Malaria and anaemia in pregnancy: importance, detection and prevention

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Malaria is an important cause of anaemia in primigravidae: evidence from a district hospital in coastal Kenya

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ABSTRACT
A study was undertaken in order to determine the prevalence and aetiology of anaemia in pregnancy in coastal Kenya, so as to establish locally important causes and enable the development of appropriate intervention strategies. 275 women attending the antenatal clinic at Kilifi District Hospital, were recruited in November 1993. The prevalence of anaemia (haemoglobin [Hb] < 11g/dl) was 75.6%, and the prevalence of severe anaemia (Hb < 7g/dl) was 9.8% among all parities. 15.3% of 73 primigravidae were severely anaemic, compared with 7.9% of 202 multigravidae (p=0.07).

In primigravidae, malaria infection (P. falciparum) was strongly associated with moderate and severe anaemia (X² test for trend p=0.003). Severe anaemia was more than twice as common in women with peripheral parasitaemia as in those who were aparasitaemic, and parasitaemia was associated with a 2.2g/dl decrease in mean haemoglobin level (p < 0.001).

In multigravidae, iron deficiency and hookworm infection were the dominant risk factors for anaemia. Folate deficiency and HIV infection were not strongly associated with anaemia. It is suggested that an intervention that can effectively reduce malaria infection in primigravidae could have a major impact on the health of these women and their infants.
INTRODUCTION
Severe anaemia in pregnancy is defined by the World Health Organisation as a haemoglobin level of less than 7g/dl (WHO, 1989). It is a major preventable contributor to maternal morbidity and mortality, being associated with a high risk of cardiac failure and shock, and rendering women less able to withstand even moderate blood loss at delivery (Lawson 1967). Anaemia in pregnancy causes considerable incapacity to women from tiredness, lassitude, breathlessness, and decreased ability to work (WHO, 1979). It is also associated with adverse perinatal outcomes (Brabin 1991).

The aetiology of anaemia in pregnancy is multifactorial, with the prevalence and causes varying considerably in different areas of the world. Causes that have been reported in sub-Saharan Africa are malaria, iron deficiency (often exacerbated by hookworm infestation), folate deficiency, haemoglobinopathies and HIV infection (Fleming 1989). Most large scale anaemia prevention programmes have concentrated on iron and folate deficiency, without addressing other potentially important "non-nutritional" causes of anaemia in pregnancy. What is still not well documented is the relative importance, from a public health point view, of malaria as a cause of maternal anaemia. We aimed to look at this by assessing the local prevalence and causes of anaemia and severe anaemia on the Kenyan Coast, with a view to determining priorities for preventative strategies.

MATERIALS AND METHODS
The study was based at Kilifi District Hospital (KDH), 50km north of Mombassa, on the Kenya Coast. This area of Kenya has perennial transmission of Plasmodium falciparum with two seasonal peaks in prevalence of the principal vectors, Anopheles gambiae and A. funestus, in June-August and during January, coinciding with the 2 rainy seasons. On average, individuals can expect to receive 10 infective bites per person per annum (Mbogo et al., 1995). The time in which the study was conducted was a period of low malaria transmission, at the end of the dry season. The study area and its malaria epidemiology have been described elsewhere (Snow et al., 1993).

