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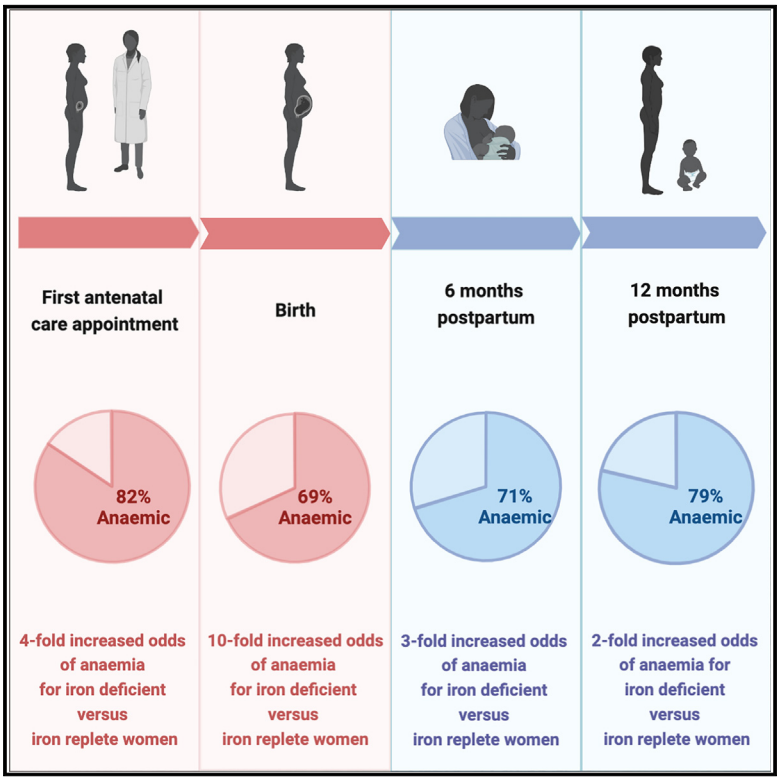
Quantifying differences in iron deficiency-attributable anemia during pregnancy and postpartum

Graphical abstract



Highlights

- Pregnant women in Papua New Guinea are at high risk of anemia and iron deficiency
- High anemia prevalence from the first antenatal care visit to 12 months postpartum
- Iron deficiency is the main cause of anemia in pregnancy but less so postpartum

Authors

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In brief

Davidson et al. report that anemia is highly prevalent in pregnant Papua New Guinean women and during the first 12 months postpartum. Iron deficiency is the main contributor to anemia in pregnancy but less so postpartum. Iron supplementation early during and between pregnancies could alleviate anemia in women of reproductive age.

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Quantifying differences in iron deficiency-attributable anemia during pregnancy and postpartum

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SUMMARY

Pregnant women in resource-limited settings are highly susceptible to anemia and iron deficiency, but the etiology of postpartum anemia remains poorly defined. To inform the optimal timing for anemia interventions, changes in iron deficiency-attributable anemia through pregnancy and postpartum need to be understood. In 699 pregnant Papua New Guinean women attending their first antenatal care appointment and following up at birth and 6 and 12 months postpartum, we undertake logistic mixed-effects modeling to determine the effect of iron deficiency on anemia and population attributable fractions, calculated from odds ratios, to quantify the contribution of iron deficiency to anemia. Anemia is highly prevalent during pregnancy and 12 months postpartum, with iron deficiency increasing the odds of anemia during pregnancy and, to a lesser extent, postpartum. Iron deficiency accounts for $\geq$72% of anemia during pregnancy and 20%–37% postpartum. Early iron supplementation during and between pregnancies could break the cycle of chronic anemia in women of reproductive age.

INTRODUCTION

Anemia in pregnancy is a major global public health problem, particularly in resource-limited regions, where every second pregnant woman is estimated to be anemic.[1,2] Anemia in pregnancy contributes significantly to maternal morbidity and mortality and increases the risk of adverse neonatal outcomes.[3–6] Consequently, reducing anemia by 50% in women of reproductive age is the second goal of the World Health Organization's (WHO) "Global Nutrition Targets for 2025."[7]

Approximately half of all anemia cases in pregnancy worldwide are attributed to iron deficiency.[2] Pregnant women have an increased susceptibility to iron deficiency due to the high iron requirements of pregnancy.[8,9] Whether women remain susceptible in the postpartum period, and for how long, is unknown. In high-income settings, the postpartum period is typically considered a time of low iron deficiency risk, as the iron stores of healthy women who take iron supplements typically return to prepregnancy levels within weeks of birth.[10,11] However, it remains unclear how hemoglobin and iron levels change from pregnancy through to the postpartum period in settings with a high burden of infections and under-nutrition.[12] Continued anemia postpartum consigns women to poor health and increases the likelihood of entering subsequent pregnancies already anemic.[13,14] Understanding hemoglobin and iron level changes

in pregnancy and into the postpartum period will inform when anemia interventions will be most effective.

The WHO's principal recommendation for the prevention of maternal anemia is universal oral iron and folate supplementation throughout pregnancy.[15] This is a widely implemented recommendation that is supported by a large Cochrane Review of iron supplementation trials in pregnancy.[16] Postpartum, the WHO recommends iron supplementation for the first 6–12 weeks in settings where anemia is a moderate or severe public health problem (population prevalence $\geq$ 20%).[12] Despite this, iron supplementation is not considered part of routine postpartum care.[12] To further understand the benefit of extending iron supplementation into the postpartum period, there is an urgent need for longer-term information on the prevalence of anemia and iron deficiency in the first year postpartum.

Furthermore, anemia has a complex etiology. In addition to iron deficiency, there are other important causes including micronutrient deficiencies (vitamins A and B12), genetic conditions (e.g., thalassemia), and infectious diseases.[17–19] In malaria endemic settings, *Plasmodium* spp. (species) infection is a major determinant of anemia in pregnancy,[19] with key prevention strategies including intermittent preventative treatment in pregnancy and use of insecticide-treated bed nets.[20] Thus, in settings with more than one cause of anemia, iron supplementation may not be enough to reduce the burden of anemia. Determining the relative contributions of these risk factors to anemia, during pregnancy and postpartum periods, is important for the planning and implementation of anemia prevention strategies.

To better understand anemia in pregnancy and postpartum and to inform anemia prevention strategies, we determined the prevalence of anemia and iron deficiency during pregnancy and the first 12 months postpartum in a prospective cohort of women in Papua New Guinea and quantified the time-varying effect of iron deficiency on hemoglobin and anemia in pregnancy and postpartum.

RESULTS

Cohort characteristics

In a cohort of 699 pregnant women recruited at first antenatal visit (Table 1), the median age was 26 years (interquartile range [IQR]: 22–30), the median gestation age was 30 weeks (IQR: 28–32 weeks), and 75% (522/699) of women were multigravida. Genetic polymorphisms were common: 94% (637/680) had a low or intermediate complement receptor 1 (CR1) expression genotype, 16% (109/673) had α^{+}-thalassemia, and 5% (36/681) had Southeast Asian ovalocytosis (SAO). Bed net use was moderate (63%, 440/698), and *Plasmodium* spp. infection detected by PCR was 12% (73/601); 42.5% (31/73) of infections were *P. falciparum*, 48% (35/73) were *Plasmodium vivax*, and 9.5% (7/73) were mixed infections (Table 1). Mean hemoglobin level at enrollment was 96.3 g/L (standard deviation [SD]: 14.5), with 82% (483/587) anemic (hemoglobin < 110 g/L). Iron deficiency (ferritin < 15 μg/L) was also highly prevalent in 81% (448/552). Enrollment sociodemographic, clinical, and diagnostic measures were similar for women who returned at birth (n = 638), 6 months postpartum (n = 552), and 12 months postpartum (n = 365) and in those who were lost to follow-up by 12 months postpartum (n = 334) (Table S1). Non-participation at each stage was due to loss-to-follow-up, relocation out of the province, or withdrawal (Figure S1).

At birth, women were asked how often they took antenatal iron folic acid supplements during their pregnancy; 65% (348/534) responded "most days"; 16% (87/534) responded "twice per week"; 7% (37/534) responded "a few times per month"; 9% (46/534) responded "only a few times during their entire pregnancy"; and 3% (16/534) "stopped taking it." Most of these women (93%, 316/343) started taking iron supplements at their first antenatal care appointment. At 6 and 12 months postpartum, 1.4% (8/550) and 1.7% (6/360) of women reported that they were taking iron folate supplements. Women were also asked about any other medication used in pregnancy; 54% (342/635) recalled taking antimalarials at least once. No women reported taking multiple micronutrient supplementation during pregnancy or postpartum.

Hemoglobin and ferritin dynamics, and burden of anemia and iron deficiency in pregnancy and postpartum

At the population level, mean hemoglobin concentration remained stable during pregnancy (adjusted mean difference of −0.44 g/L; 95% confidence interval [CI]: −2.37, 1.49; p = 0.65 at birth compared with enrollment; Figure 2; Tables S2 and S3) and then increased in the postpartum period (adjusted mean difference of 11.63 g/L; 95% CI: 9.62, 13.65; p < 0.001 and 9.63 g/L; 95% CI: 7.38, 11.87; p < 0.001 at 6 and 12 months postpartum, respectively, compared with birth). At the individual level, hemoglobin concentration showed substantial variation over time (within-woman SD = 13.02 g/L); compared with at birth, hemoglobin levels were lower at 6 months postpartum in 25% of women, higher in 74%, and did not change in 1%. The population mean ferritin concentration in pregnancy was dynamic, with a 2.3-fold increase in the geometric mean ferritin from enrollment to birth (95% CI: 2.11, 2.50; p < 0.001) and a further increase by 6 months postpartum (1.22-fold increase in adjusted geometric mean compared with birth; 95% CI: 1.12, 1.34; p < 0.001) before stabilizing (0.93-fold increase in geometric mean at 12 months compared with 6 months; 95% CI: 0.84, 1.03; p = 0.14).

