Genetic red cell disorders and severity of falciparum malaria in Myanmar

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A hospital-based survey was undertaken to investigate the relationship between the incidence and severity of malaria infection and various red cell disorders in Myanmar. The mean parasitaemia levels of patients with α- or β-thalassaemia trait or with severe glucose-6-phosphate dehydrogenase (G6PD) deficiency were lower than those of individuals with normal haemoglobin AA or with heterozygous haemoglobin E. The double genetic defect of thalassaemia trait and severe G6PD deficiency appeared to confer some degree of protection against malaria.

Introduction

The high mortality caused by falciparum malaria over many centuries has created significant selective pressure on human populations in malaria-endemic areas, which is believed to be responsible for the prevalence of deleterious genetic traits such as sickle-cell anaemia, thalassaemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency (1–4).

A combination of factors has seen the resurgence of malaria as a major disease throughout the world. Recent experience in Myanmar emphasizes the scope of the problem; it is estimated that during the malaria season (May–August), up to 40% of the total population is infected with Plasmodium falciparum (5). In 1991, for example, approximately 200 000 people required hospitalization for malaria, and 4000 of the confirmed cases, were fatal.

Despite the importance of malaria, no systematic study of the relationship between its incidence and various red cell disorders in Myanmar has been reported. Myanmar represents a particularly complex situation; in addition to the high frequency of malaria, there is a wide range of ethnic groups and a high overall incidence of red cell disorders, which vary between ethnic groups. For example, the prevalence of haemoglobin E (HbE) ranges from approximately 20% among Bamar, Shan and Mon ethnic groups to only 1% among the Chin ethnic group (6). There is also a high incidence of G6PD deficiency (hemizygous) among Bamar, with the incidence among Kayin and Mon groups being up to 16% in some areas (7, 8). The overall incidence of β-thalassaemia trait is about 4% (9) and of α-thalassaemia trait, 10% (10).

As a first step towards obtaining a more rigorous overview of the relationship between red cell disorders and the incidence of malaria, we describe here the results of an extensive hospital-based survey on the relationship between falciparum malaria and genetic red cell abnormalities in Myanmar.

Materials and methods

Study subjects

A total of 383 patients with severe falciparum malaria were selected from Thayarwady Township Hospital and the Defence Services General Hospital, Mingladon, Yangon. The hospitals are 100 km apart and patients were recruited on an individual basis. All patients were adult males, aged 19–45 years. Ethnically all were Bamar (Burmese). The following groups were excluded: under-15-year-olds; females; other ethnic groups (e.g., Shan, Kayin, Kachin, Chin, Mon, and Rakhine); and individuals who had received antimalarial treatment in the previous 7 days.

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Severity of malaria

The WHO criteria for defining the severity of the manifestations of falciparum malaria (11) were used, as outlined below.

- Category I: Essentially uncomplicated falciparum malaria, with parasitaemia <5%.
- Category II: "Hyperparasitaemia" cases, with more than 5% of red blood cells parasitized (or more than 250,000 parasites per µl of blood), but no other severe manifestation of malaria.
- Category III: Patients with impaired consciousness:
  - IIIa: Impaired consciousness but rouseable (Glasgow coma score >9/15, and no other severe manifestation); and
  - IIIb: Cerebral malaria (unrouseable coma); (Glasgow coma score ≤9/15, with or without other severe manifestations such as renal failure, severe anaemia, jaundice, etc.).

Parasite count

Both thick and thin peripheral blood smears were Giemsa-stained. The number of asexual parasites per 400 white blood cells was multiplied by 20 to give a quantitative count per µl.

Sample collection and analysis

Samples of venous blood (5–8 ml) from infected patients were collected in heparinized tubes on the day of admission. Acid–citrate–dextrose (ACD) was added (1:4) to any samples that had to be transported. Estimation of haemoglobin, erythrocyte counts (using a colorimeter), packed cell volume (PCV), and preliminary screening tests for G6PD deficiency (methaemoglobin reduction test) (12) were carried out immediately after the blood samples were collected in the hospitals. Other procedures were carried out at the central laboratory, Department of Medical Research, Yangon. G6PD variants and genotypes were assessed using starch gel electrophoresis (12) or agarose gel electrophoresis (Myint-Oo, W.J. O’Sullivan, unpublished results, 1993). Red cell indices, haemoglobin AA (HbAA) measurements, cellulose acetate electrophoresis (13), and starch gel electrophoresis (14) were carried out routinely on all samples. Screening for abnormal haemoglobins and thalassaemia traits was performed using standard methods (15).

