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Haemoglobin levels for population from Gambo, a rural area of Ethiopia, and their association with anaemia and malaria

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Abstract

Background: Knowledge of appropriate reference intervals is critical not only to provide optimal clinical care, but also to enrol populations in medical research. The aim of this study was to generate normal ranges of laboratory values for haemoglobin among healthy Ethiopian adults and children and to determine if anaemia is a possible indicator of malaria in women and children in this area of Ethiopia.

Methods: This study was carried out from January 2008 to May 2010. The reference sample population with malaria-negative consisted of 454 individuals, divided women, men and children. The malaria-infected sample population consisted of 117 individuals. The reference ranges were based on the guidelines from the Clinical and Laboratory Standards Institute. Haemoglobin concentration was determined by Hemo-Control EKF Diagnostic Analyser on whole blood. Testing for malaria-positive and negative infection was done by microscopy and by PCR.

Results: The lower limits for adult haemoglobin range obtained from this population were slightly higher than those derived from other African populations, but were equal to those established by other studies in Ethiopia and the World Health Organization (WHO). Regarding children, the minimum values were lower than those obtained from different African populations and those established by WHO. The malaria-negative group had anaemia in 35.6% of cases and in the malaria-positive group in 70.9%. There was a stronger, statistically significant association between anaemia and malaria-positive samples than between anaemia and malaria-negative samples in women and both groups of children.

Conclusions: The results from this study are a contribution in the definition of the haemoglobin parameters in African populations, which could be taken as standards for interpretation of laboratory results. The haemoglobin indices in adults from Gambo tended to be higher than other African populations and in children were lower than other studies in Africa. The results also suggest that anaemia is not useful as a supportive diagnostic criterion to monitor and evaluate malaria in women and children from Ethiopia, because a 29.1% of malaria cases will be not detected, because of not having anaemia.

Keywords: Haemoglobin, Anaemia, Malaria, Ethiopia

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Background

Locally determined reference values are of immense clinical importance when taking into account intra- and inter-population variations, so that the correct clinical interpretation of laboratory results can be made [1]. The reference intervals of haematological indices currently used in Africa are derived from data collected from Europe or North America with predominantly Caucasian populations [2-4]. Even within the African continent, it is likely that substantial differences exist because of vast diverse climate, geographic altitude, co-infections, ethnic origin and human genetics, all of which can influence laboratory reference range parameters within a population [5-10]. This emphasizes the importance of population-specific reference values for different population settings [6,11]. Therefore, it is unlikely that any one set of reference haematological values can be applied across the globe for the same purpose. For example, African populations are reported to have lower haemoglobin than their western counterparts [5,12,13]. Consequently, using reference intervals determined from Caucasian populations in clinical trials among African populations might lead to the mistaken interpretation of 'abnormal' values in these populations [5,8,14].

Reference values for various blood parameters can be derived from healthy volunteers, from subjects attending health screening clinics or having annual medical examinations [15]. Such parameters are also important for measuring disease progression and response to therapy [5,8,14]. Knowledge of appropriate reference intervals is critical not only to provide optimal clinical care, but also to enrol populations in medical research. This is particularly relevant for African countries where there is emergence and re-emergence of some infectious diseases, whose management and prognosis heavily depend on laboratory values. It is also important for research and the evaluation of clinical trial-associated toxicity and adverse events of novel interventions against tuberculosis, malaria and infection with human immunodeficiency virus (HIV) [14].

Clinical diagnosis is widely used for the diagnosis of malaria, especially in resource-poor countries. Haematological changes in malaria, such as anaemia, thrombocytopenia and leucocytosis or leucopenia are well recognized [16]. The World Health Organization (WHO) and Roll Back Malaria (RBM) partnership have recommended that anaemia should be used as an additional indicator to monitor the malaria burden at community level and interventions on a national scale [17]. To establish anaemia as a malaria indicator, it is necessary to know haemoglobin values in each population.

Anaemia is an important contributor to malaria-attributable deaths in hospitals, with severe anaemia accounting for between 17 and 54% of malaria-attributed deaths in

children under five years of age [18-20]. Therefore, the prevalence of (symptomatic and asymptomatic) anaemia in the community, as measured in population-based surveys, is used as the candidate indicator of the total malaria-related disease burden [21].

The aim of this study was to generate normal ranges of laboratory values for haemoglobin in Gambo village. To obtain the results of this study were assessed the haemoglobin parameter in healthy adults and children, and contrasted with that previously reported from African and Caucasian populations. In addition, this study suggests anaemia as a possible indicator of malaria in women and children in this area of Ethiopia as recommended by WHO and RBM.

