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Anaemia and iron deficiency in pregnancy and adverse perinatal outcomes in Southern India

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Abstract

Background/Objectives—We examined the prevalence of anaemia, iron deficiency, and inflammation during pregnancy and their associations with adverse pregnancy and infant outcomes in India.

Subjects/Methods—Three hundred and sixty-six women participating in a randomised trial of vitamin B₁₂ supplementation were monitored to assess haemoglobin (Hb), serum ferritin (SF), hepcidin, C-reactive protein (CRP), and alpha-1-acid glycoprotein (AGP) during pregnancy. Women received vitamin B₁₂ supplementation (50 µg per day) or placebo daily; all women received daily prenatal iron–folic acid supplementation. Binomial and linear regression models were used to examine the associations of maternal iron biomarkers with pregnancy and infant outcomes.

Results—Thirty percent of women were anaemic (Hb < 11.0 g/dl), 48% were iron deficient (SF < 15.0 µg/l), and 23% had iron deficiency anaemia at their first prenatal visit. The prevalence of inflammation (CRP > 5.0 mg/l: 17%; AGP > 1.0 g/l: 11%) and anaemia of inflammation (Hb < 11.0 g/dl, SF > 15.0 µg/l, plus CRP > 5.0 mg/l or AGP > 1.0 g/l: 2%) were low. Infants born to anaemic women had a twofold higher risk of low birth weight (<2500 g; risk ratio [RR]: 2.15, 95% CI: 1.20–3.84, *p* = 0.01), preterm delivery (RR: 2.67 (1.43–5.00); *p* = 0.002), underweight

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics The research protocols and study procedures were approved by the Institutional Ethical Board of St. John's Medical College and the Harvard T.H. Chan School of Public Health Human Subjects Committee, and written informed consent was obtained from all participants. A Data Safety and Monitoring Board met twice annually during the course of the trial.

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(WAZ < -2; RR: 2.20, 95%CI: 1.16–4.15, $p = 0.02$), and lower MUAC (β (SE): -0.94 (0.45)cm, $p = 0.03$). Similarly, maternal Hb concentrations predicted higher infant birth weight ($p = 0.02$) and greater gestational age at delivery (β (SE): 0.28 (0.08) weeks, $p = 0.001$), lower risk of preterm delivery (<37 weeks; RR: 0.76, 95%CI: 0.66–86, $p < 0.0001$); and higher infant MUAC (β (SE): 0.36 (0.13) cm, $p = 0.006$). Maternal SF concentrations were associated with greater birth length (β (SE): 0.44 (0.20) cm, $p < 0.03$). Findings were similar after adjusting SF concentrations for inflammation. IDA was associated with higher risk of low birth weight (RR: 1.99 (1.08–3.68); $p = 0.03$) and preterm birth (RR: 3.46 (1.81–6.61); $p = 0.0002$); and lower birth weight ($p = 0.02$), gestational age at birth ($p = 0.0002$), and infant WAZ scores ($p = 0.02$).

Conclusions—The prevalence of anaemia and iron deficiency was high early in pregnancy and associated with increased risk of adverse pregnancy and infant outcomes. A comprehensive approach to prevent anaemia is needed in women of reproductive age, to enhance haematological status and improve maternal and child health outcomes.

Introduction

Anaemia is a major public health problem worldwide, affecting over 1.6 billion people [1]. Anaemia has been associated with increased risk of maternal and infant mortality [1], impaired neurodevelopment in offspring [2] and reduced cognitive function [3, 4] and physical work capacity [3, 5] later in life. The burden of anaemia in India is among the highest in the world, where it exacts a heavy toll in terms of mortality, disability, and lost productivity [1, 6]. It is estimated that anaemia affects 56% of women of reproductive age, 59% of pregnant women, 63% of lactating women, and 70% of young children in India [1].

Iron deficiency is the leading cause of anaemia world-wide, and iron supplementation is the standard of care for prevention and treatment of anaemia [1]. It has been estimated that ~50% of anaemia is due to iron deficiency (50%, 95%CI: 47–53%) [1]. However, recent findings indicate that the proportion of anaemia due to iron deficiency may be considerably lower [7], particularly in settings where the anaemia prevalence is high, and in settings with very high inflammation exposure [7]. Other nutritional factors, such as vitamin B₁₂ and folate, and non-nutritional factors such as inflammation also contribute to the aetiology of anaemia and impact human health [8, 9]. Elucidation of the aetiology of anaemia early in pregnancy and its impact on perinatal outcomes could help to inform screening and targeted interventions for anaemia prevention.

We, therefore, conducted a prospective observational analysis among pregnant women and their infants participating in a randomised trial of vitamin B₁₂ supplementation in Bangalore, India. The objectives of this study were to: (1) determine the prevalence of anaemia, iron deficiency, iron deficiency anaemia (IDA), and inflammation in pregnant women; and (2) examine the associations between maternal biomarkers of iron status with the risks of adverse pregnancy and infant outcomes.

Subjects and methods

Study population

Participants were pregnant women, who were enrolled in a randomised, double-blind, placebo-controlled trial of vitamin B₁₂ supplementation in Bangalore, India. This trial was conducted to examine the effects of daily vitamin B₁₂ supplementation on biomarkers of maternal vitamin B₁₂ status during pregnancy. The design and main results of the trial have been previously described ([#NCT00641862](#)) [10]. Briefly, pregnant women ($n = 366$) were recruited from Hosahalli Referral Hospital in Bangalore, India, and randomised to receive vitamin B₁₂ supplementation (50 µg per day) or placebo, daily during pregnancy through 6 weeks postpartum. Findings demonstrated that maternal prenatal vitamin B₁₂ supplementation significantly increased maternal, breast milk, and infant vitamin B₁₂ concentrations [10]. All women also received daily iron (60 mg) and folic acid (500 µg) supplementation, beginning at their first prenatal visit, as per standard of care.

Pregnant women were eligible to participate in this study if they were at least 18 years of age, 14 weeks of gestation at enrolment, healthy, and carrying a single fetus. Women were excluded if they had any known medical complications, including HIV infection, hepatitis B, or syphilis. Women with a serious preexisting medical condition (defined by the need for regular medication use), or who had a previous caesarean delivery, or who were taking daily vitamin supplements in addition to iron and folic acid were also excluded.

Follow-up procedures

Structured interviews were conducted at the baseline clinic visit to collect information on sociodemographic characteristics, including maternal age, educational level, socioeconomic status, and obstetric history. A clinical examination was performed, including measurement of vital signs and blood pressure. Obstetric, reproductive, and neurological examinations were conducted. Detailed clinical, sociodemographic, and anthropometric data were collected prospectively. Weights of all the mothers were recorded using a digital balance to the nearest 0.1 kg (Salter's 9016; Tonbridge), and maternal height was measured using a stadiometer to the nearest 0.1 cm. Maternal mid-upper arm circumference and triceps, biceps, and subscapular skinfold thickness measurements were recorded by trained research assistants.

Infant weight was recorded on an electronic weighing scale to the nearest 0.01 kg; measurements were recorded by trained study staff. Infant supine length, mid-upper arm circumference, head and chest circumferences, and triceps and biceps skinfolds were recorded by trained research assistants, using standardised procedures and calibrated instruments [10–12].

