The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria

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Abstract
A randomized, double-blind, placebo-controlled community-based trial of oral iron supplementation (200 mg ferrous sulphate daily) administered to multigravid pregnant women by traditional birth attendants (TBAs) was carried out in a rural area of The Gambia. Iron supplementation led to a significant reduction in the prevalence of anaemia and of iron deficiency. Iron supplementation was not accompanied by increased susceptibility to malaria infection; there was no difference in the prevalence and severity of peripheral blood or placental malaria infection between the 2 groups of women. The birth weight of children born to women who received iron prophylaxis was increased by an average of 56 g. It is concluded that oral iron prophylaxis can be successfully delivered through TBAs integrated into a primary health care programme. This simple intervention can produce significant beneficial effects on the health of the mother without inducing increased susceptibility to malaria and has the potential for reducing perinatal mortality by increasing birth weight.

Introduction
Anaemia is a frequent consequence of pregnancy world-wide. It is particularly common in developing countries where iron deficiency is its principal cause as a result of poor intake and low bioavailability of dietary iron. In areas of the tropics where malaria is endemic, this infection is another important cause of anaemia in pregnancy, especially among primigravidae. Because of the high prevalence of iron deficiency anaemia during pregnancy it is usually recommended that all pregnant women should receive dietary supplementation with iron and folic acid (D' MAYER, 1989). However, in developing countries such as The Gambia, where health facilities are few and transport scarce, supplementation may be difficult to achieve.

Several studies have shown that administration of iron to iron-deficient individuals is associated with an increase in the incidence of clinical episodes of malaria and in the prevalence of malaria infection (BYLES & D'SA, 1970; MURRAY et al., 1978; OPPENHEIMER et al., 1986a, 1986b; SMITH et al., 1989). Two of these studies were undertaken with pregnant women (BYLES & D'SA, 1970; OPPENHEIMER et al., 1986b) and both suggested that the inclusion of iron supplementation given to iron-deficient women increased their risk of malaria. Therefore, it cannot be assumed that routine iron supplementation of pregnant women in malaria endemic areas will be beneficial and it could even be harmful, if not accompanied by malaria chemoprophylaxis. In order to investigate the potential advantages and disadvantages of routine iron supplementations during pregnancy we have undertaken a randomized controlled trial in multigravid Gambian women.

Materials and Methods
Study area and population
The trial was carried out in 18 villages near the town of Farafenni in North Bank Division, The Gambia. The geographical, demographic and climatic characteristics of the area have been described in detail elsewhere (GREENWOOD et al., 1987). The population of the study area is approximately 10 500, of whom 2500 are women between 15 and 45 years of age. Malaria is seasonal, with high transmission occurring during 4 or 5 months of the year.

The primary health care system and antenatal care
In 1982, a national village-based primary health care (PHC) programme was initiated by the Ministry of Health of the government of The Gambia. An important component of this programme has been the appointment and training of a village health worker (VHW) and a traditional birth attendant (TBA) in each village with a population of 400 or more. After selection by the community, TBAs underwent a ten-week period of training at a regional health centre where they were taught basic obstetric procedures and were issued with a delivery kit. Antenatal services support the village-based PHC system in the study area by holding clinics once a month at 2 government dispensaries. A nurse-midwife carries out a simple physical examination and performs basic screening tests for hypertension, proteinuria and glycosuria and determines the haemoglobin level. Pregnant women who attend antenatal clinics are given 2 weeks' supply of ferrous sulphate and folic acid tablets to be taken in a daily dose of 200 mg and 1 mg respectively. However, few women attend these clinics on more than 2 or 3 occasions during pregnancy.

Study design
The present study was restricted to multigravidas as, in The Gambia, malaria chemoprophylaxis is recommended only for primigravidae. Multigravid pregnant women who had been identified previously by TBAs were allocated at random by compound of residence to receive daily either 200 mg oral ferrous sulphate (60 mg elemental iron) or placebo. Iron and placebo tablets were of equal size and shape but different in colour. All pregnant women received a weekly tablet of 5 mg folic acid but no antimalarial chemoprophylaxis. Study women were asked to visit the TBA every Wednesday when each woman was issued with a plastic bag containing a week's supply of either iron or placebo tablets. Any tablets left over from the previous week were collected by the TBA and levels of compliance recorded. At the end of pregnancy the number of tablets that each woman had taken was calculated by Medical Research Council (MRC) field assistants.

