

Original Research Article

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

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 which could induce hematological complications in individuals infected with Human Immunodeficiency Virus (HIV) who are G-6-PD deficient.

Aim: Given the large number of people living with HIV in Nigeria and the study, was carried out to determine G6PD 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 .

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.

Result: 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, the immunological marker of HIV infection. Although, hemolytic anemia was absent in all G-6-PD deficient individuals in all study groups, red blood cell indices were significantly lower ($p \leq 0.05$) in the HIV group on antiretroviral therapy (ART) and prophylactic medication who were G-6-PD deficient compared with the control group and the HIV ART naïve group.

Conclusion: the high prevalence of G6PD 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 G6PD screening of patients before prescription of medications in HIV infected individuals.

Key words: Glucose 6 Phosphate Dehydrogenase, HIV, Hemoglobin, Packed Cell Volume, Hemolytic Anemia, Deficiency

INTRODUCTION

Glucose-6-phosphate dehydrogenase [G6PD, EC 1:1:1:49; D-Glucose-6-phosphate: NADP Oxidoreductase (G6PD)] 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 resulting in decreased activity of the enzyme 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 clear 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 G6PD 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

53 been described as a complication in primary HIV infection more often. (14)reported that it remains
54 unclear whether this is a rarity or whether a systematic review of cohorts that show a high prevalence
55 of G-6-DP deficiency (e.g. Sub-Saharan Africa) will reveal that hemolysis is a more common, yet
56 easily overlooked complication of primary HIV infection. This study was carried out to determine the
57 prevalence of G-6-PD deficiency in HIV infected individuals and determine its possible role in causing
58 hemolytic anemia in HIV infected individuals receiving treatment (on ARTs and prophylactics).

59 **MATERIALS AND METHODS**

60 **Study Area and Ethical Approval**

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

67 **Study subjects**

68 A total of 200 individuals were enrolled for the study comprising 150 HIV infected individuals aged 18
69 to 55 years who sought treatment in the S.S Wali Center for HIV, Aminu Kano Teaching Hospital
70 (AKTH) Kano, and 50 apparently healthy individuals (controls) were recruited from the population of
71 blood donors from the blood donor unit, staff of AKTH and students of Bayero University, Kano.
72 Informed consent was sought and obtained from the subjects and baseline clinical details obtained
73 from hospital records including basic demographic information and for the control subjects from
74 personal interviews. Subjects were divided into four groups. Group 1 (n= 50) : ART Naive HIV positive
75 individuals- (have not commenced any treatment); Group 2 (n= 50) : Stable HIV individuals – (on
76 ARTs and prophylactics for at least 3 months without any clinical episodes); Group 3(n= 50) : HIV
77 infected individuals with opportunistic infections specifically pulmonary tuberculosis and pneumonia
78 (also on ARTs) and Group 4 (n= 50) : Control group (Apparently Healthy individuals). The diagnosis of
79 OI (co- infection with tuberculosis) was based entirely on clinical grounds, where physicians involved
80 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.

Sample Collection and Analyses

Exactly 6ml of blood sample was collected from peripheral vein (antecubital vein puncture) using a 10ml syringe. Aliquots of 2ml of whole blood was then dispensed into Disodium Ethylene Diaminotetra-Acetate anticoagulant (EDTA) sample tubes, 2ml into tubes containing Acid Citrate Dextrose (ACD) and 1ml 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°C for the determination of Glucose-6-Phosphate Dehydrogenase (G-6-PD) activity. The remaining 1ml collected in the plain sample tubes without anticoagulant was allowed to clot and retract. Serum was extracted and then stored at -20°C until needed for analyses (lactate dehydrogenase assay).

Biochemical Analyses

Glucose-6-Phosphate Dehydrogenase Activity Determination

~~Glucose-6-phosphate dehydrogenase~~ (G-6-PDH) activity was measured using a quantitative G-6-PDH 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 μ mol of glucose-6-phosphate into 6-phosphoglucono- δ -lactone and generates 1.0 μ mol of NAD⁺ to NADH per minute at 37°C.). Based on the manufacturer's instruction/cut off value decreased activity or G6PD deficiency was taken as any level of enzyme activity less than 118mU/mL (Biovision Diagnostic Procedure).

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.



CD4 T lymphocyte count determination

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

Hematological Analyses

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).

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 immunoglobulins and/or complements attached to the red cell surface agglutinates in the presence of polyspecific AHG indicating a positive result.

