

Prevalence of glucose-6-phosphate dehydrogenase deficiency and haemoglobin S in high and moderate malaria transmission areas of Muheza, north-eastern Tanzania

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Abstract: Glucose-6-phosphate dehydrogenase (G6PD) deficiency and haemoglobin S (HbS) are very common genetic disorders in sub Saharan Africa, where malaria is endemic. These genetic disorders have been associated with protection against malaria and are therefore under strong selection pressure by the disease. In November-December 2003, we conducted a cross-sectional survey to determine the prevalence of G6PD deficiency and HbS in the population and relate these to malaria infection and haemoglobin levels in lowland and highland areas of differing malaria transmission patterns of Muheza, Tanzania. Blood samples from 1959 individuals aged 6 months to 45 years were collected. A total of 415 (21%) and 1181 (60%) samples were analysed for G6PD deficiency and HbS, respectively. Malarial parasite prevalence was 17.2% (114/1959) in the highlands and 39.6% (49/1959) in the lowlands. Lowlands had higher prevalence of G6PD deficiency and HbS than highlands (G6PD deficiency = 11.32% (24/212) versus 4.43% (9/203), $P = 0.01$, and HbS = 16.04% (98/611) versus 6.32% (36/570), $P = 0.0001$). Logistic regression model showed an association between G6PD deficiency and altitude [lowlands] (Odds ratio [OR] 3.4, 95% CI=1.49; 7.90, $P=0.004$). In the lowlands, G6PD deficient individuals had lower mean haemoglobin (10.9g/dl) than normal ones (12.8g/dl), $P = 0.01$. These findings show that high malaria transmission in the lowlands might have selected for G6PD deficiency and HbS.

Keywords: malaria, haemoglobin S, glucose-6-phosphate dehydrogenase, Tanzania

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency and haemoglobin S (HbS) are very common genetic disorders in Sub-Saharan Africa where malaria is endemic. These genetic disorders have been associated with protection against malaria morbidity (Moormann *et al.*, 2003). Studies have shown that children with sickle cell trait (HbAS) are protected from both mild and severe malaria (Aidoo *et al.*, 2002). HbAS is also associated with reduced parasite densities during intercurrent *Plasmodium falciparum* infections (Williams *et al.*, 2005) and enhanced acquired immunity, which suggests that HbAS probably protects against malaria infection due to increased parasite clearance and induction of antibodies (Cabrera *et al.*, 2005). These two traits are therefore under strong selection pressure by the disease (Francis & Pete, 2006).

G6PD deficiency is associated with many clinical disorders, such as neonatal jaundice, haemolytic anaemia and several cardiovascular diseases (Beutler, 1994). Some studies have shown that *P. falciparum* parasite densities are lower in G6PD deficient individuals than those with normal (Ruwende *et al.*, 1995). The enzyme is very polymorphic in many parts of the world, existing in four most common G6PD variants, namely, G6PD A, A-, B and G6PD Mediterranean. G6PD A has a normal enzyme activity and is commonly found in Africa; G6PD A- has 8 – 20% of

normal enzyme activity and is also mainly found in Africa. G6PD B has a normal enzyme activity and is distributed worldwide while G6PD Mediterranean has less than 5% of normal enzyme activity and is found in the Middle East (Mehran, 2003). Some studies in African children have shown that G6PD A- is associated with 46 – 58% reduction in risk of severe malaria for both female heterozygotes and male hemizygotes and probably homozygotes have a similar level of protection from malaria, although this genotype is quite rare (Ruwende *et al.*, 1995).

Some individuals, particularly among populations of African, Asian or those of Mediterranean origin are apt to develop haemolysis when given therapeutic doses of the antimalarials 8-aminoquinolines and sulphonamides. This defect is due to diminished activity of the enzyme glucose-6-phosphate dehydrogenase which catalyses the first and rate controlling step in the hexose monophosphate shunt metabolic pathway (Luzzatto *et al.*, 2001). Despite that, very few studies on these two genetic disorders have been conducted in north-eastern Tanzania. Therefore, we conducted this study to determine the prevalence of G6PD deficiency and HbS in the population and to relate these to malaria infection and haemoglobin levels. Results from this study will provide baseline information on G6PD deficiency and HbS in an area with difference transmission intensity which will be useful in designing and implementing different malaria interventions.

