

## Research Article

# Serum Ferritin Assessment is Comparable with Hemoglobin to Predict Adverse Pregnancy Outcomes

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## Keywords

• Hemoglobin; Iron deficiency anemia; Pregnancy outcomes; Serum ferritin

## Abstract

**Introduction:** The purpose of this study was to investigate whether serum ferritin (SF) in second trimester and hemoglobin (Hb) in third trimester and can be used to predict adverse pregnancy outcomes.

**Methods:** Retrospective study was performed on 565 women who qualified for this study between April and June 2016. Statistical analyses for iron marker status and pregnancy outcomes were tested using the Student's t-test and the x<sup>2</sup>-test.

**Results:** Prevalence of iron deficiency anemia (IDA) at time of delivery was higher (21.8%) than second trimester (14.3%). Patients with IDA at time of delivery were more likely to have lower hemoglobin (Hb) concentrations and had iron depletion (ID) in second trimester of pregnancy compared to normal group. IDA group, but not ID group, tend to have lower BMI and were more likely to suffer post-partum hemorrhage. Higher prevalence of postpartum hemorrhage was found in patients with normal iron stores in second trimester of pregnancy. IDA and ID group had significantly lower birth weight. In contrast, the rates of patients with gestational diabetes mellitus (GDM) were found significantly higher in the control group compared with IDA group.

**Conclusion:** SF testing in second trimester of pregnancy is comparable with Hb as proxy to predict adverse pregnancy outcomes. Maternal IDA and ID correlated with adverse pregnancy outcomes, no matter how "mild" the anemia is. Regarding lower prevalence of GDM in the anemic group, this should not be interpreted as evidence against iron supplementation for correction of maternal anemia.

## INTRODUCTION

According to World Health Organization (WHO), around 2 billion people, amounting to over 30% of the world's population are anemic, although prevalence rates are variable because of differences in socioeconomic conditions, lifestyles, food habits, and rates of communicable and non-communicable diseases [1]. Iron deficiency is the most common cause of anemia and is the most widespread nutritional disorder in the world [2,3]. Pregnant women are a high risk group for iron deficiency anemia (IDA) given the iron requirements of the growing baby and those of gravid mother. IDA is associated with negative outcomes for both mother and infant. There are higher risk of infections and hemorrhage which lead to maternal mortality. There also increased risk of low birth weight of the newborn, premature birth, birth asphyxia, lower Apgar score, or less cognitive development of the child [4-7]. Early iron deficiency is characterized by diminished iron stores. This becomes iron depletion (ID) when iron stores are absent. Clinical diagnosis of ID through sampling of bone marrow to identify the absence of body iron stores is impractical in most cases. Serum ferritin (SF) concentrations are the most commonly deployed indicator for determining ID, and low SF concentrations reflect a state

of ID [8]. Many women of child-bearing age have depleted iron stores. Throughout Europe and other industrialized countries between 11 and 45% of women of fertile age have been reported to have serum SF concentrations ranging between 10 and 17 mg/l [9], indicating minimal or absent iron stores. ID has been associated with reduced exercise capacity, impaired temperature regulation and impaired cognitive function in animal and human studies [10]. IDA represents the late stage of iron depletion. It occurs as tissue and cellular stores are progressively exhausted. Although mild or moderate iron deficiency and or anemia may be asymptomatic, there are a variety of blood iron indicators that exist to detect early stages of disease. The most widely used method for diagnosis of IDA is estimation of Hb and SF because of the low cost and efficiency. The guidelines suggest ferritin in testing for non-anaemic women defined at risk i.e. those with previous anemia in pregnancy, multiparity (>3), consecutive pregnancy, vegetarians, teenage pregnancies, recent history of bleeding, and finally those where estimation of iron stores is necessary as significant blood loss may occur. SF is superior to transferrin saturation or serum iron in the diagnosis of IDA as its concentration correlates with bone marrow iron stores. Also, even with iron deficiency, serum iron can be high or normal if the pregnant female is on oral iron [11]. A complete blood count is a

much less expensive test and is routinely performed all over the world. In China, not all hospitals perform SF test routinely, because it is too expensive to be done in all cases. The purpose of our study was to investigate whether indicator of iron status in second trimester of pregnancy, which is SF in this study, combined with Hb, can be used to predict adverse pregnancy outcomes.