RESULTS

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. Normal and deficient enzyme activity levels in all study groups is shown in figure 1. The HIV stable and the apparently healthy groups had lower enzyme activity than the HIV naïve and HIV-OI groups.

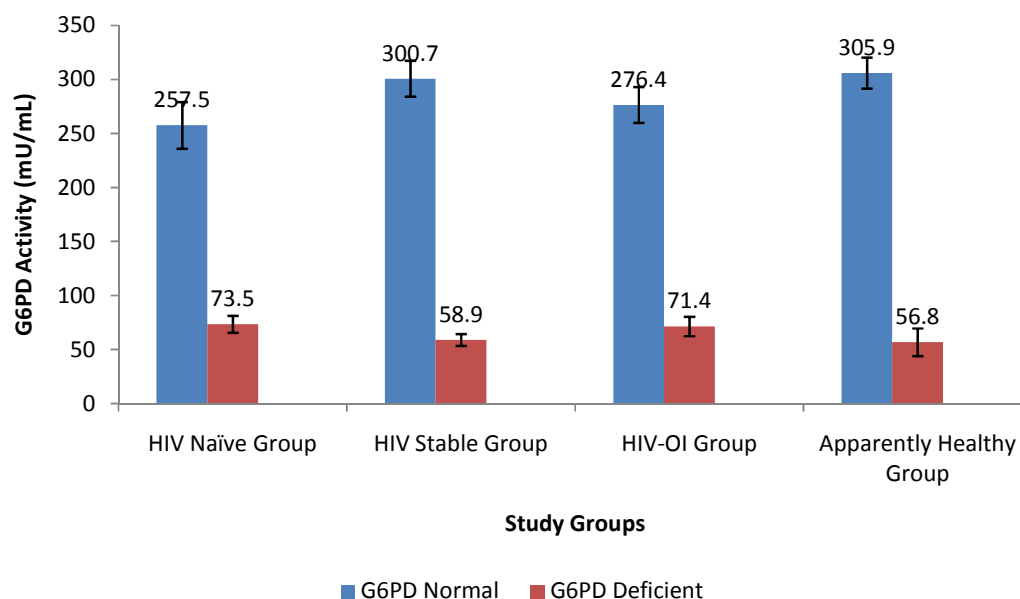


Fig. 1.G-6-PD Activity AcrossStudy Groups

Data represent mean $\pm$ SE. * $p<0.05$ and ** $p<0.01$

Prevalence of G6PD Deficiency in Study Population

Based on the manufacturer's cut off value of $< 118\text{mU/mL}$, 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.

Table 1. Prevalence of G-6-PD Enzyme Deficiency of subjects

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

Sociodemographic characteristics of G6PD Deficient subjects

Gender distribution in this study showed that 27 of the G6PD deficient individuals were females while 18 were males (table 2). A higher number of G6PD deficient individuals was found in the 3rd decade age group (table 3).

Table 2: Sex Distribution of G6PD Deficient Individuals

G6PD Activity	Male	Female	Total
Deficient (n=45)	18 (40.0%)	27 (60.0%)	45 (100.0%)
Normal (n=155)	80(51.6%)	75(48.4%)	155 (100.0%)
Total (n=200)	98 (49.0%)	102 (51.0%)	200 (100.0%)

Table 3: Age Distribution of G6PD Deficient Individuals

G6PD Activity	21-30 years	31-40 years	41-50 years	≥51 years	Percentage
Deficient (n=45)	8 (17.8%)	24 (53.3%)	11 (24.4%)	2 (4.4%)	45 (100.0%)
Normal (n=155)	50 (32.3%)	73 (47.0%)	29 (18.7%)	3 (2.0%)	155 (100%)
Total (n=200)	58 (29.0%)	97 (48.5%)	40 (20.0%)	5 (2.5%)	200 (100.0%)

CD4 Count and Hematological Parameters of G6PD Deficient Individuals

The mean values of CD4 Count and hematological parameters of the 45 G6PD deficient individuals are shown in table 4. 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. Figures 2 and 3 show mean hemoglobin and PCV levels in both normal and deficient individuals in all study groups. Lower mean values of hemoglobin and PCV were found in the G6PD deficient individuals from the HIV-OI group only when compared with individuals with normal G6PD activity.

