Under-utilized approaches to control anaemia in developing countries
Prinsen Geerligs, P.D.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Download date: 17 Jan 2019
3. Haematological Profiles of the People of Rural Southern Malawi: An Overview

B.J. Brabin\(^1\),\(^3\)  
P.D. Prinsen Geerligs\(^1\)  
F.H. Verhoeff\(^2\)  
K.A. Fletcher\(^4\)  
L.H.E. Chimsuku\(^1,\(^2\)\)\(^*\)  
B.M. Ngwira\(^2,\(^4\)\)  
O.J. Leich\(^1\)  
R.L. Broadhead\(^2\)

\(^1\) Child and Reproductive Health Group, Liverpool School of Tropical Medicine, Liverpool, United Kingdom  
\(^2\) College of Medicine, Blantyre, Malawi  
\(^3\) Emma Kinderziekenhuis, Academic Medical Centre, University of Amsterdam, The Netherlands  
\(^4\) Lymphatic Filariasis Support Centre, Liverpool School of Tropical Medicine

Correspondence: 
Professor B.J. Brabin  
Liverpool School of Tropical Medicine  
Pembroke Place  
Liverpool, L3 5QA, UK  
Email: b.j.brabin@liv.ac.uk

* Deceased
An integrative review of the results of two published and two unpublished studies of anaemia in children, adolescent females, pregnant women and adults living in southern Malawi is presented. Anaemia was universally present in all age-groups, with the higher prevalences in infants (100%) and adolescent primigravidae (93.8%). Nutritional deficits of iron and vitamin A were major contributory factors but chronic malaria haemolysis also significantly contributed to the anaemia. Among boys, anaemia was more common among those with glucose-6-phosphate-dehydrogenase (G6PD) deficiency than in those without this deficiency ($P<0.002$). This enzymopathy, which occurred in 23.5% [95% confidence interval (CI) = 16.7%-30.1%] of the male and 30% (CI = 17.3%-42.7%) of the female infants examined, was also associated with neonatal jaundice. The overall prevalences of the $-\alpha^+/\alpha^+$ and $-\alpha^+/-\alpha^+$ thalassaemia genotypes were estimated at 41.0% (CI = 28.3%-53.7%) and 8.7% (CI = 1.5%-15.0%), respectively. Haemoglobin AS was present in 18.1% (CI = 12.8%-23.4%) of the infants and haemoglobin SS in 2.5% (CI = 1.4% - 3.6%). As the prevalence of infection with Plasmodium falciparum was significantly higher in infants with haemoglobin AS than in those with AA (21.4% vs. 6.7%; $P<0.001$), an increased risk of early-onset moderate parasitaemias in young infants probably stimulates the development of immunity, protecting older heterozygotes from severe malarial infection. Innovative community approaches are required to break the cycle of ill health that anaemia supports in those living in rural areas of southern Malawi. Interventions in adolescent girls could be of particular importance, as they could break the cycle in both pregnant women and their infants.

Iron-deficiency and malaria-attributable anaemia continue to be two of the most important public-health problems in developing countries. It is estimated that iron-deficiency anaemia affects as many as 2000 million people world-wide (World Health Organization, 2000). Malaria has an equally wide global distribution but approximately 90% of the estimated 300 million–500 million, new, clinical cases of malaria that develop each year occur in sub-Saharan Africa (Goodman et al., 2000). In this region, 23 million pregnant women are exposed to malarial infection annually (Goodman et al., 2000).
Before the effectiveness of interventions for reducing iron-deficiency or malarial anaemia can be assessed, the haematological profile of the targeted population must be explored. In developing countries, there have been many studies on anaemia in particular age-groups but few profiles have been published on the haematological status of all age-groups, or of pregnant and non-pregnant women, within the same population or community. Four studies of anaemia in children, adults and pregnant women have been undertaken over the past few years amongst the people of the lower valley of the Shire river, in southern Malawi, where malaria is holo-endemic. The results of two of these investigations have already been published (Verhoeff, 2000; Prinsen Geerligs et al., 2003) but, until now, those of the other two studies have not appeared in the scientific literature. Although these studies were conducted semi-independently, each with its own primary purpose, they have provided the data used in the present, secondary and summary interpretation, with an analysis from infancy through to adulthood. The influence of genetic traits was also considered in the present study. During the period when the data were collected, anaemia control in the study area was based on case-management and vitamin-A supplementation in young children and post-partum women.

