of CD4 Count and hematological parameters of the 45 G-6-PD deficient individuals are shown in Table 4. There was significant difference (P=.05) between groups in CD4 count with the HIV ART naïve group having low CD4 count. Normal levels of hematological parameters were found in all groups though there was significant difference (P=.05) between groups in the hematological parameters. RBC, hemoglobin and PCV levels were found to be slightly lower in the HIV-OI group compared with the other groups. A significant difference (P=.05) was found between all groups in platelet count with the apparently healthy group having the lowest platelet count.

Table 1. Prevalence of G-6-PD enzyme deficiency of subjects

Subjects	Number of subjects	G-6-PD enzyme activity mU/mL (mean ± SD)
All subjects	200(100%)	236±133.2
Normal enzyme activity	155(77.5%)	286.1±108.6
Low enzyme activity (Deficient)	45(22.5%)	66.5±27.6

Table 2. Sex distribution of G-6-PD deficient and G-6-PD normal individuals across study groups

Parameter		HIV ART Naïve		HIV stable		HIV–OI		Apparently healthy	
		G-6-PD Deficient n= 12	G-6-PD Normal n=38	G-6-PD Deficient n=15	G-6-PD Normal n= 35	G-6-PD Deficient n=14	G-6-PD Normal n=36	G-6-PD Deficient n=4	G-6-PD Normal n=46
Sex	Male	4 (33.3%)	15 (39.5%)	6 (40%)	14 (40%)	4 (28.6%)	17 (47.2%)	4 (100%)	35 (76.1%)
	Female	8 (66.7%)	23 (60.5%)	9 (60%)	21 (60%)	10 (71.4%)	19 (52.8%)	0 (0%)	11 (23.9%)
	Total	12 (100%)	38 (100%)	15 (100%)	35 (100%)	14 (100%)	36 (100%)	4 (100%)	46 (100%)

Table 3. Age distribution of G-6-PD deficient and G-6-PD normal individuals across study groups

Parameter		HIV ART Naïve		HIV stable		HIV–OI		Apparently healthy	
		G-6-PD Deficient n= 12	G-6-PD Normal n=38	G-6-PD Deficient n=15	G-6-PD Normal n= 35	G-6-PD Deficient n=14	G-6-PD Normal n=36	G-6-PD Deficient n=4	G-6-PD Normal n=46
Age	20-30 yrs	3 (25%)	16 (42.2%)	4 (26.7%)	7 (20.0%)	1 (7.1%)	7 (19.4%)	0 (0.0%)	20 (43.5%)
	31-40 yrs	6 (50%)	13 (34.2%)	7 (46.7%)	18 (51.4%)	7 (50.0%)	24 (66.7%)	4(100.0%)	17 (37.0%)
	41-50 yrs	2 (16%)	9 (23.7%)	3 (20.0%)	7 (20%)	6 (42.9%)	4 (11.1%)	0 (0.0%)	9 (19.5%)
	>50 yrs	1 (8.3%)	0 (0.0%)	1 (6.6%)	3 (8.6%)	0 (0.0%)	1 (2.8%)	0 (0.0%)	0 (0.0%)
	Total	12 (100.0%)	38 (100%)	15 (100.0%)	35 (100.0%)	14 (100.0%)	36 (100.0%)	4 (100.0%)	46 (100.0)

Table 4. CD4 count and hematological parameters of G-6-PD deficient subjects across groups