Laboratory investigations. Blood sample collection

Maternal blood samples were collected at study visits at enrolment (14 weeks), and during the second and third trimesters, timed to coincide with early (median [IQR]: 10.6 (9.1, 12.6) weeks), mid- (24.1 (23.7, 25.0) weeks), and late- (33.1 (32.7, 33.6) weeks) gestation. Infant blood samples were collected at 6 weeks of age by venipuncture. The laboratory procedures

and biochemical analyses in this trial have previously been described [10]. Briefly, ~10 ml of blood was drawn from mothers during pregnancy and their infants at 6 weeks of age by venipuncture and collected in both EDTA and plain vacutainers (BD Biosciences), which were stored on ice until separated in a refrigerated centrifuge, within 4 h of being drawn. Haemoglobin and complete blood count were analysed on whole-blood samples in an automated Coulter counter (ABX Pentra C+; Horiba Medicals). Plasma and red blood cells (RBCs) were separated and stored at or below -80°C until analysis.

Biomarkers

Serum ferritin was measured by electrochemiluminescence (Elecsys 2010, Roche Diagnostics Mannheim, USA). Serum C-reactive protein and alpha-1-acid glycoprotein (AGP) were analysed via the particle-enhanced immune-turbidometric assay in a Roche Hitachi 902 analyzer (Roche Diagnostics, Germany). Hepcidin was measured by the competitive enzyme immunoassay method (Peninsula Laboratories, LLC, Bachem Group).

Anaemia was defined as low haemoglobin concentrations based on trimester-specific cutoffs (i.e., Hb < 11.0 g/dl during the first trimester, Hb < 10.5 g/dl during the second trimester, Hb < 11.0 g/dl during the third trimester) [13, 14]. Iron deficiency was defined as SF < 15.0 $\mu\text{g/l}$, and iron insufficiency was defined as SF < 30.0 $\mu\text{g/l}$. Inflammation was defined as elevated CRP or AGP using established cutoffs (CRP > 5.0 mg/l, AGP > 1.0 g/l). Anaemia of inflammation was defined as anaemia in the presence of normal serum ferritin concentrations and elevated inflammatory biomarkers (i.e., Hb < 11.0 g/dl, SF > 15.0 $\mu\text{g/l}$, plus CRP > 5.0 mg/l, or AGP > 1.0 g/l) [15].

Maternal body mass index (BMI) was defined as the ratio of weight in kilograms to height in meters squared (m^2), and categorised using World Health Organization classifications [16]. Preterm delivery was defined as less than 37 completed weeks gestation. Infant low birth weight was defined as <2.5 kg. Small for gestational age (SGA) was defined as birth weight <10th percentile of the gestational age, using the INTERGROWTH reference [17]. Infant ponderal index was defined as weight in grams divided by length in centimeters cubed (g/cm^3). Length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) z -scores were calculated using World Health Organization (WHO) standards [18–20]. Stunting, wasting, and underweight were defined as standardised, sex-specific z -scores < -2 , using LAZ, WLZ, and WAZ, respectively.

Statistical analyses

Variables were defined using conventional cutoffs where available; otherwise, medians of variables were defined based on their distributions in the population. Serum ferritin concentrations were adjusted for inflammation, considering Thurnham [21, 22] and Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) [23, 24] methods. Non-normally distributed variables were natural logarithmically transformed to ensure normality before further analysis; non-transformed values are presented in Table 1 for interpretation purposes.

Linear and binomial regression models were used to examine the associations of maternal biomarkers of iron status at enrolment (i.e., 14 weeks) with pregnancy and

infant outcomes, for continuous and categorical outcomes, respectively, including birth weight (continuous), low birth weight (categorical), gestational age at birth (continuous), preterm delivery (categorical), SGA (categorical), and infant WHO *z*-scores (continuous). Associations between maternal iron status biomarkers and perinatal outcomes were examined independently in separate models. All models included an adjustment for the intervention regimen and gestational age at sample collection, to account for the study design and timing of sample collection. The approach proposed by Rothman and Greenland was used to adjust for confounding, in which all known or suspected risk factors for the outcome that lead to a greater than 10% change-in-estimate were included in the models [25]. The missing indicator method was used for covariates, to retain observations with missing covariate data in multivariate analyses [26]. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Characteristics of participants included in this study are presented in Table 1, and a flow chart of participants is presented in Fig. 1. At enrolment (14 weeks gestation), 30% of mothers were anaemic (Hb < 11.0 g/dl), 48% were iron deficient (SF < 15.0 µg/l), 68% had insufficient iron status (SF < 30.0 µg/l), and 23% had IDA. The prevalence of inflammation (CRP > 5.0 mg/l: 17%; AGP > 1.0 g/l: 11%) and AI (Hb < 11.0 g/dl, SF > 15.0 µg/l, plus CRP > 5.0 mg/l or AGP > 1.0 g/l: 2%) was relatively low. Findings were similar after adjusting serum ferritin for inflammation (Supplementary Table I, e.g., BRINDA, Thurnham); the prevalence of iron deficiency was 59% (SF < 15.0 µg/l), and 78% of pregnant women had insufficient iron status (SF < 30.0 µg/l), after adjusting for inflammation.

Maternal serum ferritin concentrations (β (SE): 0.78 (0.07) µg/l; $p < 0.0001$), hepcidin concentrations (β (SE): 0.37 (0.05) ng/ml; $p < 0.0001$), and iron deficiency (β (SE): -1.29 (0.15); $p < 0.0001$) at baseline were significantly associated with higher maternal haemoglobin concentrations in linear regression analyses. Findings were similar after adjusting serum ferritin concentrations for inflammation (SF: (β (SE): 0.79 (0.08) µg/L, $p < 0.0001$; ID: β (SE): -1.19 (0.16), $p < 0.0001$). Similarly, higher maternal serum ferritin concentrations (Risk Ratio [RR]: 0.48, 95%CI: 0.40–0.57, $p < 0.0001$) and hepcidin concentrations (RR: 0.73, 95%CI: 0.67–0.79, $p < 0.0001$) at baseline were associated with lower risk of maternal anaemia; whereas maternal iron deficiency was associated with a 3.6 times higher risk of anaemia (RR: 3.62, 95%CI: 2.09–6.36, $p < 0.0001$). Findings were similar after adjusting serum ferritin concentrations for inflammation (SF: RR: 0.49, 95%CI: 0.41–0.58, $p < 0.0001$; ID: RR: 3.41, 2.16–5.41, $p < 0.0001$).

The associations between maternal anaemia and haemoglobin concentrations and risk of adverse pregnancy and infant outcomes are presented in Tables 2 and 3, respectively. Women who were anaemic at enrolment had infants with lower birth weight (β (SE): -166.8 (61.1) g; $p = 0.006$) and 2.15 times higher risk of low birth weight (<2500 g; RR: 2.15, 95%CI: 1.20–3.84, $p = 0.01$), compared with the women who were not anaemic, in multivariate analyses adjusting for gestational age at sample collection, vitamin B₁₂ intervention, maternal BMI, socioeconomic status, and inflammation. Infants born to women who were anaemic had

significantly lower gestational age at birth (β (SE): -0.95 (0.28); $p = 0.0007$), and 2.67 times greater risk of being born prematurely (<37 weeks; RR: 2.67, 95%CI: 1.43–5.00, $p = 0.002$). Infants born to women who were anaemic had significantly greater risk of being born underweight (WAZ < -2 ; RR: 2.20, 95%CI: 1.16–4.15, $p = 0.02$), lower WAZ scores at birth (β (SE): -0.41 (0.14), $p = 0.004$), and lower mid-upper arm circumference (β (SE): -0.94 (0.45), $p = 0.03$), compared with the mothers who were not anaemic. Similarly, maternal haemoglobin concentrations (Table 3) at the first prenatal visit were associated with significantly greater gestational age at delivery (β (SE): 0.28 (0.08) weeks; $p = 0.001$), and lower risk of preterm delivery (<37 weeks; RR: 0.76, 95%CI: 0.66–86, $p < 0.0001$). Higher maternal haemoglobin concentrations were also associated with higher infant mid-upper arm circumference (β (SE): 0.36 (0.13); $p = 0.006$) in multivariate analyses.