SUBJECTS AND METHODS

The haematological data summarized were all collected in one rural region of southern Malawi, approximately 300 m above sea level. Malaria transmission is holo-endemic in this area, with the main rains occurring from December–March. The small-scale cultivation of maize, sorghum, cotton and sugar-cane forms the primary source of food and income. There is a high level of illiteracy (71.8%) and poverty in the area. For convenience, the two unpublished investigations providing some of the data analysed were named UP1 and UP2. In all four studies, ethical approval was given by the Health Sciences Research Committee of the College of Medicine in Blantyre, and levels of malarial parasitaemia (trophozoites/μl blood) were determined for every subject. Thick bloodsmears were prepared, stained, usually with Giemsa’s stain, and examined by oil-immersion light microscopy. Trophozoites were counted against 50 leucocytes and each subject was assumed to have 8000 leucocytes/μl.

The Four Sources of Data

The oldest data analysed came from the study by Verhoeff (2000), which was undertaken, between March 1993 and June 1994, in the lower Shire valley. The main objective of this investigation was to determine the concentrations of haemoglobin and zinc protoporphyrin (ZP) in blood samples collected from pregnant adolescents and adults (at their first antenatal visit to the rural, district hospitals in Chikwawa and Montfort). The blood samples were collected by venepuncture after obtaining verbal informed consent. Haemoglobin (Hb) was measured using a cyanomethaemoglobin method and an haemoglobinometer (Biotron, Sydney). A ZP haematofluorometer (Model 206; AVIV Biomedical, Lakewood, NJ) and washed blood samples were used to measure ZP concentrations (Chimsuku, 1996). Subjects found to have >3.1 μg ZP/g Hb were considered to be suffering from iron deficiency. Serum concentrations of vitamin A were also measured, by HPLC (Catignani and Bieri, 1983), using blood samples obtained at delivery, when available, or otherwise those collected at recruitment.

The main objective of UP1, a study undertaken between July and August 1994, was to determine the prevalences of erythrocytic glucose-6-phosphate-dehydrogenase (G6PD) deficiency, malaria and anaemia in children aged 0–6 years. Most (203) of the 208 subjects were selected, by systematic sampling, from the children of non-consanguinous
marriages attending the ‘under-fives’ clinic at Blantyre; children with recent and severe haemolytic crises and high reticulocyte counts were excluded. The remaining five subjects were jaundiced infants; four neonates and a 6-month-old boy. Each blood sample was screened for G6PD deficiency using the semiquantitative, Cresyl-Blue dye test (Motulsky and Campbell-Kraut, 1964), which mainly identifies subjects with almost complete enzyme deficiency (i.e. female homozygotes, male hemizygotes and an unknown proportion of female heterozygotes). Haemoglobin concentrations were again determined by the cyanomethaemoglobin method (Dacie and Lewis, 1984), subjects with < 100 g Hb/litre being considered anaemic.

UP2 was undertaken, between March 1994 and September 1995, in the lower Shire valley, to determine the prevalences of the various α-thalassaemia polymorphisms and of sickle-cell anaemia, using cord-blood or infant-follow-up blood samples. Cord-blood samples were available from almost 700 infants born in Chikwawa District Hospital or Montfort Hospital. From these, 76 samples were selected, at random, for analysis, and 58 of these 76 were successfully characterized for α-thalassaemia genotype, by Southern-blot analysis (Old and Higgs, 1983). Screening for HbS genotypes was completed on 222 samples of infant blood, of which 95% came from subjects aged > 26 weeks. During a 12-month follow-up period, the effect of α-thalassaemia and sickle-cell haemoglobin on haematological indices and malarial-parasite prevalence was also assessed in infants who attended regularly for follow-up. Haemoglobin variants were separated and identified by standard electrophoresis (Dacie and Lewis, 1984). Haemoglobin concentrations were again estimated by the cyanomethaemoglobin method. ZP levels and transferrin saturation were determined using commercial kits (Sigma) based on a colorimetric method (Labbé et al., 1999).

