



RESEARCH ARTICLE

Prevalences of inherited red blood cell disorders in pregnant women of different ethnicities living along the Thailand-Myanmar border [version 1; referees: 2 approved with reservations]

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Abstract

Background: Inherited red blood cell disorders are prevalent in populations living in malaria endemic areas; G6PD deficiency is associated with oxidant-induced hemolysis and abnormal hemoglobin variants may cause chronic anemia. In pregnant women, microcytic anemia caused by hemoglobinopathies mimics iron deficiency, complicating diagnosis and treatment. Anemia during pregnancy is associated with morbidity and mortality. The aim of this study was to characterize the prevalence of G6PD deficiency, hemoglobinopathies, ABO and Rhesus blood groups among the pregnant population living along the Thailand-Myanmar border. Pregnant women attending antenatal clinics in this area belong to several distinct ethnic groups.

Methods: Data was available for 13,520 women attending antenatal care between July 2012 and September 2016. Screening for G6PD deficiency was done by fluorescent spot test routinely. G6PD genotyping and quantitative phenotyping by spectrophotometry were analyzed in a subsample of women. Hemoglobin variants were diagnosed by HPLC or capillary electrophoresis and molecular methods. Blood groups were diagnosed by agglutination test. The prevalence and distribution of inherited red blood cell disorders and blood groups was analyzed with respect to ethnicity.

Results: G6PD deficiency was common, especially in the Sgaw Karen ethnic group, in whom the G6PD Mahidol variant allele frequency was 20.7%. Quantitative G6PD phenotyping showed that 60.5% of heterozygote women have an intermediate enzymatic activity between 30% and 70% of the population median. HbE, beta-thalassemia trait and alpha-thalassemia trait were found in 31.2% of women. Only 0.15% of women were Rhesus negative.

Conclusions: Distribution of G6PD and hemoglobin variants varied among the

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different ethnic groups, but the prevalence was generally high throughout the cohort. These findings encourage the implementation of an extended program of information and genetic counseling to women of reproductive age and will help inform future studies and current clinical management of anemia in the pregnant population in this region.

Comments (0)

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Introduction

Inherited red cells disorders (IRD), such as hemoglobinopathies and G6PD deficient variants, are common in South-East Asian populations living in area of past and present malaria transmission (Fucharoen & Winichagoon, 2012; Howes *et al.*, 2012; Williams & Weatherall, 2012). Characterization of IRD is important for understanding the causes of anemia in the population, especially in women during pregnancy when the distinction between physiologic and pathologic anemia becomes paramount. Outcomes for inherited and acquired anemias differ by etiology and can affect required diagnostic tests and medications that can be prescribed during pregnancy.

G6PD deficiency, caused by mutations on the X-linked G6PD gene, is mainly asymptomatic unless affected individuals are exposed to certain medicines or foods that induce oxidative stress. This oxidative stress causes intravascular and extravascular hemolysis of G6PD deficient red blood cells with potentially serious clinical consequences. Heterozygous women, even with the same genotype, manifest a range of phenotypes from normal G6PD activity to severe deficiency, due to the early random inactivation of the X-chromosome (Lyonisation).

Hemoglobinopathies are caused by a number of structural variants and deletional mutations on the hemoglobin alpha and non-alpha chains. Alpha chains are encoded by four alpha genes located in pairs on the chromosome 16, while non-alpha chains are encoded by several genes on the beta-globin cluster on chromosome 11. Expression of non-alpha chains changes during embryonic, fetal and adult development; in children over one year of age and in adults, over 97% of hemoglobin is composed by two alpha and two beta chains. Mutations that cause imbalance among the four chains are called thalassemias. Hemoglobinopathies are associated with a spectrum of reduced hemoglobin levels, ranging from very mild (ca 0.5g/dL average reduction) to severe anemia. The geographic distribution of abnormal hemoglobins corresponds to that of malaria before modern times, because these abnormalities confer some protection against malaria or its pathological effects (Allison, 1954; Haldane, 1949; Hutagalung *et al.*, 1999; Siniscalco *et al.*, 1961).

The refugee and migrant populations living along the Thailand-Myanmar border, an area of low seasonal malaria transmission, are composed of several distinct ethnic groups: mainly Burman, Sgaw Karen and Poe Karen, followed by Mon, Kachin, Shan, and Rakhine. While a few studies have investigated the prevalence of hemoglobinopathies among Burman in central Myanmar (Than *et al.*, 2005; Win *et al.*, 2005), little is known about Karen and other ethnic minorities living in Karen state and along the Thailand border. There are scattered reports from Karen and Burman patients who have been resettled in resource rich countries where the capacity to determine detailed genetic traits is possible (Lee, 2012; Phylipsen *et al.*, 2010). G6PD deficiency has been studied in recent years in this border population (Bancone *et al.*, 2014; Phompradit *et al.*, 2011), but quantitative characterization of G6PD phenotypes in women has not been carried out yet at the population level.

The antenatal program of the Shoklo Malaria Research Unit (SMRU) registers over 3,000 pregnant women of mainly Burman and Karen ethnicity per year, providing free comprehensive antenatal, delivery and postpartum care. This has been described elsewhere (Hoogenboom *et al.*, 2015). During antenatal visits, women are screened routinely for anemia and malaria, and screening has expanded in the past five years to include IRD. The aim of this report is to describe the prevalence of IRD, as well as ABO and Rhesus blood groups, among the pregnant women attending routine antenatal screening at SMRU clinics along the Thailand-Myanmar border.

Methods

Study area and population

The Shoklo Malaria Research Unit is located in the north-western border of Thailand and has been providing free health assistance to the local refugee population for 30 years. The antenatal care (ANC) program was established for the early detection and treatment of malaria in 1986 for refugees and since 1998 for migrants. Women living in the catchment area of SMRU clinics are encouraged to attend the ANC as soon as they are aware of their pregnancy. At the first consultation, demographic information is collected, an obstetric and medical history recorded, and detailed clinical examination done. Antenatal screening from July 2012 to September 2016 included examination of IRB in line with Thailand national guidelines. All women had language appropriate group counseling at this first visit about the different screening tests and this was followed by one on one counseling with the option to opt out. Formal written consent was not required for the original routine blood sampling. Permission to waive consent from the individual patient for analysis of routine data was asked and obtained from the Ethic Committee of the Faculty of Tropical Medicine, Mahidol University (approval letter MUTM2017-041-01).

Definition of ethnicity

Pregnant women were asked to report the ethnicity of both parents as belonging to one of the following groups based on locally preferred terms for self-identification: Sgaw Karen, Poe Karen, Burman, "Muslim", Mon, Kachin, Pa Oh, Rakhine, Shan and "others". The ethnicity of the woman was defined "mixed" when parents' ethnicity differed. Of note, in this border area people of Islamic faith often self-identify as "Muslim" when asked about their ethnicity; the "Muslim" group is not an ethnic group, but rather a heterogeneous group of subjects with various ethnic backgrounds.

Laboratory analyses

Characterization of ABO and Rhesus blood group was performed in the clinics using agglutination method with anti-A, anti-B and anti-D sera (Plasmatec, UK).

In the central hematology laboratory, G6PD deficiency was screened by the NADPH Fluorescent Spot Test (FST, R&D Diagnostic, Greece). G6PD quantitative phenotype was assessed in selected women by spectrophotometry carried out at 37°C according to the standard WHO protocol on whole blood depleted of WBCs (Beutler *et al.*, 1977). G6PD activity was calculated

after normalization with Hb concentration and expressed as IU/gHb. The G6PD activity population median of 11.5 IU/gHb was established previously in the laboratory using the same technique on G6PD normal males (Bancone *et al.*, 2014). In the same selected women, G6PD genotyping was performed using established PCR-RFLP protocols (Bancone *et al.*, 2014). Genotyping for Mahidol was performed on all the selected women, while Chinese-4, Kaiping, Canton and Mediterranean variants were analyzed only on women with either enzymatic activity below 80% of normal in Mahidol-wild type genotype or enzymatic activity below 30% and Mahidol-heterozygous genotype. The minimum size of the sub-sample analyzed for G6PD quantitative phenotyping and genotyping was set at 300 subjects; this was calculated to allow at least 5 subjects in the smaller genotype group (homozygous mutated) based on the expected allele frequency of 15%.