The associations between maternal serum ferritin concentrations (Table 4A–C) and iron deficiency (Table 5) at enrolment and adverse pregnancy and infant outcomes are presented in subsequent tables. Maternal serum ferritin concentrations were associated with increased infant length at birth (β (SE): 0.44 (0.20) cm, $p = 0.03$) in multivariate analyses, including adjusting for gestational age at sample collection, vitamin B₁₂ intervention, maternal BMI, socioeconomic status, and inflammation. Findings were similar after adjusting maternal serum ferritin concentrations for inflammation, considering BRINDA (β (SE): 0.47 (0.20) cm, $p = 0.02$), and Thurnham (β (SE): 0.45 (0.20) cm, $p = 0.02$) methods (Tables 4B, C). Maternal iron deficiency was not significantly associated with risk of adverse pregnancy or infant outcomes, before or after adjusting for inflammation.

The associations between maternal IDA and pregnancy and infant outcomes are presented in Table 6. Women who had IDA at enrolment had infants with significantly lower birth weight (β (SE): -159.7 (67.7) g, $p = 0.02$) and increased risk of low birth weight (RR: 1.99 (1.08–3.68); $p = 0.03$) in multivariate analyses. They also had significantly lower gestational age at delivery (β (SE): -1.16 (0.31) weeks, $p = 0.0002$) and 3.46 times higher risk of preterm delivery (RR: 3.46 (1.81–6.61); $p = 0.0002$). Infants born to women who were iron-deficient anaemic also had significantly lower WAZ (β (SE): -0.39 (0.16), $p = 0.01$), compared with infants born to women without IDA.

The prevalence of elevated inflammatory biomarkers (CRP > 5.0 mg/l: 17%; AGP > 1 g/l: 11%) and anaemia of inflammation (2%) were low in pregnancy (Supplemental tables I–IV). Maternal inflammatory biomarkers during pregnancy, CRP and AGP, were not associated with maternal haematological status or risk of adverse pregnancy or infant outcomes. Maternal hepcidin concentrations during pregnancy were associated with increased infant length (β (SE): 0.31 (0.13), $p = 0.02$), LAZ (β (SE): 0.13 (0.06), $p = 0.04$), WAZ (β (SE): 0.09 (0.05), $p = 0.04$), and mid-upper arm circumference (β (SE): 0.40 (0.15), $p = 0.007$).

Discussion

In this prospective analysis among pregnant women and their infants, anaemia and iron deficiency were common early in gestation (< 14 weeks) and were associated with increased risk of adverse pregnancy and infant outcomes. Maternal anaemia at enrolment predicted a twofold higher risk of infant low birth weight, preterm delivery, and underweight;

similarly, maternal haemoglobin concentrations were associated with higher birth weight and gestational age at delivery, lower risk of preterm birth, and higher infant weight-for-age z-scores. Infants born to women with IDA had a significantly higher risk of preterm birth and low birth weight and had lower WAZ scores.

The prevalence of anaemia and iron deficiency at the first prenatal visit in this study was high: 30% of pregnant women were anaemic (Hb < 11.0 g/dl), 48% were iron deficient (SF < 15.0 µg/l), and 23% of women had IDA (Hb < 11.0 g/dl and SF < 15.0 µg/l); and 51% of infants were anaemic at 6 weeks of age. Findings were similar after adjusting for inflammation, considering Thurnham [21, 22] and BRINDA [23, 24] methods. These findings are consistent with previous studies that reported a high prevalence of anaemia and iron deficiency in pregnant women and young children in India. The National Family Health Survey in India reported 56% of women of reproductive age, 59% of pregnant women, and 70% of young children were anaemic [27]. The burden of anaemia in rural areas is estimated to be higher than in urban settings, ranging from 77% in Maharashtra state to over 90% in Northeast India [28–30]. Previous studies have also noted a high prevalence of iron deficiency and IDA in pregnancy. For example, a multicenter study among pregnant women in India reported 27% of women presented with IDA [31]. The high burden of anaemia and iron deficiency early in pregnancy, and its association with risk of adverse pregnancy and infant outcomes—despite daily iron and folic acid (and vitamin B₁₂) supplementation during pregnancy—is cause for concern. Findings are consistent with the recent Women First Pre-conception Maternal Nutrition Trial [32], and suggest that screening and interventions are urgently needed in women of reproductive age (pre-conceptionally) and early in pregnancy to prevent maternal anaemia and associated adverse pregnancy and infant outcomes.

The prevalence of elevated inflammatory biomarkers (CRP > 5 mg/l: 17%; AGP > 1 g/l: 11%) and anaemia of inflammation (2%) were low in pregnancy and were not significantly associated with risk of adverse pregnancy or infant outcomes. However, pregnant women were excluded from participating in this study if they presented with any known medical complications, including severe anaemia and acute infections which might impair vitamin B₁₂ absorption. Few studies to date have conducted comprehensive investigations of anaemia of inflammation in pregnant women early in gestation, with measurements of nutritional biomarkers for differential diagnosis, particularly in South Asia. In contrast, other studies in sub-Saharan Africa have noted a high prevalence of inflammation and anaemia of inflammation in pregnancy. For example, a cross-sectional study in pregnant women in Uganda found a high prevalence of anaemia of inflammation: over two-thirds of women who were not iron deficient had anaemia of inflammation [33]. Similarly, another study in Malawi found that 73.5% of pregnant women who were not iron deficient had anaemia of inflammation [34]. Inflammation may play an important role in the aetiology of anaemia, particularly in the context of infection or chronic diseases [35–37]. Recent studies suggest that the proportion of anaemia associated with iron deficiency may be lower in settings where the anaemia prevalence is high, and in settings with very high inflammation exposure [7].

In this current study, maternal anaemia and IDA were associated with increased risk of adverse perinatal outcomes. Maternal anaemia at enrolment predicted a twofold higher risk

of preterm delivery, infant low birth weight, and underweight. Infants born to women with IDA also had significantly higher risk of low birth weight and preterm birth and lower WAZ scores. These findings are consistent with previous studies in India and in other settings in low- and middle-income countries. For example, in a prospective cohort study in Assam, India, maternal IDA was associated with significantly increased risk of infant low birth weight and small for gestational age [38]. Similarly, a recent meta-analysis found that pregnant women with anaemia had a significantly higher risk of preterm birth and low birth weight, compared with non-anaemic women [39]. In this review, in low- and middle-income countries, 12% of low birth weight, 19% of preterm births, and 18% of perinatal mortality were attributable to maternal anaemia, highlighting the importance of targeting maternal anaemia to reduce the risk of adverse perinatal outcomes. Lower maternal haemoglobin in the first and third trimesters has also been associated with increased risk of preterm birth and low birth weight [40].