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Materials and methods

Study area and population

Muheza district is topographically divided into highland and lowland strata. Malaria is holoendemic in the lowlands with prevalence of over 75% (Lyimo, *et al.*, 1991). In the highlands, there is a modest varied level of malaria transmission whereby in some areas prevalence is 33% (Ellman *et al.*, 1998). In both strata, malaria transmission is highest during and following long rainy season, which usually extends from March through July. Ethnically, the population of Muheza comprises of the Wasambaa in the highlands and Wabondei and Wadigo in the lowlands. The study involved four villages (Kizerui, Kwelumbizi, Shambangeda and IBC Msasa) situated within the East Usambara Mountains in the highlands (600–1000 m) and four villages (Moa, Kwangena, Mwandusi and Jirihini) in the lowlands (0–300 m). Both the highlands and lowlands communities practice subsistence farming of maize, cassava, and banana. Production of coconuts and fishing is common among some communities in the lowland villages.

Study design and sample collection

This was a cross-sectional study which was conducted during the short rains in November-December 2003. A random sample of 1959 individuals aged 6 months to 45 years was selected for the survey and sub-samples were later chosen for laboratory analysis. Capillary blood samples of about 300 - 500µl were collected into EDTA anticoagulant tubes. Due to limitations of laboratory reagents, a random sample of 21% (415/1959) and 60% (1181/1959) were analyzed for G6PD deficiency and HbS, respectively.

Thick and thin blood smears for malaria parasite diagnosis were prepared. Identification and quantification of Giemsa stained blood slides for malaria parasites were done using a light microscope. Haemoglobin levels were estimated using Haemocue machine. Determination of G6PD deficiency was done using fluorescent screening test (Dacie & Lewis, 1995). Briefly, 10 µl of EDTA anticoagulated blood

was added to 50 µl of the reagent mixture and then 10 µl of the reaction mixture (D-glucose-6-phosphate, NADP, Saponin, oxidized glutathione, Tris aminomethane, HCl, MgCl₂, MTT, PMS, Azide) was applied immediately onto Whatman No. 3 filter paper at the beginning of the reaction and after 5 and 10 minutes. Control samples of known G6PD deficient and normal were run together with the tested samples. The spots were examined under Ultra Violet (UV) light. The G6PD normal samples fluoresced while deficient ones did not fluoresce. Haemoglobin S was determined using sickling test (Dacie & Lewis, 1995). Briefly, 5 µl of freshly prepared reagent was added to 1 µl of EDTA anticoagulated blood on a slide and then sealed with cover glass using petroleum jelly. Control samples of known HbS and HbA were also included with test samples and then kept overnight in a moistened chamber. Slides were examined using light microscope. Sickling took place in HbS blood samples but not on HbA blood samples.

Data analysis

All data were entered and verified in Epi Info 6.04d while statistical analysis was done using STATA software version 8 (STATA Corp., College station, TX). Prevalence of G6PD and HbAS in bivariate variables was compared using χ^2 or Fisher's exact tests. Geometric mean density (GMI) of malaria parasites and Hb concentration in G6PD deficiency and HbAS groups were compared using T and F tests. Multivariate logistic regression model was used to estimate risk factors associated with the prevalence of G6PD deficiency.

Results

Malarial parasite prevalence was 17.2% in the highlands and 39.6% in the lowlands. Among individuals examined for G6PD deficiency, the Wasambaa was the dominant ethnic group accounting for 76.9% (n = 156) in highlands while in the lowlands Wadigo accounted for 49.5% (n = 105) of the population followed by Wasegeju (20.3%; n = 43) and Wamakonde (15.6%; n=33). In the lowlands, the Wasambaa contributed to 3.3% (n = 7) only.

Table 1: Prevalence of glucose- 6-phosphate dehydrogenase (G6PD) deficiency and haemoglobin S in lowland and highland areas of Muheza

Genetic factor	Lowlands	Highlands	Test statistics
G6PD deficiency	24/212 (11.32%)	9/203 (4.43%)	$\chi^2=6.72$, p = 0.01
Haemoglobin S	98/611 (16.04%)	36/570 (6.32%)	$\chi^2=27.72$, p< 0.001

The prevalence of G6PD deficiency was significantly higher in lowland compared to highland areas (G6PD = 11.32% vs. 4.43%, $P=0.01$) (Table 1). Similarly, the prevalence of HbS was significantly higher in lowland compared to highland areas (16.04% vs. 6.32%, $P<0.001$) (Table 1). Individuals with HbS had lower level of parasites density than normal ones in both strata, although the difference was not statistically significant (Table 2). There was no significant relationship between HbS and Hb levels and sex (Table 2). Among

results showed that in highlands, sex (male) was more associated with G6PD deficiency although the difference was not significant (Table 2).