## MATERIAL AND METHODS

This study was a retrospective observational study. Approval was obtained from the institutional review board of Jiangsu Province Hospital. A manual search was performed through electronic medical records of Jiangsu Province Hospital from April 2016 to June 2016. Our inclusion criteria were: 1. Women who delivered in our hospital between April 2016 to June 2015, 2. Pregnant women who did first complete blood count (CBC) test in second trimester pregnancy, 3. CRP < 20 mg/L in second trimester pregnancy. Exclusion criteria include: 1. Multiple pregnancy, 2. Patient with Hb < 100 mg/dL at time of delivery, 3. Chronic inflammatory diseases (ie, lupus, inflammatory bowel disease), 4. Patient who lost to follow up. Among 565 women who met our study criteria, we divided into four groups: anemic group (IDA), non-anemic group (N), normal iron stores group (NIS), and iron depleted group (ID). IDA and N group were based on Hb test at third trimester of pregnancy, especially at time of delivery. ID and NID group were based on ferritin test in second trimester of pregnancy.

Maternal and neonatal aspects, also pregnancy outcomes, were analyzed for differences. Maternal characteristics include age, abortion history, gestational age, gravidity, parity, last delivery, blood pressure, and BMI. Maternal comorbidity and pregnancy outcomes include: gestational diabetes mellitus (GDM), caesarean scar pregnancy, hypertension, preterm delivery, premature rupture of membrane, mode of delivery, postpartum hemorrhage (PPH), and postpartum fever. Neonatal outcomes include fetal birth weight, APGAR score, and NICU transfer.

Gestational age was determined from the date of last menstrual period when reliable and sonographic confirmation carried out the first 20 weeks of gestation and/or the first trimester sonographic measurement of crown rump length. H is defined as a blood loss of greater than 500 mL after vaginal delivery or greater than 1,000 mL after a cesarean section. Detailed records of prenatal histories were gathered through electronic medical records. Anemia in pregnancy was defined by a Hb value less than 110 mg/dL based on World Health Organization's definition. In this study we only included patients with Hb 100-109 mg/dL. SF reflects iron stores and is the test to diagnose iron deficiency. We use a cutoff point of 20 ng/mL to classify the women in the study as having sufficient or insufficient iron deposits for pregnancy [12]. Iron depleted status defined as SF < 20 ng/mL and Hb  $\geq$  110 mg/dL. In this study women were classified into four groups: non-anemic group (Hb  $\geq$  110 mg/dL), anemic group (Hb 100-109 mg/dL), normal iron stores (SF > 20 ng/mL and Hb  $\geq$  110 mg/dL), and depleted iron stores (< 20 ng/mL and Hb  $\geq$  110 mg/dL)

### Statistical Analysis

The data were collected using Microsoft Excel 2007 (Window

XP; Microsoft Corp., Redmond, WA, USA). All statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc.). Data were expressed as the mean  $\pm$  standard deviation or rate (%). Statistical calculations and correlation were performed with the chi square test for categorical variables, Student's t-test for continuous variables. A two-sided p-value less than 0.05 was considered statistically significant.

## RESULTS

From total 565 women who qualified for this study, we identified that almost half of our patients had iron depleted status at second trimester where they first did the antenatal check-up ( $n = 277/565$ , 49%), but only approximately one fifth of them had anemic condition (Hb 100-109 mg/dL) at that time ( $n = 81/565$ , 14.3%). At time of delivery, we identified 123 (21.80%) women with anemic condition and 442 (78.20%) women with normal Hb level.

Table (1) showed correlation between Hb level at time of delivery with iron marker status in second trimester of pregnancy. Patients who developed anemic condition at time of delivery were more likely to have lower Hb concentrations ( $114.92 \pm 7.51$  vs  $119.88 \pm 7.93$ ,  $p < 0.05$ ) and had iron depleted status in second trimester of pregnancy (64.2% vs 44.8%,  $p < 0.05$ ) compared to normal group.

