

# Study of changes in red blood cell indices and iron status during three trimesters of pregnancy

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## Abstract

**Background:** During pregnancy, the hemodynamic and iron requirement changes. Several tests are used to determine iron status and anemia such as hemoglobin, hematocrit or packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin, red cell distribution width, percentage of hypochromic red cells, serum ferritin, and serum transferrin. This study was undertaken with intent to record changes in hematological profile along with transferrin and ferritin during pregnancy. The data obtained may be helpful in establishing reference range of all red blood cell indices along with serum ferritin and transferrin during pregnancy.

**Objective:** To study changes in red blood cell indices and iron status during three trimesters of pregnancy.

**Materials and Methods:** A longitudinal study was conducted on pregnant women reporting antenatal clinic in the first trimester. Data in each trimester of 57 women were collected. Birth weight of newborn delivered was recorded, and statistical analysis was done by paired *t*-test.

**Result:** Fifty-seven study subjects were divided according to the birth weight of delivered babies. Most of the parameters recorded were within the reference range across trimesters. Except for few, the changes in parameters were in accordance with expected physiological response in pregnancy. Significant drop in hemoglobin and significant rise in transferrin levels across all trimesters resulted in poor outcome of pregnancy.

**Conclusion:** This study suggests that serum transferrin level estimation in all trimesters along with hemoglobin can be advised to avoid possible poor outcome of pregnancy. This study provides additional baseline data for basic hematological parameters in pregnant women. This would benefit especially in the antenatal care and assessment of pregnant women.

**KEY WORDS:** Pregnancy, serum transferrin, serum ferritin, red cell indices, birth weight

## Introduction

Iron is the fourth most bountiful element in the earth crust. Its importance for human physiology lies in the fact that it is incorporated into a red blood cell to perform various functions. Its deficiency leads to impaired hemoglobin (Hb) synthesis which ultimately leads to anemia. There are various known causes

of anemia, but, globally, iron deficiency is the main contributor to anemia onset.<sup>[1]</sup> The main risk factors for iron deficiency anemia involve a reduced intake of iron, poor absorption of iron from foodstuffs rich in phytate or phenolic compounds. A person becomes more prone to develop iron deficiency during growth and pregnancy when iron requirements are high.

Anemia has global burden. The National Family Health Survey (NFHS-3) 2005–2006 conducted by Ministry of Health and Family Welfare, Government of India, reports that 56% of currently married women and 58.7% of pregnant women are anemic.<sup>[2]</sup> There are increased incidences of maternal and child mortality owing to severe anemia, which have been well-documented.<sup>[3,4]</sup>

During pregnancy, the hemodynamic and iron requirement changes in all trimesters. The menstrual cycle stops when pregnancy is achieved leading to decrease in iron requirements during the first trimester.<sup>[5,6]</sup> The only iron losses occur

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from the gut, skin, and urine also known as the obligatory iron loss.<sup>[7]</sup> The hemodynamic changes include generalized vasodilation and physiological increase in the plasma volume. The plasma volume can increase nearly 50% compared with the volume in nonpregnant women. This hemodilution begins in the first and second trimesters and allows better blood circulation for the placenta, thereby ensuring the well-being of the fetus.<sup>[8]</sup> During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder of pregnancy. The increase in oxygen consumption by both mother and fetus is associated with major hematologic changes. No single parameter specifically indicates iron status, and several tests are used to determine iron status and anemia such as Hb, hematocrit or packed cell volume (PCV), mean cell volume and mean cell Hb, red cell distribution width, reticulocyte Hb concentration, percentage of hypochromic red cells, serum ferritin, and serum transferrin.<sup>[9]</sup>

Our hospital is a 1,000-bed tertiary-care center catering health services in Mahakaushal region of central India. The hospital has 24 h functioning centralized laboratory equipped with various automated analyzers. The antenatal clinic of Obstetrics and Gynecology Department is attending all the pregnant women reporting at the institute. Regardless of clinical presentation and blood indices status at the time of first antenatal visit, the complete blood count is advised for the expecting mothers. Whereas the serum levels of transferrin and ferritin are not investigated as routine during pregnancy. This study was conducted with the aim to study the changes in red blood cell indices along with levels of serum transferrin and serum ferritin in all trimesters. It was also intended to study the pattern of changes in red blood cell indices in all trimesters of pregnancy. Changes in the red blood cell indices, serum transferrin, and serum ferritin were also correlated with the outcome of pregnancy in terms of birth weight of delivered babies.

