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High maternal serum ferritin in early pregnancy and risk of spontaneous preterm birth.

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ABSTRACT

Previous studies have reported inconsistent associations between maternal serum ferritin concentrations and risk of preterm birth. The aim of this study was to examine the association between iron biomarkers, including serum ferritin and risk of total, early and moderate-to-late spontaneous preterm birth (sPTB). This cohort study included women with singleton pregnancies who were attending first-trimester screening in New South Wales, Australia. sPTB births included births <37 weeks gestation following spontaneous labour or preterm premature rupture of the membranes (PPROM). Sera were analysed for iron: serum ferritin and sTfR; and inflammatory: C-reactive protein (CRP) biomarkers. Multivariate logistic regression evaluated the association between low and high iron levels and sPTB. Women with elevated serum ferritin concentrations were more likely to be older, nulliparous or have gestational diabetes. Multivariate analyses found increased odds of sPTB for women with elevated ferritin levels defined as >75th percentile (≥43 μg/L) (OR: 1.49, 95% CI: 1.06, 2.10) and >90th percentile (≥68 μg/L) (OR: 1.92, 95% CI: 1.25, 2.96). Increased odds of early and moderate-to late sPTB were associated with ferritin levels >90th (OR: 2.50, 95% CI: 1.32, 4.73) and >75th (OR: 1.56, 95% CI: 1.03, 2.37) percentiles, respectively. No association was found between sPTB, and elevated sTfR levels or iron deficiency. In conclusion, elevated early pregnancy maternal serum ferritin levels are associated with increased risk of sPTB from 34 weeks gestation. The usefulness of early pregnancy ferritin levels in identifying women at risk of sPTB warrants further investigation.

BACKGROUND

Preterm births continue to be the main cause of perinatal morbidity and mortality in developed countries, which increases the risk of neurocognitive and pulmonary deficits in surviving infants.[1] Spontaneous preterm birth (sPTB) of unknown aetiology accounts for 40-50% of all preterm deliveries,[1] with the remainder attributable to maternal or fetal indications in which labour is induced or the infant is delivered by pre-labour caesarean section.[2]

The aetiology of sPTB remains elusive.[2] There is a body of evidence which suggests that infection in pregnancy may be related to preterm birth;[3] however, the majority of randomised trials have not shown a significant reduction in preterm births following maternal administration of antibiotics.[4] Early pregnancy factors associated with sPTB remain an important area of inquiry for identifying at-risk women to study specific interventions and treatments.

The relationship between maternal iron status and risk of preterm delivery is uncertain.[5] Both low and elevated maternal iron has been associated with risk of preterm birth.[6] While some randomised trials of iron supplementation in pregnancy report a reduction in preterm births,[7] the most recent Cochrane[8]and systematic reviews[9] of intervention trials found no significant effect of iron supplementation in pregnancy on risk of preterm birth. In contrast, there are several observational studies which have found an association between elevated serum ferritin (a biomarker of iron stores) in the second trimester an increased risk of sPTB.[1; 10; 11; 12; 13; 14; 15] Potential mechanisms resulting in elevated ferritin being linked to sPTB include: intrauterine infection; failure of the maternal plasma volume to expand; and infection and inflammation.[16] Ferritin production is increased with infection and inflammation as part

of the acute phase response; therefore, interpretation of these studies is challenging. Results are conflicting with studies differing in their definition of sPTB and in the cut-off used for high ferritin (>50th, >75th, >90th centiles).[1; 10; 11; 12; 13; 14; 15] The majority of these studies do not adjust for confounding, such as age and parity and lacked information on inflammatory or iron biomarkers other than serum ferritin. Therefore the aim of the current study was to examine the association between iron biomarkers, including serum ferritin and risk of total, early and moderate-to-late spontaneous preterm birth (sPTB).

SUBJECTS AND METHODS

Design and study population

The study population included women with a singleton infant with a birth weight of at least 400 grams or at least 20 weeks gestation and who attended first trimester Down syndrome screening between January and October 2007 and had results analysed by Pathology North, a state-wide public screening service in New South Wales, Australia. Serum samples that were archived and stored at -80 ºC were thawed and used for subsequent biochemical analysis.

