



# ORIGINAL RESEARCH PAPER

# Biochemistry

## COMPARATIVE STUDY OF SERUM HEPcidIN WITH VARIOUS IRON PROFILE PARAMETERS IN DIFFERENT TRIMESTERS OF PREGNANCY.

**KEY WORDS:** pregnancy, hepcidin, hemoglobin, S. Iron, S. Ferritin.

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### ABSTRACT

**Background:** Iron deficiency and iron deficiency anemia is one of the most common nutritional deficiencies during pregnancy. Although iron status detection can be done by available well-established methods but accurate identification of maternal iron deficiency anemia with conventional markers is a challenge. Therefore, a biomarker like hepcidin which can be used for accurate identification of the risk of iron status is estimated. **Objectives:** The objective of this study is to see the effect of use of hepcidin and iron profile in pregnancy and compare it within different trimesters of pregnancy. **Methodology:** Total 300 pregnant females were included in the study, 100 in each trimester, in which patients ranging within age 18-30 years. 10 ml blood was drawn through vein puncture. From all collected blood samples hepcidin, hemoglobin S. Iron, Ferritin tests were measured. All collected data were analysed statistically to calculate p values to see the difference of significance. **Results:** The result showed that pregnant women had lower levels of hepcidin, hemoglobin, iron, and ferritin. There differences were statistically significant decrease in all parameters as trimesters of pregnancy increases, indicating that these finding further support to the risk of anemia. **Conclusion:** From our study we would like to conclude that hepcidin and iron profile are common cause of anemia in pregnancy. Anemia has a negative prognostic influence, and its correction is thought to improve prognosis; therefore, most patients are treated with transfusion and/or erythropoietin.

### INTRODUCTION

Pregnancy is a state characterized by many physiological, haematological changes, which may appear to be pathological in the non- pregnant state. In India, approximately 44,000 women die from pregnancy-related complications every year<sup>1</sup>. In India, more than half of the women are anaemic<sup>2</sup>, nearly 40% of women aged between 15-49 years are underweight, and nearly 25% of women are overweight/obese<sup>3</sup>. Iron deficiency and iron deficiency anemia during pregnancy are highly prevalent worldwide. Hepcidin is considered as important biomarker of iron status. It is the master regulator of systemic iron bioavailability in humans.

Hepcidin, a 25-amino-acids peptide hormone (Hep-25), has recently emerged as a key regulator of iron homeostasis. Hepcidin regulates intestinal absorption, macrophage release, and the placental passage of iron. This hormone inhibits the cellular efflux of iron by binding to and inducing the degradation of ferroportin, the only known cellular iron exporter<sup>4</sup>. Hepcidin expression is induced by iron overload and inflammation and is suppressed by anemia, erythropoietic activity, and hypoxia<sup>6,7</sup>.

The hemoglobin (HB) is the protein contained in red blood cells that is responsible for delivery of oxygen to the tissues. Hemoglobin concentration is often used as a pseudo marker for iron deficiency<sup>8</sup>. As pregnancy advances, maternal RBC mass increases and placental and fetal growth accelerates, which result in the rise in physiologic iron requirements to 3.0-7.5 mg/d in the third trimester<sup>9</sup>.

In pregnancy, the demand for iron increases from 0.8 to up to 7.5 mg/day of absorbed ferritin, although the exact upper limits in the third trimester are debated<sup>10</sup>. This increased demand is needed to expand maternal erythrocyte mass, fulfil foetal iron needs, and compensate for iron losses (e.g., blood loss at delivery)<sup>11</sup>.

Iron deficiency during pregnancy period continues to be a common clinical problem and is one of the most prevalent nutritional deficits both in the industrial and developing countries. Iron requirements increase significantly (near 10-

fold) over gestation; therefore, pregnant women are particularly at risk of developing iron deficiency anemia. The prevalence of anemia is dependent on nutritional status and use of sufficient prenatal supplements and ranges from 14 to 52 % women who are not taking prenatal supplements, to 0-25 % among pregnant women receiving regular multivitamin (containing iron and folic acid) preparation<sup>12-15</sup>.

