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Iron deficiency in early pregnancy using serum ferritin and soluble transferrin receptor concentrations are associated with pregnancy and birth outcomes.

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ABSTRACT

Background: There are several biomarkers for measuring iron deficiency (ID) in pregnancy, but evidence of their prevalence in association with inflammation and adverse pregnancy outcomes is inconclusive.

Objectives: To describe the prevalence and determinants of ID in women in the first trimester of pregnancy and associations with pregnancy and birth outcomes.

Design: A record-linkage cohort study of archived serum samples of women attending first trimester screening and birth and hospital data to ascertain maternal characteristics and pregnancy outcomes. Sera were analysed for iron stores (ferritin; μg/L), tissue iron (soluble transferrin receptor, sTfR; nmol/L) and inflammatory (C-reactive protein, CRP; mg/L) biomarkers. Total body iron (TBI) was calculated from serum ferritin and sTfR concentrations. Multivariate logistic regression analyzed risk factors and pregnancy outcomes associated with ID using the definitions: serum ferritin <12 μg/L, TfR ≥21.0 nmol/L and TBI<0 mg/kg.

Results: Of 4,420 women, the prevalence of ID based on ferritin, sTfR and TBI was 19.6%, 15.3% and 15.7%, respectively. Risk factors of ID varied depending on which iron parameter was used and included maternal age <25 years, multiparity, socioeconomic disadvantage, high maternal body weight and inflammation. ID was associated with reduced risk of gestational diabetes (GDM) defined using serum ferritin and TBI, but not sTfR and increased risk of large for gestation age (LGA) infants defined using TBI only.

Conclusions: Nearly 1 in 5 Australian women begin pregnancy with ID. Evidence suggests excess maternal weight and inflammation play a role in the relationships between ID and GDM and LGA infants.

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BACKGROUND

It is well established worldwide that women are at increased risk of iron deficiency (ID) during pregnancy.[1] Iron requirements increase during the second half of pregnancy due to expansion of the red blood cell mass and transfer of increasing amounts of iron to both the growing fetus and placental structures.[1] It is estimated that body iron reserves of at least 500 mg are needed at the start of pregnancy to maintain adequate iron status throughout pregnancy.[2] Currently, the best measure of ID is serum ferritin concentration.[3] Pregnant women with serum ferritin concentrations less than 12µg/L are classified as iron deficient and having depleted iron stores.[4] The concentration of serum ferritin is maximum at 12 to 16 weeks of gestation and then falls with advancing gestation, due to haemodilution and mobilization of iron stores, reaching the lowest level in the third trimester.[5] Due to these normal physiological changes in serum ferritin concentration in later pregnancy, it has been suggested that the best time to detect maternal ID is in early pregnancy.[1]

There is however, an inherent difficulty in interpreting serum ferritin concentrations because ferritin takes part in the systemic acute phase response and can increase markedly in the presence of acute or chronic infection.[6] To aid the interpretation of ferritin concentration, concurrent measurement of an acute phase response protein, which is most commonly C-reactive protein (CRP), is recommended.[4] Measurement of circulating soluble transferrin receptor (sTfR) concentrations are also reported to be useful for defining ID because sTfR is not affected by infection.[4,7] sTfR suffers from a lack of standardization of the method and significant variation in the references ranges used.[8] Another method proposed for evaluating iron status within a population is the estimation of total body iron (TBI) on the basis of the ratio of sTfR to serum ferritin.[3]

There are limited data on the prevalence of ID using these multiple iron indices from large, population-based studies.[9] Most of the information on maternal iron status in

pregnancy has been limited to monitoring anemia using hemoglobin (Hb) concentrations.[10] Hb is relatively easy and inexpensive to collect and measure in large populations.[11] There is strong evidence that maternal Hb concentration below the current cut-off value for anemia during pregnancy (110 g/L) is associated with low birth weight and preterm birth.[12] Whether there is a risk associated with ID is less certain.[12] There is some evidence that maternal ID is associated with increased risk of preterm delivery[13] and low birth weight.[14] Based on several reviews and meta-analyses, the evidence that iron supplementation in pregnancy improves clinical outcomes for the mother or infant is still inconclusive.[9,12,15,16] Further research on the prevalence of maternal ID using serum ferritin concentrations in combination with other iron and inflammatory biomarkers and its effect on pregnancy and birth outcomes, independent of confounding factors is warranted. Therefore, the aims of this study are to examine the prevalence of ID in women in the first trimester of pregnancy using various measures of iron status of serum ferritin, sTfR, TBI and CRP; and assess risk factors of ID and associations between ID and pregnancy and birth outcomes.