In this study, maternal serum ferritin concentrations were associated with increased infant length at birth, and findings were similar before and after adjusting for inflammation. The observed association of maternal haemoglobin and serum ferritin concentrations early in pregnancy and improved pregnancy and neonatal outcomes are consistent with findings from several research studies [32, 41, 42], including the recent Women First Preconception Maternal Nutrition Trial [32]. In the Women's First trial, a multi-country (India, DRC, Guatemala, Pakistan) randomized clinical trial, lipid-based maternal nutritional supplements administered pre-conception or early in pregnancy (first trimester) improved fetal growth-related birth outcomes (e.g., birth length, LAZ), compared with no supplementation [32]. In the current study, maternal iron deficiency ($SF < 15.0 \mu\text{g/l}$) was not significantly associated with increased risk of adverse pregnancy or infant outcomes, before or after adjusting for inflammation.

The aetiology of anaemia is multifactorial and complex, and includes nutritional and non-nutritional factors [43]. Findings from this study are consistent with previous evidence of the importance of iron deficiency as the leading cause of anaemia [1], as maternal serum ferritin concentrations and iron deficiency were significantly associated with maternal haemoglobin levels ($p < 0.0001$). The risk of iron deficiency is increased in pregnancy due to greater physiological demand for iron to support development of the placenta and fetus, increased maternal RBC mass volume, and requirements for maternal and infant metabolism [42, 44]. The low prevalence of inflammation and anaemia of inflammation in this study, and lack of association with risk of adverse pregnancy or infant outcomes are in contrast to previous studies among pregnant women in sub-Saharan Africa. However, the exclusion of pregnant women with active infections (and chronic conditions which might impair vitamin B₁₂ absorption) in the parent vitamin B₁₂ randomised trial constrained our ability to examine the role of infection and inflammation in the context of anaemia in this setting. This is an important study limitation which limits the generalisability of findings to healthy pregnant women. Findings suggest that a comprehensive assessment of nutritional biomarkers early in pregnancy may help to inform screening, differential diagnosis, and interventions for prevention and treatment of anaemia in pregnant women and their children [43].

There were several other limitations to this study. Although the sample was the same cohort as the parent randomised trial, these observational analyses do not inform causal interpretation of findings. Although maternal biomarkers were available for all women early in pregnancy ($n = 366$), data for pregnancy outcomes ($n = 258$), and infant blood measurements ($n = 73$) were available on a smaller subset of the total sample ($n = 366$). Although this population did not differ in terms of measured sociodemographic and nutritional variables, it is possible that they differ on other unmeasured variables. This analysis included a comprehensive assessment of iron status, including haemoglobin, serum ferritin, C-reactive protein, and AGP. However, the lack of assessment of soluble transferrin receptor and total body iron remains a study limitation.

In summary, in this large cohort of pregnant women participating in a randomised trial of vitamin B₁₂ supplementation in Southern India, maternal anaemia and iron deficiency were common in pregnancy and predicted increased risk of adverse pregnancy and neonatal outcomes. Maternal anaemia at enrolment predicted a two to four times greater risk of low birth weight and preterm delivery. Findings suggest that, although prenatal iron–folic acid supplementation improves haematological status during pregnancy, maternal anaemia and IDA early in pregnancy have an important role in determining the risk of adverse outcomes early in life. Further research is needed to understand the complex aetiology of anaemia in women of reproductive age, to inform screening and interventions to prevent anaemia and improve the health of mothers and their children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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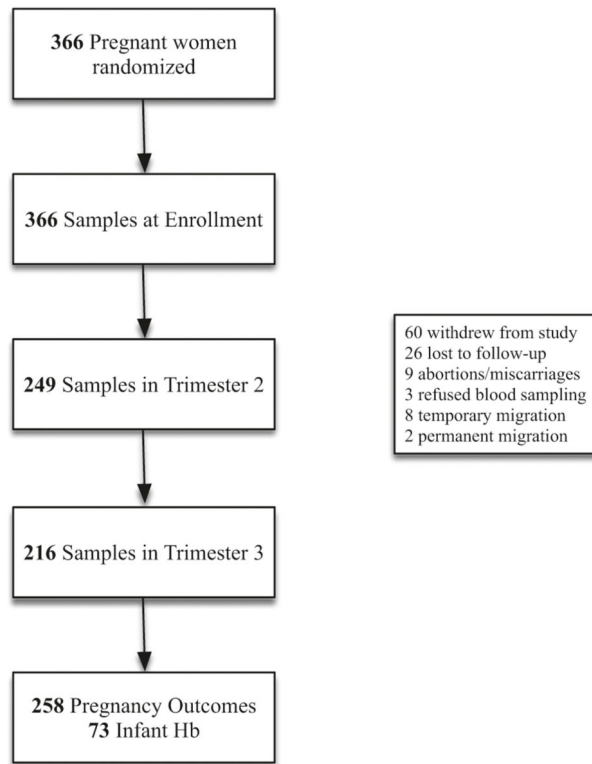


Fig. 1.
Participant flow chart

Table 1Characteristics of the study population^a

Variables	Median (IQR) or <i>n</i> (%)
Maternal characteristics	
Sociodemographic	
Age, y	22 (20, 24)
Monthly household income, INR ^b	6000 (4500, 9000)
<6000 INR, <i>n</i> (%)	161 (44)
Standard of living index	
0–22, <i>n</i> (%)	127 (35)
23–28, <i>n</i> (%)	124 (34)
29–64, <i>n</i> (%)	115 (31)
Gestational age at randomisation, weeks	10.6 (9.1, 12.6)
Parity	
Nulliparous, <i>n</i> (%)	236 (64)
Primiparous or multiparous, <i>n</i> (%)	130 (36)
Anthropometric	
Weight, kg	46.7 (41.6, 53.0)
Height, cm	153 (149, 157)
<150 cm, <i>n</i> (%)	102 (28)
Body mass index, kg/m ²	19.6 (18.1, 22.5)
<18.5 kg/m ² , <i>n</i> (%)	114 (31)
Mid-upper arm circumference, cm	23.0 (21.5, 25.5)
Biochemical, at enrolment ^c	
Haemoglobin, g/dl	11.7 (10.8, 12.6)
Hb <11.0 g/dl	109 (30)
Hb <8.5 g/dl	19 (5)
Hb <7.0 g/dl	4 (1)
Serum ferritin (SF), µg/l	16.2 (7.4, 36.4)
<30.0 µg/l	234 (68)
<15.0 µg/l	166 (48)
<12.0 µg/l	139 (40)
Serum ferritin (BRINDA adjusted), µg/l	12.4 (5.7, 28.3)
<30.0 µg/l	268 (78)
<15.0 µg/l	202 (59)
<12.0 µg/l	166 (48)
Serum ferritin (Thurnham adjusted), µg/l	14.9 (6.7, 33.5)
<30.0 µg/l	241 (70)
<15.0 µg/l	174 (51)
<12.0 µg/l	147 (43)
Iron deficiency anaemia	

