View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Digital Library of the Tanzania Health Community

Int J Mol Epidemiol Genet 2014;5(4):185-194
www.ijmeg.org /ISSN:1948-1756/IJMEG0003077

Original Article

Red blood cell indices and prevalence of hemoglobinopathies and glucose 6 phosphate dehydrogenase deficiencies in male Tanzanian residents of Dar es Salaam

Solomon Mwakasungula[1,2,3*], Tobias Schindler[1,2*], Said Jongo[3], Elena Moreno[3], Kasimu Kamaka[3], Mgeni Mohammed[3], Selina Joseph[3], Ramla Rashid[3], Thabit Athuman[3], Anneth Mwasi Tumbo[3], Ali Hamad[3], Omar Lweno[3], Marcel Tanner[2,4], Seif Shekalaghe[3], Claudia A Daubenberger[1,2]

[1]*Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland;* [2]*University of Basel, Basel, Petersplatz 1, CH-4003 Basel, Switzerland;* [3]*Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo, P.O. Box 74, Tanzania;* [4]*Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland.* [*]*Equal contributors.*

Received October 10, 2014; Accepted November 12, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Hemoglobinopathies, disorders of hemoglobin structure and production, are one of the most common monogenic disorders in humans. Glucose 6 phosphate dehydrogenase deficiency (G6PD) is an inherited enzymopathy resulting in increased oxygen stress susceptibility of red blood cells. The distributions of these genetic traits in populations living in tropical and subtropical regions where malaria has been or is still present are thought to result from survival advantage against severe life threatening malaria disease. 384 male Tanzanian volunteers residing in Dar es Salaam were typed for G6PD, sickle cell disease and α-thalassemia. The most prominent red blood cell polymorphism was heterozygous α^+-thalassemia (37.8%), followed by the G6PD(A) deficiency (16.4%), heterozygous sickle cell trait (15.9%), G6PD(A-) deficiency (13.5%) and homozygous α^+-thalassemia (5.2%). 35%, 45%, 17% and 3% of these volunteers were carriers of wild type gene loci, one, two or three of these hemoglobinopathies, respectively. We find that using a cut off value of 28.6 pg. for mean corpuscular hemoglobin (MCH), heterozygous α^+-thalassemia can be predicted with a sensitivity of 84% and specificity of 72% in this male population. All subjects carrying homozygous α^+-thalassemia were identified based on their MCH value < 28.6 pg.

Keywords: Sickle cell trait, glucose 6 phosphate dehydrogenase deficiency, α-thalassemia, screening, mean corpuscular hemoglobin, whole blood count, Tanzania, malaria

Introduction

Hemoglobinopathies are prominent in populations that have historically been or are still exposed to infection with the apicomplexan parasite *Plasmodium falciparum* [1]. The distribution of all hemoglobinopathies seems to be extremely heterogeneous within countries, and even within small geographical distances [2]. The most prevalent variant of the HbB gene carries at amino acid position 6 Glu, is named HbA, and represents the wild type, α-globin protein. The exchange from Glu to Val (E6V) gives rise to the structural α-globin variant HbS, which represents the most common pathological hemoglobin mutation worldwide inherited as an autosomal recessive Mendelian trait [3]. Heterozygote carriers of the sickle cell trait (HbAS) are distributed widely since the trait confers protection from severe malaria and malaria related death [4]. Persons with homozygote sickle cell disease (HBSS) suffer from anemia and repeated crisis, which eventually results in death at young age when left untreated reaching up to 92% of excess mortality [5]. Reduced production of the α-globin protein results in α-thalassemia that is based on a number of different of mutations in the HbA gene. In some malaria endemic countries like Papua New Guinea the α-thalassemia trait reaches carrier

frequencies up to 80%-90% [6], while in sub-Saharan Africa it is estimated to occur at frequencies of 10%-25% [7]. There are at least 40 different deletional mutations that cause α-thalassemia with the 3.7 kb deletion being the most common in Africa. The phenotype of α-thalassemia is directly related to the number of α-globin genes affected. They are divided into the α^+-thalassemias, in which one of the linked pair of a genes is deleted (-α/αα) and in the α°-thalassemia, in which both genes are deleted (--/αα) [8].

Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) is a housekeeping enzyme that catalyses the first step of the pentose phosphate pathway [9]. The reaction of this step produces NADPH which maintains high levels of reduced glutathione in cells. Reduced glutathione is a biological antioxidant which is critical in detoxification and protection of red blood cells from oxidative stress [10]. G6PD deficiency is an X-linked enzymopathy and among the most common genetic disorders caused by single amino acid exchanges in humans [11]. In sub-Saharan Africa, G6PD deficiency is present in essentially three different allelic forms. G6PD(B) is the most common allele expressing the physiological, normal enzymatic activity. G6PD(A) is associated with an amino acid substitution at position 126 where Asn is changed to Asp (N126D) resulting in about 15% loss of enzymatic activity. In the G6PD(A-) variant, the combination of the N126D polymorphism with an additional exchange at position 68 from Val to Met (V68M) leads to a loss of 90% of the enzyme function. While most individuals with the G6PD(A-) allele are asymptomatic, acute hemolytic anemia can manifest in hetero- and homozygous females as well as hemizygous males upon enhanced oxidative stress of red blood cells including the treatment with the anti-malarial drug Primaquine [12].

Currently, we perform several clinical trials testing the protection conferred by repeated intravenous application of purified, cryopreserved, live irradiation attenuated *P. falciparum* sporozoites as live malaria vaccine [13, 14]. The impact of hemoglobinopathies and G6PD deficiencies on vaccination outcome and protection against controlled human malaria infection is currently unclear and therefore only volunteers with wild type genotypes for these red blood cell polymorphisms are currently enrolled.