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}
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)

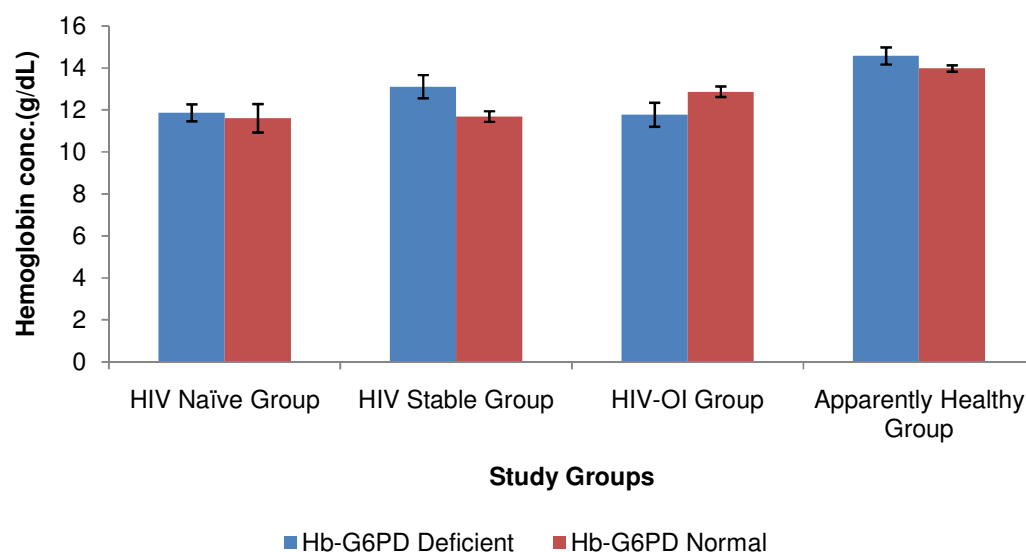


Figure 2 Hemoglobin (Hb) concentrations of G6PD deficient and G6PD Normal Individuals Across study Groups

Mean hemoglobin concentration in the G6PDdeficient individuals compared with the normal G6PDindividuals in the HIV naïve and apparently healthy groups showed no significant difference ($P=0.32$) while a significant difference ($P=0.01$; $P=0.04$) was found in the HIV stable and HIV-OI groups respectively (figure 2). Mean PCV concentration in the G6PD deficient individuals compared with thenormal G6PD individuals in the HIV naïve and apparently healthy groups showed no significantdifference ($P=0.34$; $P=0.21$) while significant difference ($P=0.001$; $P=0.05$) was found between the HIV stable and HIV-OI groups (figure 3).

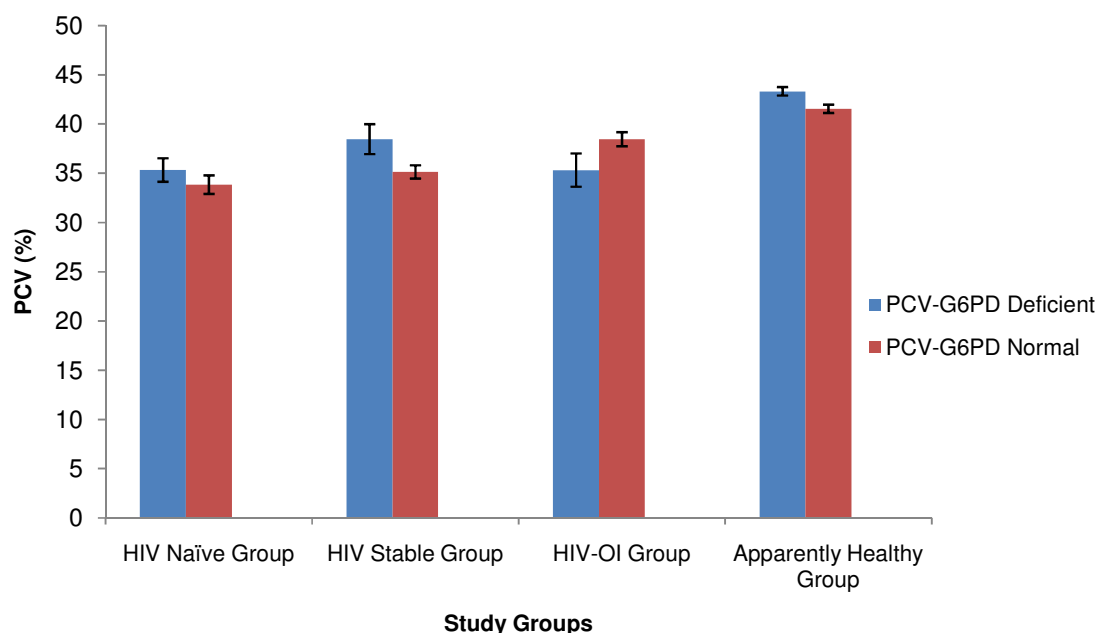


Figure 3: Packed Cell Volume (PCV) concentrations of G6PD deficient and G6PD Normal Individuals across study groups