SUBJECTS AND METHODS

Study population

This cohort study included a random sample of pregnant women who attended first trimester Down syndrome screening between January and October 2007 and had their results analysed by Pathology North, a state-wide public screening service in New South Wales, Australia. For this study, archived serum samples were thawed and analysed for serum ferritin (µg/L), sTfR (nmol/L) and CRP (mg/L) using commercial assays. Serum ferritin was measured using a solid phase direct sandwich ELISA method (Calbiotech, Inc, CA, USA) with an inter-assay coefficient of variation (CV) of 6.2%. sTfR concentrations were measured using an enzyme-linked immunosorbent assay (Quantikine IVD, Human sTfR Immunoassay,

R & D Systems, and Minneapolis, MN, USA) with an inter-assay CV of 6.4%. Total body iron (TBI; mg/kg) was calculated using the formula from Cook et al: - [log10 (sTfR/ferritin) – 2.8229]/0.1207.[3,17] Positive values of TBI represent storage iron and negative values indicate a deficient iron supply to peripheral tissues.[3,17] CRP was measured using the quantitative sandwich enzyme immunoassay technique (QUANTIKINE™, Minneapolis, USA) with an inter-assay CV of 13.3%. Three established definitions for ID were used: serum ferritin <12 μg/L,[4] TfR ≥21.0 nmol/L, according to manufacturer's guidelines,[18] and TBI<0 mg/kg.[3,19] In addition, serum ferritin concentration >70 μg/L was used to define iron replete women with adequate iron reserves to meet the estimated iron requirement of pregnancy.[2]

Data sources

The laboratory database provided information on maternal body weight and gestational age at the time of screening. Information from the laboratory database and biomarker concentrations analyzed using each woman's serum were linked to birth and hospital records to ascertain corresponding pregnancy and birth information. 'Birth data' were sourced from the NSW Perinatal Data Collection (PDC) and 'hospitalization data' from the NSW Admitted Patients Data Collection (APDC). The PDC is a statutory population-based collection of all births in NSW of at least 400-g birth weight or at least 20 weeks of gestation, and includes information on maternal characteristics, pregnancy, labour, delivery and infant outcomes at birth. The APDC is a census of all admissions in NSW public and private hospitals. Up to 50 diagnosis and procedures for each separation are coded according to the 10th revision of the International Classification of Diseases, Australian Modification (ICD-10-AM) and the Australian Classification of Health Interventions (ACHI).[20] The NSW Centre for Health Record Linkage (CHeReL) performed probabilistic record linkage between

the three datasets.[21] The CHeReL assesses the linkage quality for each study and for this study reported <5/1000 missed links and <2/1000 false positive links. Only de-identified data were provided to the researchers. The study was approved by the NSW Population and Health Services Research Ethics Committee.

Validation studies of the PDC and the APDC show excellent level of agreement with the hospital medical record and low rates of missing data.[22,23] Reporting in both datasets have high specificity (>99%) indicating few false positive reports. Only maternal, pregnancy and obstetric risk factors known to be reliably reported in birth and/or hospital data were included in the analysis. Explanatory variables included maternal age, parity, smoking during pregnancy and type of hospital (private versus public). Postcode was used to derive an indicator of socioeconomic status (SES). An Index of Relative Disadvantage produced by the Australian Bureau of Statistics was assigned to each postcode and women in the lowest 20th percentile were classified as disadvantaged.[24] Pregnancy outcomes included gestational diabetes mellitus (GDM), hypertensive disorders in pregnancy, postpartum hemorrhage (PPH), stillbirth, preterm birth, infant birthweight, small for gestational age (SGA), large for gestational age (LGA) and infant admission to neonatal intensive or special care unit. GDM was identified from hospital data (ICD10-AM codes: O24.4, O24.9) based on diagnosis by the attending clinician.[23,25,26] Hypertensive disorders in pregnancy included women with the onset of hypertension from 20 weeks including gestational hypertension, preeclampsia and eclampsia.[27] PPH was defined as blood loss of ≥500 mL following vaginal birth or ≥750 mL following caesarean section[28] and where a diagnosis of PPH was recorded in the medical record. Stillbirth (in utero fetal death after 20 weeks of gestation), preterm birth (<37 weeks gestation), infant birth weight and infant admission to a neonatal intensive or special care unit were identified from PDC data. SGA and LGA were defined respectively as those infants in

the <10th percentile and >90th percentile birth weight distribution for gestational age and infant sex.[29]