Prinsen Geerligs et al. (2003) collected their fingerprick blood samples in May 2000, with the objective of determining Hb concentrations and blood ZP levels (as measures of iron status) in rural Malawian villagers, of all ages except young infants, from the Shire valley. The two study villages, Meja and Tsamba, were selected because of their accessibility by road, the willingness of their populations to participate, and their large size. Up to six subjects — the father and mother in the nuclear family, adolescent girls and children aged 5 months–11 years — were selected from each household willing to participate. The youngest children in the household were selected in preference to older children and adolescent girls in preference to other children. Blood concentrations of Hb, measured on enrolment using a Hb photometer (HemoCue, Lake Forest, CA), and age-specific cut-off concentrations of Hb indicating anaemia (Stoltzfus and Dreyfuss, 1998) were used to calculate prevalences of anaemia. All subjects found to be anaemic were treated with a therapeutic course of iron, or referred to hospital if symptomatic. ZP was measured, for a random sample of participants, using the same methods and haematofluorometer as used by Verhoeff (2000). Again, ZP concentrations exceeding 3.1 µg/g Hb were considered indicative of iron deficiency (Labbé et al., 1999).

RESULTS

Verhoeff (2000)

Nearly all the pregnant adolescents and adults checked at their first antenatal visits [mean (s.d.) gestation = 21.6 (6.2) weeks] were anaemic (Table 1). Adolescent primigravidae had the lowest mean Hb concentration and the highest prevalence of anaemia (93.8%) but were at an equivalent risk for iron deficiency as the non-adolescent primigravidae. Adolescent primigravidae had a high prevalence of Plasmodium falciparum parasitaemia (35.2%). The seasonal prevalence of iron deficiency, anaemia and malaria parasitaemia amongst pregnant adolescents is shown in the Figure. The peak prevalence of iron deficiency occurred in February 1994
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adolescents</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primigravidae</td>
<td>Multigravidae</td>
</tr>
<tr>
<td>No. of participants</td>
<td>528</td>
<td>166</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>17.4</td>
<td>18.2*</td>
</tr>
<tr>
<td>Mean (S.D.) gestational age (weeks)</td>
<td>20.6 (5.9)</td>
<td>20.7 (6.4)</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>12–19</td>
<td>12–19</td>
</tr>
<tr>
<td>Mean (S.D.) haemoglobin (g/litre) and [no. of subjects investigated]</td>
<td>86.7 (16.5) [528]</td>
<td>92.5 (16.5)* [164]</td>
</tr>
<tr>
<td>No. and (%) of participants found anaemic*</td>
<td>495 (93.8)</td>
<td>144 (87.7)*</td>
</tr>
<tr>
<td>Mean (S.D.) zinc protoporphyrin (µg/g haemoglobin)</td>
<td>3.7 (1.9)</td>
<td>3.4 (1.7)</td>
</tr>
<tr>
<td>No. and (%) of participants found iron-deficient/no. tested*</td>
<td>291/523 (55.6)</td>
<td>76/166 (45.8)</td>
</tr>
<tr>
<td>No. and (%) of participants found smear-positive for malaria/no. tested</td>
<td>179/509 (35.2)</td>
<td>33/159 (20.3)</td>
</tr>
</tbody>
</table>

*Value significantly different from that for the primigravidae of the same age-group (P < 0.001).
†Value significantly different from that for the primigravidae of the same age-group (P < 0.01).
*Any participant with <110 g haemoglobin/litre was considered anaemic.
*Any participant with >3.1 µg zinc protoporphyrin/g haemoglobin was considered iron-deficient.
FIG. The seasonal prevalences of iron deficiency (a), malaria (b) and anaemia (c) amongst pregnant adolescents at first antenatal visit, as observed, by Verhoef (2000), between May 1993 and June 1994. Any subject with <80 g haemoglobin/litre was considered to be anaemic.