Correlation of CD4 Count and hematological parameters with G6PD Activity

The study did not show a significant correlation between G6PD 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 G6PD deficiency and hemoglobin and PCV concentrations in the HIV stable. Table 4.1 shows the correlation of G6PD activity with CD4 count , hemoglobin and PCV of G6PD deficient individuals in all groups.

Table 4.1. Pearson's correlation coefficient (r) between G6PD Activity and different variables among G6PD 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 ($p \leq 0.05$) 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 G6PD deficient individuals met the three given criteria (Table 5). All 45 G6PD individuals had negative DAT and normal LDL activity though some had low hemoglobin and PCV levels.

Table 5 Prevalence of Hemolytic anaemia 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 ($p \leq 0.05$)

(-) Indicates negative DAT test; + indicates positive DAT test

Absence of moderate or severe anemia (indicated by hemoglobin ≤ 10 g/dL) in all groups, absence of elevated LDH (≥ 460 U/L) in all groups and negative DAT indicate lack of hemolytic anemia as a result of G-6-PD deficiency.

231 DISCUSSION

232 In this study, the prevalence of G-6-PD deficiency in the study group was 22.5% which agrees with a
 233 report by (15) conducted in South West Nigeria reporting a prevalence of 28.5%.(16), reported a 20%
 234 prevalence of G6PD deficiency in his study in Nigeria. In other parts of Africa, the prevalence of G-6-
 235 PD deficiency has been reported to be 22.5% in Congo (Brazzaville), 15.7% in Mali (Bamako), 13.0%
 236 in Uganda and 9.0–15.5% in Gabon (17).The higher number of G6PD deficient femalescompared with
 237 the males was unexpected as G6PD deficiency has been reported to be sex linked with male
 238 preponderance. Most workers have reported that the deficiency is rare in females because the
 239 mutation would have to occur in both copies of the gene to cause the disorder, whereas in males only
 240 one abnormal copy of the gene is required for manifestation of the disease. Whether the females
 241 were heterozygously deficient orhomozygously deficient was not however investigated.(18)assessing
 242 the frequency of G6PD deficiency in Sardinian patients with nonarteritic anterior ischemic optic
 243 neuropathy, indicated based on sex and G6PD-deficiency interaction that sex does not have any
 244 modifier effect on G6PD deficiency. Also, another reportby (19) in Malaysia indicated that sex was not
 245 a significant predictor associated with actual G6PD enzyme levels. (19) stated that G6PD deficiency,
 246 although X-linked, is not a recessive disorder and that in female heterozygotes, redcell mosaicism
 247 arising from random X chromosome inactivation results in two populations of G6PD-deficient and
 248 G6PD-normal cells. The proportionsof these two cell types can vary enormously, ranging from
 249 completely normal activity to complete deficiency. (20) who reported a 28% prevalence found a higher
 250 number of females than males with the deficiency. However, the higher number of G6PD deficient
 251 females in this study could be attributed to the fact that a higher number of females were enrolled in
 252 the HIV study groups probably due to the fact that in some communities/societies females are more
 253 likely to seek treatment than males when encountered with medical issues. Also this study stresses
 254 the preponderance of females in the prevalence of HIV infection and supports the established fact
 255 that women are biologically more vulnerable to HIV/AIDS and more likely to contact infection from
 256 their male partners as their sexuality and gender disadvantage in terms of culture, economic and
 257 social factors place them more at risk of infection than men. The World Health Organization (WHO)
 258 reported that HIV/AIDS affects females most severely in sub-Saharan Africa and women of
 259 reproductive age make up almost 57% of adults living with HIV, accounting for up to 80% of HIV
 260 infected women in the world (21) .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 G6PD 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 (22), 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 lower levels of hemoglobin and PCV found in the G6PD deficient compared with the G6PD normal in the HIV stable and HIV OI groups could probably be attributed to individuals in those groups being on a Zidovudine (AZT) containing regimen and the use of co-trimoxazole as prophylaxis. 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 (23). (24) 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.

