The response to iron supplementation of pregnant women with the haemoglobin genotype AA or AS

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Abstract

The influence of haemoglobin genotype on the response to iron supplementation was studied in a randomized, double blind, placebo-controlled trial involving 497 multigravid pregnant women from a rural area of The Gambia. Women were randomly allocated to receive either oral iron (60mg elemental iron per day) or placebo. At 36 weeks of pregnancy, women who had received oral iron during pregnancy had higher mean haemoglobin, packed cell volume, plasma iron and ferritin levels than did women who received placebo. Iron supplementation of pregnant women with the AA haemoglobin genotype also resulted in increases in the packed cell volume (PCV) and haemoglobin level measured after delivery, and in the birth weight of the infant. However, in AS women PCV and haemoglobin level at delivery were lower in the supplemented group and supplementation was also associated with reduced birth weights. In malaria endemic areas, pregnant women with the haemoglobin genotype AS may not benefit from iron supplementation during pregnancy.

Keywords: iron supplementation, pregnant women, haemoglobin AA and AS, The Gambia

Introduction

Anaemia is a common feature of pregnancy throughout the world, but it is especially important in developing countries where it may pose a threat to the life of both the mother and her baby (FLEMING, 1989). In most developing countries, iron deficiency is the main cause of anaemia in pregnancy and therefore iron supplementation is routinely recommended. However, in endemic areas, malaria may be an equally important cause of anaemia, especially in primigravidae (BRABIN, 1983). Two studies have shown that parenteral iron administration during pregnancy increased susceptibility to malaria (BYLES & D'SA, 1970; OPPENHEIMER *et al.*, 1986). We therefore carried out a controlled trial of iron supplementation in pregnant women resident in a rural area of The Gambia where malaria is common. The effects of iron on both haematological measurements and on the prevalence of malaria were followed carefully. Because rural Gambian women may have difficulties in reaching an antenatal clinic, iron supplementation was given by traditional birth attendants (TBAs) resident in the villages of the pregnant women. The overall results of this trial, which have been presented elsewhere, were clear-cut (MENENDEZ *et al.*, 1994). Iron supplementation increased the mean haemoglobin level and packed cell volume (PCV) of pregnant women overall and did not lead to any significant increase in the prevalence of malaria. However, during the analysis of these results, it was noted that the response to iron of pregnant women with the haemoglobin genotype AS appeared to be different from that of women with the haemoglobin genotype AA. We now present the results of a detailed analysis demonstrating that iron supplementation of pregnant Gambian women with the haemoglobin genotype AS was of no value and may even have been harmful.

Subjects and Methods

Study area and population

The study was carried out in 15 villages near the town of Farafenni, North Bank Division, The Gambia. The demographic and geographical characteristics of the area have been described previously (GREENWOOD *et al.*, 1987). Its population is about 10 500, of whom approximately 2500 are women between the ages of 15 and 45 years. The area is one of seasonal malaria with high levels of transmission occurring during the 4–5 months of the rainy season.

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Study design

As described previously (MENENDEZ *et al.*, 1994), multigravid pregnant women living in study villages were allocated at random to receive daily a single tablet of oral ferrous sulphate (60 mg elemental iron) or a single tablet of placebo from the time that pregnancy was recognized until delivery. In addition, each woman received a 5 mg tablet of folic acid weekly; antimalarial drugs were not given. Primigravidae and second gravidae who were participating in a trial of antimalarial chemoprophylaxis were not included in the study. Tablets were issued weekly by TBAs living in the study villages. Tablets not used during the previous week were collected by the TBA and counted so as to provide a measure of compliance of drug consumption. Iron and placebo were coloured differently to facilitate correct administration. However, all clinical and laboratory measurements were made by investigators who did not know the treatment code.

Each woman was visited by a physician (C.M.) on 3 occasions. The first visit was made soon after a woman had reported to a TBA or Medical Research Council (MRC) field worker that she was pregnant, the second 4–6 weeks before delivery, and the final visit within a week of delivery. At the first 2 visits gestational age was assessed by palpation of the fundal height and weight, height and skin fold thickness were recorded. At each visit approximately 1 mL of blood was collected by finger-prick into a heparinized microtainer (Beckton Dickinson, Rutherford, New Jersey, USA) and thick and thin blood films were prepared. After delivery, TBAs, who had been trained previously in the technique (MENENDEZ *et al.*, 1993), collected 2 placental biopsy specimens and made 2 thick blood films from blood obtained from a cut surface of the placenta. The birth weight of each newborn infant was recorded by a field worker who did not know the treatment code, as soon as possible after birth. In the case of babies who were not seen during the first 24 h after delivery, an estimated birth weight was calculated using information obtained on weight-for-age during the first week of life in infants born to 963 Gambian multigravidae. A maximum fall in birth weight of 2·5% was observed in these babies on the second day after birth.