Here, we identified by molecular tools the prevalence and co-inheritance of the sickle cell disease, α-thalassemia traits and the G6PD deficiency in 384 unrelated, clinically healthy male Tanzanian volunteers recruited from higher education institutions in Dar es Salaam. Additionally, we analysed the impact of different combinations of hemoglobinopathies and G6PD deficiency on haematological parameters including red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The data will inform on future enrolment strategies for drug or vaccine based interventions in malaria in the coastal region of Tanzania.

Materials and methods

Ethics statement

The institutional review board (IRB) of the Ifakara Health Institute (IHI; Dar es Salaam, United Republic of Tanzania) approved the protocol of the project conducted at the Bagamoyo Clinical Trial Unit of the Bagamoyo Research and Training Center (BRTC) in the United Republic of Tanzania (IHI/IRB/No: 06-2013).

Volunteer recruitment

Volunteers were recruited from nearby colleges and/or universities located in Dar es Salaam. Through fliers and posters advertisement, interested volunteers were invited to attend a sensitization meeting conducted first within each college/university premises and later in Dar es Salaam bringing together all those who showed interest during the first meeting. After thorough explanation about the study design and procedures, interested volunteers were asked to register their contact details on the sign-up sheet and fill in an initial screening questionnaire. Volunteers, who were identified to be potentially eligible for the visit, were contacted and invited to attend a visit at the Bagamoyo Clinical Trial Unit located in Bagamoyo-Pwani, Tanzania. During this visit, more detailed information about study design, procedures, benefit and risk for participation were provided. Individual informed consent was obtained and volunteers willing to participate in the study were asked to sign the informed consent form. Potential volunteers who signed the written informed consent were

asked to provide a blood sample in order to perform different tests including the hemoglobinopathies. Volunteers who's clinical and laboratory test results were abnormal and indicated medical problems, they were referred to the closest nearby appropriate health facility (Muhimbili National Hospital) for further investigations and management.

Sample collection and DNA extraction

1 mL of venous whole blood was collected in EDTA tubes and stored at -80°C. DNA was extracted from 100 µL of whole blood with ZR Genomic DNA™-Tissue MiniPrep (Zymo Research, Irvine, USA) according to manufacturer's recommendations. Extracted DNA was then stored at -20°C for the next step of erythrocyte polymorphism screening.

Genotyping of erythrocyte polymorphisms

Depending on the a thorough literature survey [15, 16] and a genotyping dataset of about 40 volunteers from previous trials [14] PCR systems in combination with amplicon sequencing for the detection of single nucleotide polymorphisms (SNPs) such as the hemoglobin variant HbS and the G6PD variants (A) and (A-), as well as a gap-PCR system for the detection of the 3.7 kb deletion causing α-thalassemia were established. Briefly, a gene fragment containing exons 1 and 2 of the HbB gene including codon 6 that determines the HbS and HbC hemoglobin variants were amplified according to Clark et al 2004 [17] and sequenced. The G6PD(A) and G6PD(A-) were detected by amplification of the G6PD Exons 2-4 as described by Benmansour et al 2013 [9] followed by sequencing. All amplicons were shipped and sequenced by a commercial company (Inqaba Biotec, Pretoria, South Africa). The sequenced amplicons were aligned against reference genes for HbB and G6PD (NCBI Gene ID: 3043 and 2539, GRCh37.p10) and sequence polymorphisms were identified using the human single nucleotide polymorphism database (dbSNP, build 137). The sequence analysis was performed using the Geneious 6.1.5 software package (Biomatters, Auckland, New Zealand).

The α-thalassemia gap-PCR developed by Tan et al 2001 [18] was used to identify the 3.7 kb deletion within the α-globin gene loci. PCR reactions contained 1x Q-Solution for GC-rich templates, HotStarTaq DNA polymerase provided in Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 100 ng of genomic DNA, 0.1 µM primers for the amplification of the 2029 bp junction fragment for the 3.7 kb deletion and primers for the amplification of a 1800 bp α-hemoglobin control fragment. All PCR reactions were carried out on standard thermocycler (Biometra GmbH, Göttingen, Germany), with an initial 15-minute denaturation at 96°C, 30 cycles of 98°C for 45 seconds, 60°C for 90 seconds, 72°C for 135 seconds, and a final extension at 72°C for 5 minutes. Following amplification, 10 µL of product was electrophoresed through a 1.5% agarose gel with 0.6 µg/mL ethidium bromide in 1x TBE buffer first at 7 volts/cm for 1 hour followed by 3 volts/cm for additional two hours. The samples were analyzed due to the presence or absence of the junction fragment for the 3.7 kb deletion in combination with the α- hemoglobin control fragment and their genotype was assigned as wild type, heterozygous or homozygous for α-thalassemia.

Hematological analysis

Whole blood hematology of 382 participants were measured including red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Briefly, whole blood was collected in EDTA tubes, mixed and analyzed using the automated Hematology Analyzer XS-800i (Sysmex Corporation, Kobe, Japan) following calibrator and control set up as recommended by the manufacturer. Samples were analyzed within 30 min of collection.

