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ORIGINAL ARTICLE

Prospective and Longitudinal Study of Iron Metabolism Indicators During Normal Pregnancy in Chinese Women

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SUMMARY

Background: **The aim of this study was to investigate the changes in selected iron metabolism parameters during different periods of normal pregnancy and to establish reference values for these parameters related to gestational age.**
Methods: **Primipara women with a singleton pregnancy at 10 - 14 weeks of gestation were selected from women who attended the prenatal clinic at West China Second University Hospital from September 2016 to August 2017. The participants comprised 207 healthy Chinese women with a normal pregnancy. Blood samples were obtained at 10 - 14, 20 - 24, and 30 - 34 weeks of gestation, and at 6 - 12 weeks postpartum. The following parameters were measured: Ret-He, hs-CRP, serum ferritin (SF), sTfR, serum iron (SI), Tf, TIBC, and transferrin saturation (TS).**
Results: **The respective reference values for healthy pregnant women in the three trimesters of pregnancy and at 6 - 12 weeks postpartum were: Ret-He: 29.8 - 39.6, 29.2 - 40.8, 26.2 - 41.0, and 26.8 - 38.5 pg; SF: 14 - 133, 7 - 130, 5 - 110, and 10 - 140 ng/mL; sTfR: 0.4 - 2.0, 1.2 - 3.0, 2.3 - 5.2, and 0.3 - 2.4 mg/L; SI: 6.0 - 31.0, 3.7 - 30.3, 4.7 - 49.4, and 4.5 - 26.8 µmol/L; Tf: 2.0 - 3.7, 2.2 - 4.4, 2.3 - 5.3, and 1.8 - 3.7 g/L; TIBC: 40 - 84, 52 - 100, 54 - 120, and 43 - 83 µmol/L; TS: 12 - 54%, 10 - 56%, 5 - 65%, and 10 - 52%.**
Conclusions: **Significant changes in iron parameters during pregnancy and postpartum were noted. It is important to learn these alterations and establish reference values to help obstetricians make clinical decisions.**
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KEY WORDS

iron deficiency, iron test, longitudinal study, normal pregnancy, reference values

INTRODUCTION

Iron is essential for the human body, and maintenance of an adequate body iron balance is crucial [1]. The average adult body contains 2 - 4 g of iron, with more than 80% present in the hemoglobin (Hb) content of red blood cells (RBCs). While iron requirements increase significantly during pregnancy (nearly 10 times), iron deficiency (the most common nutritional deficiency worldwide), low iron stores, and low iron intake during pregnancy not only cause anemia, associated with weakness, fatigue, reduced cognitive performance, and diminished immune response, but may also increase the

risk of delivery complications. Recent research indicates that smoking affects the level of hepcidin, which may lead to subclinical iron deficiency and chronic hypoxia in mothers and fetuses. Perinatal iron deficiency can lead to maternal and (or) neonatal hypothyroxinemia, which impairs early brain development prior to any neonatal brain iron depletion, and increases and perinatal maternal and neonatal mortality [2-4]. In addition, high-speed rail during pregnancy will also affect the health of pregnant women. High hemoglobin and ferritin levels increase the risk of GMD by more than 50% and more than two-fold, respectively [5].

Diagnosis of iron depletion or iron deficiency without anemia is particularly challenging in pregnant women. Conventional laboratory tests for iron status include hematological indices such as Hb, hematocrit, and mean cell volume (MCV), and biochemical markers such as serum ferritin (SF), serum iron (SI), transferrin (Tf), transferrin saturation (TS), and serum soluble transferrin receptor (sTfR). Iron is vital for the synthesis of Hb and myoglobin as well as the function of a wide range of important iron-dependent enzymes. However, Hb and myoglobin are not suitable for iron status assessment, especially during pregnancy. There is a broad overlap between the Hb distributions in subjects with and without iron deficiency [1]. It is well known that pregnancy reflects a special physiological stage of the female body and that existing iron reference values in nonpregnant women cannot be used to evaluate the iron status of pregnant women. Biochemical markers for iron metabolism are affected by pregnancy-specific alterations in serum proteins, such as hemodilution, acute-phase responses, accelerated erythropoiesis, or effects of pregnancy hormones on protein synthesis [6]. In addition, a few studies have estimated iron status by reticulocyte indices. Therefore, earlier, reliable, noninvasive, and cost-effective indicators of iron deficiency that are routinely available in most clinical laboratories would be more practical in clinical settings.

The primary objective of the present study was to clarify the iron status during pregnancy at specified periods using conventional measurements and reticulocyte indices in healthy Chinese pregnant women. This prospective, sequential, longitudinal study investigating the changes in these iron-related parameters aimed to improve pregnancy care by determining reference values by gestational week in healthy Chinese women. It was also desired that the study findings would have implications for clinical practice.