Laboratory methods

The PCV and haemoglobin level (Hb) were measured by standard methods. The presence of abnormal haemoglobins was assessed following electrophoresis of a red blood cell haemolysate on cellulose acetate strips. Thick

blood films were stained with Giemsa's stain and examined for malaria parasites. At least 100 high power fields were examined before a film was reported to be negative. Plasma iron and iron binding capacity (UIBC) were measured by a colorimetric method (HARRISON *et al.*, 1987) using reagents obtained from the Sigma Chemical Company (Poole, Dorset, UK). Total iron-binding capacity (TIBC) was obtained by adding together the values for plasma iron and UIBC. Transferrin saturation was calculated by dividing the plasma iron value by the TIBC and multiplying by 100. Plasma ferritin was measured by an enzyme-linked immunosorbent assay (SMITH *et al.*, 1989) using a rabbit anti-ferritin antiserum obtained from Dako (UK).

Placental biopsies were fixed in 10% formol-saline or buffered formol-saline, and paraffin blocks were prepared in The Gambia within a few weeks of collection. Sections were cut subsequently in London and stained according to conventional histological techniques. Placentas were grouped into 4 categories dependent upon the presence or absence of parasites and pigment and the presence of active current and/or previous infecion during the pregnancy was determined (MENENDEZ *et al.*, 1994).

Statistical methods

Analysis of variance was used to compare characteristics at enrolment. Interaction terms were included in an analysis of covariance in order to test whether the effects of supplementation varied between genotypes. In analyses of haematological measurements, the value obtained at enrolment was treated as a covariate. In the analysis of birth weight, maternal height was treated as a covariate. Categorical variables were compared using logistic regression.

Results

Seven hundred and fifty-seven multigravidae who were potentially eligible for the study were identified; 207 were subsequently excluded because they were more than 34 weeks pregnant when first seen, delivered before a second antenatal visit was made, had an abortion, moved away, died, or declined to join the study. Thus 550 women were enrolled, with a mean gestational age of 23·8 weeks (standard deviation 5·2). At that time, 30 women (5·4%) were found to have a PCV less than 25%, treated and withdrawn from the study; 520 women completed the study and haemoglobin electrophoresis was performed for 500 of them; 98 (19·6%) had the haemoglobin genotype AS, (0·6%) had the genotype AC, and 399 (79·8%) had the genotype AA. Women with haemoglobin AS or AA are the subjects of the remainder of this report. Baseline characteristics and compliance of the 4 groups of women are shown in Table 1. The numbers of tablets consumed were very similar in all 4 groups. The baseline characteristics of the women who did not complete follow-up were also similar in all 4 groups (data not shown). Further details of compliance and follow-up have been given by MENENDEZ *et al.* (1994).

Haematological effects of iron supplementation

Haematological findings at the end of pregnancy are shown in Table 2. As described by MENENDEZ *et al.* (1994), iron supplementation was associated with significant increases in all haematological measurements and in plasma iron, plasma ferritin and transferrin saturation. The analysis of covariance (treating the initial measurements as covariates) also indicated that the AS genotype had significantly lower PCV and Hb, and higher levels of plasma iron, than AA women. None of the interaction terms was statistically significant so we cannot conclude

Table 1. Characteristics of pregnant women on enrolment into the study and number of tablets taken

	aemoglobin genotype			
	AA		AS	
	reatment group			
Characteristics	Iron[a]	Placebo[a]	Iron[a]	Placebo[a]
Age (years)	29·05±6·4 (200)	29·54±6·1 (198)	29·50±6·4 (46)	29·65±6·3 (52)
Parity	4·18±2·1 (201)	4·09±2·0 (198)	4·32±2·2 (46)	4·42±2·3 (52)
Height (cm)	161·52±6·0 (199)	160·28±6·3 (196)	161·53±5·3 (46)	161·66±5·8 (50)
Weight (kg)	55·09±7·1 (201)	54·26±7·2 (198)	55·38±8·6 (46)	55·04±7·6 (52)
Body fat (%)	28·52±4·5 (200)	28·31±4·6 (198)	28·26±4·0 (46)	28·50±5·0 (52)
No. of tablets taken	85·30±30·3 (201)	87·12±32·0 (198)	93·51±24·6 (46)	84·70±25·9 (52)

[a]Means±standard deviations (numbers of women studied are given in parentheses).