Biochemical analysis

Serum samples for this study were thawed and analysed for serum ferritin (µg/L), soluble transferrin receptor (sTfR; nmol/L) and C-reactive protein (CRP; mg/L) using commercial assays. Serum ferritin was measured using a solid phase direct sandwich ELISA method (Calbiotech, Inc, CA, USA) with an interassay CV of 6.2%. sTfR was measured using an enzyme-linked immunosorbent assay (Quantikine IVD, Human TfR Immunoassay, R & D Systems, Minneapolis, MN, USA) with an interassay coefficient of variation (CV) of 6.4%.

CRP was measured using the quantitative sandwich enzyme immunoassay technique (QUANTIKINE™, Minneapolis, USA) with an interassay CV of 13.3%.

Data sources

Laboratory records and the results of each woman's iron biomarker analyses were linked to electronic birth and hospital records sourced from the NSW Perinatal Data Collection (PDC) and the New South Wales Admitted Patients Data Collection (APDC), respectively to obtain pregnancy and birth information. The PDC is a statutory population-based collection of all births in NSW of at least 400 grams birth weight or at least 20 weeks of gestation, and includes information on maternal and infant characteristics, pregnancy, labour, delivery and infant characteristics at birth. The APDC is a census of all admissions in NSW public and private hospitals. Up to 50 diagnoses for each separation are coded according to the 10th revision of the International Classification of Diseases, Australian Modification (ICD-10-AM).[17] Validation studies of the PDC and the APDC show excellent level of agreement with the hospital medical record and low rates of missing data.[18; 19] Reporting in both datasets have high specificity (>99%) indicating few false positive reports. Only variables known to be reliably reported in birth and/or hospital data were included in the analysis. The NSW Centre for Health Record Linkage (CHeReL) performed probabilistic record linkage between the three datasets.[20] 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 (HREC/09/CIPHS/52).

Variables and definitions

The primary outcome was spontaneous preterm birth (sPTB) defined as births <37 weeks gestation after the onset of spontaneous labour or preterm premature rupture of membranes

(PPROM) and subdivided into early (<34 weeks) and moderate-to-late (34-36) preterm births.[2] Data on serum ferritin concentrations was examined continuously and as quartiles. Low ferritin was defined using the established definition for iron deficiency (serum ferritin <12 μg/L).[21] There is no standard cut-off for high ferritin, therefore commonly used cut-offs in the literature were assigned at the 50th, 75th and 90th percentiles.[1; 10; 11; 12; 13; 14; 15] As sTfR is an intercellular iron carrier protein, concentrations are inversely related to intracellular body iron concentrations. Data on TfR was examined continuously and also as iron deficient (TfR ≥21.0 nmol/L), according to manufacturer's guidelines[22] and as high iron. Again similar to ferritin, there are not standard cut-offs for high TfR, therefore cut-offs which corresponded to those used for ferritin were used for TfR (<50th, <25th and <10th percentiles).

Explanatory variables in the analysis included: maternal age, parity, gestational age at time of blood test, maternal body weight, country of birth, smoking during pregnancy, socioeconomic status, gestational diabetes, and hypertensive disorders in pregnancy. Electronic records from the laboratory database provided information on maternal body weight and gestational age at the time of screening. Postcode was used to derive an indicator of socioeconomic status (SES). An Index of Relative Disadvantage produced by the Australian Bureau of Statistics and categorized into quintiles.[23] Hospital data was used to identify PPROM and gestational diabetes mellitus (GDM) based on diagnosis by the attending clinician.[19; 24; 25] Hypertensive disorders in pregnancy included women with the onset of hypertension from 20 weeks including gestational hypertension, preeclampsia and eclampsia.[26] Missing data were infrequent with the following missing records excluded from analyses: maternal age (n= 16 records, 0.80%), smoking status (n= 11, 0.55%), country of birth (n= 29, 1.5%), and socioeconomic disadvantage (n= 12, 0.60%).