Anemia (hemoglobin < 110 g/L) was highly prevalent throughout pregnancy, with 82% (483/587) anemic at enrollment and 69% (372/537) at birth (Figure 3A). The majority of this anemia was moderate-to- severe (hemoglobin < 100 g/L) at enrollment and birth (~70%) (Figure 3B). Postpartum, anemia (hemoglobin < 120 g/L) prevalence remained high, with >70% of women anemic at 6 (375/531) and 12 months postpartum (281/356). The proportion of moderate-to-severe anemia (hemoglobin < 110 g/L) was lower postpartum, at 57% (212/375) and 63% (176/281) for 6 and 12 months postpartum, respectively. Iron deficiency (ferritin < 15 μg/L) was also highly prevalent in pregnancy (enrollment: 81%, 448/552; birth: 87%, 171/196), but prevalence declined to 26% (104/398) by 6 months postpartum and 31% (80/257) by 12 months postpartum. Similar declines were observed for iron-deficiency anemia, decreasing from 71% (325/455) at enrollment down to 27% (67/247) by 12 months postpartum.

Table 1. Cohort characteristics at enrollment

Sociodemographic details		n/N (%)[a]
Enrollment clinic	Vunapope	184/699 (26.3)
	Nonga	83/699 (11.9)
	Keravat	125/699 (17.9)
	Napapar	158/699 (22.6)
	Paparatava	149/699 (21.3)
Age (years)	median (IQR), range	26 (22–30), 16–49
Gravidity	primigravida	177/699 (25.3)
	multigravida	522/699 (74.7)
Highest level of education	primary or less	325/698 (46.6)
Employment status	not employed	531/699 (76.0)
Smoking status	never smoked	427/697 (61.3)
	current/past smoker	270/697 (38.7)
Bed net use	owns bed net	527/698 (75.5)
	net used last night	440/698 (63.0)
Clinical measures		
Gestational age (weeks)[b]	median (IQR), range	30 (28–32), 26–40
MUAC (cm)	mean (SD), range	26 (3), 13.5–43.7
	MUAC > 23 cm	592/692 (84.7)
	MUAC ≤ 23 cm	101/692 (14.4)
Body mass index (kg/m^2)	median (IQR), range	25.3 (3.2), 17·9–34·3
Fever[c]	yes	91/687 (13.2)
Diagnostic measures		
Hemoglobin level (g/L)	mean (SD), range	96.3 (14.5), 41–145
Anemia status	anemic[d]	483/587 (82.3)
Ferritin level (μg/L)	median (IQR), range	9.2 (5.3, 18.6), 0·6–292·4
Iron status[e]	iron deficient	448/552 (81.2)
	iron replete	104/552 (18.8)
Plasmodium spp. infection (PCR)	negative	528/601 (87.9)
	positive	73/601 (12.2)
	P. falciparum	31/601 (5.2)
	P. vivax	35/601 (5.8)
	mixed	7/601 (1.2)
Genetic polymorphisms		
α^+-thalassemia	wild type	564/673 (83.8)
	heterozygous	87/673 (12.9)
	homozygous	22/673 (3.3)
CR1 deficiency[f]	H/H	43/680 (6.3)
	H/L	252/680 (37.1)
	L/L	385/680 (56.6)
SAO	normal	645/681 (94.7)
	SAO	36/681 (5.3)

IQR, interquartile range; MUAC, mid-upper arm circumference; spp, species; PCR, polymerase chain reaction; CR1, complement receptor 1; H/H: high CR1 expression; H/L: intermediate CR1 expression; L/L: low CR1 expression; SAO, Southeast Asian ovalocytosis.

[a]Or otherwise stated.

[b]Gestational age was estimated from fundal height measurements using a previously published formula in women with fundal height measurements ≥24 cm.[21]

[c]Self-reported history of fever during the pregnancy prior to their first antenatal care appointment.

[d]Anemia defined as hemoglobin <110 g/L.

[e]Iron deficient: ferritin <15 μg/L; iron replete: ferritin ≥15 μg/L and C-reactive protein (CRP) ≤10 mg/L, determined by enzyme-linked immunosorbent assays.

[f]Allele abbreviations correspond to CR1 red blood cell surface expression levels: H allele, high expression; L allele, low.

Associations between iron deficiency, hemoglobin levels, and anemia

To quantify the association between iron stores and the outcomes, hemoglobin levels, and anemia, univariable and multivariable mixed-effects modeling was performed (Table 2). Models included ferritin and hemoglobin measurements from enrollment, birth, and 6 and 12 month postpartum evaluation times; thus, the effect measures represent the averages across all evaluation times. It was assumed that a concurrent measurement of ferritin/iron deficiency affects hemoglobin/anemia (see causal diagram in Figure 1). In multivariable analysis, iron deficiency was associated with a lower mean hemoglobin level over the entire study period (−8.07 g/L; 95% CI: −10.12, −6.01; $p < 0.001$). This corresponded to a 4.60-fold increased odds (95% CI: 2.79, 7.58; $p < 0.001$) of anemia in those who were iron deficient compared with iron-replete women over the entire study period. When the anemia outcome measure of moderate-to-severe anemia was used, the odds were still increased for iron-deficient individuals during pregnancy and postpartum (Table S4). Other variables associated with lower mean hemoglobin levels (and anemia) over pregnancy and postpartum included being multigravida (−2.63 g/L; 95% CI: −5.26, −0.01; $p = 0.05$; compared with primigravida), *Plasmodium* spp. infection at enrollment (−4.12 g/L; 95% CI: −7.30, −0.93; $p = 0.01$; compared with uninfected), and having α^+-thalassemia (−5.78 g/L; 95% CI: −8.48, −3.08; $p < 0.001$; compared with wild types) (Table 2).

Associations between anemia risk factors gravidity, *Plasmodium* spp. infection, α^+-thalassemia, and ferritin levels/iron deficiency were assessed though multivariable mixed-effects modeling (Table S5). In multivariable analysis, multigravida women had increased odds of iron deficiency, while *Plasmodium* spp. infection at enrollment was associated with decreased odds of iron deficiency. α^+-Thalassemia showed no significant associations with iron status, and there were no significant interactions between these risk factors and iron

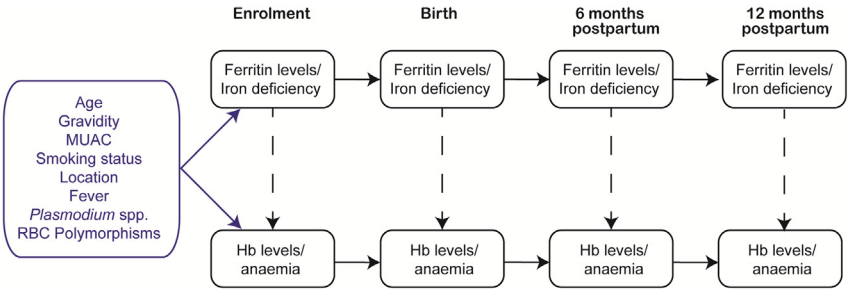

Figure 1. Causal diagram depicting the relationships between ferritin levels/iron deficiency and hemoglobin (Hb) levels/anemia at enrollment, birth, 6 months postpartum, and 12 months postpartum

Potential confounders, presented as a single node in the blue box, include age, gravidity, mid-upper arm circumference (MUAC), location of enrollment clinic, smoking status, history of fever, *Plasmodium* spp. infection, and red blood cell (RBC) polymorphisms. Vertical dashed arrows depict associations between concurrent iron stores and Hb levels/anemia—the key associations of interest. Adherence to iron supplements was not included as it is not a common cause confounder and was only recorded at a single time point (birth).

deficiency (likelihood ratio test p values ranged from 0.14 to 0.76) (Tables S6 and S7).

Differences in anemia etiology between pregnancy and postpartum periods

To investigate how anemia etiology differed between pregnancy and postpartum periods, an interaction term was included between evaluation time and iron deficiency in the regression models. Iron deficiency was associated with substantially increased odds of all anemia during pregnancy (enrollment adjusted odds ratio [aOR] = 4.18; 95% CI: 2.22, 7.90; $p < 0.001$; birth aOR 10.21; 95% CI: 3.42, 30.48; $p < 0.001$) (Table 3). A similar trend was observed for the association between iron deficiency and moderate-to-severe anemia during pregnancy (Table S8). At 6 months postpartum, the odds of anemia increased 3.28-fold (95% CI: 1.68, 6.43; p = 0.001) for those who were iron deficient versus iron replete (Table 3). By 12 months postpartum, the odds of anemia were only 2-fold increased for women who were iron deficient versus iron replete (aOR = 1.81; 95% CI: 0.75, 4.38; p = 0.19) (Table 3). The odds of moderate-to-severe anemia was increased for iron-deficient individuals at both 6 (aOR = 4.06; 95% CI: 2.34, 7.03; p = 0.001) and 12 months postpartum (aOR = 2.36; 95% CI: 1.22, 4.57; p = 0.01) (Table S8).

To further quantify the contribution of iron deficiency to anemia at enrollment, birth, and 6 and 12 month postpartum evaluation times specifically, population attributable fractions were calculated from aORs (Table 3). Iron deficiency accounted for 72% (95% CI: 49, 85) and 89% (95% CI: 66, 96) of anemia cases at enrollment and birth. Similarly, iron deficiency accounted for 62% (95% CI: 37, 78) and 84% (95% CI: 50, 95) of moderate-to-severe anemia cases at enrollment and birth (Table S8). In contrast, iron deficiency accounted for 37% (95% CI: 13, 62) and 20% (95% CI; 0, 56) of anemia cases at 6 and 12 months postpartum (Table 3). Iron deficiency accounted for 44% (95% CI: 22, 65) and 30% (95% CI: 5, 57) of moderate-to-severe anemia cases at 6 and 12 months postpartum respectively (Table S8).

To investigate how long lasting the effect of iron stores on hemoglobin levels is, iron stores were included in the mixed-effects models as lagged effects—iron stores from the preceding evaluation time (depicted in Figure S2). No discernible associations were found, indicating that concurrent iron stores are the more important determinant of hemoglobin levels and anemia (Tables S9 and S10).

DISCUSSION

Pregnant women are at risk of anemia and iron deficiency due to physiological changes and increased iron demands that occur in pregnancy. However, the burden of anemia in the postpartum period and the contribution of iron deficiency to postpartum anemia are largely unknown. In the current study of Papua New Guinean women, hemoglobin levels remained low throughout the study, with anemia prevalent in $\geq$69% from enrollment through to 12 months postpartum. Iron deficiency was associated with the greatest increase in odds of anemia throughout pregnancy and up to 12 months postpartum, demonstrating that it is a key risk factor for anemia, alongside *Plasmodium* spp. infection and α^{+}-thalassemia. Notably, the relative contribution of iron deficiency to anemia changed over time; iron deficiency contributed to 72% of anemia at first antenatal visit but only 20% of anemia by 12 months postpartum. Current anemia prevention strategies delivered during antenatal care in this setting where women present later in pregnancy are not sufficient to address the high burden of anemia during pregnancy and postpartum periods. Anemia prevention strategies delivered earlier in pregnancy, postpartum, or in women of reproductive age targeting anemia etiologies relevant for reproductive stage may also be warranted to reduce the burden of anemia.