(73.2%) whereas that of anaemia (<80 g Hb/litre) occurred in June 1994 (38.2%) and malaria prevalence peaked in April 1994 (48.3%). Of the 179 women tested for vitamin-A deficiency, 65.3% were deficient (<0.70 μmol/litre) and 21.2% severely
deficient (<0.35 μmol/litre). The women checked for vitamin-A deficiency represented approximately 13.0% of the women delivering in the two district hospitals during the study period.

UP1
Of the 153 boys from the 'under-fives' clinic who were screened, 36 tested positive for G6PD deficiency, giving a frequency of the X-linked, recessive gene responsible for the deficiency of 0.235 [95% confidence interval (CI) = 0.167–0.301] in this group. Fifteen (30.0%; CI = 17.3%–42.7%) of the 50 girls from the same clinic who were checked were also found to have this deficiency. The frequency of the deficiency in the girls was higher than expected from the relevant gene frequency in the males; assuming a Hardy–Weinberg equilibrium, the gene frequency among the boys would indicate that 36% and only 5.5% of the females from the same population should be G6PD-normal heterozygotes and G6PD-deficient homozygotes, respectively. Boys with G6PD deficiency were much more likely to be anaemic than other boys (P = 0.002; Table 2). Amongst the five clinical cases of jaundice investigated, all three male neonates (but not the one female) were severely anaemic, deeply jaundiced and G6PD-deficient, and the older jaundiced boy was also deficient (Table 3).

UP2
Although 76 cord-blood samples were selected for the detection of α-thalassaemia genes (−α^3^/−α) in UP2, sufficient DNA for the Southern-blot analysis was only obtained from 58 (76.3%). Of these 58 samples, 21 (36.2%) were heterozygous and 10 (17.2%) homozygous (all α+ homozygous). With correction for the population distribution of MCV, the prevalences of −α^3^/αα and of −α^3^/−α^3^ were estimated to be 41.0% (CI = 28.3%–53.7%) and 8.7% (CI = 1.5%–15.9%), respectively. The estimated frequency of the −α^3^ gene, calculated using the Hardy–Weinberg equilibrium, was 0.29. Twenty-two infants were followed to study the effect of their α-thalassaemia genotypes on their haematological indices (Table 4). There were no significant differences at follow-up, between genotypes, in mean Hb concentrations. The mean transferrin saturations (at approximate ages of both 10 and 26 weeks) were, however, significantly lower in the normal infants than in those with α-thalassaemia deletions (P < 0.02; Table 4).

TABLE 2. The association between glucose-6-phosphate-dehydrogenase (G6PD) deficiency and anaemia in the male infants investigated in UP1

<table>
<thead>
<tr>
<th>Category</th>
<th>G6PD-deficient</th>
<th>Normal</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of infants investigated</td>
<td>36</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>No. and (%) found anaemic (i.e. with &lt;100 g haemoglobin/litre)</td>
<td>8 (22.2)</td>
<td>5 (4.5)</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

*From a Fisher’s exact, two-tailed test.

TABLE 3. Anaemia and glucose-6-phosphate-dehydrogenase (G6PD) status of the jaundiced infants investigated in UP1

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Haemoglobin (g/litre)</th>
<th>Diagnosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>Male</td>
<td>62</td>
<td>Deep jaundice, G6PD-deficient</td>
<td>None (died 24 h after diagnosis)</td>
</tr>
<tr>
<td>2 days</td>
<td>Male</td>
<td>&lt;70</td>
<td>Deep jaundice, G6PD-deficient</td>
<td>Exchange transfusion</td>
</tr>
<tr>
<td>1 day</td>
<td>Male</td>
<td>70</td>
<td>Deep jaundice, G6PD-deficient</td>
<td>Exchange transfusion</td>
</tr>
<tr>
<td>3 days</td>
<td>Male</td>
<td>62</td>
<td>Deep jaundice, G6PD-deficient</td>
<td>Exchange transfusion</td>
</tr>
<tr>
<td>6 days</td>
<td>Female</td>
<td>102</td>
<td>Deep jaundice, G6PD-normal</td>
<td>Phototherapy</td>
</tr>
</tbody>
</table>
TABLE 4.  \( \alpha \)-Thalassaemia and haematological indices during the follow-up of the infants studied in UP2