Statistical analysis

Maternal, pregnancy characteristics and other iron and inflammatory biomarkers were examined by serum ferritin quartiles and differences were assessed using the Chi-squared (X^2) test for categorical variables and the Kruskal-Wallis Test for continuous variables. When examined continuously, serum ferritin and CRP concentrations were log-transformed. Multivariate logistic regression analysis was performed to take into account any potential confounding with explanatory variables included in the full model. Separate models were performed examining serum ferritin as a continuous variable, as quartiles, as low ferritin (<12 µg/L) and as high ferritin using three cutoffs: >50th, >75th and >90th percentiles. These cut-offs for elevated serum ferritin were examined to allow comparison with the cut-offs used in previous studies.[1; 10; 11; 12; 13; 14; 15] Separate models were also performed examining the association between sPTB with sTfR treated as continuous variable and dichotomous variables for low and high sTfR.

Final models were determined using backward stepwise selection, with variables of least significance 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

A total of 2,254 women with spontaneous labour or PPROM were included in the analysis. The median (25th, 75th) serum ferritin concentration for the total study population was 25.4

μg/L (14.5, 42.8). The association between quartiles of serum ferritin concentrations and maternal and pregnancy characteristics are shown in **Table 1**.

Table 1
Maternal serum ferritin quartiles by maternal and pregnancy characteristics and biochemical indices in women who had a spontaneous onset of labour or preterm premature rupture of the membranes (PPROM).

	Serum ferritin quartiles				
	<15 µg/L	**15-24 µg/L**	**25-42 µg/L**	**≥43 µg/L**	
	n=580	**n=536**	**n=578**	**n=560**	
	N (%)	*N (%)*	*N (%)*	*N (%)*	**P value***
Maternal characteristics					
Maternal age, years					
<25	78 (13.6)	49 (9.2)	41 (7.2)	27 (4.9)	<0.0001
25-34	342 (59.5)	344 (64.5)	391 (68.5)	353 (63.8)	
≥35	155 (27.0)	140 (26.3)	139 (24.3)	173 (31.3)	
Country of birth					
Australia	373 (64.3)	343 (64.0)	362 (62.6)	344 (61.4)	0.88
New Zealand, North and South Americas	18 (3.10)	15 (2.8)	19 (3.3)	17 (3.0)	
Europe	46 (7.9)	30 (5.6)	43 (7.4)	38 (6.8)	
Middle East and Africa	26 (4.5)	32 (6.0)	19 (3.3)	29 (5.2)	
South and Southeast Asia	56 (9.7)	58 (10.8)	59 (10.2)	61 (10.9)	
Northeast Asia	41 (7.1)	41 (7.7)	54 (9.3)	47 (8.4)	
Other	20 (3.5)	17 (3.2)	22 (3.8)	24 (4.3)	
Maternal weight quintiles (kg)[1]					
<55	87 (17.7)	93 (19.7)	89 (18.0)	100 (20.1)	0.04
55-59	106 (21.5)	75 (15.9)	88 (17.8)	85 (17.1)	
60-65	102 (20.7)	127 (26.9)	116 (23.4)	96 (19.3)	
66-73	97 (19.7)	88 (18.6)	112 (22.6)	93 (18.7)	
≥74	101 (20.5)	90 (19.0)	90 (18.2)	123 (24.8)	
Smoking during pregnancy	39 (6.8)	31 (5.8)	44 (7.7)	35 (6.3)	0.64
Socioeconomic disadvantage quintiles					
1 (most disadvantage)	119 (20.5)	113 (21.2)	110 (19.3)	130 (23.3)	0.32
2	115 (19.8)	84 (15.7)	99 (17.4)	91 (16.3)	
3	133 (22.9)	126 (23.6)	116 (20.4)	107 (19.2)	

