G6PD Deficiency in an HIV Clinic Setting in the Dominican Republic

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Abstract. Because human immunodeficiency virus (HIV)-infected patients receive prophylaxis with oxidative drugs, those with glucose-6-phosphate dehydrogenase (G6PD) deficiency may experience hemolysis. However, G6PD deficiency has not been studied in the Dominican Republic, where many individuals have African ancestry. Our objective was to determine the prevalence of G6PD deficiency in Dominican HIV-infected patients and to attempt to develop a cost-effective algorithm for identifying such individuals. To this end, histories, chart reviews, and G6PD testing were performed for 238 consecutive HIV-infected adult clinic patients. The overall prevalence of G6PD deficiency (8.8%) was similar in males (9.3%) and females (8.5%), and higher in Haitians (18%) than Dominicans (6.4%; $P = 0.01$). By logistic regression, three clinical variables predicted G6PD status: maternal country of birth ($P = 0.01$) and a history of hemolysis ($P = 0.01$) or severe anemia ($P = 0.03$). Using these criteria, an algorithm was developed, in which a patient subset was identified that would benefit most from G6PD screening, yielding a sensitivity of 94.7% and a specificity of 97.2%, increasing the pretest probability (8.8–15.1%), and halving the number of patients needing testing. This algorithm may provide a cost-effective strategy for improving care in resource-limited settings.

INTRODUCTION

The prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency is highest in populations from malaria-endemic regions, such as sub-Saharan Africa.¹ In the United States, it is most prevalent in African-American males (~10%)²; in Latin America, the prevalence is < 1% to > 15%.¹ G6PD deficiency has not been studied in the Dominican Republic, which has the highest level of African ancestry among Hispanic populations.³ The Dominican Republic is adjacent to Haiti, with a population of predominantly African ancestry. With increasing migration across this border, Dominican clinics provide care to many Haitian patients, potentially increasing the prevalence of G6PD deficiency.

G6PD catalyzes the rate-limiting step in the pentose phosphate pathway, the only source of reduced nicotinamide adenine dinucleotide phosphate (NADPH) in erythrocytes. Erythrocyte antioxidant pathways require NADPH; those with low G6PD activity are less able to withstand oxidative stress, inducing intravascular and extravascular hemolysis. A common clinical manifestation of G6PD deficiency is acute hemolysis, which is precipitated by oxidative stressors, such as fava beans, primaquine, sulfonamides, or infection.⁴ Because of its X-linked inheritance pattern, male hemizygotes and female homozygotes exhibit more severe clinical phenotypes, whereas female heterozygotes exhibit phenotypic variability due to random X inactivation.¹

Certain populations, such as those receiving malaria prophylaxis, are universally screened for G6PD deficiency.⁵ However, the benefit of screening HIV-infected patients^{5,6} is unclear. For example, although trimethoprim-sulfamethoxazole (TMP-SMX) and dapsone are commonly used in HIV-infected patients, they can precipitate life-threatening hemolysis in G6PD deficiency. Interestingly, at one HIV clinic the prevalence of G6PD deficiency was ~10%⁶; among those receiving oxidative medications, 10% developed severe hemolysis,⁶ highlighting the potential danger of these drugs.

HIV-infected, G6PD-deficient individuals may also have inherently worse outcomes.^{7–12} For example, decreased glutathione levels in HIV-infected individuals suggest that they experience chronic oxidative stress.^{7,10,12} Primary HIV infection also caused acute hemolysis in a G6PD-deficient patient.¹³ Furthermore, oxidative stress is associated with immunological dysfunction, potentially exacerbating HIV infection.⁸ Finally, CD4 T-cell glutathione levels progressively decreased during HIV progression, with decreased survival in patients with low CD4 glutathione levels.⁹ Nonetheless, another study found no survival difference between G6PD-deficient and G6PD-normal acquired immune deficiency syndrome (AIDS) patients.¹¹

Hemolysis may also predispose HIV-infected, G6PD-deficient patients to worse outcomes. For example, anemia frequently complicates HIV infection, is associated with faster progression, and is an independent risk factor for mortality.^{14–17} In addition, anemic HIV-infected patients are more likely to require hospitalization or transfusion from a hemolytic crisis.

Given these concerns regarding the role that G6PD deficiency may play in the prognosis and treatment of HIV-infected patients, and given the paucity of information regarding G6PD deficiency in the Dominican Republic, our objective was to use a comprehensive testing approach (see below) to determine the prevalence of G6PD deficiency in a set of Dominican HIV-infected patients. In addition, by evaluating patient history and chart data, we attempted to develop a cost-effective algorithm for identifying such individuals in a resource-challenged environment.