Data management and statistical analysis

All genotyping results and Complete Blood Counts (CBC) data were entered and organized in Microsoft Access 2010 electronic database (Microsoft Corporation 2010). Data were analyzed using R, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) [19]. The statistical analysis of the hematological data was done for 382 volunteers (2 volunteers had their hematological information missing), grouped according to their well-defined genotypes. Descriptive statistics, including mean

Table 1. Summary of red blood cell polymorphisms in 384 male Tanzanian volunteers analyzed

Red blood cell polymorphisms	Volunteers (n)	Prevalence (%)
αα/αα, HbAA, G6PD(B)	133	34.6
-α/αα, HbAA, G6PD(B)	86	22.4
-α/-α, HbAA, G6PD(B)	7	1.8
αα/αα, HbAS, G6PD(B)	19	5.0
αα/αα, HbAA G6PD(A)	34	8.9
αα/αα, HbAA, G6PD(A-)	27	7.0
-α/αα, HbAS, G6PD(B)	20	5.2
-α/-α, HbAS, G6PD(B)	4	1.0
-α/αα, HbAA, G6PD(A)	14	3.7
-α/αα, HbAA, G6PD(A-)	15	3.9
-α/-α, HbAA, G6PD(A)	3	0.8
-α/-α, HbAA, G6PD(A-)	4	1.0
αα/αα, HbAS, G6PD(A)	3	0.8
αα/αα, HbAS, G6PD(A-)	3	0.8
-α/αα, HbAS, G6PD(A)	7	1.8
-α/αα, HbAS, G6PD(A-)	3	0.8
-α/-α, HbAS, G6PD(A)	2	0.5

values and 95% confidence intervals (95% CIs) were used to describe hematological features such as RBC, HGB, HCT, MCV, MCH, and MCHC. Statistical significance between the wild type group and groups expressing RBC polymorphisms was assessed using Welch's t test. All *P*-values < 0.05 were considered statistically significant.

Based on the Receiver Operator Characteristic curve (ROC) and Youden's J statistic [20] the optimal MCH cut-off for prediction of the homozygous and heterozygous α^+-thalassemia trait was calculated using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium).

Results

384 unrelated male volunteers living in Dar es Salaam were invited to participate in the study. Results of the molecular typing for α-thalassemia, sickle cell trait and G6PD deficiencies are summarized in **Table 1**. When we analyzed these typing data for expression of one distinct red blood cell polymorphism, we found the following prevalence: 34.6% wild type (αα/αα, HBAA, G6PD(B), 22.4% heterozygous α^+-thalassemia (-α/αα, HBAA, G6PD(B)), 8.9% G6PD(A) deficiency (αα/αα, HBAA G6PD(A)), 7% G6PD(A-) deficiency (αα/αα, HBAA, G6PD(A-)), and 5% heterozygous sickle cell trait (αα/αα, HbAS, G6PD(B)). Homozygous α^+-thalassemia only (-α/-α, HBAA, G6PD(B) was detected in 1.8% of persons tested. Several combinations of these red blood cell polymorphisms in addition were observed. The most prevalent were -α/αα, HbAS, G6PD(B) at 5.2%, -α/αα, HBAA, G6PD(A) at 3.9% and -α/αα, HBAA, G6PD(A-) at 3.6% (**Table 1**).

Complete RBC indices among the participants with different underlying genotypes are given in **Table 2**. When comparing the hematologic features of wild type volunteers versus the hetero- and homozygous α^+-thalassemia carriers, the HGB, HCT, MCV and MCH values were significantly lower while the RBC was significantly higher. In **Figure 1**, the different RBC indices are presented as box plots with the statistically significant differences (p-value < 0.05) in relation to the wild type volunteers indicated. Clearly, α^+-thalassemia has the most prominent impact on RBC indices, followed by G6PD(A-) and G6PD(A) deficiencies (**Figure 1**).

Based on the receiver operator curve (**Figure 2**), the hematological parameter most suitable for identification of male α^+-thalassemia carriers was a cut off value of 28.6 pg MCH. The positive predictive value of MCH based on 28.6 pg was 69% and the negative predictive value was 85%. The sensitivity for heterozygous α^+-thalassemia carrier identification was 84% and the specificity was 72%. Additionally, all of the 20 homozygous α^+-thalassemia carriers were correctly identified using 28.6 pg MCH.

Discussion

The expansion and fixation of hemoglobinopathies within the human population is driven by conferring a survival advantage for heterozygote carriers against severe, life-threatening *P. falciparum* disease [21]. Our study performed with 384 unrelated, healthy, randomly selected male Tanzanian volunteers living in Dar es Salaam showed that 133 (34.6%) expressed the wild type αα/αα, HBAA, G6PD(B) genotypes for the three different red blood cell polymorphisms tested. Amongst the hemoglobinopathies, heterozygous α^+-thalassemia was most prominent with 37.8% (n = 145) of the volunteers being carriers. 20 (5.2%) volunteers were