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Genotype</th>
<th>5–14</th>
<th>24–31</th>
<th>&gt;45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (s.d.) haemoglobin (g/litre) and [no. of subjects investigated]</td>
<td>( \alpha \alpha/\alpha \alpha )</td>
<td>99 (35) [10]</td>
<td>87 (7) [10]</td>
<td>90 (11) [7]</td>
</tr>
<tr>
<td>Mean (s.d.) transferrin saturation (%) and [no. of subjects investigated]</td>
<td>( \alpha \alpha/\alpha \alpha )</td>
<td>26.7 (3.7) [10]</td>
<td>26.7 (3.4) [10]</td>
<td>26.8 (3.6) [10]</td>
</tr>
</tbody>
</table>

*Sample size was determined by subject compliance.

†Value significantly different from that for the \( \alpha \alpha/\alpha \alpha \) of the same age-group \((P < 0.02)\).

Among the 12 infants with sequential ZP measurements, mean ZP levels at 10, 26 and 50 weeks of age were consistently higher in the babies with \( \alpha \)-thalassaemia genotypes than in their normal counterparts, although the differences were not statistically significant.

Of the 222 samples used to determine the prevalence of HbS genotypes, five (2.5%; CI = 1.4%–3.6%) had HbSS, 37 (18.1%; CI = 12.8%–23.4%) had HbAS, and 18 could not be characterized. Application of the Hardy–Weinberg equilibrium gave a sickle-Hb-gene frequency of 0.11. Prior to 50 weeks of age, those with normal and sickle-cell genotypes had similar mean Hb and ZP concentrations and transferrin saturations \((P > 0.05\) for each). At 50 weeks of age, however, four infants with sickle-cell anaemia had a mean Hb concentration that was lower, by 8 g/litre, than that of their normal counterparts. At the same age, the 23 infants with HbAS then investigated also had significantly higher mean Hb levels than the 123 with HbAA \((90 v. 85 \text{ g/litre}; P < 0.05)\). Most of the children had >4.0 \( \mu \text{g} \) ZP/g Hb, indicating that they had chronic iron deficiency. A mean of 44 infants attended at each of the scheduled follow-up visits during their first 12 months. At these follow-up visits, infants with sickle-cell trait and those with sickle-cell anaemia were significantly more likely to have malaria parasitaemias than the HbAA children \((P < 0.001\) for each), with prevalences of 14.8% (48/325), 21.4% (12/56) and 6.7% (176/1735), respectively.

Prinsen Geerlings et al. (2003)
Most (95.3%) of the 753 residents of Tsamba and Meja who were asked to participate in the study by Prinsen Geerlings et al. (2003) gave their informed consent. The results for the 347 residents who, having satisfied the inclusion criteria, were enrolled in the study are summarized in Table 5. The prevalence of anaemia was generally high, varying from 100% in the infants to 32.4% in the adult males. Similarly, the prevalence of iron deficiency varied from 100% in the children aged <5 years to 80% in the adult males. The prevalence of malarial \((P. falciparum')\) parasitaemia was highest in the children aged 2–5 years (72.7%), decreasing to 26.7% in the adult males and 20.3% in the adult females.