Classification of thalassaemia was according to the protocols described by Modell & Berdoukas (16). The criteria for α-thalassaemia were as follows: HbAA, normal; haemoglobin F (HbF), normal; mean corpuscular haemoglobin, (MCH) <25 pg; at least two abnormalities in erythrocyte morphology; serum iron level, ≥60 µg/100 ml; increased osmotic resistance in 0.35–0.40% buffered saline; and absence of HbE. The following criteria for δβ-thalassaemia were used: HbAA raised (>4.0%); HbF normal or raised (>2.5%); 25 pg <MCH<27 pg; at least two abnormalities in erythrocyte morphology; serum iron level >60 µg/100 ml; increased osmotic resistance in 0.35–0.40% buffered saline; and absence of HbE. No α-thalassaemia/β-thalassaemia double heterozygotes were found in this study; however, DNA analysis was not available.

Results

Two parameters were measured in the study—the degree of clinical severity and the level of parasitaemia. These parameters were related to the nature of the infected cells.

The results shown in Table 1 illustrate the relationship between haemoglobin variants and clinical severity of malaria in the 383 study subjects. No major differences between the different groups were observed. There is an indication of a lesser degree of clinical severity for patients with β-trait, and probably for α-trait and homozygous HbE (HbEE), though the number of patients with HbEE were too few for the results to be convincing. However, a statistical analysis (Student’s t test) to compare the incidence rates of patients with various red cell genetic abnormalities classified as categories IIIa and IIIb indicated that patients with α-trait and β-trait had significantly lower incidences in category IIIb (P <0.01). One patient with β-trait was classified as category IIIb.

The relationship between the level of parasitaemia and haemoglobin variants is illustrated in Fig. 1. Attention is drawn to the very high parasitaemias observed in some subjects, particularly those with HbAA and heterozygous HbE (HbAE). Mean parasitaemia levels were as follows: 3.05 ± 0.51 for HbAA (normal); 5.29 ± 1.29 for HbAE (P >0.05); 3.30 ± 0.91 for HbEE (P >0.05); 1.77 ± 0.32 for α-trait (P <0.01); 5.01 ± 1.54 for β-trait (P >0.05); and 1.98 ± 0.59 for δβ-trait (P <0.01). Thus there were no statistically significant differences between mean parasitaemia levels in HbAA, HbAE, and HbEE, and β-thalassaemia-trait erythrocytes. This result was unexpected but it should be noted that parasitaemia levels can sometimes reach up to 40% in Myanmar. However,

* Lange, Model LP1, Bruno Lange GmbH, Berlin, Germany.
there is an indication of a trend to slightly lower parasitaemias with α- and δβ-thalassaemia traits (P <0.01).

A similar study on the relationship between G6PD deficiency and clinical severity of malaria is shown in Fig. 2. The variants tested were G6PD normal (Gdβ⁺ variant, enzyme activity, 3.24 ± 0.41 IU/gHb), G6PD mild deficiency (Gdβ⁺ variant, enzyme activity 1.87 ± 0.34 IU/gHb), and G6PD severe deficiency (GdMyanmar variant, G6PD activity 0.14 ± 0.03 IU/gHb) (Myint-Oo, unpublished results, 1993). All three types of G6PD status were found among patients with severe malaria except that three cases with GdMyanmar could be allocated to category IIIa, though there were none in category IIIb. The mean parasitaemia level of the GdMyanmar variant (1.94 ± 0.89) was significantly (P <0.01) lower than those of the other two types (Gdβ⁺: 3.69 ± 0.47 and Gdβ⁻: 4.42 ± 2.21). It should be noted that G6PD levels are affected by changes in red cell turnover in acute malaria, and inferences of genotypes can only be tentative in the absence of DNA analysis of G6PD alleles.

A high incidence of double genetic defects was detected. Of the 383 patients studied, 25 (6.5%) carried more than one defective gene, either a combination of HbE and G6PD deficiency or a combination of α/β-thalassaemia traits and G6PD deficiency. However, no α-thalassaemia/β-thalassaemia double heterozygotes were detected.

### Discussion

The survey was designed to provide basic information on two separate but related topics with respect to the relationship of red cell disorders and malaria in Myanmar: the effect of the disorders on parasitaemia levels, and their effect on the clinical severity of malaria. While significant trends in the degree of parasitaemia were observed, this was only partially reflected in the degree of clinical response to infection. In only a few situations did the genetic disorders appear to result in protection against the disease state.