Statistical analysis

The prevalence of ID was calculated using established definition for ferritin, sTfR and TBI. The concentrations of these iron biomarkers including and then excluding women with elevated CRP (>95th centile, >5 mg/L) were described using 25th, 50th (median) and 75th percentiles. Univariate analysis were performed to examine the association between maternal characteristics and pregnancy and birth outcomes with each of the three definitions of ID, using ferritin, sTfR and TBI, using Chi-squared (X^2) test, or in the case of small cell sizes, the Fisher's exact test. Multivariate logistic regression analysis was performed to take into account any potential confounding with maternal age, gestational age at blood test, body weight, parity, smoking during pregnancy, private versus public hospital, low SES and CRP levels included in full model. Using backward stepwise selection, variables with least significance were progressively dropped from each model until all remaining covariates were statistically significant (2-tailed P<0.05). Variables not selected were then added back into the selected model, one at a time to assess whether they were confounders (i.e., changed the effect by more than 10%) and final model determined. Statistical analysis was performed using SAS for Windows version 9.3 (SAS Institute Inc, Carey, North Carolina).

RESULTS

Sample characteristics

A total of 4,420 women were included in the analysis after excluding 122 women with a twin pregnancy, medical abortion, infant with a major congenital anomaly or an undetectable ferritin and sTfR concentration. The mean (±SD) age of women was 32.2 (±4.9) years (8.0% < 25 years old), 36.5% were birthed in private hospitals and 35.8% of women

were classified as disadvantaged. Nearly half (51.5%) of the women were nulliparous and 5.8% smoked during pregnancy. At the time of testing, mean (±SD) gestational age was 12.0 (±1.0) weeks and 50.8% were 10-12 weeks of gestation. The mean (±SD) maternal body weight at the time of testing was 67.0 (±14.4) kg and maternal body weight in the 75th percentile or greater was defined as ≥73 kg.

Prevalence and risk factors of iron deficiency

The prevalence of ID based on ferritin, sTfR and TBI measures was 19.6%, 15.3% and 15.7%, respectively (Table 1). Only a small proportion of women (15.3%) were defined as ID using all three iron parameters. Of the 2,781 women with detectable CRP values, 2.7% had CRP levels greater than 5 mg/L, an indication of inflammation. When women with CRP>5 mg/L were excluded there were little to no changes in the prevalence of ID using serum ferritin, sTfR and TBI or to the 25th, 50th and 75th quartiles for these iron indices (Table 1). Excluding women with elevated CRP levels, only 11.4% of women (n=331/2,893) had serum ferritin >70 µg/, indicating adequate iron reserves to meet the increased iron requirements of pregnancy.

Descriptive statistics for maternal risk factors and pregnancy outcomes and univariate association with various measures of ID are presented in Table 2. After adjusting for important confounders in multivariate analyses, univariate association of maternal risk factors with ID as defined using serum ferritin, TBI and sTfR remained. Specifically, women with serum ferritin <12 ug/L were significantly more likely to be younger with maternal age <25 years (adjusted odds ratio (AOR): 2.24; 95% CI: 1.68, 2.95, P<0.001), of low SES (AOR: 1.30; 95% CI: 1.09, 1.55, P=0.004) and multiparous (AOR: 1.67; 95% CI: 1.40, 1.99, P<0.001). Using sTfR, multivariate analyses found that women with ID were more likely to

be multiparous (AOR: 1.41, 95% CI: 1.16, 1.71, P<0.001), of low SES (AOR: 1.37; 95% CI: 1.13, 1.67, P=0.002), have higher CRP levels (AOR: 1.40; 95% CI: 1.26, 1.55, p<0.0001), and less likely to smoke during pregnancy (AOR: 0.48; 95% CI: 0.31, 0.77, P=0.002). TBI defined ID was associated with maternal age <25 years (AOR: 2.18, 95% CI: 1.60, 2.90, P<0.001) and multiparous births (AOR: 1.52; 95% CI: 1.24, 1.87, P<0.001.