DISCUSSION

There is some heterogeneity among the four studies summarized, which reflects differences in the age-groups, sample sizes and study periods. The nutritional and malarialometric studies were completed in May (Prinsen Geerlings et al., 2003) or between March and June (Verhoeff, 2000). The main rainy season in southern Malawi, which is associated with peak malaria transmission, occurs from December to March. The genetic studies (UP1 and UP2) were, however, unlikely to be influenced by study month(s). There were no droughts during any of the four studies although a severe
TABLE 5.  Haematological parameters of the various age-groups investigated by Prinsen-Goerlts et al. (2003)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infants (aged 4 months—&lt; 2 years)</th>
<th>Children aged:</th>
<th>Adolescent females (aged 10—19 years)</th>
<th>Adults</th>
<th>Non-pregnant females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>46</td>
<td>74</td>
<td>41</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.1</td>
<td>7.0</td>
<td>13.9</td>
<td>41.4</td>
</tr>
<tr>
<td>Age range</td>
<td>4–23 months</td>
<td>2–4 years</td>
<td>5–9 years</td>
<td>10–19 years</td>
<td>12–89 years</td>
</tr>
<tr>
<td></td>
<td>63.9 (24.6)</td>
<td>83.8 (12.9)</td>
<td>99.8 (18.1)</td>
<td>115.3 (12.3)</td>
<td>133.8 (20.7)</td>
</tr>
<tr>
<td>Mean (s.d.) haemoglobin (g/litre)</td>
<td>10/10 (100)</td>
<td>43/46 (93.5)</td>
<td>61/74 (82.4)</td>
<td>30/41 (73.2)</td>
<td>24/74 (32.4)</td>
</tr>
<tr>
<td>No. and (%) of participants found anaemic/no. tested*</td>
<td>10.8 (4.3)</td>
<td>9.3 (3.0)</td>
<td>7.3 (3.2)</td>
<td>5.0 (1.7)</td>
<td>4.2 (1.6)</td>
</tr>
<tr>
<td>Mean (s.d.) zinc protoporphyrin (μg/g haemoglobin)</td>
<td>6/6 (100)</td>
<td>21/21 (100)</td>
<td>28/29 (96.6)</td>
<td>29/33 (87.9)</td>
<td>36/45 (80.0)</td>
</tr>
<tr>
<td>No. and (%) of participants found iron-deficient/no. tested*</td>
<td>5/8 (62.5)</td>
<td>24/33 (72.7)</td>
<td>37/62 (59.7)</td>
<td>12/29 (41.4)</td>
<td>12/45 (26.7)</td>
</tr>
</tbody>
</table>

*Children aged < 5 years, children aged 5–9 years, adolescent and adult females, and adult males were considered anaemic if they had < 110, < 115, < 120 or < 130 g haemoglobin/litre, respectively.

†Any participant with > 3.1 μg zinc protoporphyrin/g haemoglobin was considered iron-deficient.
drought had affected the study area for a few years prior to 1992. Among the adolescent pregnant girls investigated for a year by Verhoefff (2000), the peak prevalences of iron deficiency and malaria preceded that of severe anaemia (Fig.).

In the study by Prinsen Geerligs et al. (2003), a clear age-specific trend was observed for the prevalence of anaemia, which fell sequentially from 100% in infants to 70.6% in non-pregnant, adult women and 32.4% amongst adult males. The prevalence of iron deficiency showed a similar downward trend with age but never fell below 80%. The reduction in anaemia prevalence with increasing age corresponds with a decreasing prevalence of malarial parasitaemia, from over 80% in young children to 20%–27% in adults. Despite this reduction in the prevalence of malarial infection, a high proportion of adults, especially of the women, remained anaemic. This indicates that the aetiology of anaemia amongst the people of southern Malawi is complex. In addition to iron deficiency and malaria, several other factors contribute. The seroprevalence of maternal HIV infection was high (24.0%–25.1%) among the pregnant women described by Verhoefff (2000) and the adolescent nulliparae screened as part of the same study (Brabin et al., 1998). Given its high prevalence in the study area, HIV infection is likely to contribute to anaemia in all age-groups other than children of 5–9 years (most HIV-positive neonates will have died before the age of 5 years whereas the incidence of HIV infection only becomes high during adolescence). Even though HIV infection is relatively rare in those aged 5–9 years, Prinsen Geerligs et al. (2003) still found 82.4% of children of this age-group to be anaemic and almost all to be iron-deficient (Table 5).

Hookworm infection, which could contribute to the observed iron deficiency, was not assessed in any of the four studies summarized here. Only one of 35 children admitted, with protein-energy malnutrition, to the Queen Elizabeth Central Hospital in Blantyre (the main referral hospital for the lower Shire valley) was found to have a hookworm infection (Mbewe, 1993). In a recent, unpublished, cross-sectional survey in the Shire valley, however, hookworm eggs were detected in Kato–Katz smears of faecal samples from 18.1% of the 1130 subjects (B. M. Ngwira; unpubl. obs.). Schistosoma haematobium infection is also probably common in the study area. Much of the iron that is consumed by the villagers in southern Malawi, who generally have poor diets, is probably not bio-available. The main food source is maize, complemented with ground nuts and, seasonally, with Chinese cabbage and pumpkin leaves. Without irrigation, all crops are unpredictable.