MATERIALS AND METHODS

Study design and participants

The study selected 207 primipara women between the 10th and 14th weeks of pregnancy who attended the prenatal clinic at West China Second University Hospital from September 2016 to August 2017.The participants were included consecutively and fulfilled the following inclusion criteria: primigravida, singleton pregnancy, gestational age of 10 - 14 weeks at first visit. The women were evaluated during each trimester of pregnancy (10 - 14 weeks, 20 - 24 weeks, and 30 - 34 weeks) as well as at 6 - 12 weeks postpartum.

The exclusion criteria were as follows: body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) > 30 or < 18; smoking; history of diseases such as diabetes mellitus, hypertension, liver disease, kidney disease, or diseases of the hematologic or metabolic system; multiple pregnancy; anemia during pregnancy (Hb < 110 g/L in first trimester; Hb < 105 g/L in second trimester; Hb < 110 g/L in third trimester); blood pressure higher than 140/90 mmHg; proteinuria; presence of intercurrent disease or obstetric complications (preeclampsia or eclampsia, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, placenta previa); preterm delivery or post-term pregnancy (< 37 or > 42 weeks of gestation at delivery); hs-CRP > 5 mg/L; abnormalities of the reproductive tract during pregnancy or between 12 and 16 weeks postpartum; or intrapartum hemorrhage (> 500 mL for vaginal delivery; >1000 mL for cesarean delivery).The amount of bleeding included amniotic fluid, since it was difficult to separate them accurately. The amount of bleeding during vaginal delivery is determined by the increase in gauze weight used throughout the delivery process. While the amount of bleeding during cesarean section was determined based on the volume of blood suctioned off during the operation and the increase in the weight of the gauze and packs used from the time of the skin incision to the time of wound closure [7]. Fetal growth disorders, mean Apgar score less than 8, and abnormal birth weight (< 2,500 or > 4,000 g) were also excluded. Gestational age was based on the first day of the last menstrual period and, if the pregnant woman was uncertain about the date of the last menstrual period, the ultrasound estimate was used.

We also designed a questionnaire that covered age, weight, height, race, place of residence (urban or rural area), educational level, income, previous health status, history of menorrhea, history, family history (with attention to congenital diseases, communicable diseases, and communicable diseases related to the participant), smoking history, and dietary intake of folate, iron, and vitamins. The mothers were assisted in completion of the questionnaire by nurses who ensured that all questions were answered. All women gave birth at our hospital. Data for maternal background factors (age, BMI, smoking) were obtained through the questionnaire. Pregnancy outcomes were acquired from the participants' hospital records. All involved investigators were trained to use uniform criteria and procedures. All participants provided signed informed consent. The investigation was approved by the Ethics Committee of West China Second University Hospital, Sichuan University and carried out according to the principles of the Declaration of Helsinki.

Fasting venous blood samples were taken after a

30-minute rest in the sitting position. Medical technicians obtained whole venous blood samples in vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) using standard techniques. All laboratory samples were analyzed within 4 hours after collection. All tests were performed in the Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, with appropriate calibrators, reagents, and quality control processes that followed the Westgard Rules. The indicated department was authenticated according to the ISO15189 laboratory quality management systems and the College of American Pathologists.

Measurements of biochemical indicators

The iron status of the pregnant women was evaluated by the following serum biochemical indicators: SF, sTfR, SI, Tf, total iron-binding capacity (TIBC), and TS. High-sensitivity C-reactive protein (hs-CRP), albumin, and prealbumin were also analyzed to assess the maternal stress state and nutritional state. The samples were collected into vacutainer tubes containing a separating gel. All serum biochemical indicators were measured in a Hitachi 7600-010 autoanalyzer (Hitachi Diagnosis, Tokyo, Japan). SF and hs-CRP were measured by an immunoturbidimetric assay (Ferritin, Randox Laboratories Ltd., Antrim, United Kingdom; Ultrasensitive CRP Kit; Orion Diagnostica, Espoo, Finland). SI was evaluated using an assay based on the Ferro Zine reaction (Iron Reagent Fe, Sysmex Co., Kobe, Japan), and Tf was measured by an immunoprecipitation assay (Transferrin, Randox Laboratories Ltd., Antrim, United Kingdom). TS was calculated as the ratio of SI to TIBC, expressed as a percentage. TIBC was calculated from the sum of the measured unsaturated iron-binding capacity and the measured SI. sTfR was measured by an automated immunoturbidimetric method (IDeAsTfR-IT; Orion Diagnostica). sTfR was previously reported to be a sensitive indicator of iron deficiency [8,9]. It was not stimulated by an acute-phase response. However, it has not been validated during pregnancy and postpartum in women.

Measurements of hematological indices

Routine complete blood cell counts and RBC indices were measured with a Sysmex XE-2100 electronic counter (Sysmex Co., Kobe, Japan). Hb, MCV, white blood cell count, and reticulocyte hemoglobin (Ret-He) were examined. The modern hematological analyzer with introduction of automated flow cytometry was able to provide new reticulocyte indices (Ret-He and index Ret-Y) without additional blood requirements or technical intervention, and these indices reflect the actual state of marrow erythroid activity and are not influenced by factors affecting traditional biochemical markers [10]. The samples were collected into vacutainer tubes containing ethylenediaminetetraacetic acid-dipotassium salt (EDTA-K2). In the reticulocyte channel of the Sysmex XE-2100, staining with a polymethine dye specific for RNA/DNA was analyzed by flow cytometry using a semiconductor laser. A two-dimensional distribution of forward-scattered light and fluorescence was presented as a scattergram indicating mature erythrocytes and reticulocytes. The appropriate calibrator, reagent kits, and quality control processes were provided by Sysmex Corporation.