Global estimates suggest that approximately 50% of all anemia in pregnancy is attributable to iron deficiency[1]; in the present study, 72%–89% of anemia in pregnancy was attributed to iron deficiency. This suggests that a large proportion of anemia in pregnancy in this setting would be amenable to iron supplementation. Daily prenatal iron and folic acid supplementation was recommended to the women in this study as per Papua New Guinea National guidelines.[22] In self-reported data on iron supplement use collected after birth, 65% (343/534) of women reported taking iron supplements "most days" during their pregnancy. However, almost all (93%, 316/343) of these women only started taking iron supplements at their first antenatal care appointment (enrollment), which typically occurred late on in pregnancy (median 30 [IQR 28–32] gestational weeks) where women would be likely at, or close to, the nadir of iron status in pregnancy. Iron supplementation may have contributed to the observed increase in ferritin concentration between enrollment and birth. Despite this increase, the proportion of women classified as anemic or iron deficient did not change between enrollment and birth, potentially because not enough time or

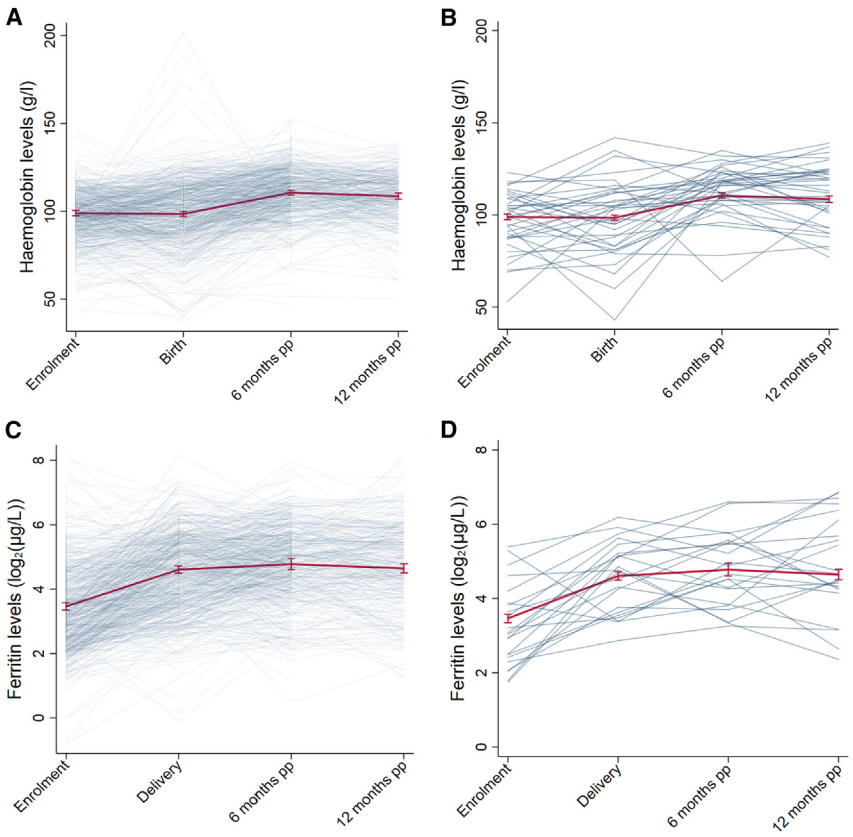

Figure 2. Hemoglobin (Hb) and ferritin dynamics over time (enrollment, birth, and 6 and 12 months postpartum [pp])

(A and B) Observed individual trajectories of Hb levels (g/L) (A) for the entire cohort and (B) for a randomly selected subset of women who have levels available at all evaluation times.

(C and D) Observed individual trajectories of ferritin levels (log2(µg/L)) (C) for the entire cohort and (D) for a randomly selected subset of women who have levels available at all evaluation times. Superimposed on the plots are the estimated mean Hb and ferritin levels over the study period (set at mean levels or prevalence of enrollment maternal factors: age, MUAC, gravidity, smoking status, clinic location, history of fever, *Plasmodium* spp. infection, and genetic polymorphisms).

doses between first antenatal care appointment were realized to achieve clinically meaningful benefits. Given that the majority of women were already iron deficient at their first antenatal care appointment and presented to this appointment during the second or third trimester of pregnancy, the WHO's primary recommendation of universal supplementation during pregnancy may not be sufficient to prevent maternal anemia in settings where first antenatal care appointments tend to occur later in pregnancy.

Another opportunity to prevent anemia exists postpartum, between pregnancies, when women are attending regular medical appointments for infant checkups and immunizations. Iron supplementation is not routinely provided as part of postnatal care in Papua New Guinea. In line with this, only ~1.5% of women in this study reported that they were taking iron folate supplements at 6 and 12 months postpartum. Iron supplementation could be extended into the postpartum period for women with moderate-to-severe anemia, through existing health systems, to improve women's hemoglobin and iron reserves before their next pregnancy. This intervention strategy could reduce the risk of anemia in subsequent pregnancies, as well as address postpartum anemia caused by iron deficiency. There is a paucity of literature on the prevalence and etiology of postpartum anemia in resource-limited settings worldwide. The high burden of postpartum anemia (>70%) observed highlights that the postpartum period should not be considered a time of low anemia risk in this setting. However, the proportion of anemia attributable to iron deficiency was lower postpartum than in pregnancy, ~20% by 12 months postpartum. Aside from iron deficiency, *Plasmodium* spp. infection was a key risk factor for anemia in this cohort. Thus, the effective implementation of malaria prophylaxis should be encouraged, as per standard of care. Bed net use in this cohort was moderate (63% reported using a net the night prior to enrollment) but could be strengthened further as an anemia prevention strategy, along with regular rapid diagnostic testing for malaria. While *Plasmodium* spp. infection (and also α^+-thalassemia) were significant risk factors for anemia, their prevalence was relatively low and could not account for all the remaining attributable risk. Other potential anemia etiologies that were not assessed here, such as infection with intestinal helminths and other micronutrient deficiencies (e.g., vitamins A and B12), may also be important.[23,24] Quantifying these intervenable risk factors will inform a potential suite of interventions that address infections and multiple nutritional needs and further reduce the significant burden of anemia in pregnancy and postpartum.

The consistently low population mean hemoglobin levels throughout the study period suggest that women of reproductive age in this setting experience a cycle of chronic anemia, with hemoglobin levels never being fully restored between pregnancies. In order to substantially reduce anemia in settings where it is highly prevalent (≥20%), the WHO recently proposed that iron supplementation be provided to all menstruating women[7] rather than just during pregnancy and postpartum periods. This strategy has not been widely implemented but has proved successful in regions of Vietnam and India, which saw anemia reductions of 20% and 24%, respectively, in women after 12 months of iron supplementation.[25,26] Another option is to target adolescent girls through schools. This approach was adopted in Ghana, where a cohort of adolescent girls received weekly iron-folic supplementation, resulting in a 26% reduction in anemia after 9 months of implementation.[27] These programs provided intermittent iron supplementation, which has similar efficacy to the

Table 2. Associations between iron stores and enrollment confounders, and hemoglobin levels and anemia over the entire study period

Variable		Hemoglobin levels (g/L)		Anemia[a]	
		Unadjusted mean difference (95% CI); p value	Adjusted mean difference (95% CI); p value	Unadjusted odds ratio (95% CI); p value	Adjusted odds ratio (95% CI); p value
Iron stores					
Ferritin (log_2(μg/l))[b]	–	1.99 (1.41, 2.58); <0.001	–	0.65 (0.57, 0.73); <0.001	–
Iron deficiency[c]	replete	Ref.	Ref.	Ref.	Ref.
	deficient	−8.29 (−10.20, −6.38); <0.001	−8.07 (−10.12, −6.01); <0.001	4.26 (2.70, 6.71); <0.001	4.60 (2.79, 7.58); <0.001
At enrollment					
Age (years)	–	−0.001 (−0.17, 0.16); 0.99	0.03 (−0.18, 0.23); 0.79	1.00 (0.97, 1.03); 0.93	1.01 (0.96, 1.05); 0.74
Gravidity	primigravida	Ref.	Ref.	Ref.	Ref.
	multigravida	−2.65 (−4.76, −0.54); 0.·01	−2.63 (−5.26, −0.01); 0.05	1.34 (0.94, 1.92); 0.11	1.18 (0.67, 2.08); 0.56
Smoking status	never	Ref.	Ref.	Ref.	Ref.
	current/past	−0.91 (−2.78, 0.95); 0.34	−1.24 (−3.28, 0.80); 0.23	1.23 (0.89, 1.69); 0.21	1.45 (0.93, 2.27); 0.11
MUAC (cm)		0.63 (0.32, 0.93); <0.001	0.51 (0.13, 0.90); 0.01	0.90 (0.86, 0.95); <0.001	0.89 (0.82, 0.96); 0.01
	>23 cm	Ref.	–	Ref.	–
	≤23 cm	−3.59 (−6.23, −0.95); 0.01	–	1.61 (1.00, 2.61); 0.05	–
Fever[d]	no	Ref.	Ref.	Ref.	Ref.
	yes	−0.95 (−3.68, 1.77); 0.49	−0.10 (−2.97, 2.78); 0.95	1.03 (0.64, 1.64); 0.91	0.84 (0.46, 1.56); 0.59
Plasmodium spp. (PCR)	negative	Ref.	Ref.	Ref.	Ref.
	positive	−6.48 (−9.41, −3.55); <0.001	−4.12 (−7.30, −0.93); 0.01	2.55 (1.44, 4.49); <0.001	1.99 (0.92, 4.26); 0.08
α^+-Thalassemia	wild type	Ref.	Ref.	Ref.	Ref.
	Het/Hom	−5.74 (−8.17, −3.32); <0.001	−5.78 (−8.48, −3.08); <0.001	2.86 (1.78, 4.62); <0.001	3.15 (1.58, 6.28); 0.001
CR1 deficiency	H/H	Ref.	Ref.	Ref.	Ref.
	H/L	2.20 (−1.71, 6.12); 0.27	2.96 (−1.19, 7.11); 0.16	0.99 (0.51, 1.93); 0.97	0.68 (0.26, 1.75); 0.42
	L/L	2.08 (−1.73, 5.89); 0.29	2.72 (−1.34, 6.79); 0.19	1.01 (0.53, 1.95); 0.97	0.69 (0.27, 1.75); 0.43
SAO	normal	Ref.	Ref.	Ref.	Ref.
	SAO	−3.22 (−7.34, 0.91); 0.13	−2.50 (−6.64, 1.64); 0.24	1.16 (0.56, 2.39); 0.70	0.90 (0.37, 2.17); 0.81

Estimated mean hemoglobin differences and odds ratios for anemia were derived from linear and logistic mixed-effects models, respectively, with a random effect for the individual-specific intercept. Adjusted models included enrollment variables listed in the table and time (enrollment, birth, and 6 and 12 months postpartum). CI, confidence interval; Het, heterozygous; Hom, homozygous; CR1, complement receptor 1; H/H, high CR1 expression; H/L, intermediate CR1 expression; L/L, low CR1 expression; SAO, Southeast Asian ovalocytosis.