Kilifi District Hospital has a busy antenatal clinic with on average of 900 women from a predominantly rural population attending every month. Women attending the antenatal clinic were recruited over a four week period in November 1993. All women who participated in the study gave full informed consent. 15 women were recruited each day, which included all first attenders, with the remainder being a random selection of re-attenders. Obstetric and a menstrual histories were taken, women were examined to establish gestational age, and a sample of venous blood was collected. A full blood cell count was performed on a model M530 Coulter Counter. Thick and thin blood films were prepared for the detection of malaria parasites, using standard Giemsa staining, counting parasites per 200 white cells, and calculating counts per microlitre from the white blood cell count; 100 high-power microscope fields were examined to confirm that a film was negative. Blood was tested for HIV antibody status using the membrane based assay 'HIVCHEK', (Ortho Diagnostic Systems, France). The testing was anonymous, with the samples being analysed only after all identifying features had been removed, making linkage of the results to individual women impossible. Additional investigations were performed on a random selection of women: 187 women were tested for red cell folate measured by radio-immuno assay, and 217 for serum ferritin, using Melisa enzyme-linked immunosorbert assay (Cambridge Life Sciences plc). Stool samples were requested from all of the study women, and 251 (91%) returned a stool specimen. Stool
microscopy was performed using the McMaster technique with egg counts estimated per gramme.

Statistical analysis was carried out using the SPSS computer programme. For continuous variables, means of the different groups were compared using regression methods. Chi-squared tests were used to investigate the association between groups of discrete variables.

RESULTS

Characteristics of the study population
Two hundred and seventy-five antenatal clinic attenders were recruited into the study, 2 women refused. Of those recruited, 73 (26.5%) were primigravidae, 155 (56.4%) were gravidity 2-5 and 47 (17.1%) were in their 6th or more pregnancy. Women of all gestational ages were recruited, ranging from 12 weeks to term, the mean and median gestational age both being 26 weeks. Primigravidae and multigravidae had similar gestational ages at recruitment. The majority of women (82.2%) attended clinic for a routine check, 16.7% because they were sick and only 1% were referred from peripheral health units. Analysis did not show any association between the reason for attendance and either haemoglobin level or malaria parasitaemia.

Figure 1  Percentage of women with severe anaemia (haemoglobin < 7g/dL) by gravidity.

Haemoglobin levels
Two hundred and eight women (75.6%) were anaemic (Hb < 11 g/dl) and 27 (9.8%) were severely anaemic (Hb < 7g/dl). Primigravidae had a somewhat higher prevalence of severe anaemia than multigravidae, 15.1% (11/73) compared to 7.9% (16/202) (p=0.07).
The approximately U-shaped distribution of severe anaemia in different parity groups (Fig. 1), showed that primigravidae and women in their 6th or more pregnancy had a higher prevalence of severe anaemia.

**Malaria**

Sixty-five women (23.6%) had *P. falciparum* in their peripheral blood. Primigravidae had higher rates of parasitaemia (33%) than multigravidae (20%) (p=0.04). When parasitaemia was present, primigravidae tended to have much higher densities of parasites than multigravidae, the geometric mean being 1749 parasites/µl in parasitaemic primigravidae compared to 371 parasites/µl in parasitaemic multigravidae (p < 0.001).

Reported fever during the pregnancy was common, with 139 (51%) women giving a history of fever at some time during the pregnancy, of whom 52 (37%) reported having had fever many times. Reported fever was not associated with the presence of either anaemia or malaria parasitaemia.

**Malaria and Anaemia**

In primigravidae, *P. falciparum* infection was strongly associated with anaemia (Table 1, $X^2$ test for trend p=0.003) and the mean haemoglobin was 2.23g/dl lower in women with parasitaemia than in those without (95% CI 1.21-3.25, p<0.001). Severe anaemia was more than twice as common in primigravidae with peripheral parasitaemia than in those without, though the difference did not reach statistical significance (rate ratio 2.45, 95% CI 0.83-7.23). In multigravidae parasitaemia was not significantly associated with anaemia (Table 1; $X^2$ test for trend p=0.62) or severe anaemia (rate ratio 0.91, 95% CI 0.27-3.03), and it was associated with a mean haemoglobin level only 0.29g/dl lower than that in those without parasitaemia (95% CI 0.31-0.89, p=0.34).