Statistical analysis

All data analyses were conducted using Statistical Package for Social Sciences software, version 13.0 (SPSS, Chicago, IL, USA). The normality of the laboratory variables was analyzed by a Kolmogorov-Smirnov test with the Lilliefors significance correction. Because most variables did not have a normal distribution, a Mann-Whitney *U* test was used to analyze differences in the data. A Mann-Whitney *U* analysis, known as the Wilcoxon rank-sum test, was used to assess differences in age within each group. Two-group independent-sample *t*-tests were used to assess the data in each group. The results were considered significant for values of $p < 0.05$. Student's *t*-test, the χ^2 test, and Fisher's exact test were used to assess the statistical significance of differences in relevant variable distributions between the groups. Reference ranges (2.5th, 50th, and 97.5th percentiles) with a 95% confidence interval were calculated for each test and gestational period.

RESULTS

Study population

Of the 207 participants recruited, 12 dropped out of the follow-up study, 2 left the study because of abortion, 6 transferred to other hospitals, and 5 withdrew. A further 49 women were excluded according to the exclusion criteria (first trimester Hb < 110 g/L; second trimester Hb < 105 g/L; hs-CRP > 5 mg/L; thyroid disease; hypertension; pruritus gravidarum; gestational diabetes mellitus; intrahepatic cholestasis of pregnancy; MCV < 89 fL; MCH < 29 pg). Thus, 133 women had a complete uncomplicated pregnancy, vaginal delivery (bleeding < 500 mL) or uterine-incision delivery (bleeding < 1,000 mL), and normal postpartum period (Table 1).

According to the definitions for the health status of pregnant women during pregnancy, there were 133 pregnant women with a mean age of 30.4 years and mean BMI of 20.4 kg/m^2. Regarding the definitions of neonatal outcomes, the mean gestational age at delivery was 271 days and the mean birth weight was 3,397.9 g. The clinical characteristics of the normal pregnant women are shown in Table 1 and the general clinical data of their newborns in Table 2.

Figure 1 shows box plots for SF, sTfR, SI, Tf, TIBC, TS, and Ret-He in the three trimesters and postpartum after outlier assessment. The concentrations and 95% reference values (95% RV; 2.5th, 50th, and 97.5th percentiles) of the selected indicators (SF, sTfR, SI, Tf, TIBC, TS, and Ret-He) in different trimesters of preg-

Table 1. Characteristic of the study participants.

Health pregnancy women (n = 133)	
Age (years)	30.4 ± 4.2
Pre-pregnancy BMI [1] (kg/m^2)	20.4 ± 2.3
10 - 14 weeks gestation systolic pressure (mmHg)	109.6 ± 8.2
10 - 14 weeks gestation diastolic pressure (mmHg)	67.9 ± 8.4
Mean days in 10 - 14 weeks gestation (ds)	94.3 ± 9.6
20 - 24 weeks gestation systolic pressure (mmHg)	109.2 ± 6.9
20 - 24 weeks gestation diastolic pressure (mmHg)	71.2 ± 7.8
Mean days in 20 - 24 weeks gestation (ds)	159.6 ± 15.4
30 - 34 weeks gestation systolic pressure (mmHg)	112.22 ± 8.5
30 - 34 weeks gestation diastolic pressure (mmHg)	71.3 ± 7.6
Mean days in 30 - 34 weeks gestation (ds)	225.4 ± 12.9
Gestational age (ds)	271 ± 7.2
6 - 12 weeks postpartum systolic pressure (mmHg)	106.2 ± 8.6
6 - 12 weeks postpartum diastolic pressure (mmHg)	70.6 ± 7.9
Weight of the placenta (g)	521 ± 89
Bleeding during childbirth (mL)[2]	278 ± 166

Data are given as mean ± SD. [1] - BMI, body mass index, [2] - Bleeding was measured by a visual method.

Table 2. Characteristics of the healthy neonates.

Health neonate (n = 133)	
Birth weight (g)	3,397.9 ± 409.1
Apgar score 1st minute	9.8 ± 0.5
Apgar score 3rd minute	9.9 ± 0.3
Apgar score 5th minute	10.0 ± 0.0

Data are given as mean ± SD.

nancy and postpartum are presented in Table 3 [11]. The means, standard deviations, medians, and 2.5th and 97.5th percentiles for all chosen indicators according to trimester are also shown in Table 3.

Regarding the trend for SF, its concentration began to decrease in the second trimester, reached its lowest point in the third trimester, and finally approached the first trimester level. Trends similar to the changes in SF concentration were seen in SI and TS. In contrast, the sTfR concentration began to rise in the first trimester, reached its highest point in the third trimester, and then decreased postpartum to approach the first trimester level. Similar trends to sTfR for changes in concentration were seen in Tf and TIBC. The Ret-He concentration was highest in the second trimester, began to decrease in the third trimester, and reached its lowest point postpartum.