As G6PD 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 (26) 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. Although no hemolysis was observed, the G-6-PD deficient individuals had lower hemoglobin, PCV and RBC levels and increased WBC levels compared with the G-6-PD normal individuals. A similar pattern was also reported by (27) 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 (28). (29) 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. (30) 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 G6PD deficient individuals in this study 62% were on co trimoxazole (CTX) though strict adherence to this medication could not be ascertained.

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 G6PD 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 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. HIV status does not appear to have any association with presence of G6PD deficiency as no association was found between CD4 count, the immunological marker of HIV infection and G-6-PD activity.

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.

REFERENCES

- 1.Carter N, Pamba A, Duparc S and WaitumbiJN.Frequency of glucose-6-phosphate dehydrogenase deficiency in malaria patients from six African countries enrolled in two randomized anti-malarial clinical trials. *Malaria Journal* .2011;10: 241.
2. Beutler E. The hemolytic effect of primaquine and related compounds: a review. *Blood*. 1959;14(2): 103-139.
3. Lui TZ, Lin TF, Hung IJ, Wei JS. and Chiu DTY. Enhanced Susceptibility of Erythrocytes Deficient in Glucose-6-Phosphate Dehydrogenase in Alloxan, GSH-induced decrease in Red Cell Deformability.*Life Sciences*; 1991:55, 155– 60.
4. CheesbroughM.The*District Laboratory Practice in tropical countries* 4thed. New York: Cambridge UniversityPress ; 2006.
5. Barnett BJ and Arduino RC. Use of fosamprenavir, a sulfa containing protease inhibitor in HIV infected patients with G6PD deficiency. *Clinical Infectious Diseases*. 2007; 44(6): 887-888.
6. Nkhoma ET, Poole C, Vannappagari V, Hall SA andBeutler E. The global prevalence of glucose-6 phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol Dis* 2009;42(3):267-78.
7. Luzzatto L and Gordon-Smith EC. Inherited haemolyticanaemia. In:Hoffbrand, AV, Lewis, SM. and Tuddenham EGD, editors. *Postgraduate Haematology*.4th ed.London: Arnold; 2001.
8. Ademowo OG. andFalusi AG. Molecular Epidemiology and activity of erythrocyte G-6-PD variants in a homogeneous Nigerian population.*East African Medical Journal*.2002;79: 42-44.
9. Egesie OJ, Joseph DE, Isiguzoro I and Esiegie UG.Glucose-6- PhosphateDehydrogenase (G6PD) Activity and Deficiency in a population of Nigerian male residents in Jos.*Nigerian Journal of Physiological Sciences*.2008;23(1-2): 9-11.
10. Omoregie R, Omokaro EU, Palmer O, Ogefere HO, Egbeobauwaye A, Adeghe JE, Osakue SI, and Ihemeje SI. Prevalence of anaemia among HIV infected patients in Benin city, Nigeria .*Tanzanian Journal of Health Research*.2009;11(1): 1-4.
11. Tungsiripat M, Drechsler H, Sarlone C, AmyotK, Laffey E, and Aberg J. Prevalence and Significance of G6PD Deficiency in Patients of an Urban HIVClinic. *Journal of International Association of Physicians in AIDS Care*. 2008; 7(2): 88-90.
12. Oshikoya KA, Lawal S, Oreagba IA, Awodele O, and Olayemi SO. Adverse Events in HIV-infected Children on Antiretroviral Therapy at a TeachingHospital in Lagos, Nigeria: A Retrospective Study. *Advances in Pharmacoepidemiology and Drug Safety*.2012; 1:117.