For pregnancy and birth outcomes, ID as defined using serum ferritin or TBI was significantly associated with decreased odds of GDM and increased odds of LGA infants (Table 2). In contrast, women with hypertensive disorders in pregnancy were more likely to high sTfR ID in early pregnancy (Table 2). After taking into account important confounders in the multivariate analyses, early pregnancy ID defined using serum ferritin or TBI remained significantly associated with reduced odds of GDM (AOR 0.43; 95% CI 0.23, 0.78 and AOR 0.39; 95% CI 0.20, 0.78, respectively) (Table 3). None of the other covariates were retained in these final models except for CRP levels which was positively associated with GDM for ID defined using serum ferritin (AOR 1.32; 95% CI 1.11, 1.57) and TBI (AOR 1.34; 95% CI 1.13, 1.59).

ID defined using TBI (AOR 1.38; 95% CI 1.03, 1.85) but not using serum ferritin (AOR 1.25; 95% CI 0.95, 1.65) remained significantly associated with increased odds of LGA infants. In the final model for ID defined using TBI and LGA, increased maternal weight (AOR 2.75; 95% CI 2.17, 3.48), multiparity (AOR 1.95; 95% CI 1.53, 2.48), and smoking during pregnancy (AOR 0.36; 95% CI 0.18, 0.72) remained significant factors associated with increased odds of LGA infants. Finally, for ID defined using sTfR, multivariate analyses found ID was no longer significantly associated with hypertensive

disorders in pregnancy (AOR: 1.20. 95% CI: 0.88, 1.88, P=0.18) or LGA infants (AOR: 0.86, 95% CI: 0.64, 1.16, P=0.32).

DISCUSSION

Results indicate that up to 1 in 5 Australian women enter pregnancy with ID and only 11% begin pregnancy with sufficient iron stores to meet the total estimated iron requirements of pregnancy. The prevalence and risk factors of ID varied depending on which iron parameter was used to define ID. Depleted iron stores, as defined by low serum ferritin occurred in 20% of women and were associated with being younger, multiparous, and more socioeconomically disadvantaged. The prevalence of ID using sTfR and TBI were both around 15% and associated with high maternal body weight, multiparity and inflammation. In terms of the consequences of maternal ID, this study found that women with first trimester ID were less likely to develop GDM and more likely to have LGA infants, based on the iron parameters serum ferritin and TBI, but not sTfR.

There are no data from Australian studies reporting on the prevalence of ID in pregnant women with which to compare our results. A recent USA study using data on 1171 pregnant women from the 1999–2006 National Health and Nutrition Examination Survey (NHANES)[10] reported the prevalence of first trimester (≤12 weeks) ID to be 7.3% for serum ferritin, 5.8% for sTfR, and 2.7% for TBI. Lower estimates of ID among American compared to Australian women may reflect differences in iron supplementation policies. All pregnant women in the USA are routinely advised to take iron supplements of 30-60 mg/d,[30] whereas, the policy in Australia[31] is to screen pregnant women for anemia and only treat those with iron deficiency anemia (IDA). It is therefore likely that fewer Australian compared to American women consume an iron supplement in the first trimester of pregnancy.

Like the current study, others have found differences in the prevalence and risk factors for ID depending on which iron measure is used to define ID.[10,32-35] Only 15.3% of women were defined as ID using all three definitions. This is not surprising given that different iron measures reflect a slightly different aspect of iron metabolism.[32] Ferritin concentrations reflect decreased storage iron but are insensitive to further change during severe ID or negative iron balance.[17] sTfR concentrations reflect functional tissue ID and generally begin to change only after iron stores (in the form of ferritin) are depleted.[17] sTfR is also elevated by ineffective erythropoiesis.[18] Concurrent measurement of sTfR substantially aids in the diagnosis of ID and allows calculation of TBI, which reflects iron status over a wider range of iron stores, from decreased storage iron to functional tissue ID.[17]