A summary of the distribution of subjects with various red cell genetic defects (a combination of haemoglobin variants, thalassaemias and G6PD variants) and clinical severity of falciparum malaria is given in Table 2. There was some evidence for a degree of protection for relatively smaller numbers of subjects with HbEE, α- and β-thalassaemia traits, and severe G6PD deficiency in category IIIb in particular. Of all individuals with double genetic defects, only one with HbAE and mild G6PD deficiency was in category IIIb. However, χ² test analysis of the data (Table 3) indicated no significant correlation between the clinical categories and red cell genetic defects (either G6PD-deficient variants or thalassaemia traits). On the other hand, combination of the results for subjects with α- and β-thalassaemia traits did indicate a significant relationship between clinical severity of malaria and thalassaemia traits genetics (Table 4).

The first in-vitro study on P. falciparum in HbE-containing erythrocytes was carried out by Nagel et al., who demonstrated a moderate decrease in growth in HbEE cells, but normal growth in HbAE cells (17). Another study from Thailand appeared to contradict this result (18). The apparent conflict between these two studies was resolved when Vernes et al. found diminished parasite growth both in homozygote HbEE cells and heterozygote HbAE cells, although the effect was small in the latter (19). On
Fig. 1. Scattergram showing the relationship between *Plasmodium falciparum* parasitaemia and haemoglobinopathies (% parasitaemias are plotted on a semi-log scale; each dot represents a single patient; standard errors are indicated). (AA = haemoglobin A; AE = heterozygous haemoglobin E; EE = homozygous haemoglobin E).

The other hand, Kruatrachue et al. also reported that HbE and thalassaemia appear to confer no advantage in respect to parasite rate, parasite density, and mortality of falciparum malaria in Thai children (20, 21). It was also observed that the parasitaemia levels did not correlate with the clinical severity of malaria.

In the present study, individuals with severe G6PD deficiency appeared to suffer less clinical fal-

ciparum malaria than those with normal or mildly deficient G6PD, although the numbers involved were too small to be statistically significant. However, a significant reduction in the mean parasitaemia level was also observed for individuals with severe G6PD deficiency. Since the deficiency state includes a wide range of variants and erythrocyte enzyme status, the relationship between this genetic disorder and falciparum malaria needs to be further explored. Nagel has pointed out that, although mortality from *P. falciparum* infection is relatively low, selection might still operate through an indirect mechanism (22).

Individuals with double genetic defects (either α-thalassaemia trait with Gd− or GdMyanmar, or β-thalassaemia trait with GdB− or GdMyanmar) were not observed in category IIIb (i.e., they did not reach the unrousable coma stage, or die), although this finding was not statistically significant owing to the small sample size. Of the 383 cases with falciparum malar-
Table 2: Relationship between overall red cell genetic defects and clinical severity of falciparum malaria

<table>
<thead>
<tr>
<th>Class</th>
<th>Genes*</th>
<th>I</th>
<th>II</th>
<th>IIIa</th>
<th>IIIb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>AA = GdB⁺</td>
<td>67</td>
<td>70</td>
<td>21</td>
<td>27²</td>
<td>185</td>
</tr>
<tr>
<td>AE + GdB⁺</td>
<td>22</td>
<td>29</td>
<td>11</td>
<td></td>
<td>11²</td>
<td>73</td>
</tr>
<tr>
<td>EE + GdB⁺</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>AA + GdE</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>AA + GdMyanmar</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>α-thal + GdB⁺</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>β-thal + GdB⁺</td>
<td>9</td>
<td>13</td>
<td>3</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>ββ-thal + GdB⁺</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>69</td>
<td>27</td>
<td>18</td>
<td>173</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>I</th>
<th>II</th>
<th>IIIa</th>
<th>IIIb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>14</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

* AA = haemoglobin AA; AE = heterozygous haemoglobin E; EE = homozygous haemoglobin E; α-thal = α-thalassaemia trait; β-thal = β-thalassaemia trait; ββ-thal = ββ-thalassaemia trait; GdB⁺ = G6PD normal; GdE = G6PD mild deficiency (10–60% of normal enzyme activity); GdMyanmar = G6PD severe deficiency (less than 5% of normal enzyme activity).
² Three patients from this group died.
³ One patient from this group died.