Vitamin-A deficiency occurred in two-thirds of the pregnant women studied by Verhoefff (2000) and was severe in one in five such women. Of the 113 non-pregnant, adolescent girls from the Shire valley checked by Fazio-Tirrozzo et al. (1998), using the retinal dose–response test (Tanumihardjo et al., 1999), 45 (40.2%) had vitamin-A deficiency. Although >88% of the pregnant women investigated by Verhoefff (2000) were found to be anaemic, only about 50% were iron-deficient. Maternal malarial infection and other nutrient deficiencies (e.g. of folate and vitamin B₁₂) may explain these findings, although ZP alone is not an ideal measurement of iron status in an area where malaria is common. No information is available on folate or vitamin-B₁₂ deficiencies in the area where Verhoefff (2000) worked but both such deficiencies are likely to be common because of the lack of animal-derived foods in the diet and the limited intake of green vegetables. Van den Broek (1998) found that 21% of the pregnant women attending clinics in a different district of southern Malawi had combined folate and vitamin-B₁₂ deficiency.

Anaemia in pregnant women is a major problem, and, as in children, is multifactorial in aetiology. Among the pregnant villagers studied by Verhoefff (2000), the highest
prevalences of anaemia, iron deficiency and malaria were all found in the adolescent girls, with a mean age of 17.4 years (Table 1). Primigravidae, whether adolescent or adult, had higher prevalences of anaemia and malaria than the multigravidae. This parity difference is well described for women living in areas where malaria is holo-endemic, with women in their first pregnancies being particularly susceptible to *P. falciparum* infection (Brabin, 1983). The high frequency of anaemia in pregnant adolescent girls, and especially the increase in prevalence of anaemia from 73.2% in the non-pregnant adolescent girls to 87.78% in the pregnant (Table 1; Verhoeff, 2000), are major causes of concern. Iron deficiency in the pregnant villagers at their first antenatal visit was no more frequent in the adolescents than in the adults. It is likely that malaria was a main contributor to the anaemia observed during pregnancy. Of the non-pregnant adolescent girls investigated by Fazio-Tirroz et al. (1998), 88.7% were anaemic and 4.4% severely anaemic (with <70 g Hb/litre). In Kenya, Leenstra et al. (2003) found that the control of malaria, by the use of insecticide-impregnated bednets, was effective in reducing the prevalence of anaemia in young adolescents. Although, by the standards of sub-Saharan Africa, the attendance at antenatal clinics by villagers from the lower Shire village might be considered reasonable, it clearly remains inadequate to provide effective anaemia control in pregnant women. From adolescence to grand multipara, anaemia remains a chronic problem in the area. Integrated strategies, that involve communities more effectively and the village-based distribution of impregnated bednets, antimalarials and haematinics, will be required if the problem of anaemia during pregnancy is to be solved.