The Mann-Whitney *U* test revealed significant differences in some indices (SF, sTfR, and SI) between two trimesters and postpartum ($p < 0.05$). There was no significant difference between the first trimester and the second trimester in the Ret-He concentration ($p = 0.65$). TIBC and TS showed no significant difference between the first trimester and postpartum ($p = 0.63$ and $p = 0.71$, respectively).

DISCUSSION

In adult women, iron deficiency is predominantly the result of iron losses with menstruation and pregnancy in excess of the amounts of bioavailable iron in the diet

Table 3. Gestational age-specific reference intervals.

Test, unit	Normal value [#] (nonpregnant state)	First trimester 10 - 14 weeks (n = 133)	Second trimester 20 - 24 weeks (n = 133)	Third trimester 30 - 34 weeks (n = 133)	Postpartum 6 - 12 weeks (n = 133)
Ret-He (pg)	> 28 [&]	29.8 - 39.6 [a]	29.2 - 40.8 [a]	26.2 - 41.0 [a]	26.8 - 38.5 [a]
[*] SF (ng/mL)	12 - 120	14 - 133 [b]	7 - 130 [b]	5 - 110 [b]	10 - 140 [b]
[*] sTfR (mg/L)	0.9 - 2.3	0.4 - 2.0 [b]	1.2 - 3.0 [b]	2.3 - 5.2 [b]	0.3 - 2.4 [b]
[*] SI (µmol/L)	6.0 - 30.0	6.0 - 31.0 [b]	3.7 - 30.3 [b]	4.7 - 49.4 [b]	4.5 - 26.8 [b]
[*] Tf (g/L)	1.9 - 3.8	2.0 - 3.7 [a]	2.2 - 4.4 [a]	2.3 - 5.3 [a]	1.8 - 3.7 [a]
TIBC (µmol/L)	40 - 77	40 - 84 [a]	52 - 100 [a]	54 - 120 [a]	43 - 83 [a]
TS (%)	20 - 55	12 - 54 [b]	10 - 56 [b]	5 - 65 [b]	10 - 52 [b]

Ret-He - reticulocyte hemoglobin, SF - serum ferritin, sTfR - serum soluble transferrin receptor, SI - serum iron, Tf - transferrin, TIBC - total iron-binding capacity, TS - transferrin saturation.
[#] - Reference intervals were defined by the 2.5th and 97.5th percentiles with a 95% confidence interval. Nonpregnant normal values are listed according to the recommendations of our local medical laboratory (Department of Laboratory Medicine, West China Second University Hospital, Sichuan University). [&] - There was no reference value for Ret-He in our medical laboratory. This value was cited from Mast [10], [*] - There was a significant difference between the data in the four groups of pregnant women during pregnancy, [a] - Data showed a normal distribution, [b] - Data showed a skewed distribution.

[12]. Advanced pregnancy and some eating habits are factors that deteriorate iron status. Regular consumption of fish and legumes, rarely drinking coffee, and milk consumption during the intervals between food intake are conditions that optimize iron status during pregnancy [13]. Therefore, diagnosis and prevention of iron deficiency is important in pregnancy. However, there is no current policy on iron supplementation for pregnant women. The decision on this supplementation remains solely based on Hb measurements in maternity care units. Although we use the CDC recommended criteria for anemia [14] at specific stages of pregnancy, development of iron deficiency anemia usually takes a long time. Staining of a bone marrow aspirate for hemosiderin is considered the gold standard for iron deficiency. However, the test is invasive and not suitable for pregnancy screening purposes.

The present results showed that ferritin levels decreased with increasing gestational periods, reached the nadir concentration at the third trimester, and then increased moderately toward postpartum, in accordance with previous findings [15]. As pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most iron accumulation occurring during the third trimester [16]. In healthy subjects, the generally accepted cutoff level for SF, below which iron stores are considered depleted, is < 15 ng/mL; in pregnancy, van den Broek et al. [11] recommended a cutoff level of 30 mg/L. However, a low SF level does not imply functional iron deficiency. It is well established that SF measurement is the preferred method for detecting depleted iron stores. Nevertheless, SF is of limited usefulness during pregnancy because it diminishes late in pregnancy, even when bone marrow iron is present [17], as noted in the study by van den Broek et al. [11]. The reference ranges specific to pregnancy obtained in the present study werein accordance with previous findings [18]. Our results for reference ranges of SF suggest a potential use in pregnant women without major medical problems and adverse pregnancy outcomes.