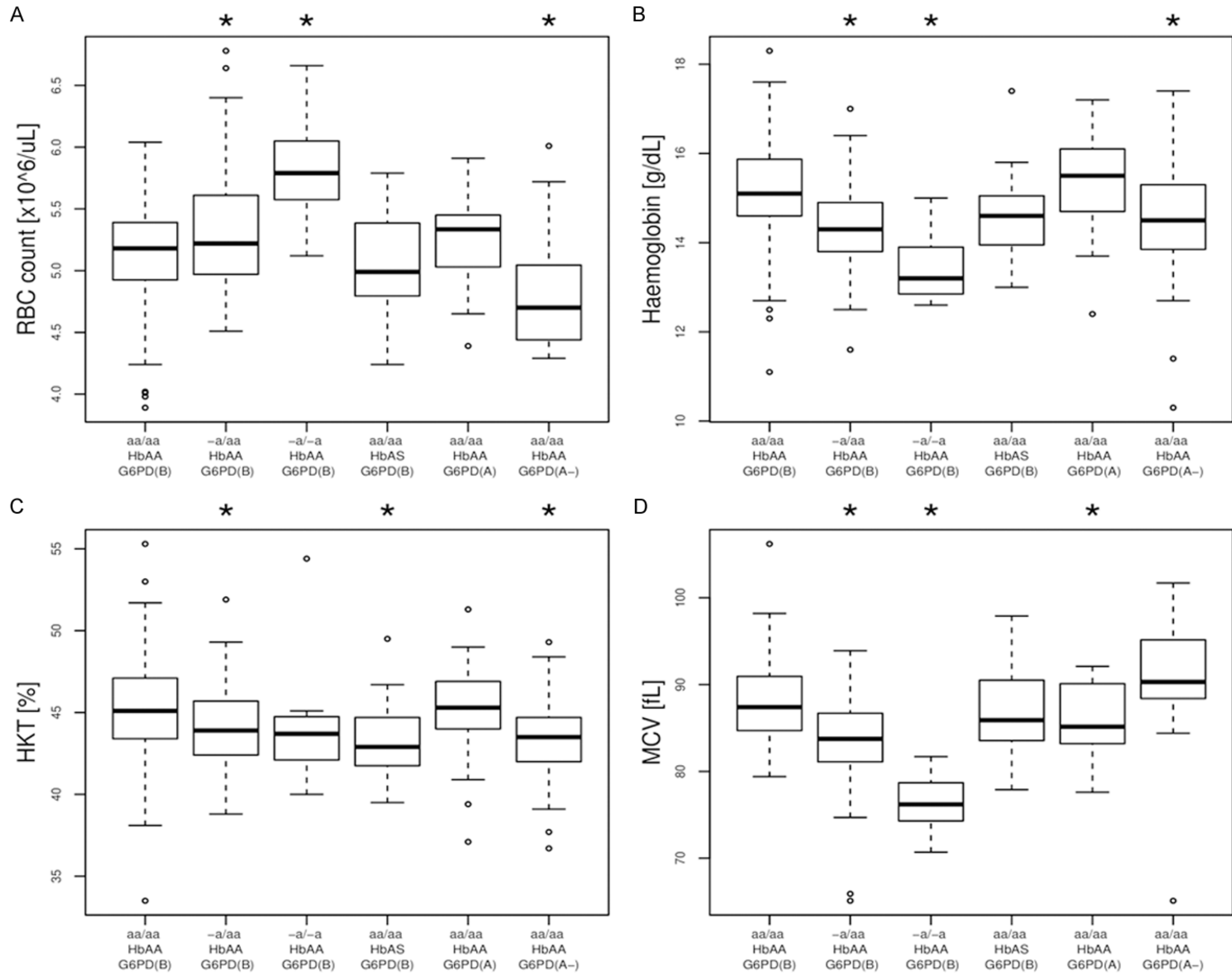
Table 2. Haematological parameters assessed in 382 male volunteers carrying different red blood cell polymorphisms

Red blood cell polymorphisms	RBC [x10^6/uL] ± 95% CI	HGB [g/dl] ± 95% CI	HCT [%] ± 95% CI	MCV [g/dl] ± 95% CI	MCH [pg] ± 95% CI	MCHC [g/dl] ± 95% CI
all volunteers	5.2 ± 0.1	14.7 ± 0.3	44.4 ± 0.6	85.7 ± 1.3	28.3 ± 0.5	33.0 ± 0.3
αα/αα, HbAA, G6PD(B)	5.1 ± 0.2	15.1 ± 0.4	45.1 ± 1.0	88.2 ± 1.6	29.5 ± 0.5	33.5 ± 0.4
-α/αα, HbAA, G6PD(B)	5.3 ± 0.2	14.3 ± 0.3	44.1 ± 1.1	83.4 ± 2.0	27.1 ± 0.7	32.5 ± 0.5
-α/-α, HbAA, G6PD(B)	5.8 ± 0.9	13.5 ± 1.7	44.5 ± 8.7	76.4 ± 7.2	23.1 ± 1.3	30.4 ± 3.2
αα/αα, HbAS, G6PD(B)	5.0 ± 0.5	14.6 ± 0.9	43.5 ± 2.3	86.9 ± 5.2	29.2 ± 1.6	33.6 ± 1.0
αα/αα, HbAA G6PD(A)	5.3 ± 0.3	15.3 ± 0.8	45.2 ± 2.0	86.0 ± 2.8	29.1 ± 0.8	33.9 ± 0.9
αα/αα, HbAA, G6PD(A-)	4.8 ± 0.4	14.5 ± 1.2	43.5 ± 2.5	91.0 ± 5.6	30.3 ± 2.4	33.3 ± 1.4
-α/αα, HbAS, G6PD(B)	5.4 ± 0.5	14.4 ± 1.1	43.8 ± 2.9	81.3 ± 5.2	26.6 ± 1.6	32.8 ± 1.1
-α/-α, HbAS, G6PD(B)	5.5 ± 1.5	13.2 ± 1.1	41.2 ± 7.2	75.9 ± 21.1	24.4 ± 6.7	32.2 ± 3.3
-α/αα, HbAA, G6PD(A)	5.4 ± 0.5	14.4 ± 2.0	44.8 ± 4.7	82.7 ± 9.6	26.6 ± 3.7	32.1 ± 1.6
-α/αα, HbAA, G6PD(A-)	5.0 ± 0.5	14.2 ± 1.4	44.1 ± 3.6	87.8 ± 4.4	28.2 ± 1.3	32.1 ± 1.5
-α/-α, HbAA, G6PD(A)	6.1 ± 0.5	13.3 ± 5.0	44.1 ± 18.4	72.2 ± 30.6	21.8 ± 8.0	30.3 ± 2.7
-α/-α, HbAA, G6PD(A-)	5.3 ± 0.8	12.4 ± 2.0	41.0 ± 7.9	77.4 ± 11.5	23.4 ± 1.6	30.3 ± 4.9
αα/αα, HbAS, G6PD(A)	5.0 ± 1.8	14.6 ± 2.8	43.9 ± 10.2	87.5 ± 18.6	29.2 ± 10.3	33.3 ± 4.9
αα/αα, HbAS, G6PD(A-)	4.9 ± 1.2	14.5 ± 5.0	42.5 ± 20.8	87.0 ± 23.4	29.7 ± 5.5	34.1 ± 6.4
-α/αα, HbAS, G6PD(A)	5.2 ± 0.3	14.4 ± 1.3	42.7 ± 5.8	82.5 ± 9.3	27.8 ± 2.8	33.7 ± 3.1
-α/αα, HbAS, G6PD(A-)	5.0 ± 0.7	14.1 ± 5.3	43.5 ± 11.2	86.7 ± 23.3	28.1 ± 13.2	32.3 ± 9.2
-α/-α, HbAS, G6PD(A)	6.1 ± 4.4	13.7 ± 25.4	43.2 ± 19.1	70.5 ± 20.4	22.3 ± 25.4	31.8 ± 44.5