G6PD deficiency was found to be common during UP1, with an estimated frequency as high as 36% for female heterozygotes. Overall hemizygote prevalence for the A− phenotype in African male populations has been reported to vary from 3% in East Africa to 22% amongst the Yoruba (Beutler, 1978). The frequency observed in UP1, in Malawian infants, is amongst the highest estimates for Africa. In a separate study of 101 pregnant women from the Shire valley, 24 (23.8%) were found to have G6PD deficiency (Howarth, 1996). The clinical importance of this polymorphism is clearly shown by its association with anaemia (22.2% of the deficient but only 4.5% of the enzyme-normal infants in UP1 had anaemia), and its presence in neonates with severe haemolytic anaemia requiring exchange transfusion. In Nigeria and Jamaica, jaundice was found more frequently in G6PD-deficient infants than in their normal counterparts (Capps et al., 1963; Gibbs et al., 1979; Dawodu et al., 1984). Although G6PD deficiency provides some protection from *P. falciparum* infection (Ruwende and Hill, 1998), this was not apparent in the results of UP1, possibly because of the use of antimalarial drugs in the study area. G6PD-deficient erythrocytes are more susceptible to oxidative stress and have a shorter life-span than enzyme-normal erythrocytes (Bernini and Latte, 1964). In those with symptomatic malarial infections, the deficient erythrocytes may therefore be more severely damaged by oxidant antimalarial drugs such as sulfadoxine-pyrimethamine (the only readily available antimalarial drug used in the first-line treatment of malaria in the lower Shire valley). G6PD deficiency may also cause chronic subclinical haemolysis in the steady state (May et al., 2000) and increased infection-induced haemolysis as a result of oxidative membrane damage (Beutler, 1978). Infection-related haemolysis in G6PD deficiency is usually mild but occasionally may be severe. The recovery of the Hb concentration to normal is also often delayed by the marrow suppression that ordinarily accompanies infection. Bacterial pneumonia is a common cause of infection-induced haemolysis in the G6PD-deficient (Tugwell, 1973), and acute respiratory infection is one of the commonest causes for health-centre attendance by young
children in Malawi. Between 5% and 10% of the children in the communities investigated in UP1 and UP2 and by Verhoef (2000) and Prinsen Geerligs et al. (2003) may have been HIV-positive, as the sero-prevalence of maternal infection with HIV was about 25% at the time of these studies.

In UP2, the infants with α+ thalassaemia genotypes were found to have a lower mean Hb concentration and significantly higher transferrin saturations than those with normal genotypes. Although α-thalassaemia is another erythrocytic variant, characterized by elevated susceptibility to oxidative stress and protection against malaria, that could lessen the risk of malarial anaemia (Yuthavong and Wilairat, 1993), there is no evidence of this in the results of UP2. The sample size in UP2 was so small, however, that the differences seen in mean Hb levels between genotypes did not reach statistical significance and may have been influenced by confounding caused by developmental changes. Nurse (1979) suggested that, in α thalassaemia, there could be a reduced rate of α-globin chain production, while transferrin is fairly saturated. There could then be a reduction in haem synthesis, a slowdown in release of iron from transferrin, and an excess of haem precursors. This might explain why, in UP2, those with the α-thalassaemia genotypes had slightly higher ZP values than the other subjects.

The frequency of sickle-haemoglobin genotypes in infants was high in UP2, as would be expected in a highly malarious area of Africa. The prevalence of malarial parasitaemia in infants was significantly higher in those with the AS and SS genotypes than in those with the AA. This contrasts with the findings from a cohort study of infants (aged 2–16 months) in western Kenya, in which those with the sickle genotypes apparently had a reduced risk of high-density parasitaemia and of severe malarial anaemia (Aidoo et al., 2002). In Ghana, however, Ringelhann et al. (1976) also reported relatively high prevalences of malarial para-
sitaemia in children (aged <5 years) with HbAS. If infants with HbAS are generally more likely to be found parasitaemic than their HbAA counterparts, the persistent moderate parasitaemias may stimulate the development of immunity, protecting older heterozygotes against severe P. falciparum infection. In UP2, mean Hb values in the children with the sickle-cell trait were, in general, not significantly different from those in the HbAA children. Only among the infants aged 12 months was the mean Hb concentration significantly higher in those with HbAS than in those with HbAA, indicating that the sickle-cell trait may offer some protection from malarial anaemia in the older infants.

This overview provides a profile of the burden of anaemia amongst a poor, essentially rural, Malawian community, and indicates that, from birth to adulthood, individuals are faced with substantial nutritional deficits and infection loads that almost preclude the achievement of a normal haematological status. Despite the knowledge of the causes and mechanisms of anaemia in tropical African communities, effective, integrated strategies for its prevention and treatment have rarely been deployed. Innovative, community-based approaches are required in order to break the cycle of ill health that anaemia holds on these communities.

ACKNOWLEDGEMENTS. The work was partly funded by the European Commission Programme for Life Sciences and Technologies for Developing Countries (contract TS3* CT920083). The DNA analysis for detecting α-thalassaemia was kindly facilitated by Dr J. Old.

REFERENCES