4 5 (least disadvantage)	102 (17.6) 111 (19.1)	111 (20.8) 100 (18.7)	115 (20.2) 130 (22.8)	116 (20.8) 114 (20.4)	
Pregnancy characteristics					
Nulliparous	268 (46.2)	292 (54.5)	315 (54.5)	340 (60.7)	<0.0001
Gestational age at blood sampling, weeks 9-10 11 12-14	 41 (12.1) 115 (34.0) 182 (53.9)	 36 (11.0) 125 (38.2) 166 (50.8)	 52 (14.2) 139 (37.9) 176 (48.0)	 64 (16.1) 145 (36.4) 189 (47.5)	 0.33
Gestational diabetes	2 (0.3)	17 (3.2)	18 (3.1)	17 (3.0)	0.003
Hypertensive disorders in pregnancy	23 (4.0)	24 (4.5)	17 (2.9)	25 (4.5)	0.51
Biochemical indices					
soluble transferrin receptor (sTfR), nmol/L Median (25^{th}, 75^{th} percentiles)	 15.6 (12.2, 19.5)	 14.5 (11.6, 18.3)	 15.0 (12.0, 18.0)	 15.3 (12.5, 18.4)	 0.01
C-reactive protein (CRP), mg/L Median (25^{th}, 75^{th} percentiles)	 0.7 (0.3, 1.4)	 0.7 (0.3, 1.4)	 0.8 (0.3, 1.6)	 0.8 (0.3,1.8)	 0.13

***** P values were determined by using the Kruskal-Wallis Test.

[1] Maternal body weight is collected by the healthcare professional referring women for Down Syndrome screening.

Serum ferritin concentrations increased with increasing maternal age and body weight. Nulliparity and GDM were associated with higher serum ferritin concentrations (**Table 1**). Compared to women with term births, women with a sPTB <37 weeks were more likely to be heavier (P=0.05) and have GDM (P=0.007) (**Table 2**). These women also had significantly higher median ferritin (25.3 vs. 26.5 µg/L, P=0.05) and CRP (0.7 vs. 1.0 mg/L, P=0.01) concentrations. There was no difference in sTfR concentrations among women with a sPTB versus term birth (P=0.61).

Univariate analysis found significant associations between elevated serum ferritin (defined >75th and >90th percentiles) and sPTB, but not sTfR concentrations (**Table 3**). No associations were found between low iron levels using either serum ferritin or sTfR. Multivariate analyses indicated increased odds of sPTB for serum ferritin concentrations >75th percentile (≥43 µg/L) and >90th percentile (≥68 µg/L) of 1.49 (95% CI: 1.06, 2.10) and 1.92 (95% CI: 1.25, 2.96), respectively (**Table 3**).

Univariate analyses examining the association between low and elevated serum ferritin and sTfR concentrations stratified by early and moderate-to-late sPTB are presented in **Supplementary Table 1**. For early sPTB, women with serum ferritin concentrations >90th percentile had a 2.54 increased odds of early sPTB (95% CI: 1.36, 4.76) and this association remained significant in the fully adjusted analyses (OR: 2.50, 95% CI: 1.32, 4.73).

Univariate analyses indicated increased odds for moderate-to-late sPTB for serum ferritin concentrations >75th and >90th percentiles (**Supplementary Table 1**). Multivariate analyses found women with serum ferritin concentrations>75th percentile had increased odds of moderate-to-late sPTB of 1.56 (95% CI: 1.03, 2.37).

Table 2
Maternal characteristics, pregnancy characteristics and biochemical indices in women who had a spontaneous onset of labour or preterm premature rupture of the membranes (PPROM) and delivered a preterm or term infant.