<table>
<thead>
<tr>
<th>Haemoglobin level (g/dl)</th>
<th>Gravida 1</th>
<th>Gravida &gt; 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>≥ 11</td>
</tr>
<tr>
<td>Parasitaemic</td>
<td>24</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Not parasitaemic</td>
<td>49</td>
<td>18 (37%)</td>
</tr>
<tr>
<td>Parasitaemic</td>
<td>41</td>
<td>8 (20%)</td>
</tr>
<tr>
<td>Not parasitaemic</td>
<td>161</td>
<td>40 (25%)</td>
</tr>
</tbody>
</table>

**Iron deficiency**

One hundred and sixty three of 217 sera tested had ferritin levels below 12ng/ml, with only 39 (18%) being in the normal range (12 - 30ng/ml) and 15 (6.9%) being above 30ng/ml. Serum ferritin levels were significantly higher in women with *P. falciparum* infection, irrespective of parity (p < 0.001): of 168 women with no parasitaemia, mean serum ferritin level was 7.03 ng/mL (95% CI 5.78-8.28), compared to the mean level in 53 women with parasitaemia, 26.42 (95% CI 17.78-35.06). Women with parasitaemia accounted for 80% of the high serum ferritin levels. Figure 2 shows the distribution of ferritin according to haemoglobin level in women with and without parasitaemia. In women with no parasitaemia, low serum ferritin was associated with a mean haemoglobin level of 9.38g/dl, compared to 11.02g/dl in women with a normal ferritin values and 9.75g/dl in those
with a high ferritin levels ($p < 0.001$). In women with malaria parasitaemia, mean haemoglobin levels in those with high, normal and low serum ferritins values were 8.64g/dl, 9.28g/dl and 8.64g/dl respectively ($p=0.52$).

**Hookworm**

Of the 251 women examined 188 (74.9%) had hookworm eggs in their stool. There was a large range in hookworm egg counts (40 - 7000 eggs/g of faeces), with 30% of women with eggs in their stool (22% of women overall) having ≥ 1,000 eggs/g. The egg counts, haemoglobin levels and gravidity are shown in Table 2. Women with higher hookworm egg counts were significantly more anaemic, with egg counts tending to be higher in multigravidae. When all gravidities were analyzed together, an egg count ≥ 1000 eggs/g was associated with a mean haemoglobin that was 0.79g/dl lower than that in those with an egg count of < 1000 eggs/g ($p=0.004$). In multigravidae the difference was 0.82g/dl, and in primigravidae 0.75g/dl.

**Table 2** Median hookworm eggs counts in relation to haemoglobin and gravidity

<table>
<thead>
<tr>
<th>Haemoglobin level (g/dL)</th>
<th>≥ 11</th>
<th>7-10.9</th>
<th>&lt; 7</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primigravidae</td>
<td>150 (n=17)</td>
<td>200 (n=38)</td>
<td>200 (n=11)</td>
<td>0.48</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>200 (n=46)</td>
<td>335 (n=126)</td>
<td>700 (n=13)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Krushkal-Wallis test

**Folate deficiency**

Eight of the 184 women tested (4.3%) had red-blood cell folate (RBCF) levels below the normal lower limit (110 ng/ml). Red-blood cell folate levels were negatively correlated with haemoglobin level ($p=0.02$; Table 3). This negative correlation was found in both parasitaemic and non parasitaemic primigravidae and multigravidae. Parasitaemic women had a higher mean red cell folate level (294 ng/ml) than those without parasitaemia (260ng/ml) though this difference was not statistically significant ($p = 0.27$).