Multiple methods exist for evaluating G6PD deficiency. The simplest are qualitative screening tests.^{18,19} These identify individuals with markedly low G6PD levels, but do not reliably identify heterozygous females with intermediate levels. Thus, quantitative tests are used for confirmation. However, due to random X inactivation, levels in female heterozygotes range from very deficient to normal, diminishing the diagnostic accuracy of these tests. Moreover, because no firm correlation exists between G6PD level and clinical phenotype, some suggest that all heterozygous females be defined as G6PD deficient.^{20–22}

Although definitive, genetic testing is complex. *G6PD* contains 13 exons encoding a 515-amino acid protein; most

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pathogenic mutations are missense mutations and span the coding region. The World Health Organization (WHO) classifies these based on enzyme activity.²³ Thus, Class I variants (< 1% activity) present with chronic nonspherocytic haemolytic anemia. Class II (< 10% activity) is associated with favism. Class III variants (10–60% activity) are most prevalent and individuals of African ancestry with the *G6PD* A- variant can experience life-threatening drug-induced hemolysis.²⁴ Although *G6PD* A- was expected to predominate in the Dominican Republic, to avoid missing unusual variants,^{25,26} we used a comprehensive sequencing strategy.

METHODS

Subject recruitment. Subjects were recruited at Clínica de Familia La Romana, a free HIV clinic in the Dominican Republic, founded as a public-private collaboration with the Columbia University International and Immigrant Family

Health and AIDS Programs. It cares for ~1,400 HIV-positive adults, with more than 15,000 visits in 2012.

In October–December 2012, all consenting HIV-infected adults undergoing a clinically indicated phlebotomy were recruited using a protocol approved by the Columbia University Institutional Review Board and the Dominican Consejo Nacional de Bioética en Salud. All patients were consented by one author (Julia Z. Xu); patients speaking Spanish were consented by Julia Z. Xu alone, those speaking only Kreyól were consented with the assistance of clinic interpreters.

Histories and chart reviews were performed for 251 enrollees, including: country of birth of the participant and parents; exposure to fava beans, naphthalene, and infection; and history of hemoglobinuria, jaundice, gallstones, transfusions, and anemia (Tables 1–3). Chart review included: medication history, changes in highly active antiretroviral therapy (HAART) regimen, complete blood count and CD4 count at enrollment, CD4 count and hemoglobin (Hb) nadir, and

TABLE 1
Demographic data

	N	%				
Prevalence of G6PD deficiency	21/238	8.8	–	–	–	–
Male	9/97	9.3	–	–	–	–
Female	12/141	8.5	–	–	–	–
Dominican	12/187	6.4	–	–	–	–
Haitian	9/50	18.0	–	–	–	–
	N	Mean	SD	P value		
Age (years)	–	41.0	12.3	–	–	–
G6PD activity level (U/g Hb)	238	8.7	2.7	–	–	–
Male	97	8.3	2.7	0.35	–	–
Female	141	8.9	2.7	–	–	–
Dominican	187	8.9	2.6	–	–	–
Haitian	50	7.7	2.8	0.001	–	–
Current Hb value (g/dL)	238	12.3	2.0	–	–	–
G6PD normal	217	12.3	2.0	–	–	–
G6PD deficient	21	12.0	1.9	0.28	–	–
Lowest Hb value (g/dL)	238	10.7	2.2	–	–	–
G6PD normal	217	10.7	2.1	–	–	–
G6PD deficient	21	10.0	3.2	0.30	–	–
Current CD4 count (cells/μL)	227	421.5	253.8	–	–	–
G6PD normal	207	424.3	255.1	–	–	–
G6PD deficient	20	393.1	244.8	0.70	–	–
Lowest CD4 count (cells/μL)	238	215.7	195.1	–	–	–
G6PD normal	217	214.2	198.2	–	–	–
G6PD deficient	21	231.9	162.9	0.35	–	–
	Total		G6PD normal		G6PD deficient	
	N	%	N	%	N	%
Medications (nonoxidative)						
HAART (ever)	194/235	82.6	181/215	84.2	13/20	65.0
HAART (current)	184/238	77.3	172/217	79.3	12/21	57.1
Azithromycin (ever)	57/238	24.0	54/217	24.9	3/21	14.3
Azithromycin (current)	18/238	7.6	16/217	7.4	2/21	9.5
Isoniazid (ever)	46/238	19.3	44/217	20.3	2/21	9.5
Isoniazid (current)	4/238	1.7	4/217	1.8	–	–
Oxidative exposures						
TMP-SMX (ever)	146/238	61.3	134/217	61.8	12/21	57.1
TMP-SMX (current)	35/238	14.7	32/217	14.8	3/21	14.3
Dapsone (ever)	2/238	0.8	2/217	0.9	–	–
Dapsone (current)	1/238	0.4	1/217	0.5	–	–
Fava beans	191/238	80.3	174/217	80.2	17/21	81.0
Naphthalene	19/238	8.0	17/217	7.8	2/21	9.5
Tuberculosis	22/238	9.2	22/217	10.1	–	–
Hepatitis	15/237	6.3	14/216	6.5	1/21	4.8
Malaria	5/237	2.1	4/216	1.9	1/21	4.8