Table (2) showed maternal characteristics between four groups which were similar, with no significant differences in maternal age, history of abortion, gestational age at delivery, gravidity, systolic blood pressure, and diastolic blood pressure ( $p > 0.05$ ). There were significant differences in terms of parity ( $0.25 \pm 0.43$  vs  $0.37 \pm 0.51$ ,  $p < 0.05$ ) and time since last delivery ( $1.49 \pm 3.02$  vs  $2.20 \pm 3.16$ ,  $p < 0.05$ ), between N-IDA group, also NIS-ID group ( $0.23 \pm 0.40$  vs  $0.32 \pm 0.47$ ,  $p < 0.05$ ;  $1.35 \pm 2.80$  vs  $1.95 \pm 3.31$ ,  $p < 0.05$ ). N group had higher BMI ( $24.31 \pm 2.84$  vs  $23.69 \pm 2.96$ ,  $p < 0.05$ ) compared to IDA group. In contrast, there were no significant difference between maternal BMI when ferritin test (NF-ID group) were used ( $p > 0.05$ ).

Maternal comorbidity and pregnancy outcomes are shown in Table (3). There were no significant difference between patients with history of caesarean section and hypertensive disorders within N-IDA group and NIS-ID group ( $p > 0.05$ ). In contrast, the rates of patients with gestational diabetes mellitus were found significantly higher in the N group compared to IDA group (27.8% vs 16.3%,  $p < 0.05$ ), which didn't occur in NIS-ID group (26.04% vs 24.55%,  $p > 0.05$ ). Regarding pregnancy outcomes, pregnant women in IDA group were more likely to suffer post-partum hemorrhage compared to N group (13.8% vs 5.4%,  $p < 0.05$ ). When ferritin test were used, pregnant women with normal iron stores were more likely to suffer post-partum hemorrhage than pregnant women with iron depleted status (9.38% vs 5.05%,  $p < 0.05$ ). No significant differences between rates of preterm delivery, PROM, mode of delivery, and postpartum fever between four groups ( $p > 0.05$ ).

From the aspect of neonatal outcomes (Table 4), infants in IDA group had a significantly lower birth weight compared with N group ( $3466.28 \pm 429.54$  vs  $3378.08 \pm 417.61$ ,  $p < 0.05$ ), as well as infants from ID group which showed significantly lower birth weight compared to mother with NIS group ( $3449.65 \pm 418.20$

**Table 1:** Correlation between Hb level at time of delivery with iron marker status in second trimester of pregnancy.

Iron status, mean ± SD or n (%)	N (n=442)	IDA (n=123)	p
Hb, mg/dL	119.88 ± 7.93	114.92 ± 7.51	0.00
Hematocrite	0.07 ± 0.33	0.05 ± 0.14	0.51
Iron depleted	198 (44.80)	79 (64.20)	0.00
Transferrin, g/L	3.82 ± 1.70	3.99 ± 0.67	0.28

**Abbreviations:** HB, Hemoglobin; IDA, Iron Deficiency Anemia; N, Normal Group; SD, Standard Deviation

**Table 2:** Maternal characteristics between N, IDA, NIS, and ID group.

Mean ± SD or n (%)	N (n=442)	IDA (n=123)	p	NIS (n=288)	ID (n=277)	p
Age, y	29.38 ± 4.13	28.74 ± 4.19	0.13	29.23 ± 4.10	29.25 ± 4.21	0.95
Abortion history	159 (36)	42 (34.10)	0.70	99(34.40)	102(36.80)	0.54
GA, w	39.35 ± 1.31	39.37 ± 1.24	0.86	39.35 ± 1.37	39.36 ± 1.22	0.93
G	1.80 ± 1.07	1.95 ± 1.18	0.18	1.76 ± 1.01	1.91 ± 1.17	0.09
P	0.25 ± 0.43	0.37 ± 0.51	0.01	0.23 ± 0.40	0.32 ± 0.47	0.02
LD, y	1.49 ± 3.02	2.20 ± 3.16	0.02	1.35 ± 2.80	1.95 ± 3.31	0.02
SBP, mmHg	115.49 ± 9.33	114.56 ± 9.77	0.33	115.80 ± 9.63	114.75 ± 9.21	0.18
DBP, mmHg	68.64 ± 8.51	67.83 ± 8.32	0.34	68.64 ± 8.37	68.30 ± 8.60	0.62
BMI, kg/m <sup>2</sup>	24.31 ± 2.84	23.69 ± 2.96	0.03	24.16 ± 3.00	24.19 ± 2.76	0.89