[a]Anemia defined as hemoglobin <110 g/L in pregnancy and hemoglobin <120 g/L in the postpartum period.

[b]Ferritin transformed to log base-2 due to positively skewed distribution, thus the coefficient/odds ratio represents the change associated with a 2-fold increase in ferritin.

[c]Iron deficient: ferritin <15 μg/L in pregnancy and postpartum; iron replete: ferritin ≥15 μg/L and CRP ≤10 mg/L in pregnancy and ferritin ≥15 μg/L and CRP ≤5 mg/L postpartum.

[d]Self-reported history of fever during the pregnancy prior to their first antenatal care appointment.

daily regime but with fewer side effects.[16] Similar interventions in Papua New Guinea may be valuable but need to consider challenges in health service access, constraints in infrastructure, and low community outreach frequency, especially in remote and rural areas. However, given the public health problem anemia poses in this setting, it is a high priority for improving the health

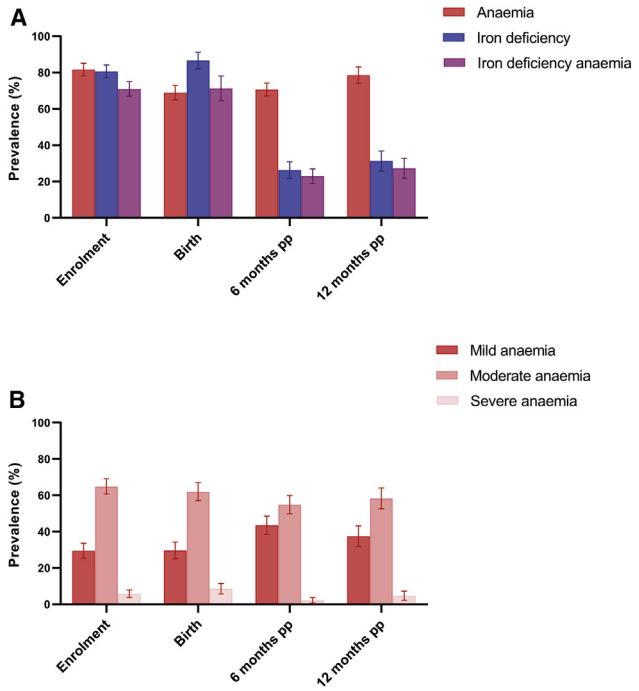

Figure 3. Prevalence (95% CI) of anemia, iron deficiency, and iron-deficiency anemia at enrollment, birth, and 6 and 12 months postpartum (pp)

(A) In pregnancy (enrollment, birth), anemia is defined as Hb <110 g/L; iron deficiency is defined as ferritin <15 μg/L; iron-deficiency anemia is defined as ferritin <15 μg/L and Hb <110 g/L. In postpartum (6 months pp, 12 months pp), anemia is defined as Hb <120 g/L; iron deficiency is defined as ferritin <15 μg/L; iron-deficiency anemia is defined as ferritin <15 μg/L and Hb <120 g/l.

(B) In pregnancy (enrollment, birth), anemia is defined as mild: Hb < 110 g/L and ≥100 g/L; moderate: Hb < 100 and ≥70 g/L; severe: Hb < 70 g/L. In postpartum (6 months pp, 12 months pp), anemia is defined as mild: Hb < 120 g/L and ≥110 g/L; moderate: Hb < 110 and ≥80 g/L; severe: Hb < 80 g/L.

Table 3. Time-varying contributions of iron deficiency to anemia at enrollment, birth, and 6 and 12 months postpartum

	Evaluation time	Anemia adjusted OR[a] (95% CI); p value	Population attributable fraction (95% CI)[b]
Iron deficiency[c]	enrollment	4.18 (2.22, 7.90); <0.001	72% (49, 85)
	birth	10.21 (3.42, 30.48); <0.001	89% (66, 96)
	6 months postpartum	3.28 (1.68, 6.43); 0.001	37% (13, 62)
	12 months postpartum	1.81 (0.75, 4.38); 0.19	20% (0, 56)

Anemia defined as hemoglobin <110 g/L in pregnancy and hemoglobin <120 g/L postpartum. Population attributable fractions for other important anemia risk factors identified in Table 2, such as *Plasmodium* spp. infection and α^+-thalassemia, were not able to be determined due to low numbers of observations in the exposed, non-anemic groups.

[a]Adjusted ORs for anemia were derived from multivariable logistic mixed-effects models with a random effect for the individual-specific intercept. Models included an interaction term included between iron deficiency and time (enrollment, birth, and 6 and 12 months postpartum) and adjusted for age, mid-upper arm circumference, gravidity, smoking status, residence, fever, and genetic polymorphisms.

[b]Population attributable fractions for anemia were calculated using the formula: [prevalence × (OR-1)]/[prevalence (OR-1) + 1] and 95% confidence limits were calculated using confidence limits for ORs.

[c]Women classified as iron deficient: ferritin <15 μg/L; replete: ferritin ≥15 μg/L and CRP ≤10 mg/L in pregnancy and ferritin ≥15 μg/L and CRP ≤5 mg/L postpartum.

and well-being of women. This is particularly relevant in light of the WHO Global Nutrition target to halve anemia in women of reproductive age by 50% in 2025[7]; a goal that is unlikely to be achieved in the near term in settings like Papua New Guinea that experience a high burden of maternal anemia without a push for further action or new intervention strategies.

As an alternative to oral iron supplementation, iron can be given intravenously. Modern intravenous iron formulations have been deemed safe and effective in preventing anemia in both pregnancy and postpartum periods in recent randomized controlled trials (reviewed in Qassim et al.,[28] Lewkowitz et al.,[29] and Sultan et al.[30]). The key advantage of this strategy is that a single infusion of intravenous iron is required, so there are no compliance concerns. However, significant health system capacity building and strengthening would need to take place to successfully deliver intravenous iron to all moderately to severely anemic pregnant and postpartum women attending health care facilities in Papua New Guinea.

Limitations of the study

In East New Britain, Papua New Guinea, ~90% of women attend antenatal care, and our study participants were representative of pregnant women attending five antenatal clinics that provide >75% of antenatal services in the province. A limitation of our study was that it did not capture women not attending antenatal care, typically women living in hard-to-reach areas with presumed poorer health, which may lead us to underestimate the true population prevalence of anemia and iron deficiency. Loss to follow-up (48% by 12 months postpartum) may also introduce bias. However, in our analyses, we used mixed-effects modeling with maximum likelihood estimation, which uses all participant outcome data regardless of completeness across time points. This estimation method provides unbiased effect estimates in the presence of attrition assuming a "missing-at-random" missing data mechanism. Furthermore, given that enrollment characteristics were similar at follow-up time points, attrition should not significantly impact study estimates or study conclusions. It should also be noted that the population attributable fractions were calculated using ORs, and given that anemia was a common outcome in our study population, this will result in an overestimation of the population attributable fractions. In terms of external validity, the relative contribution of iron deficiency, genetic polymorphisms, malaria, and other anemia risk factors will vary with the local conditions. In accordance with this, prevention and control strategies for maternal anemia will need to be tailored to the setting-specific anemia etiology. Despite these limitations, some generalizations from this study can be made. The fact that 69% of women in this study were

still anemic at birth in this cohort, coupled with the stagnant global prevalence of anemia in pregnancy over the last two decades, suggests that the WHO primary recommendation of universal antenatal iron folic acid supplementation is either not being implemented successfully, or early enough, or is not effective enough to prevent maternal anemia in resource-limited settings. Based on the high prevalence of anemia observed during the first 12 months postpartum in this study, we can postulate that populations experiencing a similarly high burden of anemia during pregnancy will continue to have high anemia prevalence in the postpartum period without intervention. Given that every second pregnant woman is estimated to be anemic in resource-limited settings,[1] anemia in the postpartum period is likely to be a widespread yet neglected condition globally.

Conclusion

Maternal anemia was highly prevalent in pregnancy and postpartum in our population, with iron deficiency a major risk factor in pregnancy, but less so in the postpartum period. Iron supplementation provided both early during pregnancy and between pregnancies, in conjunction with malaria prevention strategies, could break the cycle of chronic anemia in women of reproductive age. Research is needed to determine the effectiveness and feasibility of providing iron supplementation earlier in pregnancy, as part of routine postpartum care, and the optimal frequency and duration of supplementation required to improve the health of women in this region, as well as other settings where maternal anemia is highly prevalent.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2023.101097.