The Serum ferritin is an acute phase reactant, and marker of iron storage, has the advantage of being a sensitive indicator of iron deficiency, but because it is increased in the presence of inflammation, reflecting macrophage ferritin content<sup>4</sup>. Serum Ferritin, one of the acute phase reactants is an intracellular protein playing a role in sequestration and storage of iron<sup>16</sup>.

Thus the present study was planned to estimate hepcidin, hemoglobin, S.Iron, S. ferritin in pregnant women an aim to find out utility of these parameters in better accessing to improve maternal health and pregnancy outcomes.

### MATERIAL AND METHODS

The present study was conducted on 300 pregnant women in department of biochemistry Index medical college, hospital and research center, Indore. The ages of the patients ranged from 18-30 years of age.

An informed consent was taken from all the patients or their attendants who participated in the study after apprising them the nature and objective of study.

After getting informed consents from the subjects, 5ml of blood was collected in a red plain vacutainer tube devoid of anticoagulant under aseptic condition from the subject after overnight fasting of 12 to 14 hours. Samples were centrifuged at 3000 rpm to separate within 1 hours and following parameters were estimated.

- SERUM HEPcidIN: Enzyme Linked Immunosorbent Assay Method<sup>17</sup>.
- ESTIMATION OF HAEMOGLOBIN: 5 part fully automatic analyser<sup>18</sup>.
- ESTIMATION OF IRON: Photometric test using Ferene<sup>19</sup>.
- SERUM FERRITIN: Chemiluminescent immunoassay

method<sup>20</sup>.

- Statistical method descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as mean  $\pm$  standard deviation (SD) and student's t-test (two-tailed independent) has been used to find the significance of study parameters on continuous scale between the three groups.

### ObservationTable

**Table: 1 Mean Hepcidin, Hemoglobin, S. Iron And Ferritin Concentration Of The Subject Studied During Different Trimester Of Pregnancy**

S. No	Trimester of pregnancy	HEPCIDIN (ng/ml) Mean $\pm$ SD (Range)	Hemoglobin (gm/dl) Mean $\pm$ SD (Range)	Iron ( $\mu$ gm/dl) Mean $\pm$ SD (Range)	FERRITIN (ng/ml) Mean $\pm$ SD (Range)
1.	FIRST	16.15 $\pm$ 2.78 (11.72-20.93)	10.46 $\pm$ 0.98 (6.7-12.5)	104.98 $\pm$ 47.60 (28.54-304.03)	24.06 $\pm$ 13.93 (7.6-69.4)
2.	SECOND	11.96 $\pm$ 3.06 (6.4-17.24)	10.79 $\pm$ 1.51 (7.3-14.8)	94.50 $\pm$ 23.31 (48.82-190.25)	20.07 $\pm$ 12.22 (6.9-17.24)
3.	THIRD	9.18 $\pm$ 2.44 (4.45-13.24)	11.40 $\pm$ 1.76 (7.5-15.9)	93.38 $\pm$ 19.01 (18.28-141-13)	17.00 $\pm$ 9.62 (4.4-56.6)

**Table: 2 Statistical Evaluation Of Hepcidin, Hemoglobin, S. Iron And Ferritin Concentration Among Different Trimesters Of Pregnancy**

S. Tri- N mester O Com- pared	Hepcidin t- value	p- value	Hemoglobin t- value	p- value	S. Iron t- value	p- value	S. Ferritin t- value	p- value
1. 1st vs 2nd	10.11	p<0.001*	2.62	p<0.0093*	1.97	p<0.049*	2.15	p<0.0325*
2. 1st vs 3rd	18.79	p<0.001*	4.60	p<0.001*	2.26	p<0.024*	3.44	p<0.007*
3. 2nd vs 3rd	7.09	p<0.001*	1.78	p>0.0761**	0.37	p>0.71**	1.97	p<0.0498*