**Abbreviations:** BMI: Body Mass Index; DBP: Diastolic Blood Pressure; G: Gravidity; GA: Gestational Age; ID: Iron Deficiency; IDA: Iron Deficiency Anemia; LD: Last Delivery; N: Normal; NIS: Normal Iron Store; P: Parity; SBP: Systolic Blood Pressure; SD: Standard Deviation.

**Table 3:** Maternal comorbidity and pregnancy outcomes between N, IDA, NIS, and ID group.

n (%)	N (n=442)	IDA (n=123)	p	NIS (n=288)	ID (n=277)	p
GDM	123( 27.8)	20 (16.3)	0.00	75(26.04)	68(24.55)	0.68
Caesarean scar pregnancy	36 (8.1)	14 (11.4)	0.26	25(8.68)	25(9.03)	0.88
Hypertension	12 (2.7)	5 (4.1)	0.43	7(2.43)	10(3.61)	0.41
Preterm	13 (2.9)	1 (0.8)	0.17	7(2.43)	7(2.53)	0.94
PROM	89 (20.1)	31 (25.2)	0.22	61(21.18)	59(21.30)	0.97
MOD						
Normal	308 (69.7)	81 (65.9)	0.63	196(68.01)	193(69.68)	0.55
CS	134 (30.3)	42 (34.1)		92(31.20)	84(30.32)	
PPH	24 (5.4)	17 (13.8)	0.00	27(9.38)	14(5.05)	0.04
Postpartum fever	29 (6.6)	5 (4.1)	0.30	19(6.60)	15(5.42)	0.55

**Abbreviations:** CS: Caesarean Section; GDM: Gestational Diabetes Mellitus; ID: Iron Depletion; IDA: Iron Deficiency Anemia; MOD: Mode of Delivery; N: Normal; NIS: Normal Iron Store; PPH: Post-Partum Hemorrhage; PROM: Premature Rupture Of Membrane.

vs 3346.91 ± 419.11,  $p < 0.05$ ). In the other hand, no significant differences were observed in terms of APGAR score, amniotic staining fluid rate, and NICU transfer rate between N-IDA group and NIS-ID group ( $p > 0.05$ ).

## DISCUSSION

The present study assessed, SF (iron depleted or non-depleted) in second trimester versus Hb (anemic or non-anemic) in third trimester, in order to investigate their correlation with adverse pregnancy outcomes. As far as we are aware, there are no studies in humans on the effect of second trimester gestational depleted iron stores without anemia on adverse pregnancy outcomes. Anemia in pregnancy is a worldwide problem. WHO/CDC technical guidance based on systematic review evidence

showed that in the absence of infections, SF or serum transferrin receptor (sTfr) in combination with Hb provides the best approach to measuring iron status. However, sTfr is not widely available or used in clinical practice in many countries [13]. Our study includes only Hb and SF in order to investigate their importance in development of anemic condition especially in late pregnancy. The SF measure identifies a deficient iron status earlier than other biomarkers, such as Transferrin saturation and Hb, identifying without error the subjects without iron stores as it does not have false negatives [14]. Because SF increases not only with the iron content of the organism but also with acute or chronic inflammation, malignancy or liver disease [15], pregnant women with these conditions were excluded from this analysis. The WHO recommends that pregnant women take daily oral