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AUTHORS CONTRIBUTIONS

J.G.B., C.J.M., M.J.L.S., F.J.I.F., P.B., and B.S.C. provided major contributions to the Healthy Mothers Heathy Babies study design, with input from A.E., P.M.S., W.P., L.J.R., and E.K. M.J.L.S., E.P., P.M., H.S., P.H., W.P., D.K., K.T., R.S., R.F., and B.K. undertook field work and data collection. E.M.D. and D.H.O. performed and interpreted iron deficiency assays. E.M.D., J.A.S., and F.J.I.F. conceived and designed the statistical plan. E.M.D. and F.J.I.F. drafted the manuscript, and all authors approved the final report.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human serum samples	Healthy Mothers Healthy Babies cohort study in this paper	N/A
Human whole blood samples	Healthy Mothers Healthy Babies cohort study in this paper	N/A
Critical commercial assays		
Ferritin ELISA kit	Immunology Consultants Laboratory Immunoperoxidase Assay	Cat#E−80F
CRP ELISA kit	Elisakit,	Cat#EK-0040
QIAamp ® DNA and Blood Minikit	Qiagen	Cat#51104
Oligonucleotides		
α2/3.7 Forward CCCCTCGCCAAGTCCACCC	Geneworks	N/A
α2 Reverse AGACCAGGAAGGGCCGGTG	Geneworks	N/A
3.7 Reverse AAAGCACTCTAGGGTCCAGCG	Geneworks	N/A
4.2 Forward GGTTTACCCATGTGGTGCCTC	Geneworks	N/A
4.2 Reverse CCCGTTGGATCTTCTCATTTCCC	Geneworks	N/A
CR1 Exon 22 Forward TTCACATTGGATAGCCCAGAGC	Geneworks	N/A
CR1 Exon 22 Reverse CCCTTGTAAGGCAAGTCTGG	Geneworks	N/A
SAO Forward GGGCCCAGATGACCCTCTGC	Geneworks	N/A
SAO Reverse GCCGAAGGGGTGATGGCGGGTG	Geneworks	N/A
P. falciparum forward TATTGCTTTTGAGAGGTTTTGTTACTTTG	Bioneer Pacific	N/A
P. falciparum reverse ACCTCTGACATCTGAATACGAATGC	Bioneer Pacific	N/A
P. falciparum probe ACGGGTAGTCATGATTGAGTT	Bioneer Pacific	N/A
P. vivax forward GCTTTGTAATTGGAATGATGGGAAT	Bioneer Pacific	N/A
P. vivax reverse ATGCGCACAAAGTCGATACGAAG	Bioneer Pacific	N/A
P. vivax probe AGCAACGCTTCTAGCTTA	Bioneer Pacific	N/A
Software and algorithms		
STATA Version 15	StataCorp, College Station, TX, USA	N/A

RESOURCE AVAILABILITY

Lead contact

For further information and requests for resources should be directed to and will be fulfilled by the lead contact, Freya Fowkes (freya.fowkes@burnet.edu.au).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The data analyzed during the current study are not publicly available due to study participants' privacy, but de-identified data reported in this paper will be shared by the lead contact to applicants who provide a sound proposal to the Medical Research Advisory Council, National Department of Health, Papua New Guinea.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study setting

Papua New Guinea is a resource-limited country in the Western Pacific with a population of approximately 9 million people. The majority (87%) of Papua New Guineas population reside in rural and remote areas,[31] where rugged terrain, poor roads, and limited infrastructure restrict access to health services. A chronic shortage of health providers is another major health system constraint,[32] with an estimated nine health providers per 10,000 people nationwide.[33] At an estimated 594 per 100,000 live births, in 2013 Papua New Guinea has one of the highest maternal mortality ratios in the world.[34]

This study took place in the rural province of East New Britain province, in the Islands Region of Papua New Guinea. Five hospitals/health centers were selected as sentinel sites: Nonga Hospital, Saint Mary's Hospital (Vunapope), Keravat rural Hospital, Paparatava Health Center and Naparpar Health Center. These encompass the two largest urban hospitals and three busy health facilities in rural areas of East New Britain, and account for over 75% of antenatal care services in the province according to the Provincial Health Office.

The National Guideline for Obstetrics and Gynecology in Papua New Guinea recommends women book antenatal care after missing 2–3 menstrual periods.[35] However, a national health survey review found fewer than half of all pregnant women attended one antenatal care visit.[33] As part of routine antenatal care in Papua New Guinea, daily iron folate supplementation is provided to all women throughout pregnancy. Intermittent preventive treatment of malaria is also routinely delivered in antenatal care visits. Deworming is recommended,[35] but there are no reliable statistics on the implementation of this intervention.[36] There are no national guidelines on postnatal care after hospital discharge. For those diagnosed as anemic in pregnancy, iron folic acid supplementation is recommended for 1–3 months postpartum,[35] however, postnatal care is not recorded consistently and there is limited data on this intervention and routine care more broadly.

Subject details

This prospective cohort study of 699 pregnant women took place between March 2015 and December 2018. Pregnant women of any gravidity were randomly selected to participate through a dice roll, while attending one of the afore-mentioned five local clinics/hospitals as previously described.[37,38] Inclusion criteria required that participants be 1) at least 16 years old; 2) attending their first antenatal care appointment for the current pregnancy; 3) reside within the catchment area of the healthcare facility; 4) intending to live in East New Britain for the subsequent 12 months; and 5) be willing to participate. The original study was powered on the primary outcome of birthweight and a sample size of 700 was calculated to detect a clinically meaningful difference of at least 120g in birthweight with a power of 90% and a significance level of 5%, and where the standard deviation of birthweight was 475g for the exposures malaria, sexually transmitted infections, tuberculosis, anemia and iron deficiency (assuming prevalence of approximately 30%). The analysis of anemia and iron deficiency during pregnancy and postpartum presented here represents secondary outcomes. Follow-up visits took place at birth, 1-, 6- and 12-month postpartum, at the health facility where possible, or at their residence.

At enrollment, following informed consent, women completed interviews, covering demographic details, obstetric history, and accounts of illness and medication use in the current pregnancy. Physical examinations were performed, collecting anthropometric and fundal height measurements. Gestational age was calculated from enrollment fundal height measurements (excluding <24cm and twins) using a previously published formula.[21] Venous blood samples were collected, then again at birth, 6- and 12-month postpartum. At each timepoint, hemoglobin concentration was determined using a Hemocue haemoglobinometer (Hemocue, Ängelholm, Sweden). *Plasmodium* spp. infection was determined by ACCESSBIO *CareStart* Malaria HRP2/pLDH (Pf/PAN) Combo and women with a positive diagnosis were supported to receive appropriate treatment (Artemether lumefantrine). Intermittent preventive treatment (sulfadoxine-pyremethamine) and iron folate supplementation was provided to all women during antenatal visits by the antenatal clinic, as per national guidelines. Data on uptake and adherence of iron folate supplements during pregnancy was collected retrospectively at the birth. At 6- and 12-month postpartum women were asked to report use of any medication, but adherence data was not collected. All women had the same survey data and samples collected, and the same laboratory tests performed.

Ethics approval was obtained from Alfred Health, Australia (348/14), and the Medical Research Advisory Council, National Department of Health, Papua New Guinea (14/27).

METHOD DETAILS

Ferritin and CRP ELISAs for iron deficiency diagnosis

Ferritin (Immunology Consultants Laboratory Immunoperoxidase Assay) and C-reactive protein (CRP) (Elisakit) was determined by enzyme-linked immunosorbent assay after the cohort study was completed in serum samples at enrollment, birth, 6- and 12-month postpartum. ELISAs for Ferritin and CRP were run in 96-well plates, following the corresponding kit protocols. To obtain absolute quantification of Ferritin and CRP concentration, standard curves were established using an 8-fold dilution of standards with known concentrations, which were provided in the kits and run in duplicate. Negative controls, or 'blanks', containing ELISA reagents but no standard or serum sample, were also included in all ELISA plates. Furthermore, positive controls were incorporated in all ELISA plates, which consisted of at least two serum samples collected from Melbourne controls.

Detection of P. Falciparum and *P. Vivax* infection

Quantitative polymerase chain reaction (qPCR) was utilized to detect peripheral *P. falciparum* and *P. vivax* parasitaemia, following a previously published protocol.[39] The 18S ribosomal RNA gene was amplified with specific primers and probes for either *P. falciparum* or *P. vivax*. Standard curves were generated for absolute quantification of *P. falciparum* and *P. vivax*, using a 7-fold serial dilution of the control plasmids, ranging from 78,125 copies/μL to 5 copies/μL, in quadruplicate. To ensure reproducibility, 12% of all samples were run as repeats. Negative controls were included in all PCR reactions, which contained PCR master mix but no DNA. Assays were performed on a QuantStudio 7 Flex Real-Time PCR System (ThermoScientific) in 384-well plate format. *P. falciparum* and *P. vivax* qPCR reagent and cycling conditions specified in Additional File 1, Table S11.

Genotyping of red blood cell polymorphisms

DNA was extracted from red blood cell samples, previously separated from venous blood plasma, using the QIAamp DNA and Blood Minikit (Qiagen) manufacturer spin protocol. RBC polymorphisms α^+-thalassemia, CR1 deficiency and SAO were typed using polymerase chain reactions (PCRs). The two most common α^+-thalassemia deletions in Melanesian populations, 3.7kb and 4.2kb deletions, were typed by multiplex PCR as previously described,[40] with modifications. SAO (27bp deletion in the band 3 gene) and CR1 polymorphisms (exon 22, A/G substitution at 3650) were typed using established methods.[41,42] All PCR reagent volumes, cycling and gel conditions are specified in Additional File 1, Table S12.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed using STATA Version 15 (StataCorp, College Station, TX, USA). To determine the association between iron status (ferritin concentration, iron deficient/replete) and the outcomes hemoglobin levels or anemia, univariable and multivariable linear or logistic mixed-effects modeling were performed with time included as a categorical variable, and a random effect for the individual-specific intercepts was included to account for between-women variability at enrollment. To determine the association between iron status at preceding evaluation times and hemoglobin levels/anemia, ferritin and iron deficiency were also included as lagged effects (Figure S2). Anemia was defined as hemoglobin <110 g/L in pregnancy and hemoglobin <120 g/L in postpartum, according to WHO guidelines.[43] Iron deficiency was defined as ferritin <15 μg/L; iron replete was defined as ferritin $\geq$15 μg/L and CRP $\leq$5 mg/L or $\leq$ 10 mg/L (in pregnancy and postpartum respectively, to take into account raised ferritin due to inflammation), as per WHO recommendation.[8] Multivariable models were adjusted for enrollment confounders determined *a priori*: maternal age, gravidity, mid-upper arm circumference, location of enrollment clinic, smoking, self-reported history of fever in pregnancy, PCR detected *Plasmodium* spp. infection, α^+-thalassemia, CR1 deficiency, and SAO - informed by directed acyclic graphs (Figure 1). Effect modification by time was investigated by adding an interaction term between time and iron status. Population attributable fractions were calculated using the odds ratios from these mixed-effects multivariable models and the formula [prevalence x (OR-1)]/[prevalence (OR-1) + 1]. To determine associations between anemia risk factors: gravidity, *Plasmodium* spp. infection, α^+-thalassemia, and ferritin levels/iron deficiency, univariable and multivariable mixed-effects modellings were performed. Adherence to iron supplements was not adjusted for in the above models as it was not assumed to be a common cause confounder and was only recorded at a single time-point mid follow-up.