homozygous for α^+-thalassemia. α^+-thalassemia was exclusively due to the 3.7 kb deletion which has been reported to be the most prevalent mutation causing α^+-thalassemia in Sub-Saharan Africa [8]. In Eastern Africa, the prevalence of this mutation exceeds 50% while other known mutations have been reported to be rare [22, 23]. We have not detected the more severe form of α^+-thalassemia, the α°-thalassemia trait that is based on loss of both α-globin genes on a single chromosome. In Tanzania, the prevalence of α^+-thalassemia in different populations was shown to depend strongly on malaria endemicity levels. It ranges from 10-25% in high altitude villages with little malaria transmission up to 45-55% in populations living in low altitude villages with high malaria transmission [24]. In Dar es Salaam, malaria endemicity is highly focal [25] and the urban population is a mixture of mainly young Tanzanians that flock into town for higher education and employment.

Piel et al., estimated a prevalence of 7.5%-12.5% of HbAS in the coastal region of Tanzania [4]. Enevold et al., reported on HbAS prevalence in Tanzania to range between 0-14% [24]. Here, prevalence of the HbAS was 15.9% fitting into these estimates. The annual number of newborns with sickle cell disease, estimated to be 305,800 (CI: 238,400-398,800) globally in 2010, will likely increase by about one-third by 2050 [26]. In Jamaica, long-term cohort studies have provided invaluable information about the natural history of sickle cell disease and improvements in its control and management [5]. HBSS children need special attention like vaccination with the conjugate pneumococcal vaccine and regular penicillin treatment [5]. In contrast, very little is published about the natural history of sickle cell disease in Tanzanian children, where it is likely to contribute significantly to early childhood mortality [5, 27].

Sixty three (16.4%) and 52 (13.5%) of our volunteers were hemizygous for the G6PD(A) and G6PD(A-) enzymopathies respectively. Shekalaghe et al., showed that the prevalence of heterozygous G6PD(A) deficiency was 9.7% and the G6PD(A-) homozygous/hemizygous was 3.7% in Northern Tanzania [28]. In contrast, Segeja et al., who used a fluorescent screening test found a prevalence of up to 76.9% in some ethnic groups in Muheza district of Tanzania. These differences in prevalence could be due either to the screening method used or the population analyzed [29].


A
B
C
D
*
RBC count [x10^6/uL]
Haemoglobin [g/dL]
HKT [%]
MCV [fL]
aa/aa HbAA G6PD(B)
-a/aa HbAA G6PD(B)
-a/-a HbAA G6PD(B)
aa/aa HbAS G6PD(B)
aa/aa HbAA G6PD(A)
aa/aa HbAA G6PD(A-)