Methods

Subjects

All haemoglobin values were taken at the Gambo General Rural Hospital, from patients attending health screening. Gambo is 2,200 m above sea level and lies 245 km southeast of Addis Ababa. The prevalence of malaria for the country is 8% [22]. The reference sample population with malaria-negative consisted of 454 individuals, made up of 127 women and 84 men between 13 and 98 years of age, 126 children between one and five years of age and 117 children between six and 12 years of age. The following categories were excluded: pregnant, malaria infected, HIV-positive and intestinal helminth-positive. The malaria-infected sample population, confirmed by microscopy, consisted of 117 individuals, 40 women and 14 men (13-98 years of age), 40 children (one to five years of age) and 23 children (six to 12 years of age), non-pregnant, HIV-negative and intestinal helminths-negative.

Patients were enrolled in this study from January 2008 to May 2010 at Gambo General Rural Hospital. Oral informed consent in local languages (Oromo and Amharic) was obtained. In the case of children, their parents or a head of household permitted the inclusion in the study. Clinical histories and samples were included in the study by code and the data were analysed anonymously. The research protocol had been reviewed and approved by the Ethical Committee of Gambo General Rural Hospital.

Laboratory procedures

Haemoglobin concentration (g/dL) was determined by a Hemo-Control EKF Diagnostic analyser on whole blood. This analysis was performed within five min of collection. Testing for malaria-positive and negative infection was by microscopy and polymerase chain reaction (PCR). Haemoglobin concentration and microscopy was performed at the laboratory of the above-mentioned hospital and PCR were performed at the University Institute of Tropical Diseases and Public Health of the

Canary Islands (IUETSPC). All experiments were performed according to good laboratory practice using appropriate standard operating procedures by well-trained laboratory staff.

Microscopy and PCR testing

Microscopy for detection of *Plasmodium* species was performed using thick and thin blood smears [23], with two independent readings and a third reading for slides with discordant results. Malaria-positive infection was defined as the presence of any asexual malaria parasites detected on a thick peripheral blood smear. Finger-prick samples on 3MM filter paper were obtained for the PCR assays. Each filter paper specimen was stored in a plastic bag at room temperature and shipped to the IUETSPC. DNA extraction was performed using commercial kits (Speedtools tissue DNA Extraction Kit, Biotools, Madrid, Spain) and malaria-negative samples and *Plasmodium* species were confirmed by microscopy and nested PCR targeting the small subunit ribosomal RNA gene [24].

Statistical analysis

Statistical analysis was based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI: formerly National Council of Clinical Laboratory Services). Median and 95% reference ranges were determined for haemoglobin parameters and analysed by age and gender for adults. All calculations for determining reference ranges were based on the guidelines found in CLSI, document C28-A2 [1]. The normal distribution was tested by the Kolmogorov-Smirnov test. The sample population was grouped according to the age and gender distribution, and the median was used as a measure of central tendency. To eliminate bias due to small sample size, a bootstrap analysis method was used as a robust method to assess the 95% reference ranges from the Kolmogorov-Smirnov test. For each parameter, 10,000 bootstrap samples were selected [25] and the lower 95% reference limit was defined as the 2.5 percentile, while the upper limit was defined at the 97.5 percentile. Mann-Whitney U test was used to test for differences of means. Tests for an association between malaria and haemoglobin values were carried out by means of bivariate analysis: categorical variables were created for each group. The performance of the haemoglobin values was compared to those malaria-positive samples, in terms of their positive and negative predictive values (PPV and NPV).

An error probability (P value) of <0.05 was considered significant. All statistical calculations were performed with SPSS19 statistical and Resampling Stats for Microsoft Excel 2007 software.

Results

A total of 454 malaria-negative individuals participated in this study. Men were marginally older than women,

with mean ages of 40 and 32 years old, respectively. The mean age of child participants at study entry was two years old (one to five years of age) and nine years old (six to 12 years of age).

All haematological values were normally distributed. The analysis of haemoglobin values of these patients showed a mean, median and 95% reference range values stratified by age and gender, which are shown in Table 1. The lower haemoglobin value in non-malaria infected was 11.8 g/dL, 12.9 g/dL, 8.6 g/dL, and 9.7 g/dL in women, men and children between one and five years old and children between six and 12 years old, respectively. Women presented lower haemoglobin values than men (mean 12.2 vs 13.5 g/dL $p < 0.05$). Box and whisker plots for haemoglobin values by gender and age are shown in Figure 1.