Variables	Median (IQR) or <i>n</i> (%)
Anaemia and SF <15.0 µg/l	81 (23)
Anaemia and SF <12.0 µg/l	75 (22)
C-reactive protein (CRP), mg/l	1.7 (0.9, 3.7)
CRP >1.0 mg/l	238 (69)
CRP >5.0 mg/l	59 (17)
Alpha-1-acid glycoprotein (AGP), g/l	0.77 (0.66, 0.90)
AGP >1.0 g/l	39 (11)
CRP >5.0mg/L and AGP >1.0 g/l	21 (6)
Hepcidin, ng/ml	4.6 (1.9, 13.2)
Anaemia of inflammation ^d	6 (2)
Hematocrit, %	35.0 (32.4, 37.4)
Mean corpuscular volume, fl	83 (78, 87)
Plasma vitamin B ₁₂ , pmol/l	149 (110, 204)
<150.0 pmol/l, <i>n</i> (%)	180 (51)
Plasma MMA, µmol/l	0.47 (0.28, 0.67)
>0.26 µmol/l, <i>n</i> (%)	273 (76)
Plasma tHcy, µmol/l	9.23 (5.75, 15.08)
>15.0 µmol/l, <i>n</i> (%)	91 (25)
Erythrocyte folate, nmol/l	387 (291, 496)
<340.0 nmol/l, <i>n</i> (%)	136 (38)
Pregnancy outcomes	
Males, <i>n</i> (%)	122 (47.5)
Birth weight, kg	2.8 (2.5, 3.0)
Low birth weight (<2.5 kg), <i>n</i> (%)	38 (15.1)
Gestational age at birth, weeks	39.4 (38.3, 40.1)
Preterm (<37 weeks), <i>n</i> (%)	33 (12.8)
Preterm and low birth weight, <i>n</i> (%)	15 (6.0)
Small for gestational age ^e , <i>n</i> (%)	85 (33.9)
Infant outcomes	
Haemoglobin, g/dl	10.9 (9.9, 11.7)
Anaemia (<11.0 g/dl), <i>n</i> (%)	37 (50.7)
Length, cm	47.0 (46.0, 49.0)
Length-for-age z-score (LAZ)	-1.3 (-2.2, -0.3)
LAZ < -2	65 (28.9)
Weight-for-age z-score (WAZ)	-1.0 (-1.6, -0.5)
WAZ < -2	30 (12.0)
Weight-for-length z-score (WLZ)	-0.3 (-1.5, 0.8)
WLZ < -2	28 (15.1)
BMI z-score (BMIZ)	-0.4 (-1.6, 0.6)
BMIZ < -2	35 (15.6)
Ponderal Index, g/cm ³	0.027 (0.024, 0.030)

Variables	Median (IQR) or <i>n</i> (%)
Head circumference, cm	33.5 (32.5, 34.5)
Chest circumference, cm	31.5 (30.0, 32.5)
Waist circumference, cm	28.0 (26.0, 30.0)
Hip circumference, cm	28.0 (26.5, 29.7)
Mid-upper arm circumference, cm	9.5 (9.0, 10.5)
Biceps skinfold, mm	4.0 (3.4, 4.8)
Triceps skinfold, mm	5.3 (4.6, 6.2)
Subscapular skinfold, mm	5.2 (4.4, 6.2)

^aValues are median (IQR) and *n* (%); *n* = 366 mothers, *n* = 258 infants

^b100 INR was equivalent to approximately US\$2 at the time the study was conducted

^cEnrolment: 14 weeks gestation

^dAnaemia of inflammation was defined as anaemia (Hb < 11.0 g/dl) + serum ferritin > 15.0 µg/l + (either CRP > 5.0 mg/l or AGP > 1.0 g/l)

^eSmall for gestational age (SGA) was defined as birth weight < 10th percentile of the gestational age, using INTERGROWTH reference [17]

Table 2

Associations between maternal anaemia at enrolment and pregnancy and infant outcomes

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Pregnancy outcomes						
Birth weight, g	252	-174.7 (61.2)	0.004	243	-166.8 (61.1)	0.006
Low birth weight (<2500 g), n (%)	252	1.84 (1.03, 3.29)	0.04	243	2.15 (1.20, 3.84)	0.01
Gestational age at birth, weeks	258	-0.91 (0.28)	0.001	249	-0.95 (0.28)	0.0007
Preterm (<37 weeks), n (%)	258	2.53 (1.35, 4.74)	0.004	249	2.67 (1.43, 5.00)	0.002
Preterm and low birth weight, n (%)	252	2.01 (0.75, 5.39)	0.17	243	2.15 (0.80, 5.78)	0.13
Small for gestational age ^d , n (%)	251	1.24 (0.87, 1.76)	0.23	242	1.25 (0.87, 1.81)	0.23
Infant outcomes						
Haemoglobin, g/dl	73	0.14 (0.44)	0.76	69	-0.11 (0.44)	0.80
Anaemia (<11.0 g/dl), n (%)	73	0.83 (0.49, 1.40)	0.49	69	1.02 (0.57, 1.83)	0.95
Length, cm	227	-0.41 (0.41)	0.31	218	-0.35 (0.41)	0.39
Length-for-age z-score (LAZ)	225	-0.08 (0.20)	0.67	216	-0.03 (0.20)	0.89
LAZ < -2	225	0.96 (0.62, 1.50)	0.87	216	0.85 (0.54, 1.33)	0.47
Weight-for-age z-score (WAZ)	251	-0.41 (0.14)	0.004	242	-0.41 (0.14)	0.004
WAZ < -2	251	1.99 (1.03, 3.87)	0.04	242	2.20 (1.16, 4.15)	0.02
Weight-for-length z-score (WLZ)	185	-0.45 (0.27)	0.09	178	-0.40 (0.27)	0.15
WLZ < -2	185	1.52 (0.78, 2.97)	0.22	178	1.50 (0.76, 2.95)	0.25
BMI z-score (BMIZ)	224	-0.48 (0.22)	0.03	215	-0.45 (0.22)	0.04
BMIZ < -2	224	2.42 (1.34, 4.39)	0.004	215	2.49 (1.37, 4.56)	0.003
Ponderal index, g/cm ³	226	-0.001 (0.0007)	0.17	217	-0.001 (0.0007)	0.18
Head circumference, cm	227	-0.27 (0.24)	0.24	218	-0.021 (0.24)	0.38
Chest circumference, cm	227	-0.56 (0.29)	0.05	218	-0.61 (0.30)	0.04
Waist circumference, cm	227	-0.61 (0.40)	0.13	218	-0.61 (0.41)	0.13
Hip circumference, cm	227	0.03 (0.58)	0.96	218	-0.06 (0.57)	0.91
Mid-upper arm circumference, cm	225	-0.94 (0.46)	0.04	216	-0.94 (0.45)	0.03
Biceps skinfold, mm	226	-0.07 (0.14)	0.65	217	-0.09 (0.15)	0.54

Variables ^a	Model 1 ^b		Model 2 ^c	
	<i>n</i>	β (SE) or RR (95%CI) P-value	<i>n</i>	β (SE) or RR (95%CI) P-value
Triceps skinfold, mm	226	0.06 (0.22) 0.80	217	0.03 (0.22) 0.90

^aHb < 11.0 g/dl at enrolment (< 14 weeks gestation); beta (SE) is reported for continuous pregnancy outcomes; RR (95%CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined as birth weight <10th percentile of the gestational age, using INTERGROWTH reference [17]

Table 3 Associations between maternal haemoglobin concentrations at enrolment and pregnancy and infant outcomes