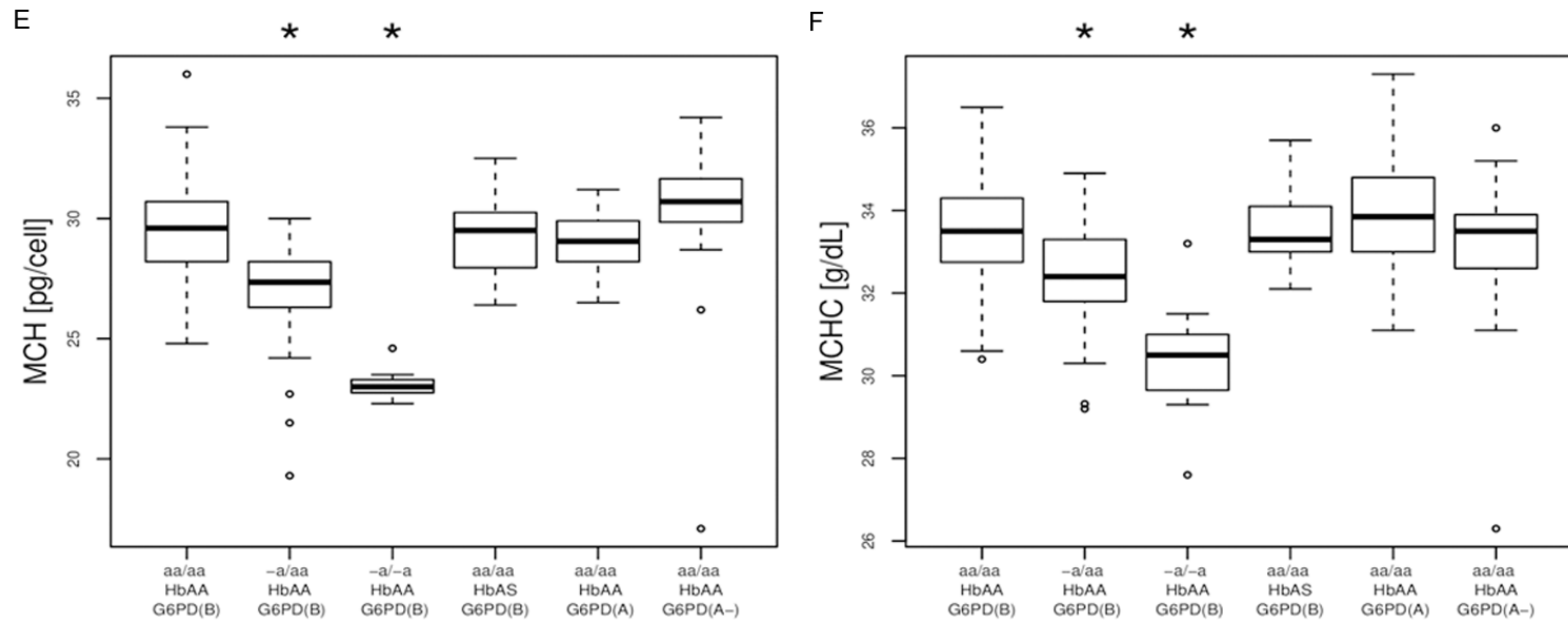


Figure 1. Box plots for RBC indices. Box plots representing the mean and lower and upper quartiles for RBC count (A), Hemoglobin (B), HKT (C), MCV (D), MCH (E) and MCHC (F) in the groups of volunteers expressing one particular RBC polymorphism given on the x-axis. Statistically significant deviations from values obtained in the wild type population are marked (*).

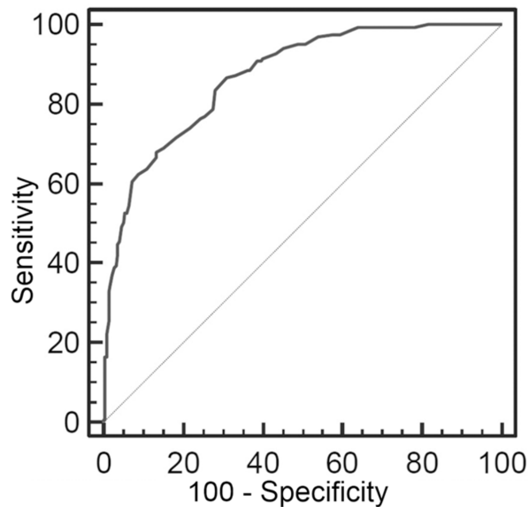


Figure 2. ROC analysis for MHC values within the α⁺-thalassemia population. ROC analysis for MCH defining 28.6 pg. as a suitable value for identification of heterozygous and homozygous male α⁺-thalassemia carriers.

In agreement with published RBC indices for α-thalassemia [8], the α⁺-thalassemia carriers had higher RBC counts compared to the wild type volunteers. Values for HBG, HKT, MCV, MCHC and MCH were all significantly reduced in comparison to wild type volunteers, typical for a microcytic, hypochromic anemia. The level of the microcytic, hypochromic anemia depends roughly on the number of α-genes mutated. This allows for using the hematology parameters as a first screening method for detection of volunteers carrying α-thalassemia. When using a cut off of 28.6 pg MCH, we were able to identify 138 of the 165 volunteers that carried homozygous or heterozygous α⁺-thalassemia. This cut off point can be used in future for preselection of male volunteers eligible for inclusion into malaria vaccine trials testing live, attenuated sporozoites in Tanzania. MCH has been described as suitable marker for hemoglobinopathy screening in US American military male personnel (27.0 pg.) [30], female Chinese (27.0 pg.) [31], male and female Iranians (27.0 pg.) [32, 33], female pregnant Taiwanese (26.5 pg.) [34], female migrant population living in the UK (25.0 pg.) [35]. The cut off values defined in other studies was lower than the 28.6 pg. proposed here, based on the fact that females tend to have generally lower MCH values [36].

Hemoglobinopathies as heritable traits have tremendous impact on public health [1]. There is growing evidence that the public health burden due to inherited red blood cell disorders like α-thalassemia, sickle cell disease and G6PD deficiencies will increase globally, including in sub-Saharan Africa [37]. Currently, more than 70% of the global burden of hemoglobinopathies occurs in Africa that still has high birth rates [38]. During the past years, malaria burden has been on the decline in many countries of sub-Saharan Africa, most likely due to scaling up of malaria prevention, proper and timely diagnosis and treatment resulting in considerable reductions of child mortality [39]. Recent surveys have indicated however, that many countries in sub-Saharan Africa have next to no facilities for the diagnosis, control and management of the common hemoglobin disorders [40].