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Supplemental information

Quantifying differences in iron deficiency-attributable anemia during pregnancy and postpartum

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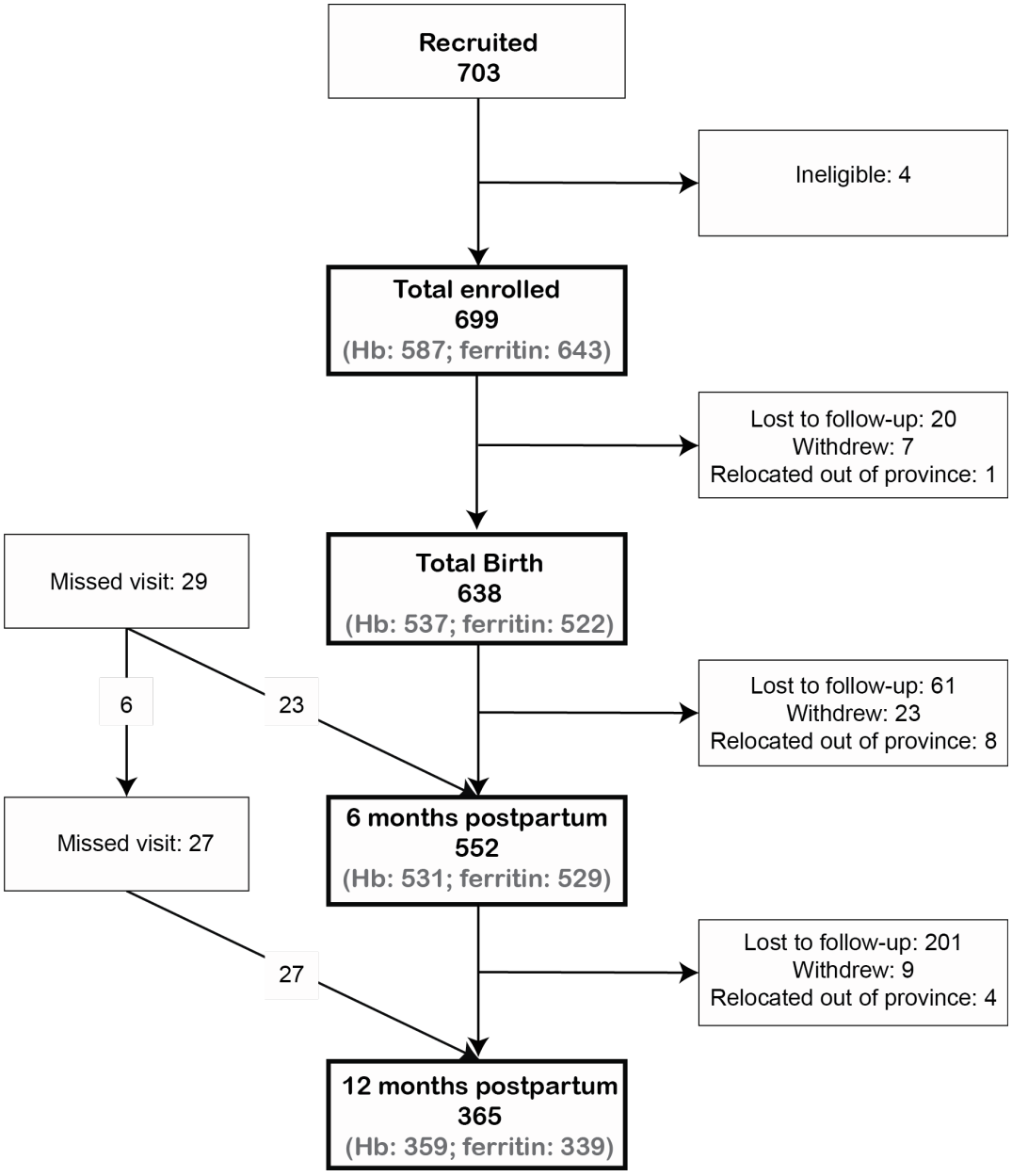
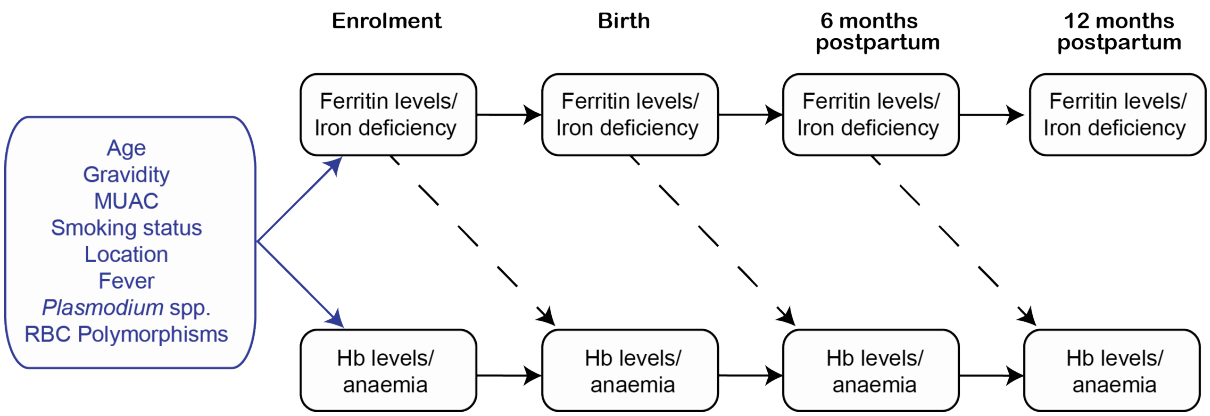
Additional File 1



Supplementary Figure 1: Flow diagram of women enrolled and the subset followed to birth, 6 months postpartum and 12 months postpartum; and the haemoglobin (Hb) and ferritin measurements available at each visit. Related to Subject Details in STAR Methods.



Supplementary Figure 2: Causal diagram depicts the relationships between ferritin levels/ iron deficiency and haemoglobin (Hb) levels/ anaemia at enrolment, birth, 6 months postpartum and 12 months postpartum. Related to Quantification and Statistical Analysis in STAR Methods. Potential confounders, presented as a single node in a blue box, include age, gravidity, mid-upper arm circumference (MUAC), location of enrolment clinic, smoking status, history of fever, *Plasmodium* spp. infection and red blood cell (RBC) polymorphisms. Angled dashed arrows depict associations between the preceding iron stores and current haemoglobin levels/anaemia (lagged effect) – the key associations of interest. Adherence to iron supplements was not included as it is not a common cause confounder and was only recorded at a single time-point (birth).

Supplementary Table 1: Enrolment cohort characteristics for the subset of women who returned at birth, and at 6 and 12 months postpartum. Related to Table 1.

		Birth	6 months postpartum	12 months postpartum	
Variable		**Included (n=638)**	**Included (n=552)**	**Included (n=365)**	**Lost to follow-up (n=334)**
Sociodemographics					
Enrolment Clinic	Vunapope	172 (27·0%)	155 (28·1%)	106 (29·0%)	78 (23·4%)
	Nonga	71 (11·1%)	63 (11·4%)	35 (9·6%)	48 (14·4%)
	Keravat	107 (16·8%)	92 (16·7%)	58 (15·9%)	67 (20·1%)
	Napapar	148 (23·2%)	123 (22·3%)	94 (25·8%)	64 (19·2%)
	Paparatava	140 (21·9%)	119 (21·6%)	72 (19·7%)	77 (23·1%)
Age (years)		26·0 (23·0-30·0); 17-49	26.0 (23·0-30·0); 17-49	26·0 (22·0-30·0);17-42	26·0 (23·0-30·0); 16-49
Gravidity	Primigravidae	154 (24·1%)	129 (23·4%)	98 (26·8%)	79 (23·7%)
	Multigravidae	484 (75·9%)	423 (76·6%)	267 (73·2%)	255 (76·3%)
Highest level education	Primary or less	292 (45·8%)	254 (46·1%)	167 (45·8%)	158 (47·4%)
Employment status	Not employed	483 (75·7%)	414 (75·0%)	271 (74·2%)	260 (77·8%)
Smoking status	Never smoked	386 (60·6%)	340 (61·6%)	228 (62·5%)	199 (59·9%)
	Current/past smoker	251 (39·4%)	212 (38·4%)	137 (37·5%)	133 (40·1%)
Bed net use	Owns bed net	479 (75·1%)	419 (75·9%)	266 (72·9%)	261 (78·4%)
	Net used last night	398 (62·4%)	351 (63·6%)	232 (63·6%)	208 (78·7%)
Clinical Measures					
Gestational age (weeks) [a]		29·8 (28·0-32·5); 26-40	29·8 (28·0-32·5); 26-40	29·8 (28·0-31·6); 26-40	29·8 (28·0-33·4); 26-39
MUAC (cm)		26·0 [3·0]; 13·5-43·7	26·0 [3·0]; 13·5-43·7	26·1 [2·8]; 14·5-37·5	25·8 [3·1]; 13·5-43·7
BMI (kg/m^2)		25·3 [3·2]; 17·9-34·3	25·3 [3·2]; 17·9-34·3	25·2 [3·1]; 18·2-34·3	25·4 [3·2]; 17·9-34·3
Fever [b]		81 (12·9%)	70 (12·9%)	37 (10·2%)	54 (16·6%)

Data are mean [SD], range; median (25th-75thpercentile), range; or n (%).

[a] Gestational age was estimated from ANC fundal height measurements in women with fundal height measurements ≥24cm.

[b] Self-reported history of fever during the pregnancy prior to their first ANC appointment.

BMI: Body mass index. MUAC: Mid-upper arm circumference

Supplementary Table 1 continued: Enrolment cohort characteristics for the subset of women who returned at birth, and at 6 and 12 months postpartum Related to Table 1.