- 357 13. Cappellini, M.D. and Fiorelli, G. (2008). Glucose-6-Phosphate Dehydrogenase deficiency.*Lancet*.
358 2008;371 :64-74.
- 359 14. Tungsiripat M, Drechsler H, Sarlone C, Amyot K, Laffey E, and Aberg J. (2008). Prevalence and
360 Significance of G6PD Deficiency in Patients of an Urban HIV Clinic.*Journal of International*
361 *Association of Physicians in AIDS Care*.2008;7(2): 88-90.
- 362 15. May J, Meyer CG, Grossterlinden L, Ademowo OG, Mockenhaupt FP, Olumese PE, Falusi AG,
363 Luzzatto L, and Bienzle U. Red cell glucose-6-phosphate dehydrogenase status and pyruvate kinase
364 activity in a Nigerian population.*Tropical Medicine and Internal Health*.2000;5:119–123.
- 365 16. Egesie OJ, Joseph DE, Isiguzoro I, and Esiegie UG.Glucose-6- PhosphateDehydrogenase
366 (G6PD) Activity and Deficiency in a population of Nigerian male residents in Jos.*Nigerian Journal of*
367 *Physiological Sciences*,2008;23(1-2):9-11.
- 368 17. Dallo A, Banni, H, Gari MA, Al-Qahtani MH, Abuzenadeh AM, and Al-Sayes F. Five novel
369 Glucose-6-Phosphate Dehydrogenase deficiency haplotypes correlating with disease severity.
370 *Journal of Translational Medicine*, 2012;10: 199.
- 371 18. Pinna A, Solinas G, Masia C, Zinellu A, Carru C, Carta A. Glucose-6-phosphate dehydrogenase
372 (G6PD) deficiency in nonarteritic anterior ischemic optic neuropathy in a Sardinian population, Italy.
373 *Invest Ophthalmol Vis Sci*. 2008;49(4):1328–1232.
- 374
375 19.Ainoon O, Alawiyah A, Yu YH, Cheong HK, Hamidah NH, Boo NY and Zaleha M.Semiquantitative
376 Screening Test for G6PD Deficiency Detects Severe Deficiency But Misses A Substantial Proportion
377 Of
378 Partially-Deficient Females Southeast Asian J Trop Med Public Health. 2003;34(2):402-414.
- 379 20. Orok, A., Fagbenna, A. Iboma, G., and Okoh, H. Glucose-6-Phosphate Dehydrogenase (G-6-PD)
380 deficiency and adverse reactions to antimalarial drugs in Lagos State, Nigeria. *Asian Journal of*
381 *Pharmaceutical and health Sciences*, 2013;3(1): 615-620.
- 382 21. UNAIDS Joint United Nations Programme on HIV/AIDS. Report on the GlobalAids Epidemic 2010.
383
- 384 22. Tamuno I. and Babashani M. Socio-demographic and symptomatic clinical profile of patients with
385 HIV infection in a tertiary health care facility in north western Nigeria. *International Journal of*
386 *Pharmaceutical and Biomedical Research* 2011;2(3): 206-210.
- 387
388 23. Takuva S, Louwagie G, Zuma K, and Okello V, “Durability of first line antiretroviral therapy:
389 reasons and predictive factors for modifications in a Swaziland Cohort,” *Journal of Antiviralsand*
390 *Antiretrovirals*.2012; 4(1):14–20.
- 391
392 24.Alem M, Kena T, Baye N, Ahmed R, Tilahun S .Prevalence of Anemia and Associated Risk
393 Factors among Adult HIV Patients at the Anti-Retroviral Therapy Clinic at the University of Gondar
394 Hospital, Gondar, Northwest Ethiopia.Scientificreports. 2013;2 (3): 662.
- 395 25. Ogbodo SO, Shu EN and Okeke AC. Vitamin antioxidants may prevent drug induced haemolysis
396 of G6PD–deficient erythrocytes.*Pharmacologyonline*.2006;1: 90-99.
- 397 26. Orok A, Fagbenna A, Iboma G, and Okoh H. Glucose-6-Phosphate Dehydrogenase (G-6-PD)
398 deficiency and adverse reactions to antimalaria drugs in Lagos State, Nigeria. *Asian Journal of*
399 *Pharmaceutical and health Sciences*. 2013;3(1): 615-620.

- 400 27. Al-Awaida W, and Akash M. Biochemical and hematological indicators of acute and chronic cases
401 of Mediterranean G-6-PD deficiency patients from Southern Jordan. *Life Science Journal*. 2014;
402 11(1):371-377.
- 403 28. Moiz B, Nasir A, Moatter T, Naqvi ZA, and Khurshid M. Population study of 1311 C/T
404 polymorphism of Glucose 6 Phosphate Dehydrogenase gene in Pakistan - an analysis of 715 X-
405 chromosomes. *BioMed Central- Genetics*.2009;10: 41.
- 406 29. Rapezzi D, Porqueddu EM, Fenu L, Racchi O, Ferraris AM, Gaetani GF, and Aceti A. Survival of
407 people who are HIV-1- positive and G6PD-deficient is unaffected by virus-induced oxidative stress.
408 *The Lancet*. 1998;4:351.
- 409
410 30. Serpa JA, Villarreal-WilliamsE, and Giordano T. Prevalence of G-6-PD deficiency in a large cohort
411 of HIV-infected patients. *Journal of Infection*. 2010;61: 399-402.