Hemoglobinopathies

For hemoglobin typing, blood samples were analyzed either by high-performance liquid chromatography (HPLC) at the local Mae Sot hospital or by Capillary Electrophoresis (CE), using a Capillarys II, Sebia, France) at the central SMRU hematology laboratory. Both techniques allow for diagnosis of Hb structural variants such as HbE, HbC, HbS (by mean of appearance of retention peaks at specific elution times), beta-thalassemia carriage (by mean of increased percentage of HbA₂), and presumptive diagnosis of alpha-thalassemia carriage (by mean of decreased percentage of HbA₂). Hemoglobin Constant Spring (HbCS), a non deletional alpha thalassemia caused by a TAA>CAA mutation on the termination codon of alpha gene, was diagnosed by CE only, with a peak ≥0.5% in the HbC/SC retention zone. Alpha-thalassemia caused by common Asian deletional mutations

(3.7, 4.2, SAE) was confirmed using a modified multiplex gap-PCR protocol (Chong *et al.*, 2000). Since data on the prevalence of alpha-thalassemia were not available, molecular analysis was performed on an arbitrarily minimum sample size of 200 subjects with suspected alpha-thalassemia defined by low percentage of HbA₂ (≤2.2% by HPLC or ≤2.1% by CE); an additional sample of at least 150 subjects with beta-thalassemia trait was studied in order to assess prevalence of alpha-thalassemia in this group and analyze variation of percentage of HbA₂ in subjects with mutations on both alpha and non-alpha globin chains.

Statistical analysis

All women with available data in the timeframe of the study were included in the analysis. Data were entered and analyzed in SPSS IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA). Calculation of ABO allelic frequency by ethnic group was performed using Bernstein method (Bernstein, 1925). Hardy-Weinberg equilibrium was tested using S2 ABOestimator, <http://webpages.fc.ul.pt/~pjns/Soft/ABOestimator/> while phenotypic differences between ethnic groups were tested using the Chi-square test. Chi-square test was also used for comparison of allelic frequencies among different ethnic groups. Normality of G6PD activity was tested by the Kolmogorov-Smirnov test.

Results

Study flow is summarized in Figure 1. A total of 15,512 pregnancies were registered at the ANC between July 2012 and September 2016. For women with more than one pregnancy during this time frame, successive pregnancies (n=1,754) were excluded from analysis. Complete demographic and ethnicity information were missing for 238 women, leaving a total of 13,520 analyzable women. ABO

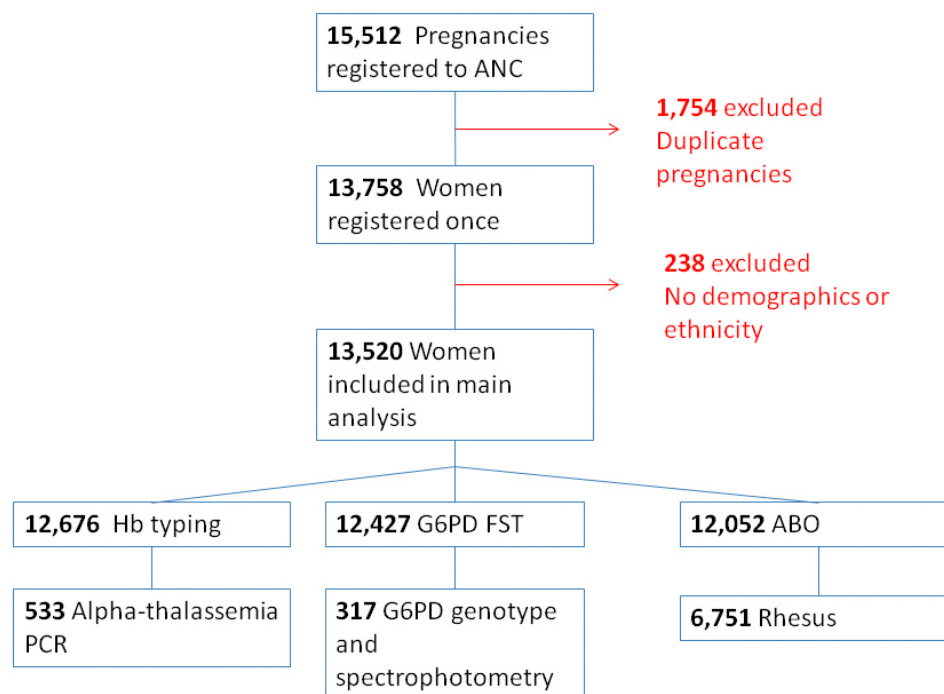


Figure 1. Study flowchart.

blood group data, G6PD FST screening and hemoglobin typing results were available in over 90% of women. Testing for Rhesus group (Rh) started systematically in June 2014; therefore data were available for 50% of women. Additional data on G6PD genotype and quantitative phenotype were analyzed in a subsample of 317 sequential women who attended the clinics between August and September 2012. Between August 2012 and August 2013, alpha deletional mutations were analyzed by PCR on 374 women presenting with a low percentage of HbA₂ ($\leq 2.2\%$ by HPLC or $\leq 2.1\%$ by CE) and on 159 women with beta-thalassemia trait.

Prevalence and distribution of ethnic groups

A total of 12,605/13,520 (93.2%) women reported both parents to be of the same ethnicity and were therefore assigned to one of the nine common ethnic groups or to the pooled category of uncommon ethnic groups called “others” (Table 1). Overall, the most represented ethnic group was the Sgaw Karen (44.3%), followed by Burman (28.5%), Poe Karen (12.4%) and “Muslim” (4.4%). The distribution of these four major ethnic groups reflected those of the general population attending the SMRU clinics with the Sgaw Karen

comprising the vast majority of women attending in the refugee camp of MLA (71.3%) and Burman women representing 39.7% and 47.6% of the population attending the clinics for migrant population of WPA and MKT, respectively. Poe Karen were 9.1% to 16.9% in the three clinics, while “Muslim” were mainly in MLA camp for displaced persons (11.9%). The other ethnic groups were in very low numbers in all clinics.

ABO and Rhesus blood groups

A total of 12,052/13,520 (89.1%) results on ABO blood groups were available among the study cohort (Table 2 and Table S1). All ethnic groups, with the exclusion of Pa-Oh, showed a higher frequency of O blood group followed by group B and group A. There were too few in the Kachin and Shan groups to be analyzed.

The four major ethnic groups were analyzed and found to be in Hardy-Weinberg equilibrium. Differences in ABO phenotypes showed that the Sgaw Karen had the highest prevalence of B and the lowest of A group, as compared to the other ethnicities, and so differed significantly from all the other three ethnic groups

Table 1. Distribution of ethnic groups in the three Shoklo Malaria Research Unit clinics.

| Ethnicity | MKT | | WPA | | MLA | | Total | |
|--------------|--------------|---------------|--------------|---------------|--------------|---------------|---------------|---------------|
| Sgaw Karen | 1,081 | 26.3% | 1,470 | 32.0% | 3,443 | 71.3% | 5,994 | 44.3% |
| Poe Karen | 456 | 11.1% | 774 | 16.9% | 441 | 9.1% | 1,671 | 12.4% |
| Burman | 1,954 | 47.6% | 1,822 | 39.7% | 77 | 1.6% | 3,853 | 28.5% |
| “Muslim” | 15 | 0.4% | 11 | 0.2% | 573 | 11.9% | 599 | 4.4% |
| Mon | 72 | 1.8% | 56 | 1.2% | 14 | 0.3% | 142 | 1.1% |
| Kachin | 2 | 0.0% | 0 | 0.0% | 12 | 0.2% | 14 | 0.1% |
| Pa Oh | 46 | 1.1% | 501 | 1.1% | 10 | 0.2% | 106 | 0.8% |
| Rakhine | 55 | 1.3% | 14 | 0.3% | 1 | 0.0% | 70 | 0.5% |
| Shan | 9 | 0.2% | 10 | 0.2% | 6 | 0.1% | 25 | 0.2% |
| “Mixed” | 348 | 8.5% | 334 | 7.3% | 234 | 4.8% | 916 | 6.8% |
| Other | 66 | 1.6% | 48 | 1.0% | 16 | 0.3% | 130 | 1.0% |
| Total | 4,104 | 100.0% | 4,585 | 100.0% | 4,827 | 100.0% | 13,520 | 100.0% |

Table 2. ABO blood groups and calculated allelic frequency in the four major ethnic groups.

| Ethnicity | Phenotypic blood group | | | | | Calculated allelic frequency | | |
|--------------|------------------------|------------|--------------|--------------|---------------|------------------------------|--------------|--------------|
| | A | AB | B | O | Total | O | A | B |
| Sgaw Karen | 997 | 369 | 1,772 | 1,868 | 5,006 | 61.1% | 14.7% | 24.3% |
| Poe Karen | 372 | 107 | 420 | 627 | 1,526 | 64.1% | 17.2% | 19.1% |
| Burman | 895 | 303 | 1,180 | 1,362 | 3,740 | 60.3% | 17.6% | 22.3% |
| “Muslim” | 109 | 38 | 122 | 186 | 455 | 63.9% | 17.7% | 19.5% |
| Total | 2,702 | 920 | 3,886 | 4,544 | 12,052 | 61.4% | 16.4% | 22.5% |

(Sgaw Karen vs Poe Karen $P < 0.001$, Sgaw Karen vs “Muslim” $P = 0.004$ and Sgaw Karen vs Burman $P < 0.001$); Poe Karen had the highest prevalence of O blood group and differed significantly from Burman ($P = 0.005$), who had the lowest prevalence of O group among ethnicities. All the other comparisons showed no statistical differences.