Each woman was visited by a physician (C.M.) on 3 occasions. The first visit was made as soon as a woman admitted to her pregnancy, the second visit 4 to 6 weeks before delivery, and the final visit during the week following delivery. During the first 2 visits, the gestational age was assessed by manual palpation of the fundal height, and the presence or absence of hepatosplenomegaly was recorded. Anthropometrical measurements were also made. Approximately one mL of blood was collected by finger-prick into a heparinized microtainer® (Becton Dickinson) at each visit. At the second and third visit, thick and thin blood films were prepared for determination of malarial parasitaemia and red blood cell morphology.
At the time of delivery, TBAs who had been trained previously in the appropriate techniques collected 2 placental biopsies and made 2 thick blood films from the placental blood (Menendez et al., 1993). The birth weight of the newborn child was recorded by a field assistant within 7 days of delivery. Sixty-five (14%) birth weights were obtained on the day of delivery, a further 302 (67%) within 3 days of delivery, and the remaining 83 (19%) within one week of birth. For babies whose weight was not recorded on the day of birth an estimated birth weight was calculated using information obtained on weight-for-age during the first week of life in babies born to 963 Gambian multigravidae. A maximum fall of 2-5% was observed in these babies on the second day after birth.

Women with a packed cell volume (PCV) less than 25% at either of the first 2 visits were excluded from the study and given ferrous sulphate, folic acid and malaria chemoprophylaxis until delivery. Any woman with a PCV less than 30% after delivery was given the same medication for 4 weeks. Women with malaria parasitaemia were treated with chloroquine phosphate (25 mg base/kg over 3 days). The presence of abnormal haemoglobins by electrophoresis was determined; 3 women with abnormal haemoglobin genotype determined; 3 women with abnormal haemoglobin genotype had AC and the rest had AS.

Laboratory methods

The PCV was measured using a haematocrit method centrifuge and the haemoglobin level was determined by spectrophotometry. Samples of placental tissues were examined for the presence of abnormal haemoglobin by electrophoresis on a cellulose acetate strip. Thick blood films were stained with Giemsa's stain and examined for malaria parasites by a single microscopist using a ×100 oil-immersion lens and ×10 eyepieces. One hundred high power fields were examined before a slide was considered negative. One parasite per high power field corresponded to a density of approximately 500 parasites/µL. Thin blood films were stained with Leishman's stain and the morphology of the red blood cells was recorded. Plasma iron and total iron-binding capacity (TIBC) were measured spectrophotometrically (Harrison et al., 1987) using a Cobus Mira®. Plasma unbound iron-binding capacity was obtained by subtracting the values for plasma iron from the TIBC. Transferrin saturation was calculated by dividing the plasma iron by TIBC and multiplying by 100 to express the result as a percentage.

Plasma ferritin was measured by an enzyme-linked immunosorbent assay (Dako Ltd). At each delivery, 2 placental biopsies were collected by TBAs. Samples were taken from the maternal surface of the placenta and fixed immediately in 10% formal saline or buffered formalin. They were processed by standard methods and 3 mm sections stained with haematoxylin and eosin, Giemsa's stain and the periodic acid-Schiff reagent. Sections were also examined under polarized light to assess deposition of malaria pigment. Placentas were classified into 4 categories according to the presence of parasites and pigment as follows: category 1, no evidence of active or past infection; category 2, evidence of past infection (pigment in fibrin and/or placental bed); category 3, active and chronic infection (parasites and pigment as for category 2); category 4, active infection (parasites and pigment confined to red cells and/or cells circulating in maternal intervillous space).

Statistical methods

Group means were calculated for haematological and iron determinations and the statistical significance of differences determined by Student's t test. Categorical variables were compared using the χ² test, with correction for 2×2 tables when necessary. Birth weights were analysed using the linear model procedure in the SAS® computer program.

The study was designed with a projected sample size of 380 so as to give it 80% power to detect at the 5% probability level a 2% increase in the PCV of 30% expected in un-supplemented women, or an increase of 10% in the 20% expected prevalence of malaria.