	Preterm birth (<37 weeks) N=175	**Term birth (≥37 weeks) N=2, 079**	
	N (%)	***N (%)***	**P-value***
Maternal age, years			
<25	16 (9.3)	179 (8.7)	0.36
25-34	118 (68.2)	1312 (63.7)	
≥35	39 (22.5)	568 (27.6)	
Nulliparous	105 (60.0)	1110 (53.4)	0.09
Country of birth			
Australia	111 (63.4)	1311 (63.1)	0.65
New Zealand, North and South Americas	6 (3.4)	63 (3.0)	
Europe	15 (8.6)	142 (6.8)	
Middle East and Africa	11 (6.3)	95 (4.6)	
South and Southeast Asia	16 (9.1)	218 (10.5)	
Northeast Asia	9 (5.1)	174 (8.4)	
Other	7 (4.0)	76 (3.7)	
Gestational age at blood sampling, weeks			
9-10	19 (15.3)	174 (13.3)	0.72
11	42 (33.9)	482 (36.9)	
12-14	63 (50.8)	650 (49.8)	
Maternal body weight quintiles (kg)			
<55	27 (17.9)	342 (18.9)	0.05
55-59	20 (13.3)	334 (18.5)	
60-65	38 (25.2)	403 (22.3)	
66-73	23 (15.2)	367 (20.3)	
≥74	43 (28.5)	361 (20.0)	
Smoking during pregnancy	14 (8.0)	135 (6.5)	0.27
Socioeconomic disadvantage quintiles			
1 (most disadvantage)	35 (20.0)	437 (21.1)	0.95
2	30 (17.1)	359 (17.4)	
3	40 (22.9)	442 (21.4)	
4	32 (18.3)	412 (19.9)	
5 (least disadvantage)	38 (21.7)	417 (20.2)	
Gestational diabetes	10 (5.7)	44 (2.1)	0.007
Hypertensive disorders in pregnancy	11 (6.3)	78 (3.8)	0.10
Serum ferritin, µg/L			
Median (25^{th}, 75^{th} percentiles)	26.5 (16.2, 52.3)	25.3 (14.3, 42.1)	0.05
soluble Transferrin receptor, nmol/L			
Median (25^{th}, 75^{th} percentiles)	15.2 (11.9, 19.0)	15.1 (12.1, 18.6)	0.61
C-reactive protein, mg/L			
Median (25^{th}, 75^{th} percentiles)	1.0 (0.4, 1.9)	0.7 (0.3, 1.5)	0.01

Table 3
First trimester serum ferritin and soluble transferrin receptor concentrations in women who had a spontaneous onset of labour or preterm premature rupture of the membranes (PPROM) and delivered a preterm versus a term infant.

	Preterm birth (<37 weeks)	**Term birth (≥37 weeks)**	**Unadjusted OR (95% CI)**	**Adjusted OR[1] (95% CI)**
	n (%)	***n (%)***		
Serum ferritin, µg/L Median (25^{th}, 75^{th} percentiles)	26.5 (16.2, 52.3)	25.3 (14.3, 42.1)	**1.24 (1.02, 1.50)**	1.17 (0.96, 1.43)
soluble transferrin receptor (sTfR), nmol/L Median (25^{th}, 75^{th} percentiles)	15.2 (11.9, 19.0)	15.1 (12.1, 18.6)	1.02 (0.99, 1.04)	1.01 (0.99, 1.04)
Elevated iron				
High serum ferritin levels, µg/L				
>50^{th} percentile (≥25)	95 (54.3)	1, 043 (50.2)	1.18 (0.87, 1.61)	--
>75^{th} percentile (≥43)	58 (33.1)	502 (24.2)	**1.56 (1.12, 2.17)**	**1.49 (1.06, 2.10)**
>90^{th} percentile (≥68)	30 (17.1)	193 (9.3)	**2.02 (1.33, 3.08)**	**1.92 (1.25, 2.96)**
Low sTfR levels, nmol/L				
<50^{th} percentile (≤15)	96 (54.9)	1202 (57.8)	0.89 (0.65, 1.21)	--
<25^{th} percentile (≤12)	50 (28.6)	663 (31.9)	0.85 (0.61, 1.20)	--
<10^{th} percentile (≤9)	24 (13.7)	221 (10.6)	1.34 (0.85, 2.10)	--
Iron deficiency				
Serum ferritin <12 µg/L	30 (17.1)	402 (19.3)	0.86 (0.57, 1.30)	--
sTfR≥21 nmol/L	29 (16.6)	318 (15.3)	1.10 (0.73, 1.67)	--

[1] Adjusted for maternal age, parity, gestational diabetes mellitus and C-reactive protein.
Empty cells indicate that adjusted analyses were not performed due to no association being found in univariate analyses.

SUPPLEMENTARY TABLE 1

First trimester serum ferritin and soluble transferrin receptor concentrations in women who had a spontaneous onset of labour or preterm premature rupture of the membranes (PPROM) and delivered an early or moderate-to-late preterm infant versus a term infant.