A total of 6,751/13,520 (49.9%) results were available for Rhesus blood grouping. Out of 2,826 Sgaw Karen and 810 Poe Karen tested, none was found to be Rh negative. Two women out of 277 “Muslim” (0.72%) and 6/2,066 Burman (0.29%) tested were Rh negative. An additional two women among the “mixed” and “Other” ethnic groups were found to be Rh negative. The overall prevalence of Rh negative group was estimated at 0.15% (Table S1).

G6PD phenotype by fluorescent spot test

A total of 12,427/13,520 (91.9%) women were screened for G6PD activity showing an overall prevalence of phenotypic deficiency of 2.9% (Table 3). Sgaw Karen and Mon ethnicities showed the

highest prevalence of deficiency (over 4%), while all the other ethnic groups had less than 2% prevalence, with the “Muslim” and Rakhine at the lower end (close to 1%).

G6PD genotypes according to ethnic group

Among the subsample of 317 women, G6PD genotyping was performed for Mahidol, Canton, Kaiping, Chinese-4 and Mediterranean variants. Since the minor ethnic groups were hardly represented in this smaller sample, they were pooled together. The major mutation found was Mahidol, representing over 95% of all mutations in all ethnic groups, with the exception of Burmans in whom Kaiping, Canton and Chinese-4 were found globally at a polymorphic frequency (Table 4).

Quantitation of G6PD activity by spectrophotometry

The distribution of quantitative G6PD activity in 317 women is displayed in Figure 2, according to G6PD genotype (all mutations are pooled). G6PD activity in homozygous women for wild type allele was not normally distributed and had a median (IQR) of 11.76 (3.05) IU/gHb, similar to the median of 11.50 IU/gHb assessed previously in males; G6PD activity in the deficient homozygotes was not normally distributed and had a median (IQR) of 0.13 (0.15) IU/gHb. Both homozygous genotypes were therefore at the extremes of the activity spectrum, while heterozygous women had a wide distribution of activities from completely normal to “completely” deficient. The G6PD activity in 74 heterozygous women for the most prevalent Mahidol variant (Figure 3) showed a normal distribution with a mean of 7.38 IU/gHb corresponding to 62.8% of the normal activity (based on population median activity in females). According to this distribution, 6.6% of women had a G6PD activity in the range of deficiency (<30% normal activity), 60.5% were in the 30–70% activity range, and the remaining 32.9% were in the normal activity range (>70% normal activity).

Hemoglobin variants

Results of the hemoglobin typing by HPLC or Capillary Electrophoresis among 12,676 women are shown in Table 5. The highest HbE allelic frequency was found among women of Rakhine (23.2%) and Burman (11.0%) followed by the Mon ethnicities (7.7%). Sgaw Karen and Poe Karen had the lowest HbE allelic frequency among all the ethnic groups (1.0% and 1.7% respectively).

Table 3. G6PD phenotype by fluorescent spot test according to ethnic group.

| | Deficient | Normal | Total | % phenotypic deficiency |
|--------------|------------|---------------|---------------|-------------------------|
| Sgaw Karen | 226 | 5,110 | 5,336 | 4.2% |
| Poe Karen | 25 | 1,525 | 1,550 | 1.6% |
| Burman | 72 | 3,618 | 3,690 | 2.0% |
| “Muslim” | 6 | 504 | 510 | 1.2% |
| Mon | 7 | 128 | 135 | 5.2% |
| Kachin | 0 | 12 | 12 | 0.0% |
| Pa Oh | 1 | 99 | 100 | 1.0% |
| Rakhine | 0 | 69 | 69 | 0.0% |
| Shan | 1 | 23 | 24 | 4.2% |
| “Mixed” | 20 | 860 | 880 | 2.3% |
| Other | 2 | 119 | 121 | 1.7% |
| Total | 360 | 12,067 | 12,427 | 2.9% |

Table 4. G6PD genotypes among 317 women of different ethnicities.

| | Wild type | Mahidol heterozygote | Chinese-4 heterozygote | Kaiping heterozygote | Mahidol homozygote | Mahidol-Canton | Total | Overall allelic frequency of all variants | Allelic frequency of Mahidol variant |
|--------------|------------|----------------------|------------------------|----------------------|--------------------|----------------|------------|---|--------------------------------------|
| Sgaw Karen | 91 | 47 | 1 | 1 | 7 | 0 | 147 | 21.4% | 20.7% |
| Poe Karen | 34 | 12 | 0 | 1 | 4 | 0 | 51 | 20.6% | 19.6% |
| Burman | 67 | 9 | 1 | 2 | 0 | 1 | 80 | 8.8% | 6.3% |
| “Muslim” | 11 | 0 | 0 | 0 | 0 | 0 | 11 | 0.0% | 0.0% |
| Other | 6 | 1 | 0 | 0 | 0 | 0 | 7 | 7.1% | 7.1% |
| “Mixed” | 16 | 5 | 0 | 0 | 0 | 0 | 21 | 11.9% | 11.9% |
| Total | 225 | 74 | 2 | 4 | 11 | 1 | 317 | 16.3% | 15.3% |

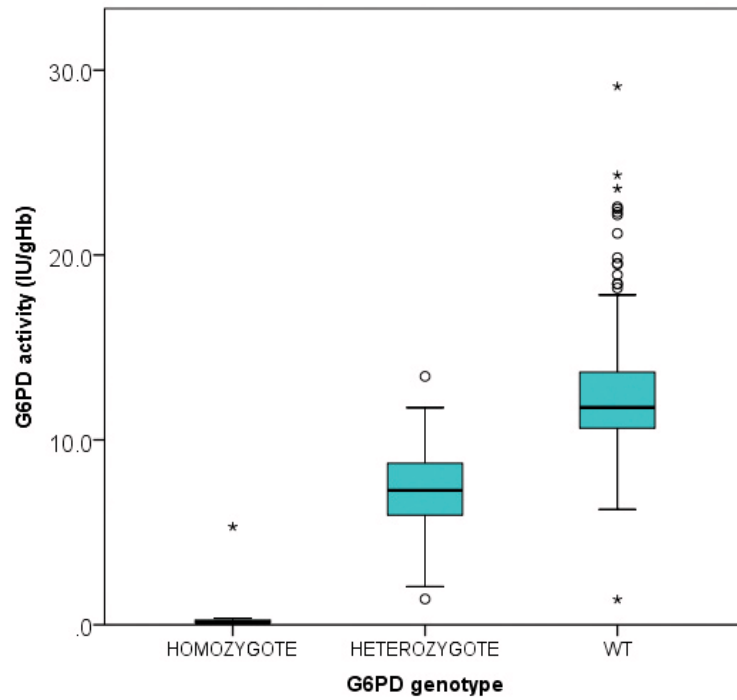


Figure 2. G6PD activity (IU/gHb) assessed by spectrophotometer in 317 women.

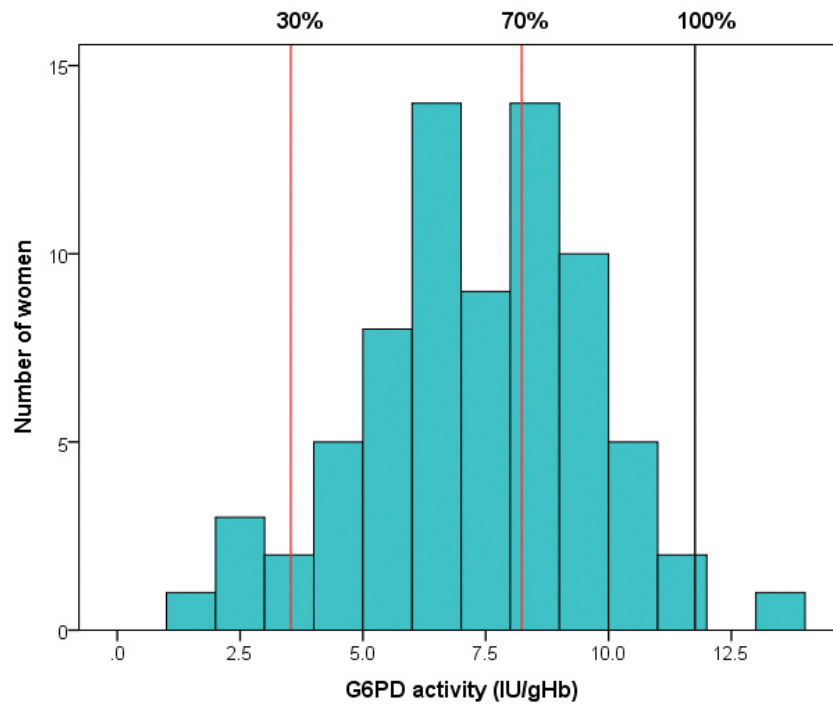


Figure 3. Distribution of G6PD enzymatic activities among 74 Mahidol heterozygous women. Vertical red lines are the 30% and 70% of normal activity (based on the female population median, 100%).