Table 2. Effect of iron supplementation on haematological and biochemical iron values adjusted for baseline values at 36 weeks of pregnancy

Genotype	Treatment	Packed cell volume (%)[a]	Haemoglobin (g/dL)[a]	Iron (μmol/L)[b]	Total iron binding capacity (μmol/L)[a]	Ferritin (μg/L)[b]	Transferrin saturation (%)[a]
AA	Iron	31·7±3·5 (197)	10·5±1·5 (163)	12·18 [1·75] (169)	89·7±3·40 (156)	99·48 [1·82] (193)	18·0±1·23 (156)
AA	Placebo	29·3±3·9 (193)	9·6±1·6 (156)	9·97 [1·75] (167)	93·4±3·09 (162)	60·34 [1·97] (189)	14·4±1·25 (161)
AS	Iron	29·9±4·4 (41)	9·8±1·6 (36)	14·88 [1·70] (38)	81·6±4·80 (38)	121·51 [1·77] (44)	22·4±2·50 (38)
AS	Placebo	28·7±3·5 (50)	9·4±1·6 (40)	11·02 [1·70] (49)	96·5±6·35 (46)	73·70 [1·86] (51)	13·9±1·82 (46)
Analyses of variance[c]							
Main effects							
Genotype							
F		7·23	4·18	4·27	0·16	2·12	0·75
P		<0·01	<0·05	<0·05	>0·5	>0·1	>0·4
Iron							
F		43·37	31·97	11·60	4·12	48·40	14·01
P		<0·001	<0·001	<0·001	<0·05	<0·001	<0·001
Two-way interaction							
F		1·25	1·08	0·57	2·27	0·06	2·45
P		>0·2	>0·2	>0·4	>0·1	>0·8	>0·1

[a]Arithmetic mean±standard deviation (numbers of measurements made are given in parentheses).
[b]Geometric mean [antilog standard deviation in square brackets] (numbers of measurements made are given in parentheses).
[c]*F*=variance ratio statistic; *P*=probability.

that the sickle-cell genotype modified the effect of iron supplementation on any of these variables by 36 weeks gestation. However, the observed effect of iron on both PCV and Hb was much greater in AA than in AS women.

Mean PCV and Hb measured shortly after delivery by genotype and treatment groups are shown in Table 3. Both Hb and PCV were lower among those with the AS genotype and iron supplementation was associated with statistically significant increases in Hb and PCV. However, the overall increases in these quantities were entirely accounted for by the individuals with AA genotype. Women with the AS genotype who received the supplements had slightly lower Hb and PCV than those who received placebo. The analysis of covariance indicated that there were highly significant interactions between the effects of iron supplementation and haemoglobin genotype on both Hb and PCV (Table 3).

Effects of iron supplementation on birth weight

Among women with the haemoglobin genotype AA, the mean birth weight of babies born to iron-supplemented women was higher than that of babies born to women who received placebo (Table 3). In contrast, among women with the haemoglobin genotype AS the mean birth weight of babies born to women who received iron supplementation was less than that of the babies of women who received placebo. The analysis of covariance, which allowed for confounding effects of maternal height, indicated that the effect of supplementation was significantly modified by the genotype.

Discussion

Most studies have shown that pregnant women with the haemoglobin genotype AS have normal fertility and normal abortion, prematurity and still birth rates in both

Table 3. Effect of iron supplementation on birth weight and haematological and biochemical values adjusted for haemoglobin genotype and baseline values shortly after delivery

Genotype	treatment	Packed cell volume (%)[a]	Haemoglobin (g/dL)[a]	Birth weight (g)[a]
AA	Iron	32·4±4·9 (183)	10·8±1·9 (148)	3·113±400 (182)
AA	Placebo	30·2±4·7 (167)	9·8±1·7 (142)	3·026±384 (164)
AS	Iron	28·6±5·6 (39)	9·5±2·0 (33)	3·010±425 (41)
AS	Placebo	30·8±3·8 (40)	10·1±1·8 (34)	3·109±402 (43)
Analysis of covariance[b]				
Main effects				
Genotype				
F		6·48	4·33	0·02
P		0·01	<0·05	0·9
Iron				
F		8·88	13·56	0·32
P		<0·01	<0·001	0·6
Two-way interaction				
F		10·91	8·76	4·54
P		<0·01	<0·01	<0·03

[a]Means±standard deviations (numbers of women studied are given in parentheses).
[b]*F*=variance ratio statistic; *P*=probability.