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95% CI)	P-value	n	β (SE) or RR (95% CI)	P-value
Pregnancy outcomes						
Birth weight, g	252	37.4 (18.2)	0.04	243	35.0 (18.2)	0.05
Low birth weight (<2500 g), n (%)	252	0.91 (0.77, 1.07)	0.27	243	0.87 (0.73, 1.04)	0.13
Gestational age at birth, weeks	258	0.27 (0.08)	0.001	249	0.28 (0.08)	0.001
Preterm (<37 weeks), n (%)	258	0.76 (0.67, 0.87)	<0.0001	249	0.76 (0.66, 0.86)	<0.0001
Preterm and low birth weight, n (%)	252	0.90 (0.68, 1.20)	0.48	243	0.86 (0.64, 1.15)	0.30
Small for gestational age ^d , n (%)	251	0.98 (0.88, 1.09)	0.72	242	0.98 (0.88, 1.09)	0.67
Infant outcomes						
Haemoglobin, g/dl	73	0.14 (0.14)	0.33	69	0.22 (0.14)	0.13
Anaemia (<11.0 g/dl), n (%)	73	1.02 (0.86, 1.22)	0.81	69	0.94 (0.78, 1.13)	0.52
Length, cm	227	0.14 (0.12)	0.27	218	0.10 (0.12)	0.42
Length-for-age z-score (LAZ)	225	0.04 (0.06)	0.47	216	0.01 (0.06)	0.81
LAZ < -2	225	0.98 (0.87, 1.11)	0.77	216	1.01 (0.90, 1.14)	0.84
Weight-for-age z-score (WAZ)	251	0.08 (0.04)	0.05	242	0.08 (0.04)	0.06
WAZ < -2	251	0.89 (0.74, 1.07)	0.22	242	0.82 (0.67, 1.01)	0.06
Weight-for-length z-score (WLZ)	185	0.07 (0.08)	0.38	178	0.07 (0.08)	0.40
WLZ < -2	185	1.02 (0.83, 1.25)	0.87	178	1.03 (0.85, 1.25)	0.78
BMI z-score (BMIZ)	224	0.09 (0.07)	0.16	215	0.09 (0.07)	0.19
BMIZ < -2	224	0.90 (0.76, 1.05)	0.18	215	0.88 (0.74, 1.05)	0.16
Ponderal Index, g/cm ³	226	0.0002 (0.0002)	0.46	217	0.0002 (0.0002)	0.44
Head circumference, cm	227	0.08 (0.07)	0.28	218	0.06 (0.07)	0.38
Chest circumference, cm	227	0.14 (0.09)	0.11	218	0.14 (0.09)	0.11
Waist circumference, cm	227	0.18 (0.12)	0.13	218	0.19 (0.12)	0.11
Hip circumference, cm	227	-0.06 (0.17)	0.72	218	-0.04 (0.17)	0.82
Mid-upper arm circumference, cm	225	0.36 (0.14)	0.008	216	0.36 (0.13)	0.006
Biceps skinfold, mm	226	0.04 (0.04)	0.32	217	0.05 (0.04)	0.26

Variables ^a	Model 1 ^b		Model 2 ^c	
	n	β (SE) or RR (95% CI) P-value	n	β (SE) or RR (95% CI) P-value
Triceps skinfold, mm	226	0.05 (0.06)	217	0.06 (0.07)
				0.35

^aHb, g/dl enrolment (14 weeks gestation); beta (SE) is reported for continuous pregnancy outcomes; RR (95%CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined as birth weight < 10th percentile of the gestational age, using INTERGROWTH reference [17]

Table 4A Associations between maternal serum ferritin concentrations at enrolment and pregnancy and infant outcomes

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Pregnancy outcomes						
Birth weight, g	244	34.0 (30.5)	0.26	242	31.6 (30.1)	0.29
Low birth weight (<2500 g), n (%)	244	0.97 (0.70, 1.33)	0.83	242	0.96 (0.70, 1.32)	0.80
Gestational age at birth, weeks	250	0.14 (0.14)	0.32	248	0.17 (0.14)	0.22
Preterm (<37 weeks), n (%)	250	0.80 (0.57, 1.13)	0.21	248	0.77 (0.55, 1.09)	0.14
Preterm and low birth weight, n (%)	244	1.06 (0.63, 1.77)	0.83	242	1.02 (0.61, 1.71)	0.93
Small for gestational age ^d , n (%)	243	0.94 (0.77, 1.14)	0.54	241	0.93 (0.76, 1.14)	0.49
Infant outcomes						
Haemoglobin, g/dl	68	0.18 (0.23)	0.42	68	0.25 (0.23)	0.28
Anaemia (<11.0 g/dl), n (%)	68	0.81 (0.61, 1.07)	0.13	68	0.82 (0.63, 1.08)	0.17
Length, cm	219	0.46 (0.20)	0.02	217	0.44 (0.20)	0.03
Length-for-age z-score (LAZ)	217	0.19 (0.10)	0.048	215	0.16 (0.10)	0.09
LAZ < -2	217	0.83 (0.67, 1.03)	0.09	215	0.86 (0.69, 1.08)	0.20
Weight-for-age z-score (WAZ)	243	0.07 (0.07)	0.35	241	0.06 (0.07)	0.36
WAZ < -2	243	0.92 (0.64, 1.33)	0.66	241	0.90 (0.63, 1.30)	0.58
Weight-for-length z-score (WLZ)	179	-0.10 (0.14)	0.46	177	-0.11 (0.14)	0.44
WLZ < -2	179	1.00 (0.69, 1.43)	0.98	177	1.03 (0.70, 1.50)	0.90
BMI z-score (BMIZ)	216	-0.13 (0.11)	0.25	214	-0.13 (0.11)	0.23
BMIZ < -2	216	1.05 (0.76, 1.44)	0.77	214	1.07 (0.78, 1.47)	0.68
Ponderal Index, g/cm ³	218	-0.0006 (0.0004)	0.10	216	-0.0006 (0.0004)	0.09
Head circumference, cm	219	-0.03 (0.12)	0.78	217	-0.05 (0.12)	0.69
Chest circumference, cm	219	-0.03 (0.14)	0.85	217	-0.06 (0.15)	0.69
Waist circumference, cm	219	-0.06 (0.20)	0.75	217	-0.08 (0.20)	0.68
Hip circumference, cm	219	-0.16 (0.27)	0.55	217	-0.20 (0.28)	0.46
Mid-upper arm circumference, cm	217	0.37 (0.21)	0.08	215	0.38 (0.22)	0.08
Biceps skinfold, mm	218	0.05 (0.07)	0.45	216	0.05 (0.07)	0.48

Variables ^a	Model 1 ^b		Model 2 ^c			
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Triceps skinfold, mm	218	-0.14 (0.10)	0.16	216	-0.15 (0.11)	0.15

^aSerum ferritin, natural log-transformed at enrolment (14 weeks gestation); beta (SE) is reported for continuous pregnancy outcomes; RR (95% CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined as birth weight < 10th percentile of the gestational age, using INTERGROWTH reference [17]

Table 4B

Associations between maternal serum ferritin concentrations at enrolment (BRINDA adjusted)^a and pregnancy and infant outcomes