## Materials and Methods

This study was conducted in the Department of Physiology and Antenatal clinic in the Department of Obstetrics and Gynecology of Netaji Subash Chandra Bose Medical College, Jabalpur, for a period of 2 years after obtaining Institutional Ethical Committee clearance. The subjects for this study were enrolled from patients attending the antenatal clinic and who volunteered after informed consent. Detailed medical and obstetrical history of enrolled subjects was taken. Gestation age was based on the last menstrual period of the subject. Iron supplementation was prescribed by the attending doctors to each subject. Those who volunteered and were willing for regular follow-up in each trimester were included in the study. Subjects included in the study were healthy, married primigravida of age group 20–30 years. Subjects residing at study area were given preference so that they could be followed easily for the whole duration of their pregnancy. Subjects who did not turn up for follow-up in the next trimester after their first visit were excluded from the study. Those who were regular

but discontinued iron supplementation were also excluded. Women with diabetes mellitus, cardiovascular disease, chronic hypertension and diseases other than anemia, and multiple pregnancies were excluded from the study. Primigravida with high-risk pregnancy were not included in the study. The enrolled subjects were followed up throughout their antenatal visits. At each visit, they were motivated to take their supplementation regularly and to undergo institutional delivery. A total of three blood samples from each trimester were collected from each subjects. Birth weight of newborn delivered at our institution by the pregnant women of the study group was recorded. Control parameters were taken from nonpregnant, nonanemic, healthy female subjects matched for age.

Taking all aseptic precautions, 7–10 mL venous blood was collected from antecubital vein. Total amount of blood collected was divided into two equal volumes.

- A. One half was mixed with EDTA anticoagulant in a vial for complete blood count estimation including Hb, erythrocyte sedimentation rate, total leukocyte count, differential leukocyte count, total red blood cell count, hematocrit, and platelet count and, based on the values obtained, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated. These hematological parameters were estimated by Arcus Hematology Analyzer.
- B. The other half of blood sample was poured in clean and dry test tube. Sample in test tubes were centrifuged to separate serum. Separated serum was used to determine values of serum ferritin and serum transferrin. Serum ferritin and serum transferrin levels were estimated by commercially available kits manufactured by Biosystems Reagents and Instruments in the analyzer model A25 of the same company. The data were statistically analyzed by software SPSS 15.

## Result

Of all the enrolled subjects at their first visit, a total of 57 subjects participated in the study throughout their pregnancy and delivered at this institute. The birth weight of delivered babies was recorded. According to the birth weight of delivered babies, the subjects were divided into two categories:

1. Group I—includes subjects who delivered low birth weight babies
2. Group II—includes subjects who delivered normal birth weight babies.

Throughout the pregnancy, group I subjects were anemic (Hb less than 11 g/dL), while the less than 11 g/dL Hb was recorded only during the second trimester in group II subjects. Mean values of different parameters including Hb, MCH, MCHC, MCV, PCV, serum transferrin, and serum ferritin in all trimesters are recorded in all subjects. Mean values were compared across trimesters in both the groups as shown in Tables 1 and 2.