G6PD = glucose-6-phosphate dehydrogenase; HAART = highly active antiretroviral therapy; Hb = hemoglobin; TMP-SMX = trimethoprim-sulfamethoxazole. All continuous variables with $P < 0.05$ are in bold.

TABLE 2
Logistic regression model (variables included in the model)*

	G6PD normal		G6PD deficient		P value
	N	%	N	%	MLE
History					
Gender (male vs. female)	88/217	40.6	9/21	42.9	0.84
Maternal COB (Haiti vs. DR)	41/216	19.0	9/21	42.9	0.01
HAART change for anemia	9/216	4.2	3/21	14.3	0.06
History of hemolysis	65/217	30.0	12/21	57.1	0.01
History of gallstones	13/217	6.0	1/21	4.8	0.82
History of transfusion	25/217	11.5	5/21	23.8	0.11
Family history of anemia	26/217	12.0	3/21	14.3	0.76
Personal history of anemia	105/217	48.4	12/21	57.1	0.45
Chart review					
Anemic (ever)	185/217	85.3	17/21	81.0	0.60
Anemic (current)	121/217	55.8	14/21	66.7	0.34
Severely anemic (ever)	16/217	7.4	5/21	23.8	0.02
Severely anemic (current)	4/217	1.8	—	—	—
Acute drop in Hb	4/217	1.8	1/21	4.8	0.39
Subacute drop in Hb	21/217	9.7	3/21	14.3	0.51

COB = country of birth; DR = Dominican Republic; G6PD = glucose-6-phosphate dehydrogenase; HAART = highly active antiretroviral therapy; Hb = hemoglobin; MLE = maximum likelihood estimate.

*Any categorical variable with MLE $P < 0.25$ was bolded and included in the initial multivariable logistic regression model.

documented declines in Hb of ≥ 2 g/dL (Tables 1–3). Anemia was defined as Hb < 12 g/dL for women and < 14 g/dL for men.^{17,27} Severe anemia was defined as a Hb nadir of < 8 g/dL.²⁷ Hb decline of ≥ 2 g/dL⁶ was defined as an acute drop if occurring within 30 days, and subacute if within 6 months. No testing was locally available to test for autoimmune hemolytic anemia or to measure HIV viral load.

G6PD levels. One patient did not undergo a blood draw and residual samples were unavailable for 12; for the remaining 238 participants, residual blood samples were available. Qualitative G6PD testing was performed locally at the clinic using the Trinity Biotech G-6-PDH Dye Reduction Kit (Bray, Ireland). Samples were incubated at 37–40°C and assessed for color change at 30 and 60 minutes.

Quantitative G6PD levels did not change due to shipping samples to Columbia University in insulated coolers, which was documented by preliminary studies before starting to enroll patients. All samples were received at Columbia within 48 hours of shipment and the cold chain was maintained in every case. Quantitative tests were performed within 1 week of collection using the Trinity Biotech G-6-PDH Kit. Results were expressed as units of activity per gram Hb (U/g Hb). The G6PD deficiency cutoff was 5.4 U/g Hb (i.e., 60% of mean normal activity, using WHO criteria).²³

G6PD sequencing. Fifty-five patient samples were sequenced: those with G6PD activity near or below the quantitative assay cutoff, those with discordant qualitative and quantitative results,