Table 4. Peripheral blood and placental malaria infection in pregnant women with haemoglobin genotype AA or AS according to whether they received iron supplementation or placebo during pregnancy

Genotype	Treatment	Peripheral blood parasitaemia 36 weeks	Peripheral blood parasitaemia Post-natal	Placental malaria
AA	Iron	50/201 (25%)	33/197 (17%)	83/158 (53%)
AA	Placebo	53/198 (27%)	23/183 (13%)	91/154 (59%)
AS	Iron	16/46 (35%)	8/44 (18%)	22/29 (76%)
AS	Placebo	13/51 (25%)	9/45 (20%)	19/33 (58%)
Logistic regression[a]				
Main effects				
Genotype				
χ^2_1		0·6	1·1	2·3
P		0·4	0·3	0·1
Iron				
χ^2_1		0·0	0·9	0·2
P		1·0	0·3	0·7
Two-way interaction				
χ^2_1		1·2	0·5	3·5
P		0·3	0·5	0·06

[a]*P*=probability.

Effects of iron supplementation on malaria

The prevalence of malaria parasites in peripheral blood was very similar in all 4 groups (Table 4). Parasite densities were also similar (results not shown). Placental malaria was most frequent in the iron-supplemented AS women, and the interaction term in the logistic regression analysis, which tested whether the effects of supplementation were different in the 2 genotypes, was of borderline significance.

malaria endemic and non-endemic areas (ADAMS *et al.*, 1953; WHALLEY *et al.*, 1963; FLEMING *et al.*, 1979). Opinion is divided as to whether women with the genotype AS are at increased risk of anaemia during pregnancy (MCCURDY, 1964; FLEMING *et al.*, 1968; BLANK & FREEDMAN, 1969; VAN DONGHEN *et al.*, 1982). In our study, the AS women had lower Hb and PCV irrespective of iron supplementation.

This increase in anaemia might reflect the different re-

sponse to malaria infection of the AS genotype. Many studies have shown that possession of the AS genotype protects African children from clinical malaria, particularly from severe forms (ALLISON, 1957). The advantage of the AS genotype is less marked in individuals with high levels of immunity and parasite rates and densities are usually similar in endemic areas in adults with either the AA or AS genotype (FLEMING *et al.*, 1979). The influence of haemoglobin genotype on malaria during pregnancy in semi-immune women is not clearcut. BRABIN & PERRIN (1985) did not find any difference in parasite rates between AA and AS pregnant women in Kenya. However, in Nigeria, FLEMING & HARRISON (1984) found that in primigravidae, whose immunity to malaria was suppressed, parasite densities were lower in women in the genotype AS than in those with the genotype AA. AS women also had lower malaria antibody levels than those with the genotype AA (CORNILLE-BROGGER *et al.*, 1979), perhaps because they were exposed to less antigenic stimulation. Our study demonstrated no overall effect of haemoglobin genotype on peripheral parasitaemia; however, the malariological surveillance was not intense and we studied only multigravid pregnant women who are less immunosuppressed than primigravidae.

Pregnant women with the AS haemoglobin genotype showed a poor haematological response to iron supplementation which was also associated with reduced birth weight of their children. The most likely explanation is that iron supplementation increased the susceptibility of these women to malaria. Two previous studies have shown that parenteral iron administration during pregnancy can increase the prevalence of malaria infection (BYLES & D'SA, 1970; OPPENHEIMER *et al.*, 1986). Our data suggested, albeit inconclusively, that there was increased placental malaria in the supplemented AS group, and hence that such effects can depend on the haemoglobin genotype. It may be that pregnant women with AS genotype rely less on the immune response against malaria than do AA women, but achieve a similar level of protection as a result of additional genotype-specific non-immunological mechanisms. If iron supplementation were to interfere with these AS specific mechanisms, then AS women receiving iron would have an enhanced risk of malaria.

A less likely explanation for the adverse effects in AS women is that administration of iron increased haemolysis of AS red blood cells by promoting auto-oxidation of haemoglobin S (but not of haemoglobin A), generation of free oxygen radicals which damage the red cell membrane, and further damage it by the deposition of ferritin-like and haemosiderin-like iron on the red cell surface (RICE-EVANS & BAYSAL, 1987; HEBBEL, 1990). Finally, it is possible that, independently of the effects of malaria, AS subjects do not utilize iron as well as subjects with the AA genotype, but we are unaware of any evidence for this.

This study provided evidence of an interaction between a micronutrient and a genotype in affecting susceptibility to a parasitic disease. The results suggest that pregnant women resident in malaria endemic areas who are known to have the haemoglobin genotype AS should not routinely be given iron supplementation during pregnancy. Further, larger studies are needed to confirm our findings and to determine the balance of advantages and disadvantages of such supplements. However, if iron is given to AS women, it should be accompanied by administration of effective malaria chemoprophylaxis regardless of maternal parity. Further studies are required to determine whether iron supplementation in pregnancy is also ineffective among pregnant women with the AS genotype resident in areas where malaria is not endemic.

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