- \*-Highly-significant
- \*\*--Non-significant

### RESULTS AND DISCUSSION

The mean serum hepcidin concentration of pregnant female in 1<sup>st</sup> trimester is 16.15  $\pm$  2.78 ng/ml, in 2<sup>nd</sup> trimester is 11.96  $\pm$  3.06 ng/ml and in 3<sup>rd</sup> trimester is 9.18  $\pm$  2.44 ng/ml. There is statistically significant difference in mean serum hepcidin in 1<sup>st</sup> vs 2<sup>nd</sup> (P-value <0.0001), 1<sup>st</sup> vs 3<sup>rd</sup> (P-value <0.0001) and in 2<sup>nd</sup> vs 3<sup>rd</sup> (P-value <0.0001). The mean hemoglobin concentration of pregnant female in 1<sup>st</sup> trimester is 11.40  $\pm$  1.79 gm/dl, in 2<sup>nd</sup> trimester is 10.79  $\pm$  1.51 gm/dl and in 3<sup>rd</sup> trimester is 10.46  $\pm$  0.98 gm/dl. There is statistically significant difference in mean haemoglobin in and 1<sup>st</sup> vs 2<sup>nd</sup> (P-value <0.0093) and 1<sup>st</sup> vs 3<sup>rd</sup> (P-value <0.0001) but no significant difference in 2<sup>nd</sup> vs 3<sup>rd</sup> (P-value 0.0761).

The mean serum iron concentration of pregnant female in 1<sup>st</sup> trimester is 104.98  $\pm$  47.60  $\mu$ gm/dl, in 2<sup>nd</sup> trimester is 94.50  $\pm$  23.31  $\mu$ gm/dl and in 3<sup>rd</sup> trimester is 93.38  $\pm$  19.01  $\mu$ gm/dl. There is statistically significant difference in mean serum iron in 1<sup>st</sup> vs 2<sup>nd</sup> (P-value <0.049) and 1<sup>st</sup> vs 3<sup>rd</sup> (P-value <0.024) but no significant difference in 2<sup>nd</sup> vs 3<sup>rd</sup> (P-value 0.71). The mean serum ferritin concentration of pregnant female in 1<sup>st</sup> trimester is 24.06  $\pm$  13.93 ng/ml, in 2<sup>nd</sup> trimester is 20.07  $\pm$  12.22 ng/ml and in 3<sup>rd</sup> trimester is 17.00  $\pm$  9.62 ng/ml. There is statistically significant difference in mean serum ferritin in 1<sup>st</sup> vs 2<sup>nd</sup> (P-value <0.0325), 1<sup>st</sup> vs 3<sup>rd</sup> (P-value <0.0007) and in 2<sup>nd</sup> vs 3<sup>rd</sup> (P-value <0.0498). Thus, we found that there is significant

decrease in all parameters as trimesters of pregnancy increases.

In consistent with our results Talabani NS (2015) also reported similar results with a statistically significant decreasing trend in haemoglobin from 1<sup>st</sup> trimester to 3<sup>rd</sup> trimester. He also observed a statistically significant difference in the values of serum iron concentration amongst the three trimesters of pregnancy<sup>21</sup>.

Tan et al (2020) observed that both hemoglobin and ferritin tend to decrease with advancing trimesters, especially among women with multiple gestations<sup>22</sup>.

Peng Sun et al (2023) Serum hepcidin levels substantially decreased from 19.39ng/mL in the first trimester to 1.32ng/mL in the third trimester<sup>23</sup>.

### CONCLUSION

From our study we would like to conclude that hepcidin and iron profile are common cause of anemia in pregnancy. Anemia has a negative prognostic influence, and its correction is thought to improve prognosis; therefore, most patients are treated with transfusion and/or erythropoietin.

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