Table 5. Distribution of Hb variants diagnosed by HPLC or CE in the different ethnic groups.

| | Sgaw Karen | Poe Karen | Burman | “Muslim” | Mon | Pa On | Rakhine | Shan | Kachin | Other | “Mixed” | Total |
|---|------------|-----------|--------|----------|-------|-------|---------|-------|--------|-------|---------|--------|
| Hb Normal | 4,072 | 1,181 | 2,217 | 361 | 83 | 76 | 25 | 20 | 7 | 84 | 595 | 8,721 |
| Beta-thalassemia trait | 421 | 123 | 60 | 17 | 4 | 4 | 0 | 1 | 1 | 3 | 49 | 683 |
| Beta-thalassemia trait with HbCS | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Beta-thalassemia/HbE disease | 1 | 1 | 3 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 9 |
| HbEE | 0 | 1 | 39 | 0 | 1 | 2 | 4 | 0 | 0 | 0 | 2 | 49 |
| HbE trait | 103 | 51 | 726 | 30 | 19 | 9 | 24 | 2 | 1 | 20 | 71 | 1,056 |
| HbE trait with HbCS | 2 | 1 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| Suspected alpha-thalassemia | 835 | 207 | 598 | 112 | 27 | 11 | 14 | 0 | 3 | 18 | 157 | 1,982 |
| Suspected alpha-thalassemia with HbCS | 34 | 17 | 58 | 7 | 2 | 2 | 0 | 0 | 1 | 1 | 8 | 130 |
| Suspected HbH disease | 2 | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Unknown Abnormal variant | 6 | 1 | 6 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 15 |
| Total | 5,479 | 1,586 | 3,727 | 528 | 136 | 104 | 69 | 24 | 13 | 126 | 884 | 12,676 |
| HbE carrier prevalence | 1.9% | 3.4% | 21.0% | 5.9% | 14.7% | 10.6% | 42.0% | 12.5% | 7.7% | 15.9% | 8.4% | 8.9% |
| HbE allelic frequency | 1.0% | 1.7% | 11.0% | 2.8% | 7.7% | 6.3% | 23.2% | 4.2% | 3.8% | 7.9% | 4.2% | 4.6% |
| Beta-thalassemia carrier prevalence | 7.8% | 7.9% | 1.7% | 3.4% | 2.9% | 3.8% | 1.4% | 8.3% | 7.7% | 2.4% | 5.7% | 5.5% |
| Beta-thalassemia allelic frequency | 3.9% | 3.9% | 0.8% | 1.7% | 1.5% | 1.9% | 0.7% | 4.2% | 3.8% | 1.2% | 2.8% | 2.7% |
| Alpha-thalassemia carrier frequency (any) | 16.0% | 14.4% | 18.1% | 22.5% | 21.3% | 12.5% | 20.3% | 0.0% | 30.8% | 15.1% | 18.7% | 16.9% |
| HbCS carrier frequency | 0.7% | 1.2% | 1.9% | 1.3% | 1.5% | 1.9% | 0.0% | 0.0% | 7.7% | 0.8% | 0.9% | 1.2% |

HbCS= hemoglobin Constant Spring

Suspected alpha-thalassemia is defined as percentage of HbA₂ ≤2.2% by HPLC or HbA₂ ≤2.1% by Capillary electrophoresis

Beta-thalassemia had an allelic frequency lower than 5% in all ethnic groups, with a carrier prevalence less than 10%; the highest allelic frequency was found in Sgaw Karen and Poe Karen (3.9%) and the lowest among women of the Rakhine and Burman ethnicities (0.9% and 0.7%, respectively).

According to the HPLC or CE results, the prevalence of presumptive alpha-thalassemia carriers was generally high in all ethnic groups, varying from 14.4% in Poe Karen women to 22.5% in “Muslim” women. Hemoglobin Constant Spring diagnosed by capillary electrophoresis was found in less than 2% of the women, with a higher prevalence among Burman (1.9%) and the lowest among

Sgaw Karen (0.7%). Molecular analyses of alpha-thalassemia deletional mutations in 533 samples with either HbA₂ ≤2.2% or beta-thalassemia trait (Table 6) showed that the most common deletion was α -3.7 followed by SEA. Among the 374 subjects diagnosed as suspected alpha-thalassemia carriers based on HbA₂, less than half (44.1%) had mutations in the alpha genes, indicating that the percentage of HbA₂ alone was not a good indicator of alpha-thalassemia carriage. The lowest HbA₂ percentage was associated with deletions on three alpha genes (HbH disease), ($P < 0.01$ vs all others), while subjects with two or one mutation had increasingly higher HbA₂ percentage, but not significantly different from those without mutations. Among the beta-thalassemia carriers the

Table 6. Alpha-thalassemia deletions among subjects with suspected alpha-thalassemia trait or beta-thalassemia trait diagnosed by HPLC or CE.

| HPLC/CE diagnosis | a-3.7/a-3.7 | a-3.7/-SEA (HbH) | aa/a-3.7 | aa/a-4.2 | aa/-SEA | Normal | Total |
|--|-------------|------------------|------------|----------|----------|------------|------------|
| Suspected Alpha-thalassemia trait (including HbCS and HbH) | 20 | 7 | 128 | 1 | 9 | 209 | 374 |
| Beta-thalassemia trait | 3 | 0 | 37 | 0 | 0 | 119 | 159 |
| Total | 23 | 7 | 180 | 1 | 9 | 335 | 533 |

prevalence of concurrent alpha-thalassemia carriage was 25.3% and in this group there was no difference in percentage of HbA₂ between subjects with alpha deletions and those without. When considering mutations on both alpha and non-alpha globin chains, Rakhine, Burman and “Muslim” showed the highest prevalence of abnormal hemoglobins.

Overall, 43.2% of women analyzed for both G6PD and Hb genotypes were found to be carrier of at least one mutation (although for alpha-thalassemia the diagnosis was only presumptive) and 9.1% of women were found to have concomitant mutations in both G6PD and hemoglobin genes.

Discussion

In Myanmar, the population is divided officially into eight main ethnicities, which the government has further classified into 135 different indigenous ethnic groups. The majority group Burman make up 68% of the country’s population of 55 million, with the Shan (9%), Karen (7%), Rakhine (or Arakanese) (4%) and Mon (2%) comprising the largest minority ethnic groups. In this area of the Thailand-Myanmar border, the Karen group predominates and this is further classified into Sgaw and Poe. Patients of Sgaw Karen, Burman, Poe Karen and “Muslim” ethnicity represent the four local major ethnic groups who seek health care at border SMRU clinics.

Some of the genetic traits analyzed in the cohort cannot be used as anthropological markers since they are under selective pressure from malaria, but the results of the study show overall a marked inter-ethnic variability. The distribution of ABO groups indicates that the Sgaw Karen is the most distinct group among the major four analyzed. Current data show similarities to previous published data on smaller sample sets from different ethnic groups living in various regions of Myanmar (Mya-Tu *et al.*, 1971) where Burman, Rakhine and Pa-On have a trend of lower group O prevalence compared to the other groups. In addition, Rhesus negativity was found only among Burman and “Muslim” groups.

G6PD deficiency is relatively common in all ethnic groups with phenotypic deficiency prevalence in women ranging from 1% of most groups (corresponding to the observed allelic frequency of 10%) to a maximum of over 4% (corresponding to the observed allelic frequency of 20%) in the Sgaw Karen. These data confirm previous observations in the male population on the Thailand-Myanmar border (Banccone *et al.*, 2014; Phompradit *et al.*, 2011),

inside Myanmar (Arnolda *et al.*, 2015; Matsuoka *et al.*, 2004; Nuchprayoon *et al.*, 2008) and in Kachin state adjacent to the Myanmar-China border (Li *et al.*, 2015). Current G6PD qualitative field tests can only detect marked deficiency, and women classified as “G6PD normal” are a rather heterogeneous group of subjects with G6PD activity ranging from 30% to 100% of normal, whose hemolytic risk when treated with oxidative drugs varies widely. Characterization of quantitative G6PD phenotype in a large population of females has never being carried out before and represents a resource for informing primaquine and antibiotic treatment in women living in an endemic area for *Plasmodium vivax* and other infectious diseases. Nitrofurantoin, a known precipitant of hemolysis in G6PD deficient patients (Youngster *et al.*, 2010), is recommended as first line treatment for urinary tract infections in pregnancy (Gupta *et al.*, 2011), due to its safety, low cost, and efficacy. Primaquine cannot be prescribed in pregnant women, but it is indicated postpartum for women infected with *P. vivax* during pregnancy and is a cornerstone of vivax malaria elimination. Our data show that over 60% of heterozygote women have a G6PD enzymatic activity over the threshold of deficiency for field tests but in the intermediate range (i.e. lower than the normal), and so could be at risk of drug induced hemolysis with certain primaquine regimens (Chu *et al.*, 2017). Furthermore, the slightly skewed distribution of G6PD activities among heterozygous women might suggest some somatic selection against deficient RBCs as seen in the severe variant Mediterranean (Rinaldi *et al.*, 1976); more investigations will be needed to establish whether this is specific to pregnancy or to the Mahidol variant.