**Table 1:** Changes in parameters during all trimesters of group I subjects, their comparison from the first trimester to the third trimester, and statistical significance

Parameters	1st trimester	2nd trimester	3rd trimester	p		
				1st and 2nd	1st and 3rd	2nd and 3rd
Hb	10.39 ± 1.39	10.44 ± 1.16	9.91 ± 1.21	>0.05	<0.05*	<0.05*
MCH	29.03 ± 4.17	32.19 ± 4.91	28.58 ± 3.86	<0.05*	>0.05	>0.05
MCHC	32.99 ± 4.11	32.17 ± 3.80	31.06 ± 3.51	>0.05	<0.05*	>0.05
MCV	89.16 ± 15.35	101.59 ± 20.39	92.87 ± 14.69	<0.01*	>0.05	<0.05
PCV	31.79 ± 4.64	33.02 ± 3.70	32.67 ± 4.82	<0.05*	>0.05	>0.05
Serum transferrin	342.11 ± 52.57	346.83 ± 57.18	374.72 ± 54.31	>0.05	<0.05*	<0.05*
Serum ferritin	49.95 ± 23.85	60.42 ± 20.24	58.49 ± 34.25	<0.05*	>0.05	>0.05

\*Statistically significant difference.

**Table 2:** Changes in parameters during all trimesters of group II subjects, their comparison from the first trimester to the third trimester, and statistical significance

Parameters	1st trimester	2nd trimester	3rd trimester	p		
				1st and 2nd	1st and 3rd	2nd and 3rd
Hb	11.37 ± 1.18	9.74 ± 1.18	11.34 ± 0.71	<0.05*	>0.05	<0.01*
MCH	32.14 ± 7.77	27.57 ± 1.91	30.38 ± 2.12	>0.05	>0.05	<0.01
MCHC	34.91 ± 4.73	31.93 ± 3.78	36.53 ± 4.54	>0.05	>0.05	<0.05*
MCV	91.92 ± 15.75	87.06 ± 8.03	84.06 ± 10.08	>0.05	>0.05	>0.05
PCV	33.01 ± 4.98	30.86 ± 5.31	31.80 ± 5.43	>0.05	>0.05	>0.05
Serum transferrin	321.18 ± 42.62	317.36 ± 60.15	301.73 ± 86.63	>0.05	>0.05	>0.05
Serum ferritin	66.25 ± 22.40	47.33 ± 13.49	59.84 ± 24.21	<0.05*	>0.05	>0.05

\*Statistically significant difference.

## Discussion

The Hb cutoff value for pregnant women is 11g/dl.<sup>[10]</sup> During pregnancy there is increase in iron requirement due to growing foetus and increase in maternal blood volume. Maternal change leads to decrease in Hb concentration in first trimester which further decline and reaches its lowest level during second trimester. During second trimester Hb concentration diminish approximately 5 g/L. It rises again during third trimester.<sup>[10]</sup> Currently, there are no WHO recommendations on the use of different Hb cutoff points for anemia by trimester.<sup>[10]</sup> In our study, we observed Hb level above cutoff value of 11g/dL in group II during the first and second trimesters. Hb level decreased in the second trimester and again rises in the third trimester in group II. In group I, throughout the duration of pregnancy, we observed Hb level below cutoff value as recommended by the WHO. As established, we did not observed drop in Hb level during the second trimester in group I. In group I, there was significant drop in Hb level in the third trimester when compared with the first and second trimesters. Maternal anemia is a risk factor for poor outcome of pregnancy.<sup>[11–13]</sup> In subjects of group I of our study, mothers who showed significant drop in Hb level in the third trimester showed poor outcome in the form of low birth weight.