and several controls with normal activity. Whole blood genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, CA) and *G6PD* exons 3–13 were amplified using previously described primers²⁸ (Table 4). Polymerase chain reaction (PCR) was performed using the TaKaRa LA Taq LongRange PCR system (Clontech Laboratories, Inc., Mountain View, CA). In brief, 50 ng of DNA was used in a 50 μ L reaction containing 8 μ L of a deoxynucleotide triphosphate (dNTP) mixture (2.5 mM each), 5 μ L of 10 $\times$ LA PCR buffer II (Mg²⁺ plus), 10 μ L of a 3 μ M forward and reverse primer cocktail, 0.5 μ L (2.5 units) of TaKaRa LA Taq[®] DNA polymerase, and 16.5 μ L of molecular grade water. PCR was performed in the PCR System 9700 (Applied Biosystems, Foster City, CA), as follows: 2 minutes at 94°C, 30 cycles of 15 seconds at 94°C and 6 minutes at 68°C, followed by 11 minutes at 68°C. *G6PD* exons 1–2 were amplified with primers designed using Applied Biosystems primer express software (v2.0 and Primer3 Input v0.4.0; frodo.wi.mit.edu/y; Table 4). PCR was performed using ~100 ng of DNA in a 50 μ L reaction, including 5 μ L of dNTP (2 mM), 5 μ L of 10 $\times$ buffer, 0.5 μ L of DNA polymerase, and 5 μ L of forward and reverse primers (2 pM). Unincorporated primers and dNTPs were eliminated using ExoSAP-IT (Affymetrix, Santa Clara, CA).

Amplification products were sequenced in both directions using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with an ABI PRISM 3100-Avant Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA). *G6PD* exons 1 and/or 2 could not be sequenced for subjects 3, 19, 20, 26, 31, 42, 47, and 55 (Table 5).

Quality control. For quantitative and qualitative enzymatic assays and for *G6PD* sequencing assays, detailed standard operating procedures were prepared, extensive testing for reproducibility and robustness were performed, and positive and negative controls were consistently used.

Statistical analysis. Clinical and laboratory data, collected using case report forms, were transformed into a database using Microsoft Access 2010 (Redmond, WA). Statistical analyses were performed using SAS 9.3 (Cary, NC). The following were analyzed using the Wilcoxon rank sum test: age, G6PD activity, current Hb value, and current CD4 count and nadir. Hb nadir was analyzed using the independent Student *t* test. Categorical variables were analyzed using maximum likelihood estimates. Statistical significance was at $P < 0.05$.

A logistic regression model was constructed to assess the predictive ability of clinical study variables on G6PD status. Univariate analyses were performed of gender, maternal country of birth, HAART regimen change due to anemia, history of hemolysis, gallstones, transfusions, or anemia, ever anemic, currently anemic, ever severely anemic, currently severely anemic, and acute or subacute Hb drop. Variables with a significance of $P < 0.25$ were included initially. The final model was built using a forward selection algorithm in SAS to select explanatory variables. Significance level of explanatory variables was $P < 0.05$.

RESULTS

Demographics and G6PD levels. At Clínica de Familia La Romana, 238 consenting patients were interviewed and tested for G6PD deficiency. Their mean age was 41.0 years, 59.2% were female, 78.6% reported the Dominican Republic

TABLE 3
Logistic regression model (beta values, odds ratios, and *P* values for the three variables in the predictive model for G6PD status)*

Variable	Beta value	Odds ratio	P value
Maternal COB (Haiti vs. DR)	1.38	3.97	0.01
History of hemolysis	1.39	4.03	0.01
Severe anemia	1.32	3.74	0.03
Model: $y = 1.38$ (country) + 1.39 (hemolysis) + 1.32 (anemia)			

COB = country of birth; DR = Dominican Republic; G6PD = glucose-6-phosphate dehydrogenase.

*Level of significance was set at $P < 0.05$ for variables included in the final model.

TABLE 4
Primer sequences

Primer name	Primer sequence for long-range PCR	Fragment size
13125-F*	GTT TAT GTC TTC TGG GTC AGG GAT GG	5,271 bp
18396-R*	AGT GTG CTG GAA GTC ATC TTG GGT	
G6PD exon1-F	AAT TGG GGA TGC AGA GCA	158 bp
G6PD exon1-R	AAG CAC AAC AAA CAG CGT GTA	
G6PD exon2-F	TGC CTT CTT AAC GAG CCT TT	155 bp
G6PD exon2-R	CAG GCA CTT CCT GGC TTT TA	
Exon	Primer sequence for individual exons	
1	Forward	AAT TGG GGA TGC AGA GCA
	Reverse	AAG CAC AAC AAA CAG CGT GTA
2	Forward	TGC CTT GTT AAC GAG CCT TT
	Reverse	CAG GCA CTT CCT GGC TTT TA
3	Forward	GCT TGT GGC CCA GTA GTG AT
	Reverse	GCA GTG GTG GGA CAC TTA
4	Forward	TAA GTG TGT CCC ACC ACT GC
	Reverse	TGG TAG AGA GGG CAG AAC CA
5	Forward	CTG AAA TCT GGC CTC TGT CC
	Reverse	CTC ATA GAG TGG TGG GAG CA
6	Forward	GAT CCT CAC TCC CCG AAG A
	Reverse	CCA GGT GAG GCT CCT GAG TA
7	Forward	GTG CAG AAC CTC ATG GTG CTG
	Reverse	GAG GAG CTC CCC CAA GAT AG
8	Forward	AGG GGG ATC AGG AAG TGA GT
	Reverse	TGT GCT CAG AGG TGG TGA CT
9	Forward	TCT CCC TTG GCT TTC TCT CA
	Reverse	CTC TCA GGG TGT GGA CCA GT
10	Forward	TTT GCA GCC GTC GTC CTC TAT G
	Reverse	CCT CCA CAC TGC TCC TTC TC
11	Forward	CCT GAC CTA CGG CAA CAG AT
	Reverse	AATATAGGGGATGGGCTTGG
12	Forward	GCA TAC CTG TGG GCT ATG GG
	Reverse	AGG TCA ATG GTC CCG GAG T
13	Forward	GTC TGT CCC AGA GCT TAT TGG
	Reverse	TGC TGC GTC TGC TTT TCT TA