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Pregnancy outcomes						
Birth weight, g	244	35.0 (30.9)	0.26	242	35.0 (30.4)	0.25
Low birth weight (<2500 g), n (%)	244	0.96 (0.69, 1.33)	0.80	242	0.96 (0.69, 1.33)	0.81
Gestational age at birth, weeks	250	0.11 (0.14)	0.44	248	0.12 (0.14)	0.38
Preterm (<37 weeks), n (%)	250	0.84 (0.60, 1.18)	0.32	248	0.82 (0.58, 1.15)	0.25
Preterm and low birth weight, n (%)	244	1.06 (0.63, 1.78)	0.84	242	1.03 (0.61, 1.74)	0.92
Small for gestational age ^d , n (%)	243	0.92 (0.76, 1.12)	0.42	241	0.90 (0.74, 1.10)	0.32
Infant outcomes						
Haemoglobin, g/dl	68	0.29 (0.23)	0.22	68	0.35 (0.23)	0.13
Anaemia (<11.0 g/dl), n (%)	68	0.78 (0.59, 1.04)	0.10	68	0.78 (0.59, 1.03)	0.08
Length, cm	219	0.49 (0.20)	0.01	217	0.47 (0.20)	0.02
Length-for-age z-score (LAZ)	217	0.19 (0.10)	0.05	215	0.17 (0.10)	0.08
LAZ < -2	217	0.84 (0.68, 1.05)	0.12	215	0.86 (0.69, 1.08)	0.20
Weight-for-age z-score (WAZ)	243	0.07 (0.07)	0.31	241	0.07 (0.07)	0.30
WAZ < -2	243	0.89 (0.61, 1.29)	0.54	241	0.89 (0.61, 1.29)	0.55
Weight-for-length z-score (WLZ)	179	-0.12 (0.14)	0.40	177	-0.11 (0.14)	0.44
WLZ < -2	179	1.00 (0.69, 1.45)	1.00	177	1.04 (0.71, 1.52)	0.84
BMI z-score (BMIZ)	216	-0.15 (0.11)	0.19	214	-0.14 (0.11)	0.20
BMIZ < -2	216	1.04 (0.75, 1.44)	0.81	214	1.05 (0.76, 1.45)	0.76
Ponderal Index, g/cm ³	218	-0.0007 (0.0004)	0.07	216	-0.0006 (0.0004)	0.07
Head circumference, cm	219	-0.04 (0.12)	0.71	217	-0.05 (0.12)	0.66
Chest circumference, cm	219	-0.06 (0.15)	0.69	217	-0.07 (0.15)	0.63
Waist circumference, cm	219	-0.10 (0.20)	0.62	217	-0.08 (0.20)	0.67
Hip circumference, cm	219	-0.22 (0.28)	0.42	217	-0.26 (0.28)	0.36
Mid-upper arm circumference, cm	217	0.38 (0.22)	0.08	215	0.42 (0.22)	0.05
Biceps skinfold, mm	218	0.06 (0.07)	0.38	216	0.06 (0.07)	0.40

Variables ^a	Model 1 ^b		Model 2 ^c	
	<i>n</i>	β (SE) or RR (95%CI)	<i>n</i>	β (SE) or RR (95%CI)
Triceps skinfold, mm	218	-0.14 (0.11)	216	-0.14 (0.11)
		0.20		0.18

^aBaseline serum ferritin concentrations were adjusted for inflammation using BRINDA [23, 24] methods; natural log-transformed; beta (SE) is reported for continuous pregnancy outcomes; RR (95%CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined by sex and gestational age < 10th percentile using INTERGROWTH methods [17]

Associations between maternal serum ferritin concentrations at enrolment (Thurnham adjusted) and pregnancy and infant outcomes

Table 4C

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Pregnancy outcomes						
Birth weight, g	242	32.1 (30.5)	0.29	242	32.3 (29.9)	0.28
Low birth weight (<2500 g), n (%)	242	0.95 (0.69, 1.31)	0.74	242	0.95 (0.69, 1.32)	0.77
Gestational age at birth, weeks	248	0.14 (0.14)	0.30	248	0.15 (0.14)	0.28
Preterm (<37 weeks), n (%)	248	0.83 (0.59, 1.16)	0.27	248	0.81 (0.57, 1.14)	0.22
Preterm and low birth weight, n (%)	242	1.04 (0.63, 1.74)	0.87	242	1.03 (0.61, 1.71)	0.92
Small for gestational age ^d , n (%)	241	0.93 (0.76, 1.12)	0.43	241	0.91 (0.74, 1.11)	0.35
Infant outcomes						
Haemoglobin, g/dl	68	0.28 (0.23)	0.23	68	0.33 (0.23)	0.16
Anaemia (<11.0 g/dl), n (%)	68	0.77 (0.57, 1.03)	0.07	68	0.79 (0.60, 1.04)	0.09
Length, cm	217	0.47 (0.20)	0.02	217	0.45 (0.20)	0.02
Length-for-age z-score (LAZ)	215	0.17 (0.10)	0.07	215	0.16 (0.10)	0.10
LAZ < -2	215	0.85 (0.69, 1.06)	0.14	215	0.87 (0.69, 1.09)	0.22
Weight-for-age z-score (WAZ)	241	0.07 (0.07)	0.34	241	0.07 (0.07)	0.33
WAZ < -2	241	0.89 (0.61, 1.28)	0.52	241	0.89 (0.62, 1.29)	0.55
Weight-for-length z-score (WLZ)	177	-0.13 (0.14)	0.35	177	-0.12 (0.14)	0.37
WLZ < -2	177	1.02 (0.71, 1.48)	0.90	177	1.06 (0.74, 1.53)	0.74
BMI z-score (BMIZ)	214	-0.15 (0.11)	0.18	214	-0.14 (0.11)	0.18
BMIZ < -2	214	1.07 (0.78, 1.47)	0.67	214	1.09 (0.80, 1.50)	0.58
Ponderal Index, g/cm ³	216	-0.0007 (0.0004)	0.06	216	-0.0007 (0.0004)	0.06
Head circumference, cm	217	-0.05 (0.12)	0.70	217	-0.05 (0.12)	0.68
Chest circumference, cm	217	-0.05 (0.14)	0.73	217	-0.06 (0.14)	0.70
Waist circumference, cm	217	-0.07 (0.20)	0.72	217	-0.05 (0.20)	0.78
Hip circumference, cm	217	-0.18 (0.27)	0.52	217	-0.20 (0.27)	0.46
Mid-upper arm circumference, cm	215	0.33 (0.22)	0.13	215	0.36 (0.22)	0.09
Biceps skinfold, mm	216	0.04 (0.07)	0.57	216	0.04 (0.07)	0.60

Variables ^a	Model 1 ^b		Model 2 ^c	
	<i>n</i>	β (SE) or RR (95%CI) <i>P</i> -value	<i>n</i>	β (SE) or RR (95%CI) <i>P</i> -value
Triceps skinfold, mm	216	-0.15 (0.11)	216	-0.15 (0.11)
		0.16		0.15

^aBaseline serum ferritin concentrations were adjusted for inflammation using Thurnham [21, 22] methods; natural log-transformed; beta (SE) is reported for continuous pregnancy outcomes; RR (95%CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined by sex and gestational age <10th percentile using INTERGROWTH methods [17]

Table 5
Associations between maternal iron deficiency at enrolment and pregnancy and infant outcomes