Hemoglobin variants are common in this population, affecting 31.2% of women overall. There are marked differences in the prevalence of HbE and beta-thalassemia in different ethnic groups (as has been described previously (Win *et al.*, 2005)) and this peculiar distribution, combined with a low rate of intermarriage between ethnic groups, suggests that the population may have partial protection from the deleterious beta-thalassemia/HbE syndrome. In fact only 9 women in the entire cohort (0.07%) were found to have beta-thalassemia/HbE syndrome, significantly below what would be expected in a random mating population ($\chi^2=23.1$, $P<0.001$). Furthermore, within the Sgaw Karen ethnicity only, the observed frequency of beta-thalassemia/HbE genotype was lower than expected ($\chi^2=9.14$, $P<0.05$) suggesting a further associated reduced survival to reproductive age. Molecular characterization of alpha-thalassemia was only performed in

subjects with suspected diagnosis by HPLC or CE and in a subsample of beta-thalassemia carriers. Data presented here confirm that, with the exclusion of HbH disease, the lower percentage of HbA₂ was not specific for alpha-thalassemia trait, as over half of the women identified by this criteria had a normal genotype. The assessed higher prevalence of alpha-thalassemia among the beta-thalassemia carriers (25.3%) seems to be closer to the true prevalence in the population and it is higher than that estimated only by the low percentage of HbA₂ (16.9%); subjects with percentage of HbA₂ over the threshold used here could be still carriers of mutations on the alpha-globin genes.

While the main reasons for investigating G6PD deficiency and Hb variants in pregnant women is the clinical management of anemia and treatment with antimicrobial agents, the results carry implications for the offspring of the women tested. Communicating this type of information to women in this population with low health literacy in meaningful language is challenging. When G6PD deficiency is diagnosed, the staff counsel the woman about which drugs and food should be avoided and give her a card explaining the diagnosis and contraindicated medications. When Hb variants are diagnosed, the woman is informed that she might experience weakness or other anemia symptoms during the pregnancy and a card with the diagnosis and a short description is given to the patient. During this simple counseling the woman is informed she might have passed her genetic trait to her present fetus and might pass it in future pregnancies; the possibility that the offspring might inherit the abnormal Hb trait from both parents and be severely affected represents an important, but difficult to convey, part of the counseling about hemoglobinopathies. Due to fragmented education affected by conflict, poverty and migration, approximately 50% of ANC attendants are illiterate (Carrara *et al.*, 2011), and the majority are totally unfamiliar with concepts of genetics and inheritance. The challenge of counseling about often asymptomatic diseases with complex implications is significant (McGready *et al.*, 2014) and could result in dire unintended consequences in some individuals. In countries where hemoglobinopathies are common, several screening approaches have been developed (for a review see Amato *et al.*, 2014); screening might happen early in secondary school (e.g. Latium region and Sardinia in Italy, Greece), mandatory pre-marital screening is practiced in countries such as Cyprus, Iran and Saudi Arabia. Couples might be offered prenatal testing on the fetus and pregnancy termination when affected. Cost/benefit studies have shown that prevention programs are highly cost effective (Koren *et al.*, 2014) and a means to reduce suffering for patients and families (Ballantyne *et al.*, 2009). In Myanmar, there is no routine practice of prenatal screening for Hb variants, and this kind of testing would only be available to private patients consulting at specialty clinics in major urban centers. In Thailand, the screening is performed by HPLC on voluntary basis at the first ANC appointment. When the mother is found to be a carrier, the father of the baby is also tested and if found to be carrier, a deeper molecular investigation is performed. In the low-resource setting of SMRU, Hb testing for the mother alone costs approximately the

same amount as all other investigations performed at the first ANC consultation combined. Hb testing for the father is not routinely performed due to cost, and pre-natal fetal diagnosis is not possible. Furthermore, SMRU is not equipped to offer long-term clinical care for subjects with transfusion-dependent thalassemia, and the costs for refugees or migrants would be a limiting factor even when referral to a reference center in Thailand is possible. Safe termination of pregnancy is not accessible to most migrant and refugee women in this border area and unsafe abortions are a cause of maternal morbidity and mortality in these vulnerable communities (Turner *et al.*, 2012). Despite these challenges and limitations, the current data on the high prevalence of HbE, alpha and beta-thalassemia warrant a continuation of screening and encourage the implementation of a more extended program of early information and counseling to girls and women of reproductive age among the population. The pioneering and remarkable example of the thalassemia screening carried out by the “Centro Studi Microcitemie Roma” during 37 years among young students in Latium, Italy, (Amato *et al.*, 2014) shows that information, screening of relatives, and counseling are major factors in the success of programs for the prevention of severe hemoglobinopathies. For long term sustainability, laboratory testing can be carried out with cheaper techniques, such as single-tube osmotic fragility test (OFT), dichlorophenolindophenol (DCIP) for HbE, and microscopic examination following a step-wise approach whereby the most expensive tests are only performed on samples that result abnormal by initial screening. This approach, especially if offered as part of a strong preconception healthcare package, including partner testing where appropriate, genetic counseling, folic acid supplementation, and effective and acceptable family planning provision, could substantially decrease the suffering of vulnerable families. Such a program would require community engagement and human resource development to equip local staff with the specialized skills of sensitive and responsive genetic counseling for individuals with limited background science knowledge.

In conclusion, anemia is one of the most common clinical problems in pregnancy, and has been associated with severe adverse pregnancy outcomes, including maternal deaths (McGready *et al.*, 2012). In this area of the Thai-Myanmar border, anemia is multi-factorial, with hemolysis, hemoglobinopathies, hemorrhage, micronutrient deficiencies, intestinal parasites, and malaria being some of the most common causes. These complex and often concurrent contributors are superimposed on the physiological hemodilution of pregnancy, making interpretation of individual measurements of hemoglobin and hematocrit challenging. In women with congenital Hb variants, anemia can be present before pregnancy and might worsen during gestation, and requirement for iron supplementation can be difficult to assess. The current population data will help inform ongoing efforts to optimize the clinical management of anemia in the local pregnant population by investigating newer marker of iron deficiency anemia (for example, hepcidin), which might be more reliable in such context as compared to classic markers (Bah *et al.*, 2017; Wray *et al.*, 2017).

Data availability

Due to ethical and security considerations, the data that supports the findings in this study can be accessed only through the Data Access Committee at Mahidol Oxford Tropical Medicine Research Unit (MORU). The data sharing policy can be found here: <http://www.tropmedres.ac/data-sharing>. The application form for datasets under the custodianship of MORU Tropical Network can be found in [Supplementary File 1](#).

Ethical statement

Ethical approval for the study was granted by the Faculty of Tropical Medicine, Mahidol University (MUTM 2017-041-01) and by Oxford University (OXTREC#583-16).

Competing interests

No competing interests were disclosed.

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Supplementary material

Table S1. ABO blood groups and calculated allelic frequency in minor ethnic groups.

[Click here to access the data.](#)

Supplementary File 1: Application form for datasets under the custodianship of Mahidol Oxford Tropical Medicine Research Unit (MORU) Tropical Network.

[Click here to access the data.](#)

References

- Allison AC: **Protection afforded by sickle-cell trait against subtertian malarial infection.** *Br Med J.* 1954; 1(4857): 290–294.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Amato A, Cappabianca MP, Lerone M, *et al.*: **Carrier screening for inherited haemoglobin disorders among secondary school students and young adults in Latium, Italy.** *J Community Genet.* 2014; 5(3): 265–268.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Arnolda G, Nwe HM, Trevisanuto D, *et al.*: **Risk factors for acute bilirubin encephalopathy on admission to two Myanmar national paediatric hospitals.** *Matern Health Neonatol Perinatol.* 2015; 1: 22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bah A, Pasricha SR, Jallow MW, *et al.*: **Serum Hepcidin Concentrations Decline during Pregnancy and May Identify Iron Deficiency: Analysis of a Longitudinal Pregnancy Cohort in The Gambia.** *J Nutr.* 2017; 147(6): 1131–1137.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ballantyne A, Newson A, Luna F, *et al.*: **Prenatal diagnosis and abortion for congenital abnormalities: is it ethical to provide one without the other?** *Am J Bioeth.* 2009; 9(8): 48–56.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bancone G, Chu CS, Somsakchaicharoen R, *et al.*: **Characterization of G6PD Genotypes and Phenotypes on the Northwestern Thailand-Myanmar Border.** *PLoS One.* 2014; 9(12): e116063.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Berstein F: **Zusammenfassende Betrachtungen über die erblichen Blutstrukturen des Menschen.** *Z Indukt Abstamm Vererbungsl.* 1925; 37(1): 237–370.
[Publisher Full Text](#)
- Beutler E, Blume KG, Kaplan JC, *et al.*: **International Committee for Standardization in Haematology: recommended methods for red-cell enzyme analysis.** *Br J Haematol.* 1977; 35(2): 331–340.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Carrara VI, Hogan C, De Pree C, *et al.*: **Improved pregnancy outcome in refugees and migrants despite low literacy on the Thai-Burmese border: results of three cross-sectional surveys.** *BMC Pregnancy Childbirth.* 2011; 11: 45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chong SS, Boehm CD, Cutting GR, *et al.*: **Simplified multiplex-PCR diagnosis of common southeast asian deletional determinants of alpha-thalassemia.** *Clin Chem.* 2000; 46(10): 1692–1695.
[PubMed Abstract](#)
- Chu CS, Bancone G, Moore KA, *et al.*: **Haemolysis in G6PD Heterozygous Females Treated with Primaquine for Plasmodium vivax Malaria: A Nested Cohort in a Trial of Radical Curative Regimens.** *PLoS Med.* 2017; 14(2): e1002224.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fuchareon S, Winichagoon P: **New updating into hemoglobinopathies.** *Int J Lab Hematol.* 2012; 34(6): 559–565.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gupta K, Hooton TM, Naber KG, *et al.*: **International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases.** *Clin Infect Dis.* 2011; 52(5): e103–120.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Haldane JBS: **The rate of mutation of human genes.** *Hereditas Suppl.* 1949; 35(S1): 267–273.
[Publisher Full Text](#)
- Hoogenboom G, Thwin MM, Velink K, *et al.*: **Quality of intrapartum care by skilled birth attendants in a refugee clinic on the Thai-Myanmar border: a survey using**