In this study, multivariate analyses found women with ID based on serum ferritin and TBI concentrations were more likely to be younger, multiparous and socioeconomically disadvantaged. These risk factors of ID have been reported previously in the NHANES study[10] and elsewhere.[35,36] Multiparity may reflect depleted iron supply with increasing pregnancies, while younger age and low SES are thought to reflect poorer diets and lower intake of dietary iron and supplements. For women with ID defined using sTfR, multiparity and low SES were also risk factors. However, these women were also more likely to be heavier, and have high CRP concentrations. There are several postulated explanations for the association between greater maternal weight, a marker of obesity and ID, including dilutional hypoferremia, poor dietary iron intake, increased iron requirements, and/or impaired iron absorption in obese individuals.[37] There is also recent evidence that obesity-related inflammation may play a central role through its regulation of hepcidin, such that iron absorption is reduced.[37]

In the present study, higher CRP concentrations in women with high sTfR suggests that greater body weight resulted in chronic immune activation, leading to alterations in iron homeostasis and impaired erythropoiesis.[38] sTfR only acts as a marker of erythropoiesis when iron stores are adequate.[31] The majority of women in this study with ID based on sTfR levels (70%) had adequate iron stores (serum ferritin levels ≥ 12 μg/L). These findings suggest that ID defined using sTfR (high sTfR concentrations) reflect impaired erythropoietin production as a result of an immune response by inflammatory cytokines[39] rather than inadequate iron nutrition.

In terms of pregnancy and birth outcomes, our study did not detect a significant association between ID and preterm birth or SGA infants. The previous literature on the association between ID and these pregnancy outcomes is inconsistent;[12,15,16] with some reporting no association between ID and preterm birth,[14] while others have found an association with low ferritin.[40,41] Inconsistencies in the literature may be explained by studies conducted in high- risk or different populations and/or settings and lack of adjustment for important confounders such as, body weight and low-grade inflammation, in analyses.

Women with ID defined using serum ferritin and TBI were less likely to develop subsequent GDM. These findings confirm those by Lao et al, who in a retrospective study of 242 pregnant women with ID found that women with iron deficiency anemia (IDA) were less likely to have GDM (AOR: 0.46; 95% CI: 0.23, 0.90) after adjusting for multiparity and BMI≥25 kg/m^2.[42] Women with IDA had significantly lower gestational weight gain throughout pregnancy, which the authors interpreted as suggestive of lower dietary energy and iron intakes. One of the limitations of the study by Lao et al. is the lack of data on

inflammatory biomarkers. In our study, increased CRP levels, suggestive of increased inflammation, were significantly associated with increased odds of GDM. GDM is increasingly being recognized as an inflammatory condition that involves unbalanced inflammatory cytokine production.[43] An important component of innate immunity during infection and inflammation is redistribution of iron, whereby iron is shifted from the circulation into cellular stores to decrease iron bioavailability to invading microorganisms.[38] Redistribution of iron from the circulation into cellular stores may explain why an association with GDM was only found for serum ferritin and TBI and not sTfR. It may be that being iron deficient offers some advantage in that less iron is available to invading pathogens . However, it is still uncertain whether elevated serum ferritin concentrations reflect excess iron or inflammation.[44-47] Further studies to elucidate this pathway are required.

The finding that first trimester ID defined using TBI was associated with increased risk of LGA is also consistent with Lao et al.[33] We found excessive maternal body weight but not CRP levels to be associated with LGA, suggesting that the previously stated explanations for the association between ID and obesity, poor dietary iron intake or impaired iron absorption in obese individuals may be contributing.[37] It could also be that CRP levels were not associated with having an LGA infant because they were measured early in pregnancy. It is possible that CRP levels may have changed later in pregnancy and showed evidence of inflammation. It is also possible that women in our study with first trimester ID were diagnosed at their first antenatal booking and recommended to take iron supplements. A recent systematic review and meta-analysis of iron supplement use in pregnancy found that a daily dose of iron was associated with a significant increase in birth weight by 15.1g (95% CI 6.0 to 24.2) (P for linear trend=0.005) and decrease in risk of low birth weight by 3% (relative risk 0.97; 95% CI: 0.95 to 0.98) for every 10 mg increase in dose/day (P for linear

trend<0.001).[15] However, without information on iron supplement use or the iron status of these women later in pregnancy, it is uncertain whether improved maternal iron status led to increased risk of LGA infants among these women.