Parameter	HIV ART Naïve n=12 (mean ± SD)	HIV stable n=15 (mean ± SD)	HIV-OIs n=14 (mean ± SD)	Apparently healthy n=4 (mean ± SD)
CD4 (cells/µl)	308.5±194.9^{a}	454.9±237.8^{b}	425.7±167.1^{c}	756.5±37.9^{ab}
RBC (10^6/µL)	4.3±0.6^{a}	4.2±0.8^{a}	3.2±2.9^{b}	5.3±0.4^{c}
MCH (pg)	26.2±2.5^{a}	31.3±4.8^{b}	29.5±5.7^{b}	27.4±1.2^{a}
MCHC(g/dL)	32.4±1.5^{b}	34.0±1.5^{c}	33.3±2.0^{a}	33.6±1.0^{a}
MCV (fL)	81.9±7.0^{a}	90.1±32.8^{b}	88.7±13.9^{b}	81.4±1.9^{a}
PLT×10^3/µL	292.7±82.1^{a}	259.4±74.2^{b}	287.3±115.2^{c}	180.3±50.6^{ac}
HB (g/dL)	11.6±1.4^{a}	13.1±2.2^{b}	11.8±2.1^{a}	14.6±0.8^{c}
PCV (%)	35.8±4.1^{b}	38.5±5.9^{bc}	35.3±6.3^{b}	43.3±2.0^{c}
WBC×10^3/µL	4.5±1.6^{a}	4.8±1.3^{a}	5.1±1.8^{b}	5.2±1.5^{b}
LYM (%)	37.6±14.1^{a}	45.2±12.4^{b}	37.0±8.5^{a}	48.3±11.8^{b}
NEUT (%)	50.2±16.8^{a}	44.7±13.3^{b}	53.4±11.6^{c}	43.3±13.8^{b}

Mean blood count parameters of the 45 G-6-PD deficient subjects across groups
a, b, c =Data along the same row with different superscript alphabets are significantly different (P=.05)

Figs. 2 and 3 show mean hemoglobin and PCV levels in both normal and deficient individuals in all study groups. No significant difference (*P*=.28) was found in hemoglobin concentration between the individuals with normal enzyme activity and those with deficient activity in the HIV naïve group and apparently healthy group respectively while a significant difference (*P*=.000;*P*=.05) was found in the HIV stable and HIV-OI groups respectively (Fig. 2). Mean PCV concentration in the G-6-PD deficient individuals compared with the normal G-6-PD individuals in the HIV naïve and apparently healthy groups showed no significant difference (P=0.34; P=0.21) while significant difference (*P*=0.001; *P*=0.05) was found in the HIV stable and HIV-OI groups (Fig. 3). Lower mean values of hemoglobin and PCV were found in the G-6-PD deficient individuals from the HIV-OI group when compared with individuals with normal G-6-PD activity.

3.5 Correlation of CD4 Count and Hematological Parameters with G-6-PD Activity

The study did not show a significant correlation between G-6-PD deficiency with CD4 count, hemoglobin concentration and PCV concentration in all groups except the HIV stable group. A significant correlation (r= -.78,*P*=.001; r=.75,*P*=0.001) was found between G-6-PD deficiency and hemoglobin and PCV concentrations in the HIV stable. Table 5 shows the correlation of G-6-PD activity with CD4 count, hemoglobin and PCV of G-6-PD deficient individuals in all groups.

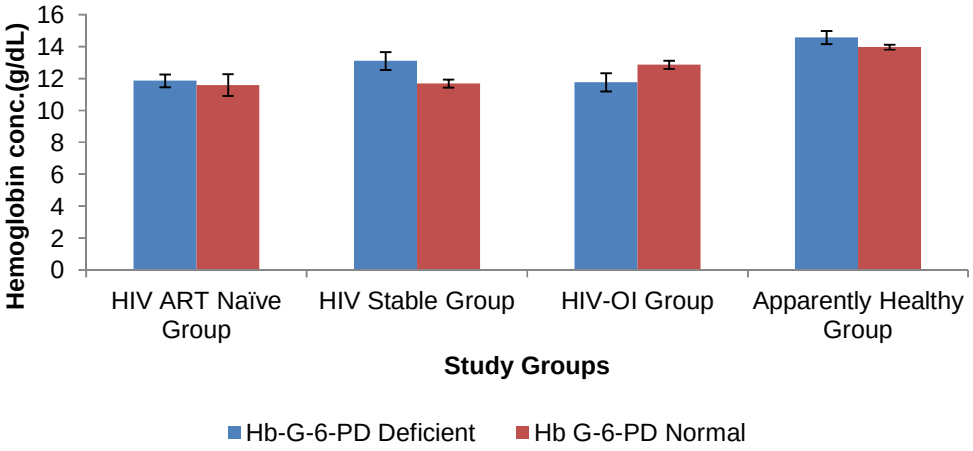

Fig. 2. Hemoglobin (Hb) concentrations of G-6-PD deficient and G-6-PD normal individuals across study groups