**Table 4:** Neonatal outcomes between N, IDA, NIS, and ID group.

Means ± SD or n (%)	N (n=442)	IDA (n=123)	p	NIS (n=288)	ID (n=277)	p
Fetal birth weight, g	3466.28 ± 429.54	3378.08 ± 417.61	0.04	3449.65 ± 418.20	3346.91 ± 419.11	0.00
APGAR 1'	9.98 ± 0.15	9.91 ± 0.53	0.16	9.91 ± 0.57	9.94 ± .34	0.53
APGAR 5'	9.99 ± 0.09	9.95 ± 0.30	0.16	9.96 ± 0.30	9.96 ± 0.24	0.94
Amniotic fluid staining	77(17.4)	16(13)	0.24	50(17.40)	43(15.50)	0.55
NICU transfer	31(7)	3(2.4)	0.059	16(5.6)	18(6.5)	0.64

**Abbreviations:** ID: Iron Deficiency; IDA: Iron Deficiency Anemia; N: Normal; NICU: Neonatal Intensive Care Unit; NIS: Normal Iron Store; SD: Standard Deviation.

iron-folic acid (IFA) supplementation as part of antenatal care to reduce the risk of low birth weight, maternal anemia, and ID [16]. According to regulations of our hospital, all pregnant women are supplemented with multiple micronutrient supplementation containing 60 mg of iron. Only pregnant women with Hb 100-109 mg/dL were included in this analysis based on our hypothesis that no additional iron supplementation needed for "these" Hb level. An adequate iron status during pregnancy implies body iron reserves of at least 500mg or SF concentrations higher than 70ug/L at conception, but only 15% to 20% of women have reserves of such magnitude [7].

Our study showed that the prevalence of pregnant women with IDA was higher among third trimester (21.8%) than second trimester (14.3%). Our study also showed that patients who developed anemic condition at time of delivery were more likely to have lower Hb concentrations and had iron depleted status in second trimester of pregnancy compared to normal group ( $p < 0.05$ ). In our hospital, ferritin test only be done once, which is for pregnant women who decide to deliver in our hospital, usually in second trimester. Based on the result, ferritin test can be done more frequently in order to prevent iron depletion "fall" into IDA. This suggests Hb and SF can be used as a proxy to predict development of iron deficiency anemia later in the third trimester of pregnancy, especially at the time of delivery.

Regarding maternal characteristics, our study showed that IDA group tends to have lower BMI compared to normal group. Our findings were similar with the one reported by Rasmussen, S et al. [17], Research conducted by Charles, A.M et al. showed that pregnant women with a BMI of 25-29 were 60% less likely to be anemic than those with a BMI less than 25 [18]. Anemia is considered one of the main nutritional deficiency disorders affecting a large fraction of the population not only in developing but also in developed countries. Lack of education about the importance of intake of balanced and iron-rich diet contribute to it. Obstetricians have an important role to play by making women aware of the importance of a balanced diet, because it would lower the risk of the women having low body weight when they begin to have children, thus making them less likely to become anemic [19]. However, we observed no significant differences in BMI between iron depleted and normal iron store group. SF is an acute phase reactant and is potentially higher in any infective or inflammatory process. Previous studies have concluded that overweight and obese people are in a state of an ongoing subclinical inflammation that can secondarily lead to more catastrophic events like iron deficiency [20,21]. Study by Khan, A et al. found that people with BMI  $\geq 30$  kg/m<sup>2</sup> had significant

higher ferritin level than BMI 18-25kg/m<sup>2</sup> [22]. The mean BMI pregnant women in our study were still in normal range (NIS vs ID group:  $24.16 \pm 3.00$  vs  $24.19 \pm 2.76$ ). Also, there was no classification of high ferritin group in this study. This explained why there's no correlation between BMI and iron stores in our study.

In contrast, we found that pregnant women with IDA were significantly having lower prevalence of GDM. Our findings suggest that the decreased prevalence of GDM in women with IDA is likely to be consequent to the combined effects of iron deficiency, which also reflected nutritional deficiency in general and reduced gestational weight gain. Analysis on 736 pregnant women conducted by Lao and Ho, LF also showed that the likelihood of GDM is significantly reduced with maternal IDA [23]. Based on iron stores, no significant differences between prevalence of GDM from pregnant women with normal iron stores and iron-depleted status. SF is postulated to be involved in diabetogenesis as a marker of iron stores. Several studies have shown that excess deposition of iron, indicated by elevated ferritin levels, may cause elevated insulin levels by suppressing hepatic glucose production in the liver, interfering with insulin synthesis and secretion in the pancreas, and decreasing glucose uptake in the muscle [24-26]. Those accumulations may lead to insulin resistance and beta cell function impairment and result in the development of type 2 diabetes. In prospective cohort study of 1456 healthy pregnant women conducted by Chen, X et al., found that women who developed GDM had a higher concentration of serum ferritin than women who did not develop GDM [27]. Again, because there were no classification of high ferritin group on this study, we found no correlation between iron stores and prevalence of GDM.