	Early preterm vs. Term			**Moderate-to-late preterm vs. Term**		
	Early preterm (<34 weeks) N=63	**Term (≥37 weeks) N=2079**	**Unadjusted odds ratio (95% CI)**	**Moderate-to-late preterm (34-36 weeks) N=112**	**Term (≥37 weeks) N=2079**	**Unadjusted odds ratio (95% CI)**
	n (%)	***n (%)***		***n (%)***	***n (%)***	
Serum ferritin, µg/L						
Median (25^{th}, 75^{th} percentiles)	26.4 (17.2, 47.4)	25.3 (14.3, 42.1)	1.28 (0.93, 1.75)	27.0 (15.4, 52.5)	25.3 (14.3, 42.1)	1.22 (0.96, 1.54)
soluble transferrin receptor, nmol/L						
Median (25^{th}, 75^{th} percentiles)	15.2 (11.5, 18.8)	15.1 (12.1, 18.6)	0.99 (0.94, 1.04)	15.0 (12.7, 19.2)	15.1 (12.1, 18.6)	**1.03 (1.00, 1.06)***
Elevated iron						
High serum ferritin levels, µg/L						
>50^{th} percentile (≥25)	34 (54.0)	1043 (50.2)	1.17 (0.70, 1.93)	61 (54.5)	1043 (50.2)	1.19 (0.81, 1.74)
>75^{th} percentile (≥43)	20 (31.8)	502 (24.2)	1.46 (0.85, 2.51)	38 (33.9)	502 (24.2)	**1.61 (1.08, 2.42)***
>90^{th} percentile (≥68)	13 (20.6)	193 (9.3)	**2.54 (1.36, 4.76)****	17 (15.2)	193 (9.3)	**1.75 (1.02, 299)***
Low sTfR levels, nmol/L						
<50^{th} percentile (≤15)	34 (54.0)	1202 (57.8)	0.85 (0.52, 1.41)	62 (55.4)	1202 (57.8)	0.90 (0.62, 1.33)
<25^{th} percentile (≤12)	21 (33.3)	663 (31.9)	1.07 (0.63, 1.82)	29 (25.9)	663 (31.9)	0.75 (0.48, 1.15)
<10^{th} percentile (≤9)	9 (14.3)	221 (10.6)	1.40 (0.68, 2.88)	15 (13.4)	221 (10.6)	1.30 (0.74, 2.28)
Iron deficiency						
Serum ferritin <12 µg/L	9 (14.3)	402 (19.3)	0.70 (0.34, 1.42)	21 (18.8)	402 (19.3)	0.96 (0.59, 1.57)
sTfR≥21 nmol/L	9 (14.3)	318 (15.3)	0.92 (0.45, 1.89)	20 (17.9)	318 (15.3)	1.20 (0.73, 1.98)

* P-value<0.05; **P-value <0.005; ***P-value <0.001

The association between serum ferritin concentrations >90th percentile did not reach statistical significance in the fully adjusted analyses (OR: 1.62, 95% CI: 0.93, 2.84).

sTfR concentrations by specific cut-offs were not associated with increased odds of early sPTB. The significant association between sTfR and moderate-to-late sPTB found in the univariate analysis (OR: 1.03, 95% CI: 1.00, 1.06) was no longer significant in the fully adjusted analysis (OR: 1.02; 95% CI: 0.99, 1.06).

DISCUSSION

Preterm birth is a major public health concern. This study found that early pregnancy serum ferritin concentrations are significantly elevated in pregnant women with subsequent spontaneous preterm labour or preterm premature rupture of membranes (PPROM). We also found that women with sPTB had significantly higher first trimester CRP concentrations and that there was no association between sTfR concentrations (a biomarker of iron supplied to tissues) and sPTB. These results suggest that serum ferritin levels are elevated as part of acute phase reaction and that the inflammatory process associated with sPTB is apparent from the first trimester of pregnancy.