The study was approved by the Gambia Government/MRC ethical committee.

Results

demographic findings

Seven hundred and fifty-seven multigravid pregnant women resident in PHC villages were identified; 121 (16%) were excluded because they were more than 34 weeks pregnant when first seen, had an abortion, moved away, or died before the second visit, or because they declined to join the investigation. A further 86 women (49 on iron supplementation, 37 placebo) who delivered before the second visit and thus had an incomplete follow-up have also been excluded. There was no significant difference in the characteristics of the women in these 2 groups.

The characteristics of the 2 groups of multigravid women on entry into the trial were very similar except that the plasma iron and ferritin levels were slightly but significantly higher in the women of group scheduled to receive iron supplementation (Table 1). Immediately after enrolment, 30 women were found to have a PCV less than 25% (17 in the iron group and 13 in the placebo group; difference not significant) and so they were treated and excluded from the rest of the study. A further 29 women were found to have a PCV less than 25% at the second visit 4 to 6 weeks before delivery (7 in the iron group and 22 in the placebo group, P<0.01) and they were then excluded. Estimated tablet consumption was similar in the 2 groups (mean 81.1, standard deviation [SD] 35.4 for the iron group and 81.7, SD 35.6 for the placebo group).

Response of haematological values to iron supplementation

Mean haemoglobin and PCV values were significantly higher, and the prevalence of anaemia was significantly lower, in the iron treatment group than in the placebo group at both 36 weeks of pregnancy and one week after delivery. Similarly, at the end of the pregnancy, plasma iron, plasma ferritin, and transferrin saturation were significantly higher, and the TIBC significantly lower, in

| Table 1. Baseline data for eligible women who completed the study |
|-----------------|-----------------|
|                  | Iron supplement* | Placebo* |
| No. of subjects  | 273             | 277     |
| Age at entry (years) | 28.9 (6.3)  | 29.4 (6.1) |
| Parity           | 4.13 (2.09)     | 4.12 (2.04) |
| Gestational age at enrolment (years) | 24.0 (5.2)  | 24.5 (5.1) |
| Weight (kg)      | 55.4 (7.8)      | 54.5 (7.1) |
| Height (cm)      | 161.6 (5.9)     | 160.5 (6.1) |
| Body fat (kg)    | 15.8 (4.4)      | 15.6 (4.2) |
| Haemoglobin genotype  |               |
| AA               | 208             | 212     |
| Abnormal         | 57              | 51      |

*No. of subjects refers to the number of subjects who completed the study and were included in the statistical analysis.

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591
Effect of iron supplementation on the outcome of pregnancy

Table 2. Haematological and iron measurements at the end of the pregnancy and within a week of delivery by treatment group

<table>
<thead>
<tr>
<th>Measure</th>
<th>Iron supplement</th>
<th>Placebo</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Mean†</td>
<td>No. Mean†</td>
<td></td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 36 weeks</td>
<td>251 31.4 (3.66)</td>
<td>259 29.3 (3.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After delivery</td>
<td>251 31.7 (5.18)</td>
<td>259 30.4 (5.54)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 36 weeks</td>
<td>231 10.4 (1.43)</td>
<td>229 9.5 (1.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After delivery</td>
<td>207 10.4 (1.99)</td>
<td>203 9.9 (1.77)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma iron (μmol/L)</td>
<td>219 14.6 (8.04)</td>
<td>211 11.9 (7.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma ferritin (μg/L)</td>
<td>249 117 (67)</td>
<td>258 81 (58)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>211 18.8 (12.8)</td>
<td>251 14.5 (11.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total iron binding capacity (μmol/L)</td>
<td>211 88 (30.5)</td>
<td>232 93.9 (27.5)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Standard deviation in parentheses.
†Significance of difference between groups.
‡At 36 weeks.

women who had received iron supplements compared to those who had received placebo (Table 2).

At the end of pregnancy, significantly fewer women who took iron had anisocytosis, polychromasia or target cells than did women who took placebo (data not shown). Similar findings were obtained with samples collected within one week after delivery.