WHO Safe Motherhood Needs Assessment. *BMC Preg Childbirth.* 2015; 15: 17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Howes RE, Piel FB, Patil AP, *et al.*: **G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map.** *PLoS Med.* 2012; 9(11): e1001339.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Hutagalung R, Wilairatana P, Looareesuwan S, *et al.*: **Influence of hemoglobin E trait on the severity of Falciparum malaria.** *J Infect Dis.* 1999; 179(1): 283–286.
[PubMed Abstract](#) | [Publisher Full Text](#)

Koren A, Profeta L, Zalman L, *et al.*: **Prevention of β Thalassemia in Northern Israel - a Cost-Benefit Analysis.** *Mediterr J Hematol Infect Dis.* 2014; 6(1): e2014012.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Lee AC: **Transfusion-dependent anaemia of undetermined origin: a distinctive syndrome in paediatric medical tourism.** *Ann Acad Med Singapore.* 2012; 41(7): 305–308.
[PubMed Abstract](#)

Li Q, Yang F, Liu R, *et al.*: **Prevalence and Molecular Characterization of Glucose-6-Phosphate Dehydrogenase Deficiency at the China-Myanmar Border.** *PLoS One.* 2015; 10(7): e0134593.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Matsuoka H, Wang J, Hirai M, *et al.*: **Glucose-6-phosphate dehydrogenase (G6PD) mutations in Myanmar: G6PD Mahidol (487G>A) is the most common variant in the Myanmar population.** *J Hum Genet.* 2004; 49(10): 544–547.
[PubMed Abstract](#) | [Publisher Full Text](#)

McGready R, Boel M, Rijken MJ, *et al.*: **Effect of early detection and treatment on malaria related maternal mortality on the north-western border of Thailand 1986–2010.** *PLoS One.* 2012; 7(7): e40244.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

McGready R, Kang J, Watts I, *et al.*: **Audit of antenatal screening for syphilis and HIV in migrant and refugee women on the Thai-Myanmar border: a descriptive study [version 2; referees: 2 approved].** *F1000Res.* 2014; 3: 123.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Mya-Tu M, May-May-Yi, Thin-Thin-Hlaing, *et al.*: **Blood groups of the Burmese population.** *Hum Hered.* 1971; 21(5): 420–430.
[PubMed Abstract](#) | [Publisher Full Text](#)

Nuchprayoon I, Louicharoen C, Charoenvej W: **Glucose-6-phosphate dehydrogenase mutations in Mon and Burmese of southern Myanmar.** *J Hum Genet.* 2008; 53(1): 48–54.
[PubMed Abstract](#) | [Publisher Full Text](#)

Phompradit P, Kuesap J, Chaijaroenkul W, *et al.*: **Prevalence and distribution of glucose-6-phosphate dehydrogenase (G6PD) variants in Thai and Burmese populations in malaria endemic areas of Thailand.** *Malar J.* 2011; 10: 368.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Phylipsen M, Prior JF, Lim E, *et al.*: **Two new alpha1-globin gene point mutations: Hb Nedlands (HBA1:c.86C>T) [alpha28(B9)Ala->Val] and Hb Queens Park (HBA1:c.98T>A) [alpha32(B13)Met->Lys].** *Hemoglobin.* 2010; 34(2): 123–126.
[PubMed Abstract](#) | [Publisher Full Text](#)

Rinaldi A, Filippi G, Siniscalco M: **Variability of red cell phenotypes between and within individuals in an unbiased sample of 77 heterozygotes for G6PD deficiency in Sardinia.** *Am J Hum Genet.* 1976; 28(5): 496–505.
[PubMed Abstract](#) | [Free Full Text](#)

Siniscalco M, Bernini L, Latte B, *et al.*: **Favism and Thalassemia in Sardinia and their Relationship to Malaria.** *Nature.* 1961; 190: 1179–1180.
[Publisher Full Text](#)

Than AM, Harano T, Harano K, *et al.*: **High incidence of 3-thalassemia, hemoglobin E, and glucose-6-phosphate dehydrogenase deficiency in populations of malaria-endemic southern Shan State, Myanmar.** *Int J Hematol.* 2005; 82(2): 119–123.
[PubMed Abstract](#) | [Publisher Full Text](#)

Turner P, Willemse C, Phakaudom K, *et al.*: **Aeromonas spp. bacteremia in pregnant women, Thailand-Myanmar border, 2011.** *Emerg Infect Dis.* 2012; 18(9): 1522–1523.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Williams TN, Weatherall DJ: **World distribution, population genetics, and health burden of the hemoglobinopathies.** *Cold Spring Harb Perspect Med.* 2012; 2(9): a011692.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Win N, Lwin AA, Oo MM, *et al.*: **Hemoglobin E prevalence in malaria-endemic villages in Myanmar.** *Acta Med Okayama.* 2005; 59(2): 63–66.
[PubMed Abstract](#)

Wray K, Allen A, Evans E, *et al.*: **Hepcidin detects iron deficiency in Sri Lankan adolescents with a high burden of hemoglobinopathy: A diagnostic test accuracy study.** *Am J Hematol.* 2017; 92(2): 196–203.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Youngster I, Arcavi L, Schechmaster R, *et al.*: **Medications and glucose-6-phosphate dehydrogenase deficiency: an evidence-based review.** *Drug Saf.* 2010; 33(9): 713–726.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Referee Status: ? ?

Version 1

Referee Report 25 September 2017

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Issarang Nuchprayoon

Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

This study is a valuable study based on its large sample size of pregnant women of rarely studied ethnic group, namely Sgaw Karen in comparison with Burman.

The subject of study; inherited red cell variants are valid in itself. It is controversial whether heterozygous G6PD mutation, thalassemia traits, and ABO, Rh blood groups are 'disorders'. People with all of these conditions do not have anemia in their usual state. Only people with compound heterozygous thalassemia 'diseases', homozygous HbE (HbEE), and HbH will have some degree of anemia. The causes of anemia in pregnant women are by far commonly caused by iron deficiency, which is not addressed in this study. Therefore the reference to anemia in pregnancy should be de-emphasized in the rationale and conclusion of this study.

G6PD results in this report does confirm other studies in Myanmar ethnic groups, many of which were reported in males. One additional citation that the author should consider is a report from Louicharen *et al*, 2009¹, who studied prevalence of G6PD mutations in Karen of suan peung, at a Thai-Myanmar border. In this paper in which details are reported in supplementary article in Science website. the prevalence of G6PD deficiency 20%, with predominantly (94%) Mahidol variant.

The methodology for estimating prevalence of alpha-thalassemia trait in this paper is controversial. Using low HbA2 is known to be unreliable and in fact the authors showed only 44.1% specificity. Since it is known how much is alpha-tha are missed in people with normal A2, the total prevalence is therefore not known for certainty. The prevalence of alpha-thalassemia in subset of studies in known beta-thal trait population may be more accurate estimation assuming all subjects were tested, but need to be stratified by each ethnic group. The author may wish state a conservative estimate in each ethnic group, like at least 25%, instead of a specific proportion.

Due to large number of Karen and Burmans in this study, the author may wish to point out the value of this paper in population genetics. Their difference in prevalence was a founder effect and due to estimation of G6PD Mahidol variant emerging around 1500 years ago¹ as protective factor against malaria, the Burman may be a more recent population in this region.

In the final conclusion paragraph, the author should made a summary statement of their key findings on prevalence of variants but not about anemia since this paper did not address the issue. A mention of newer marker for iron deficiency detection is irrelevant and not useful in this resource-limited region.