**Table 3** Mean red cell folate levels (ng/ml) in relation to haemoglobin and malaria parasitaemia

<table>
<thead>
<tr>
<th>Haemoglobin level (g/dL)</th>
<th>≥ 11</th>
<th>7-10.9</th>
<th>&lt; 7</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>240</td>
<td>264</td>
<td>359</td>
<td>0.02</td>
</tr>
<tr>
<td>Parasitaemic</td>
<td>273</td>
<td>293</td>
<td>340</td>
<td>0.884</td>
</tr>
<tr>
<td>Not parasitaemic</td>
<td>234</td>
<td>256</td>
<td>365</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**HIV infection**

Nineteen of the 274 women tested (6.9%) were HIV positive and 3 (15.8%) of them had severe anaemia compared with 9.4% (24/255) of women with no HIV infection ($p = 0.41$). 6 of the women who were HIV positive (31.6%) had malaria parasitaemia compared with 66 (25.5%) of those who were HIV negative ($p = 0.56$).
**DISCUSSION**

This study confirmed that anaemia is a major problem among pregnant women in the Coast of Kenya, primigravidae and grand-multigravidae having the highest prevalence of severe anaemia.

The causes of anaemia are multifactorial. Malaria was the main cause of anaemia and severe anaemia among primigravidae, being associated with a reduction of haemoglobin of more than 2g/dl.

In areas where malaria is endemic, infection with *P.falciparum* in pregnancy is greatest in primigravidae with the prevalence and intensity of parasitaemia decreasing with increasing gravidity (McGregor & Smith, 1952; McGregor, 1984; Steketee & Breman, 1988; Greenwood *et al.*, 1989). The reasons why primigravidae have a greater predisposition to *P.falciparum* infection remains uncertain. The importance of malaria as a cause of anaemia in pregnancy in this Kenyan population is supported by the tendency of primigravidae to have more severe anaemia than any other gravidity group. This has been found in other malarious areas (Van Dongen and Van’t Hof, 1983; Brabin *et al.*, 1990; Jackson *et al.*, 1991). By contrast, in non-malarious areas, anaemia usually increases as gravidity increases, with primigravidae being the least anaemic group. This is presumably because iron deficiency tends to increase as gravidity increases (Isah *et al.*, 1985).

Several studies have shown that, in areas where malaria is endemic, effective antimalarial chemoprophylaxis started before 24 weeks of gestation, prevents the development of severe anaemia in primigravidae (Gilles *et al.*, 1969; Kortmann 1972; Fleming & Ghatoura 1986; Mutabingwa *et al.*, 1993). The antimalarial drugs shown to be effective in these studies were chloroquine and pyrimethamine (Gilles *et al.*, 1969), chloroquine alone (Kortmann 1972), chloroquine and proguanil (Fleming *et al.*, 1986) and proguanil with and without chloroquine (Mutabingwa *et al.*, 1993). In a large community-based...
study in The Gambia, comparing antenatal administration of maloprim or placebo every two weeks, an overall improvement of haemoglobin level was observed in the chemoprophylaxis group among primigravidae but not multigravidae (Greenwood et al., 1989). Where the prevalence of malaria parasitaemia is lower, chemoprophylaxis has not effect on maternal haemoglobin level (Hamilton et al., 1972, Jackson and Lathan 1982). A recent review of randomised controlled trials of chemoprophylaxis of malaria in pregnancy has been published by Garner and Brabin in (1994).

Despite evidence for its important role in the prevention of severe anaemia in primigravidae, most countries currently lack policies for the delivery of effective antimalarial drugs to pregnant women. This is mainly due to the difficulty in identifying drugs which fulfil the requirements of being safe for the mother and fetus, effective as an antimalarial compound, acceptable to the women and affordable. With increasing chloroquine resistance in many parts of the world, compounded by frequently poor compliance due to its bitter taste, chloroquine prophylaxis is often no longer effective. In Malawi, compliance with a weekly chloroquine regime was only 36%, and its efficacy 8% (Heyman et al., 1990). Daily proguanil may be an effective alternative which was shown to have good compliance in the studies discussed above, but it remains expensive for routine distribution. Many of the newer antimalarial drugs (such as mefloquine) are prohibitively expensive for routine use in developing countries, or remain untested in pregnancy (such as halofantrine). On presently available evidence, fortnightly maloprim seems to offer the most feasible and effective means of preventing malarial anaemia. However, in Malawi a two doses’ regimen of sulphadoxine-pyrimethamine, one dose given in the second trimester and one in the third, was found to be effective at reducing placental parasitaemia (Schultz 1994). Further studies to examine the impact of sulphadoxine-pyrimethamine on malaria are required, and two such studies are being carried out in Kenya, one in Kisumu (in an area of high transmission) and one in Kilifi (by the authors of this paper), with the main outcome measure being the effectiveness of the regime in preventing severe maternal anaemia in primigravidae.