The means and 95th reference intervals for the Gambo General Rural Hospital population evaluated in the present study were compared with parameters obtained from other African populations and with parameters set by WHO. In general, the lower limits for adult haemoglobin range obtained from this population were slightly higher than those derived from Kenya [12], Uganda [5] and Gambia [14], but were equal to those established by another study in Ethiopia [26] and the haemoglobin range established by WHO (2011) [27]. In the child populations, the minimum values were lower than those obtained from different African populations and those established by WHO (Table 2).

In the 117 malaria-positive samples, asexual malaria parasites were observed in the blood smears. The mean of haemoglobin values for the malaria-positive individuals group was determined and stratified by sex and age (Table 3). Of the malaria-positive slides, 51.3% ($n = 60$) were *Plasmodium vivax*, 40.2% ($n = 47$) were *Plasmodium falciparum* and 8.5% ($n = 10$) were mixed (*P. vivax* and *P. falciparum*).

Haemoglobin values of the malaria-positive individuals were compared with those of the sex and age-specific malaria-negative groups using Mann-Whitney U test. For the different groups, the mean values of haemoglobin were significantly ($P < 0.05$) lower for infected groups than for the uninfected groups among women, men and children.

According to the results of the study, new anaemia values are defined, in the Gambo population, as a haemoglobin level under 11.8 g/dL, 12.9 g/dL, 8.6 g/dL, and 9.7 g/dL in women, men and in both groups of children, respectively. The malaria-negative group had anaemia in 35.6% of cases and the malaria-positive group in 70.9%. There was a stronger, statistically significant association between anaemia and malaria-positive samples than between anaemia and malaria-negative samples in women, and both groups of children (Table 4).

Table 1 Mean, median and 95th percentile reference ranges of haemoglobin level for 454 Ethiopian malaria-negative adults and children

	Subject group			
	Female (n = 127)	Male (n = 84)	Children 1-5 years (n = 126)	Children 6-12 years (n = 117)
Haemoglobin (g/dL)				
Mean ± SD	12.2 ± 2.7	13.5 ± 2.4	9.1 ± 3.0	10.3 ± 3.1
Median	12.8	14 (<0.05)*	9.5	10.5
95% range	11.8-12.7	12.9-14.0	8.6-9.6	9.7-10.8

*P value (Mann-Whitney U test) for comparison of medians for adult subjects.

The PPV of three age groups (women, children between one to five years of age, and children between six to 12 years of age) were 38%, 38.6% and 25%, respectively. In the cases of NPV were 85%, 90% and 90% respectively.

If the malaria-positive group with anaemia is analysed, it observed in women that 45% infected by *P. vivax* and 78% by *P. falciparum* showed anaemia. In children, between one to five years of age, 74% infected by *P. vivax*, and 82.3% by *P. falciparum* showed anaemia and in children, between six to 12 years of age, showed anaemia in 54% infected by *P. vivax*, and 85.7% by *P. falciparum*. In women and both groups of children infected by different *Plasmodium* species, their mean values of haemoglobin were not significantly different ($p > 0.05$). In men, the haemoglobin value mean in malaria produced by *P. falciparum*, or *P. vivax* was not calculated due to the low number of malaria-positive samples.

Discussion

Clinical laboratory values provide important data to help assess the health of an individual. In the absence of locally derived reference values for African populations, clinicians and researchers have had to use reference values from Caucasian populations. Differences in haematological values among distinct populations have been known for a long time and are discussed by several authors. Previous studies have shown that such values vary with age, ethnic origin, sociodemographic characteristics, genetic differences and environmental context [5,9,12,29]. The use of only Caucasian reference ranges for clinical management could lead to unnecessary treatment. The development of region- and age-specific reference values is thus essential for efficient patient management and the proper performance of clinical research.

WHO has designated haemoglobin, among other values, as essential to be measured by laboratory services in sub-Saharan Africa [30]. This study is the first description of haemoglobin ranges in the Gambo General Rural Hospital (Ethiopia). This haemoglobin range may serve in this area as standard for interpretation of laboratory results. This study has not only allowed the definition of haemoglobin ranges for a rural area in Ethiopia, but also provided evidence that reference intervals from one population should not be applied universally [31].