bp = base pair; G6PD = glucose-6-phosphate dehydrogenase; PCR = polymerase chain reaction.

*These primers, obtained from previously published work,²⁸ were used to amplify exons 3–13.

as their maternal country of birth, 21% reported Haiti, and 0.4% reported another country.

The cutoff for G6PD deficiency was 5.4 U/g Hb (i.e., < 60% of the mean normal level). Mean male and female G6PD levels were similar: 8.3 and 8.9 U/g Hb, respectively (Table 1). Overall, 8.8% were G6PD deficient with similar prevalence in males and females: 9.3% and 8.5%, respectively (Table 1). Subjects with Haitian, as compared with Dominican, ancestry had a higher prevalence: 18.0% and 6.4%, respectively ($P = 0.01$). Furthermore, G6PD levels were lower in patients of Haitian, as compared with Dominican, ancestry: 7.7 and 8.9 U/g Hb, respectively ($P < 0.05$). Although not designed to compare G6PD-normal and G6PD-deficient cohorts, the Hb and CD4 counts did not differ between these groups (Table 1).

Oxidative exposures. Because treatment protocols were similar for all patients, who were not previously screened for G6PD deficiency, we did not expect oxidative exposures to differ between G6PD-deficient and G6PD-normal groups (Table 1). Most patients (82.6%) received HAART previously; 77.3% were receiving HAART at enrollment. Both groups had significant oxidative exposures. For example, > 60% of subjects received TMP-SMX, with 57.1% of G6PD-deficient subjects receiving TMP-SMX previously and 14.3% currently receiving it. Two patients also received dapsone after discontinuing TMP-SMX, but both were G6PD normal. Finally, 81% of G6PD-deficient patients reported ingesting

fava beans; < 10% reported naphthalene exposure, hepatitis, tuberculosis, or malaria.

Risk factors predicting G6PD deficiency. By interview, 57.1% of G6PD-deficient and 30.0% of G6PD-normal patients reported a history of hemolysis ($P = 0.01$; Tables 2 and 3). By chart review, G6PD deficient, as compared with G6PD normal, patients had 3.9 times higher odds of a history of severe anemia ($Hb < 8$ g/dL; $P = 0.02$), but were not more likely to be anemic at interview (Tables 2 and 3). Five had an acute Hb drop, and 24 a subacute drop, but neither was associated with G6PD deficiency. In addition, of 12 patients with a HAART regimen change for “drug-induced” anemia, all were initially on zidovudine (AZT), which was discontinued in all, whereas nine received TMP-SMX, which was discontinued in five; three of these 12 were G6PD deficient.

A logistic regression model was built to help identify individuals at increased risk for G6PD deficiency (Tables 2 and 3). The final model identified three significantly predictive variables: maternal country of birth ($P = 0.01$), history of hemolysis ($P = 0.01$), and severe anemia ($P = 0.03$), with each showing a ~4-fold increase in the odds of G6PD deficiency (Tables 2 and 3). Two positive parameters increased the odds ~15-fold; three increased them 60-fold. These three criteria provide a simple screening questionnaire to assess risk; thus, in our cohort, 126 patients met any one criterion (19 G6PD deficient), 20 met any two criteria (five G6PD deficient), and two met all three (both G6PD deficient).