It has long been known that inflammation can mimic some aspects of iron deficiency by impairing the utilization of existing iron stores for RBC production and inducing iron-sequestration syndromes and low SI. The iron-related indicator SF is widely recognized as an acute-phase reactant and a marker of acute and chronic inflammation and is nonspecifically elevated in a variety of inflammatory conditions [19]. Therefore, we measured SF in combination with hs-CRP in the present study. Hs-CRP is used as a marker of inflammation, even in chronic inflammation. As ferritin is also an acute-phase reactant, it is often elevated in disease conditions. In this study, a normal hs-CRP was used to exclude elevated ferritin caused by acute-phase reactions, and the cutoff value was defined as no more than 5 mg/L to minimize the impact of acute-phase reactions. To assess the iron status, we use a combination of measurements (hs-CRP, ferritin, and sTfR) in our hospital. Identified in 1986, sTfR was shown to be a single polypeptide chain with a molecular mass of 84.9 kDa [20]. It can be detected in serum, and its concentration is closely related to erythroid TfR turnover. It is controlled by bone marrow erythropoietic activity and iron status [21]. In other words, the prime determinants of the sTfR concentration are cellular iron demand and erythroid proliferation rate [22]. sTfR is examined because erythroblasts in the bone marrow increase their presentation of membrane TfR under iron deficiency. Circulating sTfR is a soluble form of the membrane re-

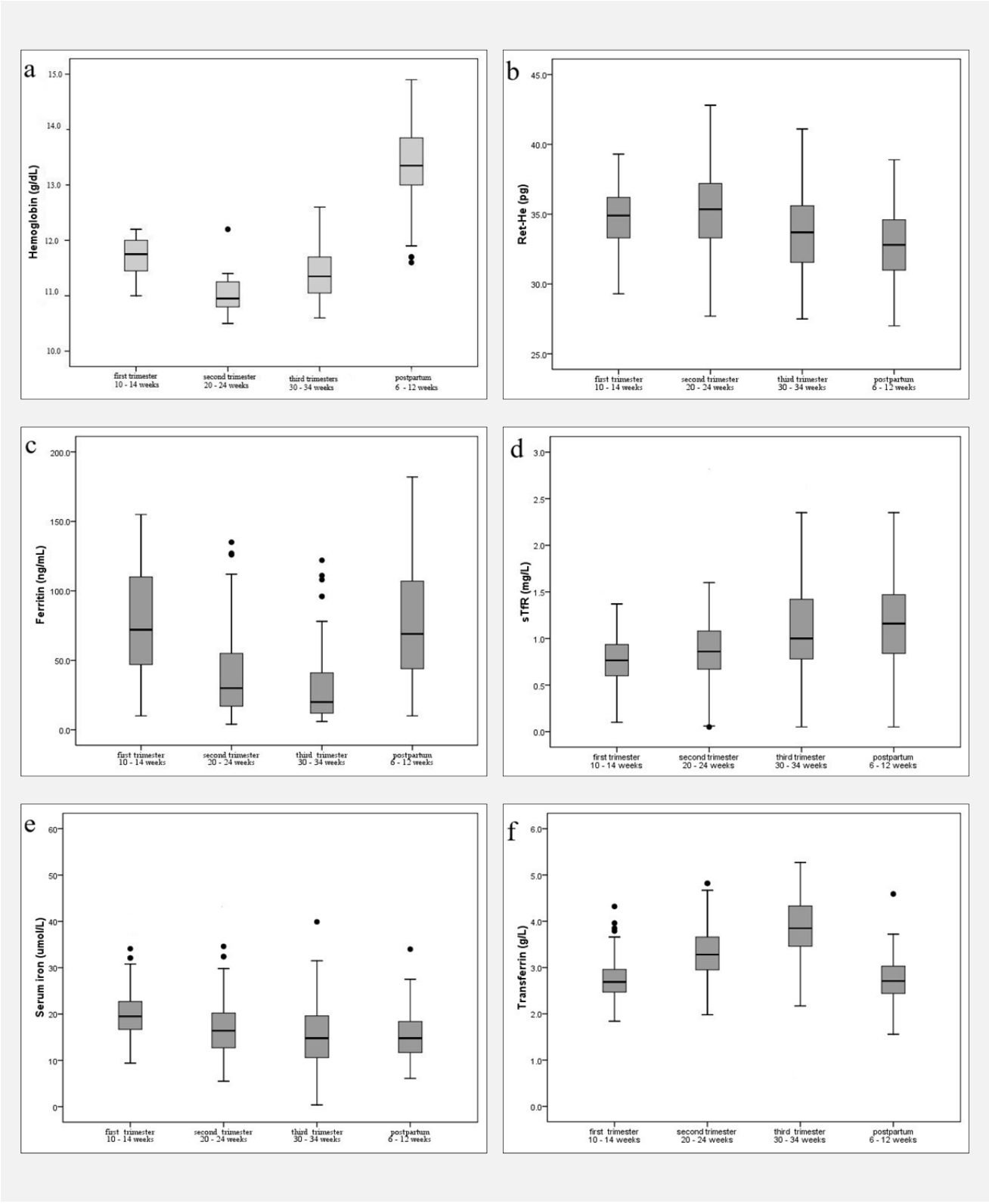

Figure 1. Box and whisker plots of (a) hemoglobin, (b) Ret-He, (c) serum ferritin, (d) sTfR, (e) serum iron, (f) transferrin, (g) TIBC, and (h) TS during each trimester and at postpartum.