The haemoglobin indices in women and men from Gambo tended to be similar to Caucasian population

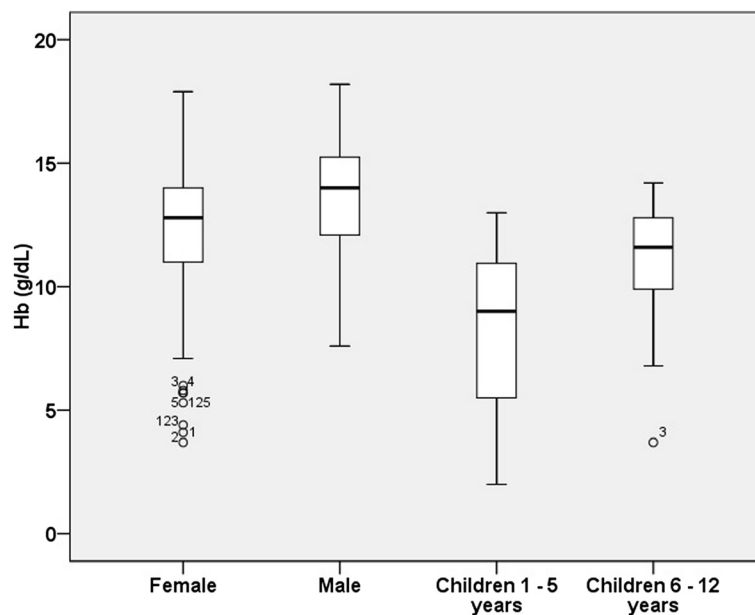


Figure 1 Box and whisker plots showing variation in haemoglobin (Hb) values in malaria-negative Ethiopian adults and children.

n = 127 for female samples, n = 84 for male samples, n = 126 for child samples between one and five years old, n = 117 for child samples between six and 12 years old.

Table 2 Comparison of haemoglobin values

Subjects	Haemoglobin values by country and reference						
	Present study	Ethiopia [26]	Kenya [12]	Uganda [5]	Gambia [14]	Tanzania [28]	WHO [27]
Female	11.8-12.7 (12.2)	12.2-16.6 (14.3)	5.9-10.0 (8.4)	9.9-13.7 (12.4)	9.8-15.0	NA	12.0-15.0
Male	12.9-14.0 (13.5)	13.9-18.3 (16.1)	8.3-11.3 (9.9)	11.5-17.1 (14.4)	11.1-16.6	NA	13.0-17.0
Children 1-5 years	8.6-9.6 (9.1)	NA	NA	8.8-12.5 (10.8)	8.7-13.4	8.1-13.9 (11.3)	11.0-14.0
Children 6-12 years	9.7-10.8 (10.3)	NA	NA	10.0-13.7 (11.8)	9.5-14.4	10.3-14.7 (12.6)	11.5-15.5

Values are the 95th reference intervals and in brackets are means.
 WHO data are from above sea level.
 NA: not available.

intervals or higher than other African populations. This finding is consistent with previous studies in healthy and HIV-uninfected populations in other Ethiopian regions [12,32]. These findings could be attributable to the geographic location of Gambo area (altitude 2,200 m above sea level). This speculation is corroborated by findings from other studies that reported higher haemoglobin levels among people living at high altitudes [32]. Altitude-induced erythropoiesis and/or dietary factors could play a role in causing these variations [12].

However, haemoglobin values for children between one and five years of age (8.6-9.6 g/dL) and children between six and 12 years of age (9.7-10.8 g/dL) were lower than those reported from other studies in Africa and among Caucasian populations (see Table 2). Surprisingly, the 2.5th and 97.5th percentile for haemoglobin ranges for Gambo children is lower than standard in industrialized and African countries. This wider range may be attributable to several factors, including poor nutritional status resulting in iron deficiency, genetic disorders (eg, thalassemia, sickle cell trait) [9], which was not checked in the population included in this study, or an important deficit of haemoglobin from living at over 2,000 m above sea level. There was no evidence of a difference between boys and girls in the haemoglobin parameter, which is consistent with previous studies, showing that differences between genders become evident only at about 12 years of age [33,34].