Table 3: Frequency of red cell variants and severity of malaria

<table>
<thead>
<tr>
<th>G6PD variants*</th>
<th>No. in clinical category:</th>
<th>I</th>
<th>II</th>
<th>IIIa</th>
<th>IIIb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GdB⁺</td>
<td></td>
<td>114</td>
<td>(117.0)²</td>
<td>127</td>
<td>(122.0)</td>
<td>40</td>
</tr>
<tr>
<td>GdB⁻</td>
<td></td>
<td>11</td>
<td>(13.5)</td>
<td>11</td>
<td>(14.1)</td>
<td>8</td>
</tr>
<tr>
<td>GdMyanmar</td>
<td></td>
<td>15</td>
<td>(9.5)</td>
<td>8</td>
<td>(9.9)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>140</td>
<td>146</td>
<td>51</td>
<td>46</td>
<td>383</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combined defects*</th>
<th>No. in clinical category:</th>
<th>I</th>
<th>II</th>
<th>IIIa</th>
<th>IIIb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>67</td>
<td>(67.6)</td>
<td>70</td>
<td>(70.5)</td>
<td>21</td>
</tr>
<tr>
<td>Single defect</td>
<td></td>
<td>59</td>
<td>(63.2)</td>
<td>69</td>
<td>(65.9)</td>
<td>27</td>
</tr>
<tr>
<td>Double defect</td>
<td></td>
<td>14</td>
<td>(9.1)</td>
<td>7</td>
<td>(9.5)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>140</td>
<td>146</td>
<td>51</td>
<td>46</td>
<td>383</td>
</tr>
</tbody>
</table>

* x² test = 10.6 (P >0.10; not significant).
² Figures in parentheses are the expected frequency.
³ x² test = 7.73 (P >0.10; not significant).
Table 4: Statistical analysis of subjects with thalassemia traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Clinical category (χ² test):</th>
<th>Illa</th>
<th>Illb</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td></td>
<td>4 (3.3)b</td>
<td>0 (3.0)</td>
</tr>
<tr>
<td>β</td>
<td></td>
<td>4 (4.3)</td>
<td>0 (3.8)</td>
</tr>
</tbody>
</table>

a Data for α- and β-thalassemia traits were pooled because of the small numbers. χ² Test = (6.8 – 0)²/6.8; P <0.01 (1 degree of freedom).

b Figures in parentheses are the expected frequency (under the null hypothesis that haemoglobin trait and clinical category are not associated), e.g., 3.3 = (25 × 51)/383.

The four who died with cerebral malaria had normal genes or single genetic defects.

The main objective of this study was to look for evidence that, within the Myanmar population, some of the erythrocyte disorders present conferred significant protection against malaria, such as that observed for sickle-cell anaemia (HbS) (23) and Melanesian ovalocytes (24, 25). While no definitive evidence was found, some trends indicative of protection against severe falciparum malaria were observed.

Acknowledgements

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Résumé

Relation entre les troubles génétiques des érythrocytes et la gravité du paludisme à falciparum au Myanmar

Des échantillons de sang veineux ont été prélevés chez quatre groupes de malades entrant dans diverses catégories de paludisme à falciparum grave et compliqué: 140 sujets de la catégorie I, 146 de la catégorie II, 51 de la catégorie IIIa et 46 de la catégorie IIIb. Les examens suivants ont été réalisés afin d’étudier les divers types d’anomalies génétiques des érythrocytes: morphologie des érythrocytes; hémoglobine globulaire moyenne; électrophorèse de l’hémoglobine; hémoglobine AA (HbAA), hémoglobine F (HbF), fer sérique; fragilité osmotique; glucose-6-phosphate déshydrogénase (G6PD); électrophorèse de la G6PD. Des numérations parasitaires ont été faites sur frottis minces réalisés le jour de l’admission et colorés au Giemsa.

La parasitémie moyenne chez les sujets porteurs d’hémoglobine AA et d’hémoglobine E hétérozygote était sensiblement plus élevée que chez les sujets porteurs des gènes des traits thalassémiques α ou β (P <0,01). La parasitémie moyenne chez les sujets atteints d’un déficit sévère en G6PD était également, de façon significative, plus faible que chez les sujets ayant soit une G6PD normale soit un déficit léger en G6PD (P <0,01). Les sujets présentant le double défaut génétique d’un trait thalassémique et d’une forme grave de déficit en G6PD semblaient posséder une meilleure protection contre le paludisme grave que les sujets normaux ou ceux présentant un défaut génétique unique. Cependant, les résultats n’étaient pas statistiquement significatifs, si l’on excepte le fait qu’une analyse par χ² indique un certain degré de protection chez un petit groupe rassemblant les porteurs des traits thalassémiques α et β.

References

Malaria and red cell genetics in Myanmar