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Pregnancy outcomes						
Birth weight, g	244	-54.3 (58.6)	0.35	242	-45.4 (57.9)	0.43
Low birth weight (<2500 g), n (%)	244	1.38 (0.76, 2.51)	0.30	242	1.36 (0.75, 2.46)	0.31
Gestational age at birth, weeks	250	-0.29 (0.26)	0.28	248	-0.35 (0.26)	0.18
Preterm (<37 weeks), n (%)	250	1.61 (0.84, 3.09)	0.15	248	1.75 (0.91, 3.37)	0.09
Preterm and low birth weight, n (%)	244	1.19 (0.44, 3.16)	0.73	242	1.26 (0.47, 3.35)	0.64
Small for gestational age ^d , n (%)	243	0.96 (0.67, 1.38)	0.84	241	0.97 (0.68, 1.38)	0.85
Infant outcomes						
Haemoglobin, g/dl	68	-0.41 (0.44)	0.36	68	-0.64 (0.44)	0.15
Anaemia (<11.0 g/dl), n (%)	68	1.17 (0.70, 1.95)	0.54	68	1.20 (0.72, 1.98)	0.48
Length, cm	219	-0.77 (0.39)	0.048	217	-0.71 (0.39)	0.07
Length-for-age z-score (LAZ)	217	-0.29 (0.19)	0.12	215	-0.25 (0.19)	0.18
LAZ < -2	217	1.45 (0.94, 2.23)	0.09	215	1.41 (0.92, 2.18)	0.11
Weight-for-age z-score (WAZ)	243	-0.12 (0.14)	0.38	241	-0.10 (0.13)	0.46
WAZ < -2	243	1.49 (0.74, 2.98)	0.26	241	1.46 (0.74, 2.88)	0.27
Weight-for-length z-score (WLZ)	179	0.07 (0.26)	0.79	177	0.10 (0.26)	0.69
WLZ < -2	179	1.30 (0.66, 2.53)	0.45	177	1.19 (0.62, 2.31)	0.60
BMI z-score (BMIZ)	216	0.21 (0.21)	0.33	214	0.25 (0.21)	0.24
BMIZ < -2	216	0.99 (0.54, 1.83)	0.98	214	0.90 (0.50, 1.65)	0.74
Ponderal Index, g/cm ³	218	0.001 (0.0007)	0.11	216	0.001 (0.0007)	0.09
Head circumference, cm	219	-0.03 (0.23)	0.89	217	0.01 (0.23)	0.97
Chest circumference, cm	219	0.18 (0.28)	0.52	217	0.23 (0.28)	0.41
Waist circumference, cm	219	0.13 (0.39)	0.73	217	0.10 (0.39)	0.79
Hip circumference, cm	219	0.33 (0.53)	0.54	217	0.41 (0.54)	0.45
Mid-upper arm circumference, cm	217	-0.29 (0.42)	0.50	215	-0.32 (0.42)	0.45
Biceps skinfold, mm	218	0.12 (0.14)	0.39	216	0.13 (0.14)	0.34

Variables ^a	Model 1 ^b		Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	β (SE) or RR (95%CI)	P-value
Triceps skinfold, mm	218	0.56 (0.20)	0.01	0.59 (0.20)	0.004

^aSerum ferritin < 15.0 $\mu\text{g/l}$ at enrolment (14 weeks gestation); beta (SE) is reported for continuous pregnancy outcomes; RR (95%CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined as birth weight <10th percentile of the gestational age, using INTERGROWTH reference [17]

Table 6 Associations between maternal iron deficiency anaemia at enrolment and pregnancy and infant outcomes

Variables ^a	Model 1 ^b			Model 2 ^c		
	n	β (SE) or RR (95%CI)	P-value	n	β (SE) or RR (95%CI)	P-value
Pregnancy outcomes						
Birth weight, g	244	-169.7 (67.9)	0.01	243	-159.7 (67.7)	0.02
Low birth weight (<2500 g), n (%)	244	1.77 (0.96, 3.27)	0.07	243	1.99 (1.08, 3.68)	0.03
Gestational age at birth, weeks	250	-1.02 (0.31)	0.001	249	-1.16 (0.31)	0.0002
Preterm (<37 weeks), n (%)	250	2.90 (1.56, 5.39)	0.001	249	3.46 (1.81, 6.61)	0.0002
Preterm and low birth weight, n (%)	244	2.20 (0.79, 6.10)	0.13	243	2.43 (0.88, 6.71)	0.09
Small for gestational age ^d , n (%)	243	1.16 (0.79, 1.73)	0.45	242	1.18 (0.80, 1.76)	0.40
Infant outcomes						
Haemoglobin, g/dl	68	-0.35 (0.48)	0.47	69	-0.49 (0.47)	0.30
Anaemia (<11.0 g/dl), n (%)	68	1.05 (0.61, 1.81)	0.87	69	1.25 (0.79, 2.00)	0.34
Length, cm	219	-0.87 (0.44)	0.05	218	-0.86 (0.45)	0.06
Length-for-age z-score (LAZ)	217	-0.29 (0.22)	0.18	216	-0.25 (0.22)	0.25
LAZ < -2	217	1.28 (0.82, 2.00)	0.28	216	1.17 (0.74, 1.83)	0.51
Weight-for-age z-score (WAZ)	243	-0.40 (0.16)	0.01	242	-0.39 (0.16)	0.01
WAZ < -2	243	1.73 (0.85, 3.53)	0.13	242	1.88 (0.95, 3.70)	0.07
Weight-for-length z-score (WLZ)	179	-0.16 (0.31)	0.61	178	-0.08 (0.31)	0.80
WLZ < -2	179	1.15 (0.53, 2.49)	0.72	178	1.10 (0.50, 2.44)	0.81
BMI z-score (BMIZ)	216	-0.27 (0.25)	0.27	215	-0.21 (0.25)	0.40
BMIZ < -2	216	1.23 (0.63, 2.39)	0.54	215	1.18 (0.61, 2.30)	0.62
Ponderal Index, g/cm ³	218	-0.0002 (0.0008)	0.78	217	-0.0001 (0.0008)	0.93
Head circumference, cm	219	-0.31 (0.26)	0.24	218	-0.26 (0.27)	0.33
Chest circumference, cm	219	-0.39 (0.32)	0.23	218	-0.32 (0.33)	0.33
Waist circumference, cm	219	-0.16 (0.44)	0.73	218	-0.11 (0.45)	0.80
Hip circumference, cm	219	0.02 (0.61)	0.97	218	0.12 (0.62)	0.84
Mid-upper arm circumference, cm	217	-0.82 (0.48)	0.09	216	-0.88 (0.49)	0.07
Biceps skinfold, mm	218	0.01 (0.16)	0.94	217	0.01 (0.16)	0.97

Variables ^a	Model 1 ^b		Model 2 ^c	
	β (SE) or RR (95%CI)	P-value	β (SE) or RR (95%CI)	P-value
Triceps skinfold, mm	218 0.32 (0.23)	0.17	217 0.34 (0.24)	0.15

^aHb < 11.0 g/dl and serum ferritin < 15.0 µg/l at enrolment (14 weeks gestation); beta (SE) is reported for continuous pregnancy outcomes; RR (95%CI) is reported for binary pregnancy outcomes

^bModels adjusted for gestational age of sample and vitamin B12 intervention

^cModels adjusted for gestational age of sample, vitamin B12 intervention, maternal BMI, standard of living index (SLI) >28, educational level, and ln(CRP)

^dSmall for gestational age (SGA) was defined as birth weight <10th percentile of the gestational age, using INTERGROWTH reference [17]