		Birth	6 months postpartum	12 months postpartum	
Variable		**Included (n=638)**	**Included (n=552)**	**Included (n=365)**	**Lost to follow-up (n=334)**
Diagnostic Measurements					
Haemoglobin level (g/l)		96·3 [14.7], 41-145	95·9 [14·3], 41-137	96·3 [13·9], 41-143	96·4 [15·1], 44-145
Anaemia [c]		443 (82·2%)	383 (82·9%)	245 (84·5%)	238 (80·1%)
Ferritin level (µg/l)		9·1 (5·3-17·7), 0·6-292·4	8·9 (5·3-16·9), 0·6-292·4	9·2 (5·1-18·2), 0·6-292·4	9·3 (5·4-19·2), 0·6-264·5
Iron status [d]	Iron deficient	417 (82·6%)	363 (83·1%)	236 (81·1%)	212 (81·5%)
	Iron replete	88 (17·4%)	74 (16·9%)	55 (18·9%)	48 (18·5%)
Plasmodium spp. (PCR)	Negative	485 (87·9%)	415 (87·7%)	266 (87·8%)	262 (87·9%)
	Positive	67 (12·1%)	58 (12·3%)	37 (12·2%)	36 (12·1%)
	P. falciparum	28 (5·1%)	29 (6·1%)	14 (4·6%)	17 (5·7%)
	P. vivax	32 (5·8%)	36 (7·4%)	18 (5·9%)	17 (5·7%)
	Mixed	7 (1·3%)	7 (1·5%)	5 (1·7%)	2 (0·7%)
Genetic polymorphisms					
α^{+}-thalassemia	Wildtype	512 (83·0%)	446 (84·0%)	292 (83·2%)	272 (84·5%)
	Heterozygous	84 (13·6%)	67 (12·6%)	49 (14·0%)	38 (11·8%)
	Homozygous	21 (3·4%)	18 (3·4%)	10 (2·8%)	12 (3·7%)
CR1 deficiency [e]	H/H	40 (6·4%)	34 (6·3%)	21 (5·8%)	22 (6·9%)
	H/L	232 (37·1%)	208 (38·4%)	133 (37·0%)	119 (37·1%)
	L/L	354 (56·5%)	299 (55·3%)	205 (57·1%)	180 (56·1%)
SAO	Normal	593 (94·6%)	513 (94·8%)	335 (93·1%)	310 (96·6%)
	SAO	34 (5·4%)	28 (5·2%)	25 (6·9%)	11 (3·4%)

Data are mean [SD], range; median (25th-75thpercentile), range; or n (%)

[c] Anaemia defined as haemoglobin <110g/l at birth and haemoglobin <120g/l at 6 and 12 months postpartum

[d] Women classified as iron deficient: ferritin <15µg/l in pregnancy and postpartum. Women classified as iron replete: ferritin ≥15µg/l and CRP≤10mg/l in pregnancy and ferritin ≥15µg/l and CRP≤5mg/l postpartum.

[e] Allele abbreviations correspond to CR1 red blood cell surface expression levels: H allele: high expression; L allele: low.

spp: species. RDT: Rapid Diagnostic Test. PCR: Polymerase Chain Reaction. CR1: Complement Receptor 1. SAO: Southeast Asian Ovalocytosis

Supplementary Table 2: Unadjusted and adjusted estimated mean differences in haemoglobin levels (g/l) over pregnancy and postpartum periods. Related to Figure 2.		
Model Parameter	**Unadjusted estimate (95% CI); p-value**	**Adjusted estimate [a] (95% CI); p-value**
Estimated mean at enrolment	96·42 (95·15, 97·69)	98.94 (97.43, 100.45)
Estimated mean difference [b]		
Enrolment	Reference	Reference
Birth	2·59 (1·04, 4·14); 0·001	-0·44 (-2·37, 1·49); 0·65
6 months postpartum	15·29 (13·73, 16·85); <0·001	11·63 (9·62, 13·65); <0·001
12 months postpartum	12·17 (10·38, 13·95); <0·001	9·63 (7·38, 11·87); <0·001
Between-women SD	9·32	7·32
Within-woman SD	12·93	13·02

Unadjusted or adjusted estimated mean differences (95% CI), p-value, were derived from linear mixed-effects models with a random effect for the individual-specific intercept. CI- Confidence Interval.

[a] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, location of enrolment clinic, history of fever, *Plasmodium* spp. infection and red blood cell polymorphisms (α^+-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

[b] The estimated mean haemoglobin level at enrolment is derived with confounders set to mean values of continuous variables or the prevalence of categorical variables.

Supplementary Table 3: Unadjusted and adjusted relative difference in the geometric mean of ferritin levels (µg/l) over pregnancy and postpartum periods. Related to Figure 2.		
Model Parameter	**Unadjusted estimate (95% CI); p-value**	**Adjusted estimate [a] (95% CI); p-value**
Estimated geometric mean at enrolment (µg/l)	10·28 (9·62, 10·98)	5·87 (3·26, 10·61)
Ratio of geometric means [b]		
Enrolment	Reference	Reference
Birth	2·31 (2·13, 2·49); <0·001	2·30 (2·11, 2·50); <0·001
6 months postpartum	2·81 (2·60, 3·03); <0·001	2·81 (2·58, 3·05); <0·001
12 months postpartum	2·64 (2·41, 2·88); <0·001	2·60 (2·35, 2·87); <0·001
Between-women SD (log_2 (µg/l))	0·57	0·54
Within-woman SD (log_2 (µg/l))	0·65	0·65

Unadjusted or adjusted estimated ratio of geometric means (95% CI), p-value, were derived from linear mixed-effects regression models with a random effect for the individual-specific intercept. CI- Confidence Interval.

[a] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, location of enrolment clinic, history of fever, *Plasmodium* spp. infection and red blood cell polymorphisms (α^+-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

[b] The estimated geometric mean ferritin level at enrolment is derived with confounders set to mean values of continuous variables or the prevalence of categorical variables.

Supplementary Table 4: Associations between iron stores and enrolment confounders; and the outcome, moderate-to-severe anaemia, over the entire study period. Related to Table 2.			
		Moderate-to-severe anaemia [a]	
Variable		**Unadjusted odds ratio (95% CI); p-value**	**Adjusted odds ratio (95% CI); p-value**
Iron stores			
Ferritin (log_2(µg/l)) [b]		0·65 (0·59, 0·72); <0·001	-
Iron deficiency [c]	Replete	Reference	Reference
	Deficient	3·61 (2·56, 5·09); <0·001	3·86 (2·64, 5·65); <0·001
At enrolment			
Age (years)		1·00 (0·98, 1·03); 0·99	0·99 (0·96, 1·02); 0·55
Gravidity	Primigravida	Reference	Reference
	Multigravida	1·66 (1·20, 2·29); 0·002	1·93 (1·23, 3·01); 0·004
Smoking status	Never	Reference	Reference
	Current/ past	1·18 (0·89, 1·56); 0·26	1·22 (0·87, 1·72); 0·26
MUAC (cm)		0·92 (0·87, 0·96); <0·001	0·93 (0·87, 1·00); 0·04
	>23cm	Reference	-
	≤23cm	1·46 (0·97, 2·19); 0·07	-
Fever [d]	No	Reference	Reference
	Yes	1·20 (0·80, 1·82); 0·38	1·31 (0·81, 2·13); 0·27
Plasmodium spp. (PCR)	Negative	Reference	Reference
	Positive	2·75 (1·73, 4·37); <0·001	2·45 (1·40, 4·29); 0·002
α^+- thalassemia	Wildtype	Reference	Reference
	Het/Hom	2·69 (1·83, 3·94); <0·001	3·03 (1·87, 4·91); <0·001
CR1 deficiency	H/H	Reference	Reference
	H/L	0·88 (0·48, 1·61); 0·68	0·99 (0·50, 1·97); 0·99
	L/L	0·89 (0·49, 1·59); 0·68	1·00 (0·51, 1·95); 0·99
SAO	Normal	Reference	Reference
	SAO	1·35 (0·70, 2·57); 0·37	1·19 (0·59, 2·39); 0·63

Odds ratios were derived from logistic mixed-effects models with a random effect for the individual-specific intercept. Adjusted models included enrolment variables listed in the table and time (enrolment, birth, 6 months and 12 months postpartum). CI- Confidence Interval.

[a] Moderate-to-severe anaemia defined as haemoglobin <100g/l in pregnancy and haemoglobin <110g/l in the postpartum period.

[b] Ferritin transformed to log base-2 due to positively skewed distribution, thus the odds ratio represents the change associated with a two-fold increase in ferritin.

[c] Iron deficient: ferritin <15µg/l in pregnancy and postpartum; iron replete: ferritin ≥15µg/l & CRP≤10mg/l in pregnancy and ferritin ≥15µg/l & CRP≤5mg/l postpartum.

[d] Self-reported history of fever during the pregnancy prior to their first antenatal care appointment.

Het: heterozygous. Hom: homozygous. CR1: complement receptor 1; H/H: high CR1 expression; H/L: intermediate CR1 expression; L/L: low CR1 expression. SAO: Southeast Asian ovalocytosis.

Supplementary Table 5: Associations between enrolment exposures of interest, ferritin levels and iron deficiency over the entire study period. Related to Table 2.					
		Ferritin levels (µg/l)		**Iron deficiency ***	
		Unadjusted geometric mean ratio (95% CI); p-value	**Adjusted geometric mean ratio (95% CI); p-value**	**Unadjusted odds ratio (95% CI); p-value**	**Adjusted odds ratio (95% CI); p-value**
Gravidity	Primigravida	Reference	Reference	Reference	Reference
	Multigravida	-0·27 (-0·44, -0·10); 0·002	-0·43 (-0·64, -0·22); <0·001	1·74 (1·04, 2·90; 0·04	2·66 (1·41, 5·02); 0·002
Plasmodium spp. (PCR)	Negative	Reference	Reference	Reference	Reference
	Positive	0·31 (0·06, 0·55); 0·01	0·40 (0·13, 0·63); 0·003	0·50 (0·24, 1·04); 0·07	0·47 (0·22, 1·00); 0·05
α^{+}- thalassemia	Wildtype	Reference	Reference	Reference	Reference
	Het/Hom	-0·02 (-0·22, 0·18); 0·84	-0·08 (-0·29, 0·14); 0·49	1·30 (0·69, 2·43); 0·42	1·35 (0·71, 2·60); 0·36

Estimated mean ferritin differences and odds ratios were derived from linear and logistic mixed-effects models with a random effect for the individual-specific intercept. Adjusted models included gravidity, *Plasmodium* spp. infection, maternal age, smoking status, mid-upper arm circumference, birth clinic location, fever, genetic polymorphisms (α^{+}- thalassemia, complement receptor 1 deficiency and Southeast Asian ovalocytosis), and time (enrolment, birth, 6 months and 12 months postpartum).

* Iron deficiency in pregnancy defined as ferritin <15µg/l; iron replete was defined as ferritin ≥15µg/l and CRP ≤10mg/l. Iron deficiency postpartum defined as ferritin <15µg/l; iron replete was defined as ferritin ≥15µg/l and CRP ≤5mg/l.
Het: heterozygous. Hom: homozygous.