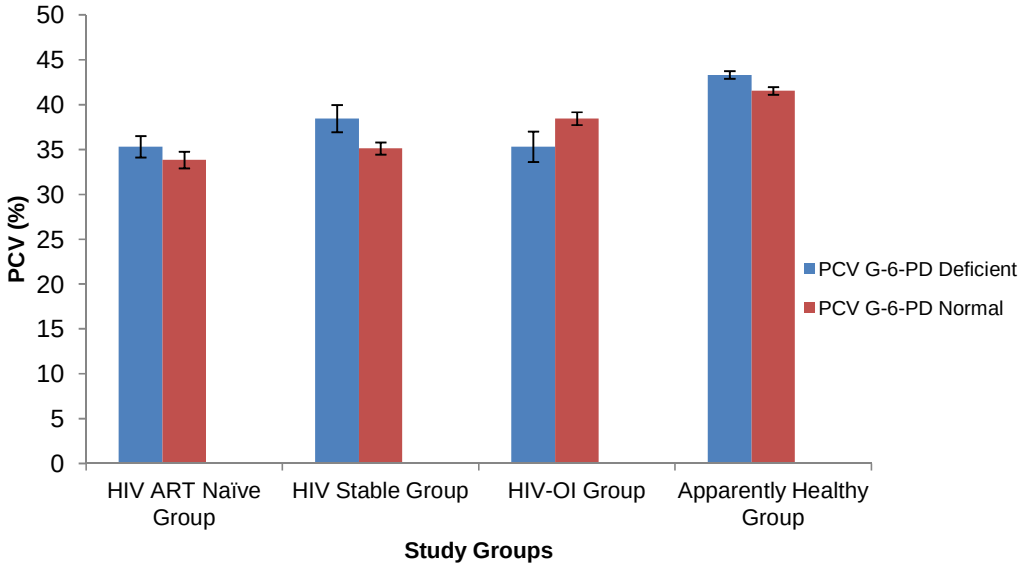

Fig. 3. Packed Cell Volume (PCV) concentrations of G-6-PD deficient and G-6-PD normal individuals across study groups

Table 5. Pearson's correlation coefficient (r) between G-6-PD activity and different variables among G-6-PD deficient individuals

Group	HIV ART Naïve		HIV stable		HIV-OI		Apparently healthy	
Parameter	**r- value**	**p value**	**r- value**	**p-value**	**r-value**	**p-value**	**r-value**	**p-value**
CD4	.17	.44	-.14	.32	.02	.89	.14	.35
HB	-.09	.54	-.78**	.000	.19	.18	.09	.53
PCV	-.02	.87	.75**	.000	.17	.19	-.06	.69

*** = Correlation is significant at P=0.01*
**= Correlation is significant at P=0.05*

The mean LDH value for the study population was 259.6±77.1 U/l while within the different groups, both the G-6-PD deficient and G-6-PD normal individuals had mean values of 270.4±67.6 U/l, 230.2±89.2 U/l, 274.8±66.7 U/l and 263±76.9 U/l for the HIV ART Naïve, HIV stable, HIV-OIs and apparently healthy groups respectively. There was significant difference (p≤0.05) between the HIV groups and the apparently healthy group with mean value for the HIV groups of 258.5±77.4 U/l and the apparently healthy 262.9±76.9 U/l.

An absence of hemolytic anemia was observed in this study. Based on the premise that a combined presence of moderate or severe anemia characterized by low hemoglobin level (≤10 g/dl), elevated LDH activity(≥460 U/l) and positive Direct Antiglobulin Test (DAT) are indicators of hemolytic anemia, there was no individual with hemolytic anemia as none of the 45 G-6-PD deficient individuals met the three given criteria (Table 6). All 45 G-6-PD individuals had negative DAT and normal LDL activity though some had low hemoglobin and PCV levels.