Suggestion of counseling is irrelevant and impractical here.

References

1. Louicharoen C, Patin E, Paul R, Nuchprayoon I, Witoonpanich B, Peerapittayamongkol C, Casademont I, Sura T, Laird NM, Singhasivanon P, Quintana-Murci L, Sakuntabhai A: Positively selected G6PD-Mahidol mutation reduces Plasmodium vivax density in Southeast Asians. *Science*. 2009; **326** (5959): 1546-9 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Galanello R, Cao A: Gene test review. Alpha-thalassemia. *Genet Med*. 2011; **13** (2): 83-8 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: molecular genetics, G6PD, blood groups, clinical care of thalassemia

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Oct 2017

Germana Bancone, Shoklo Malaria Research Unit, Thailand

We thank the reviewer for his useful comments and suggestions. Below the answer to his questions.

Q1) The subject of study; inherited red cell variants are valid in itself. It is controversial whether heterozygous G6PD mutation, thalassemia traits, and ABO, Rh blood groups are 'disorders'. People with all of these conditions do not have anemia in their usual state. Only people with compound heterozygous thalassemia 'diseases', homozygous HbE (HbEE), and HbH will have some degree of anemia. The causes of anemia in pregnant women are by far commonly caused by iron deficiency, which is not addressed in this study. Therefore the reference to anemia in pregnancy should be de-emphasized in the rationale and conclusion of this study.

A1) Anaemia is the most common medical problem of the pregnant population under study and is associated with maternal mortality (McGready, Boel et al. 2012) but a systematic analysis of genetic causes of anemia has never been undertaken. We think that, although analyzing these markers is interesting in itself, we cannot avoid highlighting that our primary interest is their contribution to anaemia during pregnancy; our plan is also to analyse soon clinical data in relation to the findings presented in this manuscript. Following the reviewers suggestions, we have re-phrased some of the text in the introduction and conclusion to clarify our message.

Q2) G6PD results in this report does confirm other studies in Myanmar ethnic groups, many of which were reported in males. One additional citation that the author should consider is a report from Louicharen *et al*, 2009¹, who studied prevalence of G6PD mutations in Karen of suan peung, at a Thai-Myanmar border. In this paper in which details are reported in supplementary article in Science website. the prevalence of G6PD deficiency 20%, with predominantly (94%) Mahidol variant.

A2) We have added the suggested citation

Q3) The methodology for estimating prevalence of alpha-thalassemia trait in this paper is controversial. Using low HbA2 is known to be unreliable and in fact the authors showed only 44.1% specificity. Since it is known how much is alpha-tha are missed in people with normal A2, the total prevalence is therefore not known for certainty. The prevalence of alpha-thalassemia in subset of studies in known beta-thal trait population may be more accurate estimation assuming all subjects were tested, but need to be stratified by each ethnic group. The author may wish state a conservative estimate in each ethnic group, like at least 25%, instead of a specific proportion.

A3) As we answered also to the other reviewer, we have modified all the text and Tables to improve accuracy and clarity on this point.

Q4) Due to large number of Karen and Burmans in this study, the author may wish to point out the value of this paper in population genetics. Their difference in prevalence was a founder effect and due to estimation of G6PD Mahidol variant emerging around 1500 years ago¹ as protective factor against malaria, the Burman may be a more recent population in this region.

A4) Although this is an interesting point to consider, the current data were not collected or analyzed to support or contradict evolutionary dynamics of Karen and Burman populations, therefore we prefer not to address the topic.

Q5) In the final conclusion paragraph, the author should made a summary statement of their key findings on prevalence of variants but not about anemia since this paper did not address the issue. A mention of newer marker for iron deficiency detection is irrelevant and not useful in this resource-limited region. Suggestion of counseling is irrelevant and impractical here.

A5) We have revised the text of the conclusive paragraph. We strongly believe that, having found a pretty high prevalence of abnormal Hb variants, a discussion on counselling pregnant women is warranted in this setting. Though this counselling is time-intensive and challenging, it is feasible in low resource settings with appropriately trained and sensitive local staff.

Furthermore, better diagnostic tools for iron-deficient anaemia during pregnancy have been shown to be particularly useful in populations with a high prevalence of haemoglobin disorders (Wray, Allen et al. 2017); our data bring evidence on the widespread distribution of IRD and on the need for testing in resource-limited areas.

Competing Interests: I have no competing interests

Referee Report 04 September 2017

doi:[10.21956/wellcomeopenres.13359.r25454](https://doi.org/10.21956/wellcomeopenres.13359.r25454)**Thomas N. Williams**  1,2

¹ Centre for Geographic Medicine Research-Coast, Kenyan Medical Research Institute (KEMRI)-Wellcome Trust Research Programme, Kilifi, Kenya

² Imperial College, London, UK

This is a nice summary of an impressive study of red cell disorders in more than 13,000 pregnant women by the team at the Shoklo Malaria Research Unit on the Thai-Myanmar border.

Major comments

1) The background to the study is rationalized on the basis of the potential importance of inherited red cell disorders as causes and confounders of anaemia in pregnant women within the region. The inclusion of data on ABO and Rhesus in this paper, though interesting, does not fit well within this rational framework. Neither are particularly relevant to the main story. I would either drop those data or alter the rationale to justify their inclusion.

2) The referencing could be improved throughout the introduction. For example, a reference for Lyonisation and G6PD activity could be added, and the third paragraph, which contains a lot of technical information, should be referenced along the way rather than through the bunching of 4 (slightly random) references at the end.

3) Introduction paragraph 3 could be tightened up. The first sentence is a bit confused: “..structural variants and deletional mutations on the hemoglobin chains” is a bit of a mixture of nomenclature more usually specific to either the protein or the gene. The “deletional mutations” more commonly refer to the gene than the protein and probably more correctly should be referred to as “genetic deletions” rather than mutations (which more commonly relate to sequence changes than deletions). Further down, “...97% of hemoglobin is composed of two alpha and two beta chains” – the authors should clarify that they’re talking about ADULT haemoglobin (HbA). The references on the geographic distribution with malaria are a bit random and refer to different IRDs. A recent authoritative review would probably be a better way to go.

4) Methods: Lab analyses. It would be very helpful if the authors explained here on behalf of the non-expert what the G6PD spot test is designed to do and at what level of normal G6PD activity the test turns positive. A major issue with this test, as the authors acknowledge, is that it is a binary test that shows sufficient / deficient only. It is not designed to pick up heterozygotes and is only partially sensitive for homozygotes, depending on the genotype involved and the degree to which it perturbs G6PD production. Some discussion of this in the methods section would help the non-expert reader.

5) In the same section, the authors state that the quantitative test was conducted in “selected women”. It took me some digging to find that it was actually conducted in “a sub-set of sequentially recruited [and therefore “unselected”] women”. This could be better phrased at this point.

6) It would help the reader if the flow chart (Figure 1) could be amended to make the above more clear with regard to the quantitative test and the genotyping for both G6PD and other conditions.

7) Methods; haemoglobinopathies. The authors used an HbA2 of <2.2 or <2.1% (depending on the method) to define “presumptive” carrier status for alpha-thalassaemia. As the authors state later in the paper, this is neither a sensitive nor specific approach to the diagnosis of the alpha-thalassaemias overall. It’s almost useless for the carrier forms of the common minor single gene -3.7 and -4.2kb deletional forms of the condition. As such, the authors’ focus on this sub-group for further work up for alpha thalassaemias constitutes a biased sample. It would really have been better from the perspective of trying to determine

the prevalence of these conditions if the authors had centred their genetic testing on an unselected sample of women. This is the approach they took for G6PD genotyping which therefore produced figures that were more understandable. The figures given for the prevalence of the various alpha thalassaemias are difficult to interpret and probably very misleading because of the way in which the patients were sampled.

8) For this reason, I would remove the rows regarding “suspected alpha thalassaemia” from table 5 – the authors say later on that half of the sub-sample they tested were normal genetically, so this row will mislead the casual reader. HbCS and HbE are more legitimate as these have their own HPLC peaks.

9) Discussion, paragraph 2 seems a bit odd and comes rather out of the blue. This is the first mention of anthropology that I can find in the paper and the use of these IRDs as anthropological tools is put up and shot down in a single sentence!

10) Authors could be more clear that their estimate of G6PDd on the basis of their spot test result will be an underestimate, since homozygosity for less severe variants will not always be picked up (as shown by the outlier on Fig 2).

11) Discussion page 10 paragraph 2 left column. The authors say that all women with “Hb variants” are informed of the results and counseled that they may experience weakness etc in pregnancy. I assume that this is not the case for “suspected alpha thalassaemia”? This would give rise to a great deal of unnecessary anxiety if half don't actually have the condition anyway and in the case of the -3.7 and -4.2 deletions they are clinically silent when inherited alone. The whole area of how to approach antenatal counseling in populations with a high prevalence of multiple conditions is highly complex and the discussion of this element could be tightened up a bit.