The impact of malaria on the haemoglobin level at 36 weeks of pregnancy, compared with that at the start of the study, was more marked among those who took iron than among those who took placebo (10.5 g/dL vs. 9.9 g/dL, P<0.01) and 9.6 g/dL vs. 9.4 g/dL (P=0.43) respectively. However, the haemoglobin level of women receiving iron supplementation who experienced malaria recovered more rapidly than did that of women who were receiving placebo. After delivery, the mean haemoglobin level of women who had received iron supplements was higher than that of women who had received placebo, regardless of whether or not they had had malaria during their pregnancy (Figure). The prevalence of overall malaria parasitaemia and of high density peripheral blood parasitaemia was similar in the 2 groups, both at the end of the pregnancy and after delivery (Table 3). TBAs collected 392 placental specimens for histology, and placental blood smears were obtained from 370 women. There was no significant difference in the prevalence of infected placentas between the 2 groups for each category of infection. Analysis of placental blood smears also showed no difference in the proportion or density of infections between the 2 groups.

The prevalence of anaemia at the end of pregnancy was 96.2% (926/971) among those who took iron and 91.4% (914/996) among those who took placebo (P=0.04). In a long period of time, as in pregnancy (BONNAR et al., 1969), TBAs were highly effective in administering iron supplements, especially when required for short periods after delivery. They did not find increased susceptibility to malaria infection associated with iron administration, as has been noted in some other studies. One possible explanation for this finding is the high level of immunity of the women in our study who had been exposed frequently to malaria since early childhood. It is possible that a different effect
might have been observed in primigravidae who were more susceptible to malaria. A second possible explanation for our findings is that the dose of iron given was not sufficient to show any adverse effect. Both factors may have acted simultaneously. Although we did not measure the incidence of malaria infection, it is unlikely that we missed many episodes since there was no significant difference in the prevalence of placental malaria for any category of infection between the 2 groups and pigment deposition in the placenta probably lasts for several months after an attack of malaria (Watkinson & Ruston, 1983).

The results of the study suggest that iron prophylaxis improved haematological characteristics without increasing susceptibility to malaria infection. Among those women who did have a malaria infection, chloroquine treatment and iron supplementation allowed them to recover their haemoglobin level within 4 weeks. Women who had a malaria infection for which they received curative treatment, but who were receiving placebo, failed to improve similarly.

Several studies have reported an association between maternal anaemia and foetal birth weight (MacGregor, 1963; Harrison & Ibeziako, 1973). However, most were observational, retrospective studies that established correlations between perinatal haemoglobin or haematocrit and subsequent outcome. In these studies no correction was made for possible confounding variables associated with both anaemia and birth weight such as malarial infection and caloric intake (pregnancy weight gain).

Two randomized trials of iron supplementation (60 mg/d elemental iron) found no effect of supplementation on either gestational age or birth weight. One of these studies was carried out on a small group of 73 Australian women with a mean haemoglobin level at entry of 12.7 g/dL (Fleming et al., 1974). Therefore, its results are difficult to extrapolate to developing countries. The second study was carried out on 200 Nigerian primigravidae living in an urban area (Fleming et al., 1986). It has been shown that iron deficiency increases with increasing parity, so the results of the Nigerian study are not incompatible with the existence of a beneficial effect of iron supplementation in women at a higher risk of iron deficiency.

We believe that ours was the first randomized trial of iron supplementation to pregnant women in which there seemed to be a positive effect on birth weight. The increase in birth weight found, although small and just not statistically significant (56 g, 95% confidence interval 12-128), may have been important since infant mortality is very strongly influenced by birth weight (Kramer, 1987). The fact that there was a stronger correlation between birth weight and the number of iron tablets taken, while this correlation was absent for the placebo group, suggests that there may be a dose-response effect. Indeed, when analysis was restricted to women who had received the equivalent of 3 months' supply of tablets, the difference in birth weight between groups increased and was statistically significant (96 g; P=0.04).

Routine iron supplementation of multigravid Gambians was found to be beneficial by increasing haemoglobin level and PCV, and probably birth weight, and had no deleterious effect by increasing susceptibility to malaria infection; thus it has the potential of reducing both maternal and infant mortality. Administration of iron by TBAs proved to be an effective way of achieving this supplementation.

References


Received 2 June 1993; revised 7 December 1993; accepted for publication 12 January 1994.