Information on iron supplement use among pregnant women in Australia is limited.[48,49] Only one Australian study has examined iron supplementation in the 1st, 2nd and 3rd trimesters and reported use to be 14%, 13% and 27%, respectively.[49] Worldwide, there is no consensus regarding the optimum iron dose for supplementation during pregnancy, with variations between 30 to 120 mg/day.[50] A range of pregnancy supplements with iron alone or in combination with other minerals and vitamins are available on the market and each preparation varies in the amount of contained elemental iron (from 5 to 105 mg). Contemporary data on the number of supplements, timing, frequency and dose of iron consumed by pregnant women by gestational age is needed to ensure that some women are not consuming excessive amounts leading to higher infant birth weights than desired.

The strengths of this study are the large population-based cohort design with thorough measurement, high ascertainment and reporting of first trimester iron status and pregnancy and birth outcomes. Limitations include lack of data on maternal anemia, iron supplement use, maternal diet, and insulin and glucose concentrations.

In conclusion, results indicate that a significant proportion of women experience early pregnancy ID due to inadequate iron reserves. These findings reinforce the importance of routine screening of pregnant women for anemia and performing iron studies among those suspected of ID. More research is needed to understand how to best interpret information from multiple iron measurements taking into consideration the complex changes that occur in

these concentrations during pregnancy. An association between ID and decreased risk of GDM and increased risk of LGA for the iron parameters serum ferritin and TBI but not sTfR, supports the possible role of ferritin in an inflammatory response.[51-53] Further research examining the complex relationship between iron and the immune response is needed. And finally, while difficult to substantiate without information on iron supplement use, further research is needed to ensure excess iron supplement use is not contributing to undesired outcomes in women with increased body weight and other risk factors for LGA infants.

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Contribution to authorship

AK, NN, CLR, JM, VT conceived and designed the study; NN, CLR, JM, VT acquired data; AZK was responsible for the integrity of data and statistical analysis; AZK drafted the manuscript; and all authors approved the manuscript and critically reviewed the manuscript for important intellectual content.

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Declaration of Competing Interests

None of the authors have a conflict of interest to declare.

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Table 1
Description of median, 25th and 75th percentiles and proportion of iron deficient women based on first trimester serum ferritin, transferrin receptor and total body iron measurements for all women and for women without inflammation (n=4420).

	N	25th percentile	Median	75th percentile	Percent Deficient[1]
All women (n=4,420)					
Serum ferritin (μg/L)	3,795	14.2	25.1	42.7	19.6
Transferrin receptor (TfR; nmol/L)	4,406	12.2	15.1	18.7	15.3
Total body iron (TBI; mg/kg)	3,781	1.2	3.4	5.4	15.7
Women with CRP>5mg/L (n=2,502)					
Serum ferritin (μg/L)	3,603	14.2	25.4	42.8	19.7
Transferrin receptor (TfR; nmol/L)	3,884	12.2	15.2	18.7	15.5
Total body iron (TBI; mg/kg)	3,589	1.2	3.4	5.4	15.8

[1]Percent deficient defined as serum ferritin <12 μg/L; transferrin receptor ≥21.0 nmol/L and total body iron <0 mg/kg.

Table 2: Univariate analysis of maternal and pregnancy characteristics and pregnancy and birth outcomes by different definitions of maternal iron deficiency using serum ferritin, soluble transferrin receptor and total body iron concentrations.