4. DISCUSSION

In this study, the prevalence of G-6-PD deficiency in the study group was 22.5% which agrees with a report by [14] conducted in South West Nigeria reporting a prevalence of 28.5%. [9], reported a 20% prevalence of G-6-PD deficiency in his study in Nigeria. In other parts of Africa, the prevalence of G-6-PD deficiency has been reported to be 22.5% in Congo (Brazzaville), 15.7% in Mali (Bamako), 13.0% in Uganda and 9.0–15.5% in Gabon [15]. The higher number of G-6-PD deficient females compared with the males was unexpected as G-6-PD deficiency has been reported to be sex

Table 6. Prevalence of hemolytic anemia in relation to G-6-PD deficiency

Parameter	HIV ART Naïve	HIV stable	HIV-OIs	Apparently healthy
HGB (g/dL)	11.6±1.4[a]	13.1±2.2[b]	11.8±2.1[a]	14.6±0.8[c]
LDH (U/l)	298.0±68.4[a]	232.3±96.0[b]	321.7±70.5[c]	250.5±93.2[b]
DAT (+/-)	-	-	-	-

a, b, c =Data along the same row with different superscript alphabets are statistically significant (p≤0.05)
(-) Indicates negative DAT test; + indicates positive DAT test
Absence of moderate or severe anemia (indicated by hemoglobin≤10g/dL) in all groups, absence of elevated LDH (≥ 460U/l) in all groups and negative DAT indicate lack of hemolytic anemia as a result of G-6-PD deficiency

linked with male preponderance. Most workers have reported that the deficiency is rare in females because the mutation would have to occur in both copies of the gene to cause the disorder, whereas in males only one abnormal copy of the gene is required for manifestation of the disease. Whether the females were heterozygously deficient or homozygously deficient was not however investigated. [16] assessing the frequency of G-6-PD deficiency in Sardinian patients with non arteritic anterior ischemic optic neuropathy, indicated based on sex and G-6-PD-deficiency interaction that sex does not have any modifier effect on G-6-PD deficiency. Also, another report by [17] in Malaysia indicated that sex was not a significant predictor associated with actual G-6-PD enzyme levels. [17] stated that G-6-PD deficiency, although X-linked, is not a recessive disorder and that in female heterozygotes, red cell mosaicism arising from random X chromosome inactivation results in two populations of G-6-PD-deficient and G-6-PD-normal cells. The proportions of these two cell types can vary enormously, ranging from completely normal activity to complete deficiency. [18] who reported a 28% prevalence found a higher number of females than males with the deficiency. However, the higher number of G-6-PD deficient females in this study could be attributed to the fact that a higher number of females were enrolled in the HIV study groups probably due to the fact that in some communities/societies females are more likely to seek treatment than males when encountered with medical issues. Also this study stresses the preponderance of females in the prevalence of HIV infection and supports the established fact that women are biologically more vulnerable to HIV/AIDS and more likely to contact infection from their male partners as their sexuality and gender disadvantage in terms of culture, economic and social factors place them more at risk of infection than men. The World Health Organization (WHO) reported that HIV/AIDS affects females most severely in sub-Saharan Africa and women of reproductive age make up almost 57% of adults living with HIV, accounting for up to 80% of HIV infected women in the world [19]. It could not however be ascertained in this study whether the high level of G-6-PD deficiency found in the HIV infected individuals was preexisting before HIV infection or occurred as a consequence of the infection. In the apparently healthy group, the higher number of males than females was not surprising as the apparently healthy group comprised 98% males probably as a result of screening criteria of the donors usually excluding females from blood donation. The higher number of individuals with low enzyme activity found in the third decade age range (31- 40 years) could also be due to the fact that the study population comprised of more HIV infected individuals and a larger number of the G-6-PD deficient individuals were in the HIV groups. Most reports by several authors recorded mean age of HIV patients in the third decade range. A study in Kano Nigeria by [20], showed that about 86% of the study population fell within the 20 – 49 years age brackets which is known to be the sexually active age group with highest peak percentage observed within the 30 – 39 age groups for both sexes. The researchers stated that this pattern was noted in their study where over three-quarters of the patients studied were in the age group of 20 – 39 years as was also observed in several other studies done in Nigeria. The absence of association between CD4 count, an immunological marker used to monitor disease progression in HIV infected individuals suggests that G-6-PD deficiency plays no direct role in HIV disease progression. The lower levels of hemoglobin and PCV found in the G-6-PD deficient individuals compared with the G-6-PD normal individuals in the HIV OI group could probably be attributed to individuals in those groups being on a Zidovudine (AZT) containing regimen and the use of co trimoxazole or other antimicrobials as prophylaxis and also as treatment for opportunistic infections. Some workers have reported that being on AZT containing regimen was also a risk factor for developing anemia. Studies have shown that