Education is required for future parents in a situation whereby both partners are carriers for homozygous or heterozygous thalassemia traits and/or the sickle cell trait. These couples have higher chance of having severely affected off springs such as in the case of α-thalassemia the Hb Bart's hydrops foetalis syndrome [8]. Voluntary screening programs have been implemented in some of the Middle Eastern countries and they have reduced child mortality and morbidity due to hemoglobinopathies significantly [41]. One good example is that neonatal screening programs followed by early prophylactic treatment for cases of sickle cell disease with penicillin and vaccination programs against common pathogens have reduced early deaths from infections [5]. In Tunisia, due to the availability of demographic and epidemiological data, successful prevention strategies based on prenatal diagnosis, neonatal screening, and premarital screening and counseling have been implemented [38]. Development of economical and cost-effective screening strategies, case management guidelines and education of the general public are cornerstones of future prevention programs of hemoglobinopathies. In sub-Saharan African countries, such measures will have great impact on public health in these fast growing populations in the time of malaria elimination [37, 42].

Acknowledgements

We would like to thank the participants of this study. The generous support of this study by Biolytix (http://www.biolytix.ch) is acknowledged. This study was made possible through

financial support of the Swiss Tropical and Public Health Institute and Ifakara Health Institute.

Disclosure of conflict of interest

The authors report no conflicts of interest. The authors share the responsibility for the content and writing of this article.

Address correspondence to: Claudia A Daubenberger, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland. Tel: +41 61 284 82 17; Fax: +41 61 284 81 01; E-mail: claudia.daubenberger@unibas.ch

References

[1] Williams TN and Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. Cold Spring Harb Perspect Med 2012; 2: a011692.

[2] Weatherall DJ. Thalassemia as a global health problem: recent progress toward its control in the developing countries. Annals of the New York Academy of Sciences 2010; 1202: 17-23.

[3] Bunn HF. Pathogenesis and treatment of sickle cell disease. N Engl J Med 1997; 337: 762-769.

[4] Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Williams TN, Weatherall DJ and Hay SI. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. Nat Commun 2010; 1: 104.

[5] Serjeant GR. The natural history of sickle cell disease. Cold Spring Harb Perspect Med 2013; 3: a011783.

[6] Oppenheimer SJ, Higgs DR, Weatherall DJ, Barker J and Spark RA. Alpha thalassaemia in Papua New Guinea. Lancet 1984; 1: 424-426.

[7] Higgs DR and Weatherall DJ. The alpha thalassaemias. Cell Mol Life Sci 2009; 66: 1154-1162.

[8] Harteveld CL and Higgs DR. α-thalassaemia. Orphanet J Rare Dis 2010; 5: 13.

[9] Benmansour I, Moradkhani K, Moumni I, Wajcman H, Hafsia R, Ghanem A, Abbes S and Prehu C. Two new class III G6PD variants [G6PD Tunis (c.920A>C: p.307Gln>Pro) and G6PD Nefza (c.968T>C: p.323 Leu>Pro)] and overview of the spectrum of mutations in Tunisia. Blood Cells Mol Dis 2013; 50: 110-114.

[10] Mason PJ, Bautista JM and Gilsanz F. G6PD deficiency: the genotype-phenotype association. Blood Rev 2007; 21: 267-283.

[11] Beutler E. G6PD deficiency. Blood 1994; 84: 3613-3636.

[12] Howes RE, Battle KE, Satyagraha AW, Baird JK and Hay SI. G6PD deficiency: global distribution, genetic variants and primaquine therapy. Adv Parasitol 2013; 81: 133-201.

[13] Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, Holman LA, James ER, Billingsley PF, Gunasekera A, Richman A, Chakravarty S, Manoj A, Velmurugan S, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Plummer SH, Hendel CS, Novik L, Costner PJ, Mendoza FH, Saunders JG, Nason MC, Richardson JH, Murphy J, Davidson SA, Richie TL, Sedegah M, Sutamihardja A, Fahle GA, Lyke KE, Laurens MB, Roederer M, Tewari K, Epstein JE, Sim BK, Ledgerwood JE, Graham BS and Hoffman SL. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science 2013; 341: 1359-1365.

[14] Shekalaghe S, Rutaihwa M, Billingsley PF, Chemba M, Daubenberger CA, James ER, Mpina M, Ali Juma O, Schindler T, Huber E, Gunasekera A, Manoj A, Simon B, Saverino E, Church LW, Hermsen CC, Sauerwein RW, Plowe C, Venkatesan M, Sasi P, Lweno O, Mutani P, Hamad A, Mohammed A, Urassa A, Mzee T, Padilla D, Ruben A, Sim BK, Tanner M, Abdulla S and Hoffman SL. Controlled Human Malaria Infection of Tanzanians by Intradermal Injection of Aseptic, Purified, Cryopreserved Plasmodium falciparum Sporozoites. Am J Trop Med Hyg 2014; 91: 471-480.

[15] Veenemans J, Andang'o PE, Mbugi EV, Kraaijenhagen RJ, Mwaniki DL, Mockenhaupt FP, Roewer S, Olomi RM, Shao JF, van der Meer JW, Savelkoul HF and Verhoef H. Alpha+ -thalassemia protects against anemia associated with asymptomatic malaria: evidence from community-based surveys in Tanzania and Kenya. J Infect Dis 2008; 198: 401-408.

[16] Veenemans J, Jansen EJ, Baidjoe AY, Mbugi EV, Demir AY, Kraaijenhagen RJ, Savelkoul HF and Verhoef H. Effect of alpha(+)-thalassaemia on episodes of fever due to malaria and other causes: a community-based cohort study in Tanzania. Malar J 2011; 10: 280.