Increase in maternal blood volume during the first and second trimesters did not affect the PCV values obtained in both the study groups. Significant difference in PCV was

observed only in the first and second trimesters in group I. Rest of the PCV values comparison across trimester in both the groups was insignificant. The PCV decreases during pregnancy, and it has been reported in various previous studies.<sup>[14–16]</sup> The PCV values obtained in group II of our study was similar to the findings of earlier studies.<sup>[14,15]</sup> Hb and hematocrit tests are essential to diagnose iron deficiency, but the limitation with these tests is that changes in Hb concentration and hematocrit occur only at the late stages of iron deficiency.<sup>[17]</sup>

The normal reference range for MCH, MCHC, and MCV used in this study are 26.7–31.9 pg/cell, 32.3–35.9 g/dL, and 79–93.3 fL, respectively.<sup>[18]</sup> In our study, MCH and MCHC values were within limits or on borderline in both the groups. We observed significant increase in MCV during the second trimester in group I. Increase in MCV values could be owing to physiological macrocytosis induced by iron supplementation as reported in previous studies.<sup>[19,20]</sup> One possible mechanism for poor outcome in group I could be increased viscosity of blood owing to iron-induced macrocytosis, which might have impaired placental perfusion.<sup>[21]</sup>

The suggested value of serum ferritin to classify individual as iron deficient is <12 µg/L. During latent/second phase of anemia, there is decrease in iron availability for erythropoiesis and, during the third phase of anemia, the circulation of red blood cell parameters decreases. The suggested value of serum ferritin during these phases of iron deficiency anemia is <12 µg/L.<sup>[22,23]</sup> Throughout the pregnancy in all subjects

included in our study, the serum ferritin levels remain well above the suggested value of iron deficiency state that is above 12 µg/L. As the pregnancy advances, the subjects of group I in this study showed increase in serum ferritin level with respect to the first trimester level. On the other hand, the subjects of group II showed decrease in serum ferritin level with respect to the first trimester level.

The suggested normal range for serum transferrin is 200–400 mg/dL.<sup>[18]</sup> There is a strong correlation between the value of total iron-binding capacity and level of serum transferrin.<sup>[24]</sup> The increased demands of iron during pregnancy leads to increased total iron-binding capacity, and, accordingly, serum transferrin level also rises.<sup>[25,26]</sup> The serum transferrin level recorded were within reference range. The mean serum transferrin level observed in both the groups was more than 300 mg/dL [Tables 1 and 2]. We recorded continuous rise in serum transferrin levels in subjects of group I in all three trimesters. The rise in the third trimester was significant when compared with the first and second trimesters in group I ( $p < 0.05$ ). The levels of serum transferrin obtained in group I of our study is in accordance with previous studies of Chang *et al.*<sup>[27]</sup> and Chaudhari *et al.*<sup>[28]</sup> The blood samples collected in both the studies for estimating serum transferrin level across different trimesters were from pregnant females reporting at different trimesters/period of gestation. While in our study, the pregnant women were inducted in first trimester and followed up through all trimesters till delivery. Thus, the results obtained in our study are from the same subjects obtained in their different trimesters.

Important finding of our study is that the levels of Hb and serum transferrin correlated well with the birth weight of delivered babies. It has been established that maternal anemia is a risk factor for poor outcome of pregnancy.<sup>[11–13]</sup> The group of subjects in our study who showed significant drop in Hb and significant rise in serum transferrin levels delivered low birth weight babies.

Limitations in the form of drop out of subjects after the first antenatal visits restricted our sample size. In this study, we considered that all the subjects were following advice and were taking adequate iron supplementation as well as on adequate diet. But, it cannot be ignored that they might lack regular diet as well as iron supplementation, which is not strictly followed in this study. So, it is further recommended that studies carrying larger cohort, considering all the confounding factors as well as strict follow-up on diet and iron supplementation are required, which is the further aspect of our study.

## Conclusion

From the results of this study, it can be summarized that there occurs physiological iron deficiency state in pregnancy. It also suggests that serum transferrin level estimation in all trimesters along with Hb can be advised to avoid possible poor outcome of pregnancy. As we are serving at tertiary level health institution, there are still more possibilities available for

research to be done in this field. Subjects tend to drop out during follow-up antenatal visits and at the time of delivery limits the number of subjects included in the study. The data obtained may be helpful in establishing reference range of all red blood cell indices along with serum ferritin and transferrin during different trimesters in Indian population. Further efforts are required to make these tests affordable to all segments of our community.

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