Interestingly, this study found that greater maternal body weight and GDM were both independently associated with elevated ferritin levels and sPTB and included as confounders in the adjusted analyses. Both overweight and GDM are inflammatory conditions. While there is some evidence that maternal overweight and obesity during pregnancy is associated with increased risks of preterm birth,[27] the association between GDM and sPTB is inconsistent and controversial.[28]

Results from previous studies examining the association between elevated ferritin and preterm birth have been inconsistent.[1; 10; 11; 12; 13; 14; 15] Only one other study measured serum ferritin levels in the first trimester in a small sample of 30 cases and 90 controls and found no significant difference in the proportion of women with ferritin levels > 75th percentile in the early preterm vs. term delivery groups (36.7% vs. 25.6%, P = 0.251).[29] Thresholds for elevated serum ferritin have varied as either >50th, 75th or 90th percentiles.[1; 10; 11; 12; 13; 14; 15] In order to compare our study to previous findings, we examined all three of these thresholds. We found that serum ferritin levels >75th percentile (≥43 µg/L) were associated with increased odds of sPTB (<37 weeks) and the sub-category of moderate-to-late sPTB (34-36 weeks). However, only the higher threshold (>90th percentile) for serum ferritin levels (≥68 µg/L) was significantly associated with early sPTB in the current study. This is in agreement with a few studies which have found levels above 30 µg/L are associated with preterm birth.[11; 14] Inconsistent findings across studies may be related to differences in study populations and the severity of sPTB, reduced numbers of women in certain categories of the exposure and/or outcome and the types of confounders included in adjusted analyses. Previous studies have mostly been cross-sectional and limited to serum ferritin measurements later in pregnancy or at the time of birth.[10; 11; 13; 14; 15]

The usefulness of serum ferritin as a marker for sPTB is uncertain. Significant associations between elevated ferritin and sPTB were not found across all the thresholds or subtypes for sPTB (i.e. early versus moderate-to-late). Before routine screening of serum ferritin for the prediction of sPTB can be recommended, further research is needed to establish normative values, to understand the variability in ferritin as an early pregnancy biomarker and to determine its accuracy, reliability, interpretability, and feasibility. While the current study found a increased odds of sPTB in association with elevated ferritin and this does not

demonstrate that ferritin will function well as a diagnostic test unless ferritin is shown to be a manifestation of sPTB.[30]

It has been proposed that high ferritin concentrations may be a marker of clinical and subclinical vaginal infection which, in turn, may be triggers in the preterm delivery pathway.[10; 13] There is evidence of an association between vaginal infections and preterm delivery from longitudinal studies[31; 32] and a single randomised controlled trial which found second trimester antenatal screening and treatment for asymptomatic vaginal infections reduced the rate of preterm births by 50%.[33] The association between maternal iron status and vaginal infections in early pregnancy has not been well studied. Studies have observed an increase in various bacterial and non-bacterial infectious diseases in genetic iron overload diseases, such as hemochromatosis, where iron levels in serum are increased.[34] Given that a notable adaptations of bacterial growth is enhanced virulence secondary to acquiring a supply of iron from the host,[34] future studies are needed that examine the relationship between maternal iron status and early pregnancy infections.

Strengths of this study include a longitudinal design, one of the largest sample sizes to date with measurement of serum ferritin in the first trimester of pregnancy as well as other iron and inflammatory biomarkers, adjustment for confounders, and a sensitivity analysis using a range of cut-offs for elevated serum ferritin. Limitations include the lack of data on anaemia, other medical conditions which impact iron status, such as haemochromatosis, early pregnancy infections and placental iron biomarkers such as serum placental isoferritin.

In summary, results from this study provide further support of an association between elevated ferritin concentrations in early pregnancy and risk of sPTB. importantly, this

suggests that an inflammatory process which is detectable in early pregnancy may be a plausible biological mechanism for this association. Further research investigating the pathophysiological processes between elevated ferritin and sPTB, which consider the links between inflammation, obesity, GDM and vaginal infections are warranted.

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Contribution to authorship

AZK, CEC, CLR, JM, NN conceived and designed the study; CLR, JM, KP, VT, NN 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. All authors have approved the final article.

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