AZT can inhibit bone marrow activity, resulting in decreased production of blood cells and platelets, previous research has demonstrated its association with incident anemia [21]. [22] found in their study in North West Ethiopia that anemic individuals using Anti-retroviral therapy were higher in numbers than non-users which indicated that the drug is one factor for being anemic. Furthermore, the strong association found between hemoglobin and PCV with G-6-PD activity in the HIV stable group suggests that administration of some ARTs may have some hematological effects in G-6-PD deficient individuals. Other hematological parameters including RBC, MCV, MCH, WBC and platelet count were found to be within normal range among the G-6-PD deficient individuals in all groups. However the lower platelet count found in the G-6-PD deficient individuals from the apparently healthy group compared with the HIV groups could be gender related. The G-6-PD deficient individuals in the apparently healthy group were all males while most of the G-6-PD deficient individuals in HIV groups were females. Several studies have shown that platelet count is higher in females and that prevalence of thrombocytopenia is higher among males than among females. [23] found in their study gender-related differences in platelet count and confirmed that, on average, women have significantly more platelets than men. [24] demonstrated that platelet count has been reported to be about 20% higher in females than in males. As G-6-PD deficiency causes increased susceptibility of erythrocytes to hydrogen peroxide and other reactive oxygen species that can lead to hemolytic anemia, an attempt was made to investigate the possibility of hemolytic anemia in G-6-PD deficient individuals within the study population particularly the HIV groups on medications (ARTs /prophylactics especially sulphonamides like co-trimoxazole and anti malarials). This was to determine whether administration of these sulphonamides and antimalarials containing sulfa moieties caused hemolytic effects on the G-6-PD-deficient erythrocytes. [25] reported that drugs that have been implicated in induction of hemolysis of G-6-PD-deficient erythrocytes are mainly sulphonamide containing antimalarials and antimicrobials, non-steroid anti inflammatory drugs (NSAID) and quinines. However results from this study showed no hemolysis of G-6-PD deficient erythrocytes in all groups. This agrees with a study by [18] in Lagos Nigeria undertaken to evaluate the effect of G-6-PD enzyme activity and its correlation to adverse drug reaction to anti- malarial drugs which showed no relationship between G-6-PD activity and adverse drug reaction. A similar pattern was also reported by [26] in a study conducted in Jordan. They reported that some drugs are harmless to mild G-6-PD A variant but could cause hemolysis to those who have the Mediterranean variant. This could probably explain the lack of hemolytic anemia found in this study since Nigerians carry the G-6-PD A variant. The most common variant in Africans is regarded as G-6-PD A-(202A>G) whereas G-6-PD 563C>T is the most frequent genotype in southern Europe, Middle East countries and Indian subcontinent [27]. [28] suggested that more than one mechanism may be involved in the metabolism of glutathione in HIV-1-infected cells, with oxidative stress playing a small part. [29] found a 6.8% G-6-PD deficiency among a total of 1110 patients with higher rates among African Americans (9.7%) and Hispanics (2.9%) with similar hemoglobin concentrations at baseline among subjects with or without G-6-PD deficiency. Among patients with G-6-PD deficiency in their study, 53.3% were prescribed trimethoprim-sulfamethoxazole or dapsone and during follow-up, 6.7% of these patients developed acute hemolytic anemia. These results provided by the workers, suggested a strong clinical evidence for recommending G-6-PD testing in HIV-infected subjects from susceptible ethnic backgrounds. Among the G-6-PD deficient individuals in this study 62% were on co trimoxazole (CTX) though strict adherence to this medication could not be ascertained.

5. CONCLUSION

The high prevalence of G-6-PD deficiency (22.5%) found in this study agreed with reports from many authors in Nigeria and some parts of the world. The high number of G-6-PD deficient females in the study indicates the need for more attention and awareness of this enzyme disorder in both males and females. The absence of hemolytic anemia and other hematologic complications should not deter clinicians from careful consideration of medications administered to patients particularly HIV infected patients on ARTs and also with hematological complications and opportunistic infections. G-6-PD deficiency does not seem to play a role in HIV disease progression.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the

appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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