TABLE 5
Comparison of qualitative, quantitative, and molecular results for 55 selected subjects ordered by value of quantitative result*

Number	Gender	Maternal country of birth	Qual. result	Quant. result (U/g Hb)	G6PD genotype	Genotype interpretation
1	Male	Dominican	Deficient	0.8	G6PD A-	Hemizygous male
2	Male	Dominican	Deficient	1	G6PD A-	Hemizygous male
3	Male	Dominican	Deficient	1.1	G6PD A-	Hemizygous male
4	Female	Dominican	Deficient	1.3	G6PD A-/A-	Homozygous female
5	Male	Haitian	Deficient	1.3	G6PD A-	Hemizygous male
6	Male	Haitian	Deficient	1.6	G6PD A-	Hemizygous male
7	Male	Haitian	Deficient	1.7	G6PD A-	Hemizygous male
8	Male	Haitian	Deficient	1.7	G6PD A-	Hemizygous male
9	Male	Dominican	Deficient	1.7	G6PD A-	Hemizygous male
10	Female	Dominican	Deficient	2.1	G6PD A-/B+	Heterozygous female
11	Male	Dominican	Deficient	2.2	G6PD A-	Hemizygous male
12	Female	Dominican	Deficient	2.8	G6PD A-/B+	Heterozygous female
13	Female	Dominican	Deficient	3	G6PD A-/B+	Heterozygous female
14	Female	Haitian	Normal	3.9	G6PD A-/B+	Heterozygous female
15	Female	Dominican	Normal	4	G6PD A-/B+	Heterozygous female
16	Female	Dominican	Equivocal	4.6	G6PD A-/B+	Heterozygous female
17	Female	Haitian	Equivocal	4.7	G6PD A-/A+	Heterozygous female
18	Female	Haitian	Equivocal	4.8	G6PD A-/B+	Heterozygous female
19	Female	Haitian	Equivocal	5	G6PD A-/B+	Heterozygous female
20	Female	Dominican	Equivocal	5.1	G6PD A-/B+	Heterozygous female
21	Female	Haitian	Equivocal	5.3	G6PD A-/A+	Heterozygous female
22	Female	Haitian	Normal	5.6	G6PD A-/A+	Heterozygous female
23	Female	Haitian	Normal	5.6	G6PD A-/B+	Heterozygous female
24	Female	Dominican	Equivocal	5.6	G6PD A-/B+	Heterozygous female
25	Female	Dominican	Equivocal	5.6	G6PD A-/B+	Heterozygous female
26	Male	Haitian	Normal	5.7	G6PD A+	Normal male
27	Female	Haitian	Normal	6	G6PD A-/B+	Heterozygous female
28	Male	Dominican	Normal	6.1	G6PD A+	Normal male
29	Female	Dominican	Normal	6.2	G6PD A-/B+	Heterozygous female
30	Female	Dominican	Deficient	6.2	G6PD A-/B+	Heterozygous female
31	Female	Haitian	Normal	6.4	G6PD A+/B+	Normal female
32	Female	Dominican	Normal	6.4	G6PD B+/B+	Normal female
33	Female	Dominican	Equivocal	6.5	G6PD A+/B+	Normal female
34	Female	Dominican	Normal	6.5	G6PD B+/B+	Normal female
35	Female	Dominican	Normal	6.6	G6PD A+/B+	Normal female
36	Female	Dominican	Normal	6.6	G6PD A+/B+	Normal female
37	Female	Dominican	Normal	6.7	G6PD A+/A+	Normal female
38	Female	Dominican	Normal	6.8	G6PD B+/B+	Normal female
39	Female	Dominican	Normal	6.9	G6PD B+/B+	Normal female
40	Female	Dominican	Normal	6.9	G6PD A+/B+	Normal female
41	Female	Dominican	Deficient	6.9	G6PD B+/B+	Normal female
42	Female	Dominican	Normal	6.9	G6PD A-/B+	Heterozygous female
43	Male	Dominican	Normal	7	G6PD B+	Normal male
44	Male	Dominican	Normal	7.1	G6PD B+	Normal male
45	Female	Haitian	Normal	7.7	G6PD B+/B+	Normal female
46	Male	Haitian	Normal	8.8	G6PD B+	Normal male
47	Female	Dominican	Normal	9.2	G6PD B+/B+	Normal female
48	Male	Dominican	Normal	10	G6PD B+	Normal male
49	Female	Dominican	Normal	10.5	G6PD B+/B+	Normal female
50	Male	Dominican	Normal	11.1	G6PD B+	Normal male
51	Male	Haitian	Normal	11.3	G6PD B+	Normal male
52	Female	Dominican	Normal	12.3	G6PD B+/B+	Normal female
53	Female	Dominican	Normal	13.4	G6PD B+/B+	Normal female
54	Female	Haitian	Normal	15.6	G6PD A+/B+	Normal female
55	Female	Dominican	Normal	20.1	G6PD A+/B+	Normal female

G6PD = glucose-6-phosphate dehydrogenase.