Supplementary Table 6: Effect modification of the associations between iron measurements and haemoglobin levels (g/l) over the entire study period. Related to Table 2.			
Potential effect modifier	**Enrolment iron store**	**Adjusted estimated mean difference (95% CI) †**	**Likelihood ratio test p-value**
***Plasmodium* spp. infection∫**			
Negative	Iron deficient * vs. replete	-7·84 (-9·99, -5·70)	0·57
Positive		-9·39 (-14·54, -3·25)	
Gravidity			
Primigravida	Iron deficient * vs. replete	-6·16 (-9·87, -2·46)	0·23
Multigravida		-8·62 (-10·87, -6·36)	
α^{+}-thalassemia genotype			
Wildtype	Iron deficient * vs. replete	-8·37 (-10·57, -6·18)	0·45
Heterozygous/Homozygous		-6·55 (-10·97, -2·12)	

Estimated mean differences were derived from multivariable linear mixed-effects models with random effects for the individual-specific intercept and interaction terms between the iron store and potential effect modifier. Likelihood ratio test p-values were derived from comparing the likelihood of the models with and without the interaction terms.

† Adjusted for age, mid-upper arm circumference, gravidity, smoking status, residence, history of fever, *Plasmodium* spp. infection and RBC polymorphisms (α^{+}-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

∫ *Plasmodium* spp. infection detected by PCR at enrolment.

* Women classified as iron deficient: ferritin <15μg/l; replete: ferritin ≥15μg/l and CRP≤10mg/l in pregnancy / CRP≤5mg/l postpartum.

Supplementary Table 7: Effect modification of the associations between iron measurements and anaemia over the entire study period. Related to Table 2.				
Potential effect modifier	**Enrolment iron store**	**Adjusted odds ratio** [†]	**(95% CI)**	**Likelihood ratio test p-value**
***Plasmodium* spp. infection**∫				
Negative	Iron deficient [*] vs. replete	4·46 (2·77, 7·43)		0·76
Positive		5·63 (1·29, 24·55)		
Gravidity				
Primigravida	Iron deficient [*] vs. replete	2·67 (1·14, 6·24)		0·14
Multigravida		5·30 (3·08, 9·12)		
α^+- thalassemia genotype				
Wildtype	Iron deficient [*] vs. replete	5·17 (3·03, 8·81)		0·15
Heterozygous/Homozygous		2·06 (0·63, 6·74)		

Odds ratios were derived from logistic mixed-effects models with random effects for the individual-specific intercept and interaction terms between the iron store and potential effect modifier. Likelihood ratio test p-values were derived from comparing the likelihood of the models with and without the interaction parameters.

Anaemia defined as haemoglobin <110g/l in pregnancy and <120g/l postpartum.

[†] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, residence, history of fever, *Plasmodium* spp. infection and RBC polymorphisms (α^+-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

∫ *Plasmodium* spp. infection detected by PCR at enrolment.

[*] Women classified as iron deficient: ferritin <15µg/l; replete: ferritin ≥15µg/l and CRP≤10mg/l in pregnancy / CRP≤5mg/l postpartum.

Supplementary Table 8: Time-varying contributions of iron deficiency to moderate-to-severe anaemia at enrolment, birth, 6 months postpartum and 12 months postpartum. Related to Table 3.			
	Evaluation time	**Moderate-to-severe anaemia adjusted OR [a] (95% CI); p-value**	**Population attributable fraction (95% CI)**
Iron deficiency [b]	Enrolment	3·04 (1·74, 5·33); <0·001	62% (37, 78)
	Birth	6·81 (2·23, 20·81); 0·001	84% (50, 95)
	6 months postpartum	4·06 (2·34, 7·03); <0·001	44% (22, 65)
	12 months postpartum	2·36 (1·22, 4·57); 0·01	30% (5, 57)

CI- Confidence Interval.

[a] Adjusted odds ratios for moderate-to-severe anaemia, were derived from multivariable logistic mixed-effects models with a random effect for the individual-specific intercept. Models included an interaction term included between exposure and time (enrolment, birth, 6 months postpartum and 12 months postpartum) and included age, mid-upper arm circumference, gravidity, smoking status, residence, fever and genetic polymorphisms.

Moderate-to-severe anaemia defined as haemoglobin <100g/l in pregnancy and haemoglobin <110g/l postpartum.

[b] Women classified as iron deficient: ferritin <15µg/l; replete: ferritin ≥15µg/l & CRP≤10mg/l in pregnancy and ferritin ≥15µg/l & CRP≤5mg/l postpartum.

Supplementary Table 9: Associations between lagged iron status and maternal haemoglobin levels (g/l) over the entire study period. Related to Table 2.		
	Unadjusted estimated mean Hb difference (95% CI); p-value	**Adjusted estimated mean Hb difference (95% CI); p-value** [a]
Lagged ferritin (log_2(µg/l)) [b]	1·33(0·57, 2·09); 0·001	0·31 (-0·64, 1·27); 0·52
Lagged Iron deficient vs. replete [c]	-2·91 (-5·98, 0·15); 0·06	1.79 (-2·82, 6·37); 0·45

Unadjusted and adjusted estimated mean differences were derived from linear mixed-effect models with a random effect for the individual-specific intercept.

[a] Included one of the iron deficiency and lagged counterparts; along with initial haemoglobin measure, time, age, mid-upper arm circumference (MUAC), gravidity, smoking status, residence, fever, *Plasmodium* spp. infection and red blood cell polymorphisms.

[b] Ferritin transformed to log base-2 due to positively skewed distribution, thus the coefficient represents the change associated with a two-fold increase in ferritin.

[c] Women classified as iron deficient: ferritin <15µg/l in pregnancy and postpartum. Women classified as iron replete: ferritin ≥15µg/l and CRP≤10mg/l in pregnancy and ferritin ≥15µg/l and CRP≤5mg/l postpartum.

Supplementary Table 10: Associations between lagged iron status and maternal anaemia over the entire study period. Related to Table 2.		
	Unadjusted anaemia odds ratio (95% CI); p-value	**Adjusted anaemia odds ratio (95% CI); p-value** [a]
Lagged ferritin (log_2(µg/l)) [b]	0·90 (0·78, 1·03); 0·12	1·09 (0·94, 1·27); 0·26
Lagged Iron deficient vs. replete [c]	1·12 (0·68, 1·85); 0·65	0·62 (0·28, 1·40); 0·25

Unadjusted and adjusted odds ratios were derived from logistic mixed-effect models with a random effect for the individual-specific intercept.

[a] Included one of the iron deficiency and lagged counterparts; along with initial anaemia status, time, age, mid-upper arm circumference (MUAC), gravidity, smoking status, residence, fever, *Plasmodium* spp. infection and red blood cell polymorphisms.

Anaemia defined as haemoglobin <110g/l in pregnancy and haemoglobin <120g/l in the postpartum period.

[b] Ferritin transformed to log base-2 due to positively skewed distribution, thus the odds ratio represents the change associated with a two-fold increase in ferritin.

[c] Women classified as iron deficient: ferritin <15µg/l in pregnancy and postpartum. Women classified as iron replete: ferritin ≥15µg/l and CRP≤10mg/l in pregnancy and ferritin ≥15µg/l and CRP≤5mg/l postpartum.

Supplementary Table 11: *P. falciparum* and *P. vivax* qPCR reagent and cycling conditions. Related to Method details in STAR Methods.			
µL/reaction		**Cycling parameters**	
Taqman master mix	5	50°C 2 minutes	
Forward primer	0.4	95°C 10 minutes	
Reverse primer	0.4	95°C 15 second	45 cycles
Probe	0.15	58°C 60 seconds	
DNase free water	1.05	40°C 60 seconds	
DNA	3		

P. falciparum: Plasmodium falciparum. P. vivax: Plasmodium vivax. qPCR: quantitative polymerase chain reaction. DNase: deoxyribonuclease. DNA: deoxyribonucleic acid.

Supplementary Table 12: Red blood cell polymorphism PCR reagent, cycling and gel conditions. Related to Method Details in STAR Methods.

Polymorphism	Reagent µL/reaction		Cycling parameters	Gel conditions
α^+- thalassemia	Taq polymerase	0.1	98°C 3 minutes 98°C 10 seconds 65°C 30 seconds 60°C 50 seconds 72°C 90 seconds (38 cycles [a]) 72°C 5 minutes	1.5% agarose gel 100V for 1.5 hours
	α2/3.7 Forward primer	0.4		
	α2 Reverse primer	0.1		
	3.7 Reverse primer	0.6		
	4.2 Forward primer	0.4		
	4.2 Reverse primer	0.4		
	PCR buffer	2		
	dNTPs	0.25		
	Betaine	2		
	DMSO	0.5		
	DNase free water	2.25		
	DNA	1		
CR1 deficiency (exon 22)	Taq polymerase	0.2	94°C 60 seconds 94°C 15 seconds 63°C 15 seconds 72°C 90 seconds (35 cycles) 72°C 10 minutes	2% agarose gel 80V for 1.5 hours
	Forward primer	0.2		
	Reverse primer	0.2		
	PCR buffer	1		
	dNTPs	0.2		
	$MgCl_2$	0.5		
	DNase free water	6.7		
	DNA	1		
CR1 deficiency (intron 27)	Taq polymerase	0.2	94°C 60 seconds 94°C 15 seconds 54°C 15 seconds 72°C 90 seconds (35 cycles) 72°C 10 minutes	2% agarose gel 80V for 1.5 hours
	Forward primer	0.2		
	Reverse primer	0.2		
	PCR buffer	1		
	dNTPs	0.2		
	$MgCl_2$	0.5		
	DNase free water	6.7		
	DNA	1		
SAO	Taq polymerase	0.2		

	Forward primer	0.2	95°C 2 minutes		
	Reverse primer	0.2	95°C 15 seconds	35 cycles	1% agarose gel
	PCR buffer	1	70°C 15 seconds		80V for 1.5 hours
	dNTPs	0.2	72°C 90 seconds		
	$MgCl_2$	0.5	72°C 10 minutes		
	DNase free water	6.7			
	DNA	1			

[a] Touchdown cycling used: temperature decreased by 0.5˚C/cycle for the first 8 cycles.

PCR: polymerase chain reaction. dNTP: deoxynucleoside triphosphates. DMSO: dimethyl sulfoxide. DNase: deoxyribonuclease. DNA: deoxyribonucleic acid.