Other important preventable causes of anaemia in pregnancy in this population are iron deficiency and hookworm infestation. More than 75% of women tested were iron depleted (ferritin < 12ng/ml). Serum ferritin is both an indirect measure of iron stores and an acute phase reactant. We found that both low and high serum ferritin were associated with a lower mean haemoglobin level. Malaria parasitaemia was strongly positively associated with serum ferritin, and 80% of women with high serum ferritin values had malaria parasitaemia, so malaria is likely to confound the association between serum ferritin and haemoglobin. Interpretation of these results is therefore difficult, and serum ferritin levels probably underestimate the prevalence of iron deficiency. Additionally, it is possible that iron replete individuals are more prone to malaria infection, for which there is some evidence in young children (Smith et al., 1989; Oppenheimer et al., 1986). In aparasitaemic women, low serum ferritin levels were associated with haemoglobin levels on average 1.64g/dl lower then those in subjects with normal serum ferritin values. The majority of women (89%) with low serum ferritin were not severely anaemic, though it is possible that many may have become more anaemic as their pregnancies progressed.

Hookworm infection was highly prevalent in this community, with 75% of women having hookworm eggs in their stools. Over 22% of women had an egg count of 1000 eggs/g or more, and this was associated with a mean haemoglobin level of 0.75g/dl lower than in those with egg counts of less than 1000 eggs/g. Interestingly, multigravidae had
higher egg counts than primigravidae, presumably due to increased exposure with increasing age, and an increasing number of infected children in the compound. In pregnant women, many of whom are already iron deficient, hookworm infection is probably a significant contributor to anaemia.

Malaria infection has been shown to be an important risk factor for folate deficiency, as haemolysis stimulates erythroid hyperplasia and so increases the requirement for folate (Fleming 1989). However, only 4.3% of women in this study had folate deficiency. This low level of folate deficiency may be explained by a diet that is rich in folic acid, with dark green leafy vegetables being eaten regularly by most women. Alternatively it may also be that red cell folate levels in such a population do not give a true picture of folate status. We observed an association between high red cell folate levels and parasitaemia and this has been found previously (Fleming et al., 1984, 1986; Bradley-Moore et al., 1985). It has been suggested that malaria parasites may synthesize folate (Bradley-Moore et al., 1985). We also observed an association between anaemia and high red cell folate in both parasitaemic and aperasitaemic women of all gravities. It is likely that in this severely iron deficient population, this association was due to iron deficiency increasing red cell folate level, as has been reported by Omer et al., (1970) and Saraya et al., (1973).

HIV infection was detected in 6.9% of the population tested. The proportion of women with malaria or severe anaemia was higher in the group of women who were HIV positive, although the association did not reach statistical significance. In Malawi, HIV infection has been shown to be associated with malaria parasitaemia (Wirima et al., 1993), and HIV infection has frequently been shown to be associated with anaemia (Doukas, 1992).

CONCLUSION
This population has high prevalences of anaemia and severe anaemia in pregnancy. Iron deficiency and hookworm were the main factors associated with anaemia in multigravidae, whereas in primigravidae malaria parasitaemia was the most important risk factor identified. The prevention of severe anaemia could have a major impact on the health of pregnant women and could contribute to a reduction in maternal mortality. There is an urgent need for studies to identify effective means of preventing the severe anaemia secondary to malaria infection in pregnancy.

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Malarial anaemia in primigravidae