1st trimester: 10 - 14 weeks, 2nd trimester: 20 - 24 weeks, 3rd trimester: 30 - 34 weeks, postpartum: 6 - 12 weeks after delivery. The boxes display the 75th and 25th percentiles and the median, and the whiskers indicate the 5th and 95th percentiles. Values below the 5th percentiles and above the 95th percentiles are indicated as points outside the whiskers.
Ret-He - reticulocyte hemoglobin, sTfR - serum soluble transferrin receptor, TIBC - total iron-binding capacity, TS - transferrin saturation.

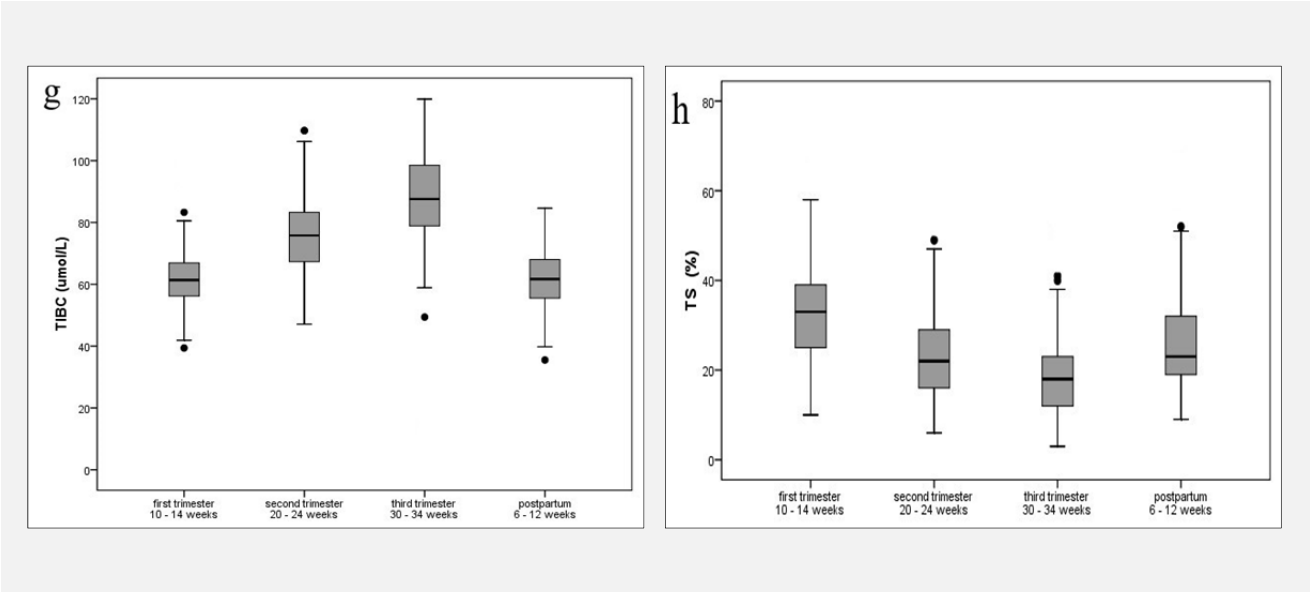

Figure 1. Box and whisker plots of (a) hemoglobin, (b) Ret-He, (c) serum ferritin, (d) sTfR, (e) serum iron, (f) transferrin, (g) TIBC, and (h) TS during each trimester and at postpartum (continued).

1st trimester: 10 - 14 weeks, 2nd trimester: 20 - 24 weeks, 3rd trimester: 30 - 34 weeks, postpartum: 6 - 12 weeks after delivery. The boxes display the 75th and 25th percentiles and the median, and the whiskers indicate the 5th and 95th percentiles. Values below the 5th percentiles and above the 95th percentiles are indicated as points outside the whiskers.
Ret-He - reticulocyte hemoglobin, sTfR - serum soluble transferrin receptor, TIBC - total iron-binding capacity, TS - transferrin saturation.