Minor

1) Though it's an editorial decision, I would have assumed that since this is a UK based journal the spelling should be in UK English throughout.

2) Abstract Methods: Blood groups were “diagnosed” makes it sound as if these are a disease! I would change to “determined” or similar.

3) “resource rich countries” – preferred current term is “high-income countries”

4) Final paragraph of the introduction could probably be condensed and moved to join the methods section. I would replace by a short sentence summarizing the aims of the current study.

5) Please give the make and model of the HPLC machine used for the haemoglobin phenotyping.

Could do with a good proof-read throughout. A few examples I picked up:

1) Introduction line 3 “...living in area of past...” should read “...living in areas of past...”

2) Abstract Methods: Data were available rather than data was available (data are plural in this context).

3) page 4 line one column 2 – “SAE” should read “SEA”.

4) Table 6 – use the Greek symbol for alpha rather than the letter A.

5) page paragraph 2 left column “found to be carrier..” should read “found to be carriers..”

References

1. Bancone G, Gilder M, Chowwiwat N, Gornsawun G, Win E, Cho W, Moo E, Min A, Charunwatthana P, Carrara V, White N, Nosten F, McGready R: Prevalences of inherited red blood cell disorders in pregnant women of different ethnicities living along the Thailand-Myanmar border. *Wellcome Open Research*. 2017; **2**. [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Haemoglobinopathies, epidemiology, clinical research, malaria, infectious diseases

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Oct 2017

Germana Bancone, Shoklo Malaria Research Unit, Thailand

We thank the reviewer for his useful comments and suggestions. Below the answers to his questions.

Major comments

Q1) The background to the study is rationalized on the basis of the potential importance of inherited red cell disorders as causes and confounders of anaemia in pregnant women within the region. The inclusion of data on ABO and Rhesus in this paper, though interesting, does not fit well within this rational framework. Neither are particularly relevant to the main story. I would either drop those data or alter the rationale to justify their inclusion.

A1) Although the description of ABO and Rhesus blood groups was meant to add information on the genetic background of ethnic groups, we agree with the reviewer that it does not fit well with the framework of the manuscript. We have therefore decided to drop this part.

Q2) The referencing could be improved throughout the introduction. For example, a reference for Lyonisation and G6PD activity could be added, and the third paragraph, which contains a lot of technical information, should be referenced along the way rather than through the bunching of 4 (slightly random) references at the end.

A2) We have added more references in the introduction. The 4 references mentioned by the reviewer only concerned the selection by malaria of haemoglobinopathies.

Q3) Introduction paragraph 3 could be tightened up. The first sentence is a bit confused: “..structural variants and deletional mutations on the hemoglobin chains” is a bit of a mixture of nomenclature more usually specific to either the protein or the gene. The “deletional mutations”

more commonly refer to the gene than the protein and probably more correctly should be referred to as “genetic deletions” rather than mutations (which more commonly relate to sequence changes than deletions). Further down, “...97% of hemoglobin is composed of two alpha and two beta chains” – the authors should clarify that they’re talking about ADULT haemoglobin (HbA). The references on the geographic distribution with malaria are a bit random and refer to different IRDs. A recent authoritative review would probably be a better way to go.

A3) We have reviewed and corrected the paragraph to include the reviewer’s suggestions.

Q4) Methods: Lab analyses. It would be very helpful if the authors explained here on behalf of the non-expert what the G6PD spot test is designed to do and at what level of normal G6PD activity the test turns positive. A major issue with this test, as the authors acknowledge, is that it is a binary test that shows sufficient / deficient only. It is not designed to pick up heterozygotes and is only partially sensitive for homozygotes, depending on the genotype involved and the degree to which it perturbs G6PD production. Some discussion of this in the methods section would help the non-expert reader.

A4) We have revised the text to better explain the G6PD fluorescent spot test.

Q5) In the same section, the authors state that the quantitative test was conducted in “selected women”. It took me some digging to find that it was actually conducted in “a sub-set of sequentially recruited [and therefore “unselected”] women”. This could be better phrased at this point.

A5) We agree with the reviewer that this part of the method was not phrased clearly and we have modified it.

Q6) It would help the reader if the flow chart (Figure 1) could be amended to make the above more clear with regard to the quantitative test and the genotyping for both G6PD and other conditions.

A6) The Flow chart has been modified to exclude results of ABO and Rh blood groups and to clarify G6PD and Hb genotyping.

Q7) Methods; haemoglobinopathies. The authors used an HbA₂ of <2.2 or <2.1% (depending on the method) to define “presumptive” carrier status for alpha-thalassaemia. As the authors state later in the paper, this is neither a sensitive nor specific approach to the diagnosis of the alpha-thalassaemias overall. It’s almost useless for the carrier forms of the common minor single gene -3.7 and -4.2kb deletional forms of the condition. As such, the authors’ focus on this sub-group for further work up for alpha thalassaemias constitutes a biased sample. It would really have been better from the perspective of trying to determine the prevalence of these conditions if the authors had centred their genetic testing on an unselected sample of women. This is the approach they took for G6PD genotyping which therefore produced figures that were more understandable. The figures given for the prevalence of the various alpha thalassaemias are difficult to interpret and probably very misleading because of the way in which the patients were sampled.

A7) We thank the reviewer for pointing out this issue that we got wrong in the first version of the manuscript. At the time we started the screening we were not fully aware of the low specificity of the Hb typing for alpha-thalassaemia and one of our aims was to assess the diagnostic power of CE/HPLC for all hemoglobin variants. Nevertheless we agree that the way we presented the data was not appropriate and that the prevalence of alpha gene mutations in the subgroup of women with low HbA₂ is not representative of the general population. We have therefore modified the text and the Table. This has also allowed correcting the numbers of women with low HbA₂ tested by PCR which is 354 and not 374 as reported previously.

Q8) For this reason, I would remove the rows regarding “suspected alpha thalassaemia” from table 5 – the authors say later on that half of the sub-sample they tested were normal genetically, so this row will mislead the casual reader. HbCS and HbE are more legitimate as these have their own HPLC peaks.

A8) This has been done.

Q9) Discussion, paragraph 2 seems a bit odd and comes rather out of the blue. This is the first mention of anthropology that I can find in the paper and the use of these IRDs as anthropological tools is put up and shot down in a single sentence!

A9) Since we have compared all these genetic markers between ethnic groups, one might argue that they could be used to estimate genetic differences between the ethnicities. We have removed this sentence together with the analysis of ABO/Rh blood groups.

Q10) Authors could be more clear that their estimate of G6PDd on the basis of their spot test result will be an underestimate, since homozygosity for less severe variants will not always be picked up (as shown by the outlier on Fig 2).

A10) We have added and clarified a few sentences to emphasize this point which is already well described in the literatures. With this analysis we wanted to concentrate the attention on the distribution of G6PD activities among heterozygous women which has not been studied extensively before.

Q11) Discussion page 10 paragraph 2 left column. The authors say that all women with “Hb variants” are informed of the results and counseled that they may experience weakness etc in pregnancy. I assume that this is not the case for “suspected alpha thalassaemia”? This would give rise to a great deal of unnecessary anxiety if half don’t actually have the condition anyway and in the case of the -3.7 and -4.2 deletions they are clinically silent when inherited alone. The whole area of how to approach antenatal counseling in populations with a high prevalence of multiple conditions is highly complex and the discussion of this element could be tightened up a bit.

A11) It is very difficult to carry on a proper ante-natal screening and counseling in our setting and we are fully aware of the limitations of our approach. At the beginning all women diagnosed with abnormal Hb were counseled. As explained already in A7 the first aim for genotyping subjects with low %HbA₂ was to rule out alpha thalassaemia carriage; when genotyping became available and the results showed that half of women did not have mutations on the alpha genes and another 40% were silent carriers, we modified the counseling accordingly.

We are aware the discussion is quite long but we also wanted to touch on the several aspects that make this setting peculiar.

Minor

1) Though it’s an editorial decision, I would have assumed that since this is a UK based journal the spelling should be in UK English throughout.

We agree and have changed the spelling to UK English; more than an editorial choice, the first author is an Italian who has a relaxed approach towards spelling.

2) Abstract Methods: Blood groups were “diagnosed” makes it sound as if these are a disease! I would change to “determined” or similar.

We have cut this part.

3) “resource rich countries” – preferred current term is “high-income countries”

This has been changed

4) Final paragraph of the introduction could probably be condensed and moved to join the methods section. I would replace by a short sentence summarizing the aims of the current study

This has been done.

5) Please give the make and model of the HPLC machine used for the haemoglobin phenotyping. We have added it.

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 - 3) page 4 line one column 2 – "SAE" should read "SEA".
 - 4) Table 6 – use the Greek symbol for alpha rather than the letter A.
 - 5) page paragraph 2 left column "found to be carrier.." should read "found to be carriers.."
- We have revised the whole manuscript and hopefully corrected all typos and incorrect terms.

Competing Interests: I have no competing interests