Malaria in children and G6PD Deficiency in an endemic area for malaria in Bengo province, Angola.

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Stae of art

Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway, important in the protection of cells against oxidative stress. The G6PD deficiency is the most common enzymopathy X linked worldwide. The majority of the G6PD deficient do not manifest any symptoms, however, acute hemolytic anemia may be trigger by several agents, such as primaquine. Current WHO guidelines state that in elimination areas a single 0.25 mg base/kg primaquine dose should be given, as a gametocytocide, to all patients with parasitological-confirmed P. falciparum malaria on the first day of treatment in addition to an ACT. In face of this recommendation, endemic malaria countries should be informed of the prevalence G6PD deficiency, in order to make safe and appropriate decisions regarding the use of potentially unsafe drugs for G6PD deficient individuals.

Objectives

The aim of this study is to determine the prevalence of G6PD deficiency in an holoendemic region in Africa for P falciparum, evaluating the genotype and the phenotype of the enzyme.

Methods

The sampling was performed at the General Hospital of Bengo between Abril 2014 and November 2016, involving the population living in the area covered by CISA's (the Centro de Investigação em Saúde de Angola) Health Demographic Surveillance System (Fig 1).

This is a longitudinal prospective cohort study, involving 1692 children. Children were selected from maternity, and all children born in the term in which mothers signed informed consent, where included. Blood was obtaind by puncturing the heel and conserved in filter paper. All children were followed in quarterly medical consultation (Fig 2).

The G202A, A376G genotypes were determined through Real Time PCR methods in a Bio-Rad CFX Connect - Real-Time PCR Instrument(Fig 3).

For the enzyme activity NeoLISA kit was used for Neonatal screening of G6PD deficiency.

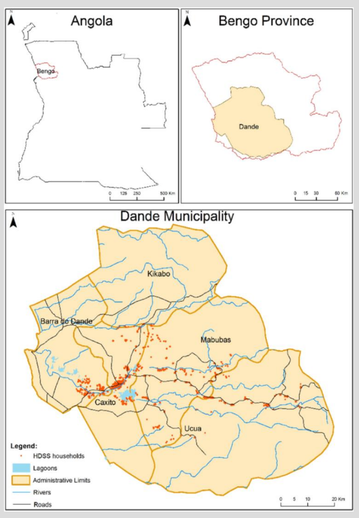

Fig 1. Study area

ANOVA was deployed to test for enzyme activity differences between genotypes. The exact test served to compare the mutation frequencies and the frequencies of clinical conditions associated to different genotypes. Statistical analysis was performed in SPSS 21.0 .

Fig 3. Real-Time PCR Instrument

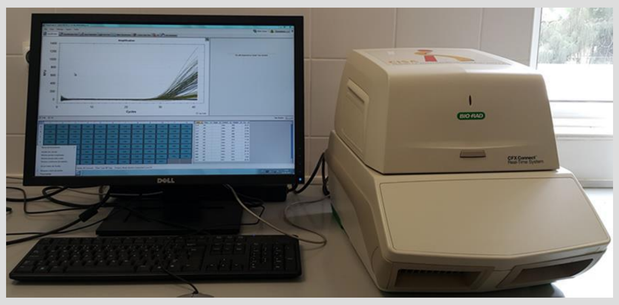

***Fig 2.* Medical consultation**

Results

Table 1. Genotypic frequencies (A) and Allelic frequencies for G6PD

A

Gender	Male (N=649)			Female (N=695)					
Genotype	B	A	A-	B/B	B/A	A/A	B/A-	A/A-	A-/A-
N	395	131	123	222	167	64	154	57	31
Freq (%)	(60.8%)	(20.2%)	(19.0%)	(31.9%)	(24.0%)	(9.2%)	(22.2%)	(8.2%)	(4.5%)

B

Allele	B	A	A-
N	1160	483	396
Freq	0,569	0,237	0,194

Results (cont.)

The prevalence of the G6PD A- allele was 19.4%, with 19% of hemizygous males and 4.5 % of homozygous females (table 1). Moreover 22.2% and 8,2% of heterozygous B/A- and A/A- heterozygous females respectively were observed. The enzyme activity was low in G6PD deficient in both sexes and with statistical significance from A and B alleles respectively for boys ($p < 0.001$) and for girls ($p < 0.003$).(table 2)

Table 2. G6PD enzyme activity by genotype and by sex

	Class				Activity		
	Normal	Intermediate	Deficiency	p [a]	U/g Hb	95% Conf	p [b]
Male							
B	25 (23.6%)	55 (51.9%)	26 (24.5%)		4.80	[4.15-5.46]	
A	7 (18.4%)	23 (60.5%)	8 (21.1%)		4.47	[3.49-5.44]	
A-	1 (3.7%)	4 (14.8%)	22 (81,5%)	p<0.001	1.66	[0.55-2.76]	p<0.001
Total	33	82	56				
Female							
B/B	18 (28.6%)	32 (50.8%)	13 (20.6%)		5.22	[4.21-6.22]	
B/A	7 (17.9%)	22 (56.4%)	10 (25.6%)		4.51	[3.58-5.43]	
A/A	4 (21.1%)	8 (42.1%)	7 (36.8%)		4.15	[2.78-5.50]	
B/A-	8 (19.5%)	19 (46.3%)	14 (34.1%)		3.62	[2.75-4.49]	
A/A-	3 (21.4%)	7 (50.0%)	4 (26.8%)		4.51	[2.84-6.18]	
A-/A-	0 (0.0%)	2 (18.2%)	9 (81.8%)	p=0.037	0.97	[0.32-1.62]	p=0.003
Total	40	90	57				

a- Pearson Chi-square, b - ANOVA

There seems to be a protection against malaria in heterozygous girls, since the lowest number of confirmed cases (7.7%) was observed in the class B/A- in a 18 months period (table 3).

Table 3. Association between genotype and clinical features

	Visit Hopsital	p [a]	Fever occurence	p [a]	Malaria (reported)	p [a]	Malaria (TDR)	p [a]
Male	(n=1349)		(n=1349)		(n=1349)		(n=770)	
B	329 (41.7%)		292 (37.0%)		100 (12.7%)		53 (11.5 %)	
A	169 (34.3%)		110 (34.6%)		37 (11.6%)		18 (10.5%)	
A-	78 (32.2%)	0.007**	72 (29.8%)	0.115	29 (12.0%)	0.880	19 (13.8%)	0.657
Total			474		166		90	
Female	(n=1413)		(n=1413)		(n=1413)		(n=765)	
B/B	184 (38.3%)		165 (34.3%)		47 (9.8%)		21 (8.0%)	
B/A	106 (30.9%)		101 (29.4%)		34 (9.9%)		24 (13.9%)	
A/A	46 (34.1%)		43 (31.9%)		15 (11.1%)		8 (12.1%)	
B/A-	88 (32.0%)		83 (30.2%)		24 (8.7%)		12 (7.7%)	
A/A-	37 (33.6%)		39 (35.5%)		13 (11.8%)		6 (9.4%)	
A-/A-	33 (47.8%)	0.052	28 (40.6%)	0.373	13 (18.8%)	0.241	11 (24.4%)	0.014*
Total			459		146		82	

a- Pearson Chi-square

Conclusions

The prevalence of G6PD deficiency among children in Bengo is considerable and is similar to that found in other parts of Africa. This data, can be used by Minestry of Health in order to make safe and appropriate decisions regarding the use of potentially unsafe drugs for G6PD deficient individuals.

Acknowledgements

We are grateful to the community members who participated in this study, and the hospital staff who helped collect the data.

Funding entities