[17] Clark BE and Thein SL. Molecular diagnosis of haemoglobin disorders. Clin Lab Haematol 2004; 26: 159-176.

[18] Tan AS, Quah TC, Low PS and Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha-thalassemia. Blood 2001; 98: 250-251.

[19] R Foundation for Statistical Computing. Development Core Team. R: A language and environment for statistical computing. home page; http://www.R-project.org. 2008.

[20] Ruopp MD, Perkins NJ, Whitcomb BW and Schisterman EF. Youden Index and optimal cutpoint estimated from observations affected by

a lower limit of detection. Biom J 2008; 50: 419-430.
[21] Taylor SM, Cerami C and Fairhurst RM. Hemoglobinopathies: slicing the Gordian knot of Plasmodium falciparum malaria pathogenesis. PLoS Pathog 2013; 9: e1003327.
[22] Williams TN. Human red blood cell polymorphisms and malaria. Curr Opin Microbiol 2006; 9: 388-394.
[23] Williams TN, Wambua S, Uyoga S, Macharia A, Mwacharo JK, Newton CR and Maitland K. Both heterozygous and homozygous alpha+ thalassemias protect against severe and fatal Plasmodium falciparum malaria on the coast of Kenya. Blood 2005; 106: 368-371.
[24] Enevold A, Alifrangis M, Sanchez JJ, Carneiro I, Roper C, Borsting C, Lusingu J, Vestergaard LS, Lemnge MM, Morling N, Riley E and Drakeley CJ. Associations between alpha+-thalassemia and Plasmodium falciparum malarial infection in northeastern Tanzania. J Infect Dis 2007; 196: 451-459.
[25] Maheu-Giroux M and Castro MC. Impact of community-based larviciding on the prevalence of malaria infection in Dar es Salaam, Tanzania. PLoS One 2013; 8: e71638.
[26] Piel FB, Hay SI, Gupta S, Weatherall DJ and Williams TN. Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions. PLoS Med 2013; 10: e1001484.
[27] Pranpanus S, Sirichotiyakul S, Srisupundit K and Tongsong T. Sensitivity and specificity of mean corpuscular hemoglobin (MCH): for screening alpha-thalassemia-1 trait and beta-thalassemia trait. J Med Assoc Thai 2009; 92: 739-743.
[28] Shekalaghe S, Alifrangis M, Mwanziva C, Enevold A, Mwakalinga S, Mkali H, Kavishe R, Manjurano A, Sauerwein R, Drakeley C and Bousema T. Low density parasitaemia, red blood cell polymorphisms and Plasmodium falciparum specific immune responses in a low endemic area in northern Tanzania. BMC Infect Dis 2009; 9: 69.
[29] Segeja MD, Mmbando BP, Kamugisha ML, Akida JA, Savaeli ZX, Minja DT, Msangeni HA and Lemnge MM. Prevalence of glucose-6-phosphate dehydrogenase deficiency and haemoglobin S in high and moderate malaria transmission areas of Muheza, north-eastern Tanzania. Tanzan J Health Res 2008; 10: 9-13.
[30] Pierce HI, Kurachi S, Sofroniadou K and Stamatoyannopoulos G. Frequencies of thalassemia in American blacks. Blood 1977; 49: 981-986.
[31] Liao C, Xie XM, Zhong HZ, Zhou JY and Li DZ. Proposed screening criteria for beta-thalassemia trait during early pregnancy in southern China. Hemoglobin 2009; 33: 528-533.
[32] Karimi M and Rasekhi AR. Efficiency of premarital screening of beta-thalassemia trait using MCH rather than MCV in the population of Fars Province, Iran. Haematologia (Budap) 2002; 32: 129-133.
[33] Hashemizadeh H and Noori R. Premarital Screening of Beta Thalassemia Minor in northeast of Iran. Iran J Ped Hematol Oncol 3: 210-215.
[34] Pranpanus S, Sirichotiyakul S, Srisupundit K, Tongsong T. Sensitivity and specificity of mean corpuscular hemoglobin (MCH): for screening alpha-thalassemia-1 trait and beta-thalassemia trait. J Med Assoc Thail Chotmaihet Thangphaet 2009; 92: 739-743.
[35] Sorour Y, Heppinstall S, Porter N, Wilson GA, Goodeve AC, Rees D and Wright J. Is routine molecular screening for common alpha-thalassaemia deletions necessary as part of an antenatal screening programme? J Med Screen 2007; 14: 60-61.
[36] Saathoff E, Schneider P, Kleinfeldt V, Geis S, Haule D, Maboko L, Samky E, de Souza M, Robb M and Hoelscher M. Laboratory reference values for healthy adults from southern Tanzania. Trop Med Int Health 2008; 13: 612-625.
[37] Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010; 115: 4331-4336.
[38] Fattoum S. Evolution of hemoglobinopathy prevention in Africa: results, problems and prospect. Mediterr J Hematol Infect Dis 2009; 1: e2009005.
[39] Noor AM, Kinyoki DK, Mundia CW, Kabaria CW, Mutua JW, Alegana VA, Fall IS and Snow RW. The changing risk of Plasmodium falciparum malaria infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity. Lancet 2014; 383: 1739-1747.
[40] Weatherall DJ. The challenge of haemoglobinopathies in resource-poor countries. Br J Haematol 2011; 154: 736-744.
[41] Zlotogora J. Population programs for the detection of couples at risk for severe monogenic genetic diseases. Hum Genet 2009; 126: 247-253.
[42] White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA and Dondorp AM. Malaria. Lancet 2014; 383: 723-735.