Following recommendations of the CLSI guidelines for laboratory indicators and Canadian laboratory initiative

Table 3 Mean, median and 95th percentile reference ranges of haemoglobin levels for 117 malaria-positive adult and children from Gambo (Ethiopia)

Haemoglobin (g/dL)	Subject group			
	Female (n = 40)	Male (n = 14)	Children 1-5 years (n = 40)	Children 6-12 years (n = 23)
Mean ± SD	9.3 ± 3.4	10.4 ± 2.7	6.4 ± 2.5	7.7 ± 3.2
Median	10.2	10.3	6.6	8.2
95% range	8.3-10.5	8.8-11.9	5.6-7.2	6.4-9.1

on paediatric reference intervals [1,35], a robust bootstrap analysis was used to eliminate bias. In addition, this study included only ambulatory subjects who were otherwise apparently healthy, their individual health status was assessed for the same subclinical condition as HIV, intestinal helminths, pregnancy, and malaria, known to interfere with the obtained parameters. In the context of resource-limited settings, the methods included in this study were sufficient to determine reference ranges for use in this population.

This study presents the first description of haemoglobin ranges for adults and children in the Gambo General Rural Hospital (Ethiopia). The development of this reference range may provide guidelines to be used by local health practitioners in patient management within this region and for the design, performance and evaluation of clinical research. The findings obtained in this study were comparable to those of other studies within Africa [5,12,14,26-28]. Thus, they could be used as reference ranges for adults and children within the Gambo General Rural Hospital that presents a radius of action 100 km. These haemoglobin ranges provide anaemia values, which can be used to monitor the malaria burden at the population level, and that use Gambo General Rural Hospital as a reference hospital, as recommended by WHO and RBM.

Finally, WHO defines anaemia as a haemoglobin concentration of <12.0 g/dL, <13.0 g/dL in females and males, and 11.0 g/dL and 11.5 g/dL in children between one and five years old and those between six and 12, respectively [36]. Anaemia was defined in this study as haemoglobin values of <11.8 g/dL, <12.9 g/dL in women and men, which are similar to WHO. In children, anaemia was defined as haemoglobin values of <8.6 g/dL

Table 4 Adjusted odds ratios (OR) and 95% confidence intervals (CI) for haemoglobin values associated with malaria infection

	Malaria-positive		
	Female	Children 1-5 years	Children 6-12 years
OR	3.5	5.8	2.9
95% CI	(1.7, 7.3)	(2.5, 13.7)	(1.1, 7.3)
P-value	0.001	0.001	0.022

and <9.7 g/dL for the two groups mentioned above, respectively. Child values are lower than WHO values, as explained above. Malaria-negative groups presented anaemia in 35.6% of cases of all analysed sample. Malaria-positive groups did not have anaemia in 29.1% of cases of all analysed sample. The anaemia in malaria-positive groups was presented in 70.9% of the cases: this anaemia could be divided into 35.6% of anaemia cases caused by unknown reason, as occurred in the malaria-negative groups, and into 35.3% of anaemia attributed to malaria. Hence, the presence of malaria doubles the presence of anaemia. Malaria can produce the same percentage of anaemia as other pathologies that cause anaemia. Women and both groups of children with malaria were 3.5, 5.8 and 2.9 times, respectively, more likely to have anaemia than women and children with malaria-negative data. This association between anaemia and malaria in women and children is consistent with previous reports, which indicate that anaemia values is a sensitive indicator to scaling up malaria interventions and may thus be used as a proxy indicator to track the burden of malaria [37]. Previous articles indicate that anaemia in Ethiopia is mainly due to malaria [38,39]. The PPV of 38%, 38.6% and 25% in each groups, could be in accordance with a malaria low prevalence in study area, nevertheless, despite of the NPV of 85%, 90% and 90% obtained here, a negative anaemia result does not rule out malaria-positive [40]. Based on these results, anaemia values may have limited utility in this area as indicator of malaria infection.

Conclusions

The results from this study are a contribution in the definition of haemoglobin parameters in African populations, which could be taken as standard for interpretation of laboratory results. The haemoglobin indices in adults from Gambo tended to be higher than in other African populations and in children were lower than other studies in Africa.

The results also suggest that anaemia is not useful as a supportive diagnostic criterion to monitor and evaluate malaria in women and children from Ethiopia, because 29.1% of malaria cases will be not detected, because of not having anaemia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MASM carried out the sample collection in Gambo (Ethiopia), the microscopy and molecular study, the analysis and interpretation of data and prepared the manuscript. MAQ, RNAL and JLM helped with the statistical analysis and interpretation. PB and AB helped with the performance of molecular studies. FR and GT helped with the collection of samples and analysed haemoglobin values. BV helped to draft the manuscript and EMC coordinated and funded the study and drafted the manuscript. All authors read and approved the final manuscript.

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