*All three assays correlate well at low G6PD activity levels, but discordant results increase near the quantitative cutoff for G6PD deficiency (i.e., 5.4 U/g Hb).

The questionnaire's sensitivity with one positive criterion is 90.5% (19/21 G6PD-deficient patients identified) and the specificity is 50.5% (109/216 G6PD-normal patients identified). Requiring two positive criteria lowers sensitivity (23.8%), but increases specificity (93.1%). As a screening tool, the higher sensitivity using one positive criterion is preferred; however, qualitative biochemical testing may be needed to exclude false positives.

Decreasing incubation time improves qualitative screen sensitivity. Qualitative, quantitative, and molecular tests for

G6PD deficiency were compared. The qualitative test²⁹ differentiates G6PD normal from markedly deficient samples. Normal G6PD levels produce sufficient NADPH to reduce the dye within 60 minutes. Because G6PD-normal samples may decolorize at earlier times, we evaluated samples at 30 and 60 minutes to detect intermediate activity. At 60 minutes, 14/238 samples did not decolorize, 13 of which had deficient G6PD activity. There were eight false negatives, all females, six of which were "equivocal," with little or no color change at 30 minutes, but color changed by 60 minutes

FIGURE 1. Glucose-6-phosphate dehydrogenase (G6PD) activity histograms in males and females. Individual patients are arranged from left to right on the x axis in ascending order of enzyme activity. There is a clear difference in the activity level between G6PD-deficient and G6PD-normal males, but no clearly identifiable cutoff in females.

(Table 5). Thus, the sensitivity of the 60-minute assay is 61.9% (13/21), but increases to 90.5% (19/21) when read at 30 minutes; nonetheless, the specificity is similar: 99.5% and 97.7%, respectively.

The “gold standard” quantitative assay has limitations in detecting heterozygotes. Several females had levels surrounding the cutoff of 5.4 U/g Hb, evident on the activity histograms (Figure 1); G6PD-deficient and G6PD-normal males are easily distinguished, but females have a smooth distribution making a cutoff difficult to identify.

Therefore, to diagnose heterozygotes definitively, *G6PD* exons were sequenced for all deficient, “equivocal,” and borderline subjects, and for selected controls. Of 55 patients sequenced, 29 carried at least one *G6PD* A[−] allele (i.e., containing the nonpathogenic A376G and pathogenic G202A substitutions). The *G6PD* A⁺ allele (A376G alone), exhibiting normal enzymatic activity,³⁰ was the only other variant detected. All biochemically deficient males were hemizygous for the A[−] variant; all biochemically deficient females were heterozygotes, except for one homozygous deficient female, whose enzyme level resembled that of male hemizygotes. In comparison with the quantitative assay, sequencing had 100% sensitivity and 96.3% specificity, detecting eight heterozygotes with normal activity. The three methods agreed well when subjects had low G6PD activity (19/21 samples; 90.5%), with uncertainty near the quantitative activity cutoff.

DISCUSSION

G6PD deficiency is highly prevalent (8.8%) in HIV-infected patients in the Dominican Republic. All of our G6PD-deficient subjects carried the “African” variant (*G6PD* A[−]). In addition, 14 subjects carried the A⁺ allele, also associated with African ancestry.

Many factors can induce hemolysis in G6PD-deficient individuals.⁴ Because HIV-infected patients frequently receive oxidative medications and often develop anemia, awareness of G6PD deficiency is particularly important. In this study,

G6PD-deficient subjects were more likely to report a history of hemolysis or severe anemia. Nonetheless, many HIV-infected G6PD-normal patients also experienced hemolysis. We could not determine whether clinic-prescribed medications, such as TMP-SMX and dapsone, caused hemolysis, which requires a prospective study; however, these can precipitate severe hemolysis in G6PD deficiency.^{24,31–34}

Anemia in HIV-infected patients is often multifactorial, complicating the identification of G6PD deficiency. Although laboratory tests for hemolysis are helpful, they are rarely performed in low-resource settings. Thus, it was not possible to differentiate acute hemolysis from other etiologies (e.g., iron deficiency anemia, AZT-induced bone marrow suppression). Because this study was not intended to change treatment, additional laboratory tests were not requested.