Our study found that IDA significantly increased prevalence of PPH. These results showed even "slight" decreased in Hb level could lead to maternal morbidity. In contrast, pregnant women with normal iron store at second trimester of pregnancy were more likely to suffer postpartum hemorrhage. This result could be related with our sample which weren't large enough. Prospective study on 467 low risk mothers by Lao, et al., found no correlation between postpartum hemorrhage and SF level at gestational age 28-30 weeks [28].

No significant correlation was found between IDA and preterm birth. The possible explanation is that we only included patient with Hb level between 100-109 mg/dL into our study, which we believed not enough to reach significance. Our findings consistent with cohort study of 160.700 patients by Zhang, Q et al., although they used different cut-off point (Hb < 10 g/dL)

[29]. Study by Marti, A et al., however, showed that maternal anemia was found to be significantly associated with prematurity [30]. Large retrospective analysis of 96,066 deliveries conducted by Drukker, L et al., also showed that anemia at birth was significantly associated with preterm delivery [31].

In this study we found no correlation between iron depleted status with preterm birth. In contrast, study by Bhutta, A.T et al., have shown preterm infants born from iron depleted mother are associated with short- and long-term adverse health consequences including an increased risk of infant mortality, as well as increased risk of cognitive dysfunction and CVD later in life [32]. Iron supplementation is necessary to prevent iron deficiency in preterm infants. Preterm infants certainly require more iron, both in absolute amount and on a per kilogram bodyweight basis, than full-term infants because their iron stores are lower at birth and their total body iron content has to increase at a greater rate postnatally [33].

Regarding neonatal outcomes, as expected neonatal in the anemic group had statistically significant lower birth weight compared to control group ( $p < 0.05$ ). A retrospective population-based study of 153,396 deliveries by Levy A et al., also showed significant association between maternal anemia and rates of low birth weight [34]. Low iron status in second trimester of pregnancy were found to be associated with low birth weight, which is consistent with study by Scholl, TO [35].

Limitation of this study is that the time of anemia diagnoses were recorded but not the true onset of anemia in the prenatal maternity logbook. SF test is not a routine in our hospital, so no follow up were documented especially in pregnant women with low iron store. The other limitation of this study is its retrospective nature, and our findings should be confirmed with larger prospective studies.

## CONCLUSION

Iron deficiency during pregnancy continues to be a common clinical problem and is one of the most prevalent nutritional deficits both in the industrial and developing countries. Based on results of our study, we believe that SF and Hb testing in second and third trimester can be used as proxy to predict development of iron deficiency anemia later in third trimester of pregnancy, especially at the time of delivery. SF can be done frequently in third trimester as follow up in pregnant women who had low iron store. We realized that Hb was the most effective and cost-friendly test to detect IDA, so we recommend SF test in high risk pregnant women. Once again, as stated in the literature, our study showed that maternal IDA correlated with adverse pregnancy outcomes, included lower birth weight and higher rate of post-partum hemorrhage, no matter how "mild" the anemia is. We believe that maintaining a healthy body weight, and frequently visiting an antenatal clinic, will help to lower the prevalence of anemia. Although in this study there were negative correlation between iron depleted status and postpartum hemorrhage, SF remains an important indicator of iron status. Starting pregnancy with good iron status can pre-empt adverse pregnancy outcomes. Regarding lower prevalence of GDM in the anemic group, this should not be interpreted as evidence against iron supplementation for correction of maternal anemia, the importance of which is indisputable.

## AUTHOR CONTRIBUTIONS

LZS: corresponding author, manuscript editing. IC: data collection, analysis, writing manuscript, manuscript editing.

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