ceptor produced by proteolytic cleavage. Both the expression of TfR on the cell surface and the concentration of sTfR are inversely related to the level of intracellular iron [23]. sTfR is not affected by inflammation [24], thus making it a more reliable measurement than SF when inflammation is present. sTfR and the derived sTfR/log ferritin (ferritin index) are reliable markers of iron deficiency in mixed situations. Measurement of sTfR was introduced as a powerful tool to evaluate the iron status of pregnant women in a variety of studies [6, 7,10,19]. Measurement of sTfR may provide more specific information and has an advantage over SF because it can distinguish iron deficiency anemia from anemia associated with chronic inflammation, as well as identify iron depletion and functional iron depletion in patients with concurrent inflammation [25]. The sTfR:SF ratio was considered the gold standard marker for the iron status and was used to assess the iron status in pregnant populations in the United States National Health and Nutrition Examination Survey [26]. The sTfR concentration in pregnant women in the first trimester was very close to the reference values in non-pregnant women. However, the sTfR concentration in the second trimester was significantly higher than that in the first trimester ($p = 0.02$). The mean sTfR value increased gradually from the second trimester and reached the maximal concentration in the third trimester. The sTfR concentration decreased abruptly after delivery. Our data for the sTfR concentration during pregnancy are in accordance with another study showing that sTfR increased from early to late pregnancy [8]. It has been demonstrated that the low sTfR in the first trimester of pregnancy is caused by reduced erythropoiesis and iron requirements. Among the changes during pregnancy, physiological changes occur in the sTfR concentration. Elevated sTfR may reflect tissue iron deficiency and pregnancy-associated erythropoiesis [27]. Laboratory diagnosis of absolute iron deficiency has been based on low SI, low percent TS, and low ferritin [28]. The limitations of using TS reflect those of using SI, i.e., wide diurnal variation and low specificity. TS is also reduced in inflammatory disease [29]. SI is of limited use because of diurnal variation, and there is more variation during pregnancy because of physiological changes. Thus, the reference values of SI may be migratory and less available for clinical diagnosis. TIBC, Tf, and TS are measures of iron transport. The present results showed that TIBC and Tf decreased toward the end of the third trimester, where the nadir of TIBC and Tf concentrations were reached. The clinically relevant associations between these two indicators (TIBC and Tf) and iron status still exist because, as pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most iron accumulation occurring during the third trimester. Therefore, the trends in TIBC and Tf can explain the iron requirements during pregnancy. TS is less reliable as an indicator of iron status because of the in-

traday and interday variability in SI. Thus, we do not recommend using SI and TS to evaluate iron status during pregnancy, because the physiological changes in pregnancy are much greater than those in the nonpregnant state.

The reticulocyte channel of the Sysmex XE-2100 counter provides a parameter called RET-Y by measuring the forward light scatter histogram, expressed in arbitrary units (channel numbers). This parameter is dependent on the Hb content of reticulocytes, and a high correlation between RET-Y and the reticulocyte hemoglobin content (CHr) parameter (ADVIA-120) has been shown [30]. Ret-Y can be transformed into Ret-He, expressed in picograms, by applying a recently published regression plot (Ret-He = $5.5569e^{0.001\text{Ret-Y}}$) [31]. Ret-He, generated by all Sysmex XE analyzers, has been recognized as a direct assessment of iron incorporation into erythrocyte Hb and a direct estimate of the recent functional availability of iron and, thus, provides the same information as CHr [31]. The reticulocyte count reflects the erythropoietic activity of the bone marrow and Ret-He could be a sensitive marker for early detection of a negative balance between body iron content and demand for erythropoiesis. Starting in the mid-1990s, automated flow cytometric analysis has replaced traditional microscopic quantification of reticulocytes [32]. This parameter is dependent on the Hb content of reticulocytes, Ret-He, which can be used as a marker for iron status evaluation because reticulocytes exist in the circulation for only 1 - 2 days. The results for Ret-He revealed that the zenith Ret-He was reached during the second trimester of gestation. Subsequently, Ret-He decreased toward the end of the third trimester. However, the regulatory mechanism for the Ret-He content during pregnancy remains unclear, and we cannot fully explain the trend for the Ret-He content during gestation.

The limitation of our study is that it was impossible to ascertain the exact amounts of iron supplementation, because the current family planning policy has remained stable, and people pay more attention to providing good prenatal and postnatal care in China. About 95% of the pregnant women stated that they had used some iron supplementation or multivitamin products containing iron (approximately 150 mg elemental iron for unspecified times) from the 20th week to the end of pregnancy. But we found that they were unable to describe the exact dosage and duration of iron supplementation in the questionnaire survey, and it was also difficult to assess their dietary iron intake.

CONCLUSION

The conventional gold standard for assessment of iron status is bone marrow examination, but this has limited ability for screening of iron status during pregnancy. We consider that the best combination of measurements would be Hb, sTfR, and SF or bone-marrow iron. This combination would reflect functional impairment, tissue avidity for iron, and iron storage, respectively. An appropriate combination of laboratory tests can provide evidence of iron depletion and reflect iron-restricted RBC production and can thus help toward establishment of a correct assessment paradigm for iron status and appropriate treatment.

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Declaration of Interest:

The authors declare that they have no conflicts of interest related to this study.

References:

1. Milman N. Iron and pregnancy--a delicate balance. Ann Hematol 2006;85:559-65 (PMID: 16691399).
2. Haas JD, Brownlie T 4th. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nutr 2001;131:676S-88S (PMID: 11160598).
3. Hu X, Wang R, Shan Z, et al. Perinatal Iron Deficiency-Induced Hypothyroxinemia Impairs Early Brain Development Regardless of Normal Iron Levels in the Neonatal Brain. Thyroid 2016;26: 891-900 (PMID: 27231981).
4. Chelchowska M, Ambroszkiewicz J, Gajewska J, Jablonska-Glab E, Maciejewski TM, Oltarzewski M. Hepcidin and Iron Metabolism in Pregnancy: Correlation with Smoking and Birth Weight and Length. Biol Trace Elem Res 2016;173:14-20 (PMID: 26785 641).
5. Fernandez-Cao JC, Aranda N, Ribot B, Tous M, Arija V. Elevated iron status and risk of gestational diabetes mellitus: A systematic review and meta-analysis. Matern Child Nutr 2017;13(4) (PMID: 27966840).
6. Choi JW, Im MW, Pai SH. Serum transferrin receptor concentrations during normal pregnancy. Clin Chem 2000;46:725-7 (PMID: 10794761).
7. Mimura T,Hasegawa J, Nakamura M, Okai T.Correlation between the cervical length and the amount of bleeding during cesarean section in placenta previa. J Obstet Gynaecol Res 2011;37:830-5 (PMID: 21450025).
8. Akesson A, Bjellerup P, Berglund M, Bremme K, Vahter M. Serum transferrin receptor: a specific marker of iron deficiency in pregnancy. Am J Clin Nutr 1998;68:1241-6 (PMID: 9846853).
9. Park G, Park CY, Jang SJ, et al. Soluble transferrin receptor-ferritin index and estimated body iron in iron-deficiency anemia in "select" chronic diseases. Ann Hematol 2009; 88:913-5 (PMID: 19137299).
10. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood 2002;99:1489-91 (PMID: 11830506).
11. van den Broek NR, Letsky EA, White SA, Shenkin A. Iron status in pregnant women: which measurements are valid? Br J Haematol 1998;103:817-24 (PMID: 9858238).

12. Mei Z, Flores-Ayala RC, Grummer-Strawn LM, Brittenham GM. Is Erythrocyte Protoporphyrin a Better Single Screening Test for Iron Deficiency Compared to Hemoglobin or Mean Cell Volume in Children and Women? Nutrients 2017;9(6). pii: E557 (PMID: 28561801).

13. Bivolarska AV, Gatseva PD, Maneva AI. The Role of Eating Habits on the Iron Status of Pregnant Women. J Am Coll Nutr 2016;35:118-14 (PMID: 26259505).

14. Recommendations to prevent and control iron deficiency in the United States. Centers for Disease Control and Prevention. MMWR Recomm Rep 1998;47:1-29 (PMID: 9563847).

15. Milman N, Agger AO, Nielsen OJ. Iron supplementation during pregnancy. Effect on iron status markers, serum erythropoietin and human placental lactogen. A placebo-controlled study in 207 Danish women. Dan Med Bull 1991;38:471-6 (PMID: 1802636).

16. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000;72:257S-64S (PMID: 10871 591).

17. WHO. Iron Deficiency Anaemia Assessment, Prevention, and Control A guide for programme managers. WHO 2001. https://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/

18. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynecol 2009;114:1326-31 (PMID: 19935037).

19. Kalantar-Zadeh K, Kalantar-Zadeh K, Lee GH. The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease? Clin J Am Soc Nephrol 2006;1 Suppl 1:S9-18 (PMID: 17699375).

20. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta 2003;329:9-22 (PMID: 12589962).

21. Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clin Chem 1998; 44:45-51 (PMID: 9550557).

22. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 1990;75:102-7 (PMID: 2294984).

23. Baynes RD. Assessment of iron status. Clin Biochem 1996;29: 209-15 (PMID: 8740506).

24. Beerenhout C, Bekers O, Kooman JP, van der Sande FM, Leunissen KM. A comparison between the soluble transferrin receptor, transferrin saturation and serum ferritin as markers of iron state in hemodialysis patients. Nephron 2002;92:32-5 (PMID: 12187081).

25. Allen J, Backstrom KR, Cooper JA, et al. Measurement of soluble transferrin receptor in serum of healthy adults. Clin Chem 1998;44:35-9 (PMID: 9550555).

26. Mei Z, Cogswell ME, Looker AC, et al. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. Am J Clin Nutr 2011;93:1312-20 (PMID: 21430118).

27. Kaestel P, Aaby P, Ritz C, Friis H. Markers of iron status are associated with stage of pregnancy and acute-phase response, but not with parity among pregnant women in Guinea-Bissau.Br J Nutr 2015;114:1072-9 (PMID: 26285696).

28. Goodnough LT, Skikne B, Brugnara C. Erythropoietin, iron, and erythropoiesis. Blood 2000;96:823-33 (PMID: 10910892).

29. Coyne D. Iron indices: what do they really mean? Kidney Int Suppl 2006:S4-8 (PMID: 16830699).

30. Buttarello M, Temporin V, Ceravolo R, Farina G, Bulian P. The new reticulocyte parameter (RET-Y) of the Sysmex XE 2100: its use in the diagnosis and monitoring of posttreatment sideropenic anemia. Am J Clin Pathol 2004;121:489-95 (PMID: 15080300).

31. Garzia M, Di Mario A, Ferraro E, et al. Reticulocyte hemoglobin equivalent: an indicator of reduced iron availability in chronic kidney diseases during erythropoietin therapy. Lab Hematol 2007;13:6-11 (PMID: 17353176).

32. Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. Clin Chem Lab Med 2010;48:1369-80 (PMID: 20666695).