	Serum ferritin (<12 µg/L)		**Soluble transferrin receptor (≥21 nmol/L)**		**Total body iron (<0 mg/kg)**	
	Deficient	**Replete**	**Deficient**	**Replete**	**Deficient**	**Replete**
	n=742 (19.6%)	n=3,053 (80.5%)	n=676 (15.3%)	n=3,730 (84.7%)	n=594 (15.7%)	n=3,187 (84.3%)
Maternal and pregnancy risk factors						
Maternal age ≤25 years	90 (13.0)**	185 (6.6)	46 (7.2)	280 (8.1)	70 (12.5)**	204 (6.9)
Gestational age ≤12weeks	198 (47.3)	965 (51.1)	204 (51.7)	1156 (50.7)	154 (46.0)	1006 (51.2)
Body weight ≥75th percentile (73 kg)	192 (30.1)	697 (26.8)	211 (37.0)**	805 (25.3)	170 (33.9)**	717 (26.3)
Multiparous	397 (57.2)**	1305 (46.2)	366 (57.1)**	1618 (46.9)	321 (57.4)**	1376 (46.7)
Smoking during pregnancy	45 (6.5)	158 (5.6)	23 (3.6)**	212 (6.2)	33 (5.9)	168 (5.7)
Private hospital maternity care	242 (32.6)*	1135 (37.2)	262 (38.8)	1343 (36.0)	189 (31.8)*	1181 (37.1)
Low socioeconomic status	305 (42.2)**	1031 (34.8)	278 (42.1)**	1245 (34.3)	266 (38.9)	1102 (35.7)
Inflammation (CRP>5 mg/L)	8 (1.1)**	89 (3.0)	36 (5.7)**	72 (2.2)	9 (1.6)	88 (2.8)
Pregnancy and birth outcomes						
Gestational diabetes	12 (1.6)**	117 (3.8)	27 (4.0)	120 (3.2)	9 (1.5)**	120 (3.8)
Hypertensive disorders in pregnancy	30 (4.0)	174 (5.7)	47 (7.0)*	190 (5.1)	24 (4.0)	179 (5.6)
Postpartum haemorrhage	20 (2.7)	120 (85.7)	27 (4.0)	136 (3.7)	19 (3.2)	121 (3.8)
Stillbirth	5 (0.7)	7 (0.2)	2 (0.3)	12 (0.3)	3 (0.5)	9 (0.3)
Preterm birth (<37 weeks)	28 (4.0)	112 (4.0)	31 (4.8)	131 (3.8)	23 (4.1)	117 (4.0)
Infant birthweight (percentiles)						
≤10%	46 (6.6)	213 (7.6)	53 (8.3)	254 (7.4)	35 (6.3)	224 (7.6)
11-90%	558 (80.4)	2321 (82.3)	514 (80.2)	2830 (82.2)	445 (79.6)	2422 (82.3)
>90%	90 (13.0)*	287 (10.2)	74 (11.5)	361 (10.5)	79 (14.1)**	298 (10.1)
Admitted to NICU or special care nursery	35 (15.6)	117 (14.7)	34 (16.7)	139 (14.1)	34 (18.2)	118 (14.2)

* P<0.05, **P<0.01: Association between maternal characteristics, pregnancy outcomes and iron deficiency status.

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Table 3
Multivariate analysis of pregnancy and birth outcomes by different definitions of maternal iron deficiency using serum ferritin, soluble transferrin receptor and total body iron concentrations.

	Serum ferritin (<12 µg/L)	**Soluble transferrin receptor (≥21 nmol/L)**	**Total body iron (<0 mg/kg)**
	OR (95% CI), P-value		OR (95% CI), P-value
Gestational diabetes			
Univariate	0.41 (0.23, 0.75)**	1.25 (0.82, 1.92)	0.39 (0.20, 0.78)**
Full model	0.41 (0.18, 0.89)*	Not required	0.34 (0.14, 0.84)*
Final model	0.43 (0.23, 0.78)**	Not required	0.38 (0.19, 0.76)**
Large for gestational age			
Univariate	1.32 (1.02, 1.69)*	1.12 (0.86, 1.45)	1.46 (1.12, 1.91)**
Full model	1.42 (1.01, 2.01)*	Not required	1.46 (1.02, 2.10)*
Final model	1.25 (0.95, 1.65)	Not required	1.38 (1.03, 1.85)*
Hypertensive disorders in pregnancy			
Univariate	0.70 (0.47, 1.04)	1.39 (1.00, 1.94)*	0.71 (0.46, 1.09)
Full model	Not required	1.14 (0.67, 1.93)	Not required
Final model	Not required	1.29 (0.88, 1.88)	Not required

*P<0.05, **P<0.01: Association between maternal characteristics, pregnancy outcomes and iron deficiency status
Full model: multivariate analysis adjusting for maternal age, gestational age at blood test, body weight, parity, smoking during pregnancy, private versus public healthcare, low SES and log CRP levels.