Discussion

Like many other studies in Muheza, Tanzania, malaria parasite prevalence was higher among individuals living in the lowlands than highlands (Kamugisha, 2005; Maxwell *et al.*, 2003). The prevalence of G6PD deficiency and HbS was significantly higher in lowland

Table 2: Distribution of *P. falciparum* parasite prevalence, geometric mean parasite density, (GMPD) haemoglobin level and sex with respect to G6PD and HbS in lowland and highland areas of Muheza

Stratum	Lowlands (n=212)			Highlands (n=203)			Lowlands (n=611)			Highlands (n=570)		
	G6PD-	G6PD+	Overall	G6PD-	G6PD+	Overall	HbS	HbAA	Overall	HbS	HbAA	Overall
Prevalence (%)	10.7	39.9	39.6	0	18.0	17.2	38.2	31.7	37.2	8.3	18.5	17.9
Test Statistic	$\chi^2=0.005, P=0.82$			$\chi^2=1.96, P=0.161$			$\chi^2=1.52, P=0.217$			$\chi^2=2.39, P=0.122$		
GMPD (parasites/ μ l)	657	644	645	0	647	647	363	579	543	225	448	439
Test statistic	F=0.04, P=0.841			F=1.82, P=0.179			F=1.92, P=0.168			F=0.49, P=0.484		
Haemoglobin (g/dl)	10.9	12.8	11.01	12.0	11.5	12.0	11.1	10.9	11.0	12.1	11.8	11.8
Test statistic	T=2.768, P=0.01			t=1.104, P=0.297			t=0.49, P=0.622			t= 0.69, P=0.488		
Sex (% of males)	54.2	40.4	42.0	66.7	37.1	38.2	40.7	43.9	41.24	47.2	41.6	41.9
Test statistic	$\chi^2=1.65, P=0.199$			$\chi^2=3.18, P=0.075$			$\chi^2=0.33, P=0.563$			$\chi^2=0.44, P=0.506$		

Key: G6PD- = G6PD deficiency; G6PD+ = G6PD normal; HbS = abnormal haemoglobin; HbAA = normal haemoglobin

individuals sampled for G6PD analysis (Table 2), *P. falciparum* parasite prevalence was lower in G6PD deficient than in normal G6PD individuals in both strata. Parasite density was lower in G6PD deficient individuals in the highland. In the lowlands, haemoglobin levels were significantly higher in individuals with normal G6PD (Table 2) compared with those with G6PD deficiency (12.8 vs. 10.9g/dl; $P=0.01$).

Results from a logistic regression model fitted for strata, sex, Hb levels and parasite prevalence, showed that the significant variable associated with G6PD deficiency was strata [lowlands](Odds ratio [OR] 3.4, 95% CI=1.49; 7.90, $P=0.004$). Sex (female) had reduced effect on the G6PD deficiency and Hb levels. However, the difference was not statistically significant (OR=0.49, $P=0.069$ and OR=1.19, $P=0.081$) for sex and Hb, respectively. A logistic regression model was also fitted for the two strata separately where

compared to highland areas. Individuals with HbS had lower level of parasites density than normal ones in both strata, although the difference was not statistically significant. In both the highlands and lowlands villages, *P. falciparum* parasite prevalence was lower in G6PD deficient than in normal G6PD individuals. Parasite density was lower in G6PD deficient individuals in the highland. The G6PD deficiency is known to provide partial protection against malaria, by providing defective environment in the affected red cells. In the lowlands, haemoglobin levels were significantly higher in individuals with normal G6PD.

A previous study (Awah & Uzoegwu, 2006) showed that less severe clinical malaria symptoms were observed more in G6PD deficient HbAS when compared to G6PD normal HbAA subjects during malaria attack. Therefore inheriting both genetic disorders reduces malarial anaemia, parasitaemia and

severe malarial symptoms. Like in our study, a recent study in Kenya (Moorman *et al.*, 2003) observed higher prevalence of HbS and G6PD deficiency in the lowlands compared to the highlands. Another study in north-eastern Tanzania, using a more robust screening technique (Enevold *et al.*, 2007) found prevalence of HbS of around 16.0% in Mgome village in the lowlands of Muheza district. Using the rapid screening test, we observed a similar prevalence rate of HbS in lowland Muheza.

The observation of a higher prevalence of HbS and G6PD deficiency genes in the lowlands is likely to be directly linked to the high malaria transmission. The reduced parasites density seen in individuals with HbS in both lowland and highland areas suggest that malaria has already exerted a strong selective pressure in the human populations at both sites. In conclusion, G6PD deficiency and HbS genes may provide a natural protection against malaria.

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