Interestingly, one patient, identified as G6PD deficient, had started on HAART (containing AZT) and TMP-SMX, and presented 6 weeks later with symptomatic anemia, dark urine, jaundice, and a 7-g/dL Hb drop. Two weeks following hospitalization, transfusion, and discontinuation of AZT and TMP-SMX, the Hb returned to baseline. This patient reported frequent episodes of dark urine and jaundice after ingesting fava beans. Other G6PD-deficient patients also reported dark urine or jaundice after ingesting fava beans or having contact with mothballs, suggesting that the *G6PD* A[−] variant can be severe.²⁴

Although these clinic patients had not heard of G6PD deficiency, they were knowledgeable about sickle cell disease. Because optimal management involves patient recognition of hemolysis and avoidance of oxidative triggers, it is essential to increase their awareness. Providers would also benefit from improved understanding. In this setting, treatment typically starts with AZT-based HAART and TMP-SMX. Patients often return 1 month later with a significant Hb drop by routine testing. Because AZT can cause bone marrow suppression, they are switched to a different HAART regimen. In our cohort, AZT was discontinued in all patients presenting with “drug-induced” anemia, whereas TMP-SMX was discontinued in only ~50% of patients. When TMP-SMX was discontinued, providers were primarily concerned about bone marrow suppression, rather than hemolysis due to G6PD deficiency. Providers reported anecdotal cases of TMP-SMX prophylaxis with refractory anemia, despite multiple HAART regimen changes; in such cases, provider awareness of oxidative drugs and G6PD deficiency may prevent morbidity.

Although well characterized, G6PD deficiency presents diagnostic challenges. Uncertainty remains in identifying heterozygotes with intermediate G6PD levels, and females with skewed X inactivation can have normal activity. Various methods may improve heterozygote detection.^{20,22} For example, we improved qualitative screening sensitivity by assessing decolorization at 30 minutes, which detected most heterozygotes, including six of eight with normal activity. However, this also identified two normal subjects. In addition, genotype does not directly correlate with clinical severity and, though the quantitative assay is not perfect, low activity remains the diagnostic gold standard. Thus, molecular and modified qualitative assays may identify heterozygotes with normal activity, who are less likely to have clinical manifestations.

Although we identified G6PD deficiency using multiple modalities, only qualitative screening is readily performed in resource-limited settings. Although guidelines recommend screening for patients with relevant racial or ethnic

FIGURE 2. Proposed algorithm for evaluating HIV+ Dominican patients in a resource-challenged environment. The patient questionnaire identifies (A) the maternal country of birth (i.e., Haiti vs. the Dominican Republic), (B) a history of hemolysis, and (C) a history of severe anemia, to determine whether glucose-6-phosphate dehydrogenase (G6PD) enzymatic activity should be measured. Based on this historical information and G6PD activity, the patient can or cannot receive dapsone and trimethoprim-sulfamethoxazole (TMP-SMX), along with appropriate counseling.

backgrounds,³⁵ these are not routinely followed because of controversy regarding cost-effectiveness.^{5,6,36} The few published studies involved patients with the *G6PD* A- allele, who are thought to be less sensitive to drug-induced hemolysis,^{35,36} which may not be true.²⁴ Nonetheless, the frequency of this complication in HIV clinics using dapsone or TMP-SMX prophylaxis has not yet been determined.

Another limitation of G6PD screening in resource-limited settings is the lack of suitable alternatives for prophylaxis; as an example, atovaquone is either not available or too expensive.³⁶ In addition, the risk of discontinuing prophylaxis may outweigh the risk of hemolysis. Nonetheless, changing or stopping prophylaxis is not the only alternative; patient education about hemolysis symptoms and oxidative trigger avoidance can be readily provided.

Although screening all patients for an X-linked disorder may not be cost-effective, screening male patients alone, who typically exhibit a more severe phenotype, may be worthwhile.⁵ However, we found no gender differences in prevalence, and several females had markedly low activity. Another strategy would only test anemic patients. However, because reticulocytes have higher G6PD levels, actively hemolyzing patients may have “normal” G6PD activity.

An ideal approach would identify patients benefitting most from screening and/or close monitoring after starting oxidative drugs (Figure 2). Our screening questionnaire identified a subpopulation at higher risk of G6PD deficiency (53%). Screening the entire cohort with the qualitative test interpreted at 30 minutes, yielded acceptable sensitivity (90.5%). Performing this assay only on subjects identified by the questionnaire is highly sensitive (94.7%) and specific (97.2%) and increases the pretest probability from 8.8% to 15.1%. However, this predictive model is limited by small sample size: only 20 patients were positive for two questionnaire parameters and only two patients had all three. Therefore, this algorithm should be validated prospectively. Nonetheless,

improved recognition and management of G6PD deficiency may enhance safe and cost-effective care for these patients.

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