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Research Article

THE EFFECT OF MATERNAL OBESITY ON FETAL IRON TRANSFER

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Abstract- Objectives: 1.To demonstrates the effect of maternal obesity and fetal iron transfer by taking pregnant women in three groups based on their BMI. 2. To correlate the effect of inflammatory mediators in early pregnancy on cord blood iron status. 3. To sensitize the women to reduce their weight before contemplating for next pregnancy. **Study Design:** Prospective cross sectional cohort study. **Population and Sample:** All pregnant women with singleton pregnancy without known medical complications. **Methods:** In first trimester during first visit after confirmation of pregnancy, the pregnant women were divided into three groups based on the BMI. 90 eligible pregnant women were taken and it was conducted for one year. The pregnant women with complications inherent to pregnancy were excluded. In second trimester maternal CRP was done and during delivery cord blood iron profile was done. OUTCOME: The effect of inflammatory mediators on cord blood iron profile in relation to BMI of pregnant women was established.

Key Words- Cord blood iron profile, inflammatory mediators, Body mass index.

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Introduction

Most of the women of reproductive age group were overweight or obese; this burden is increasing rapidly in working population due to stress. [1] According to National survey of family health report overall prevalence of overweight among women of 20-44 years was 24.4% and 23% were obese [3].

The body mass index is a simple index of weight for height measure defined as weight in kilograms and height in square metres (kg/m2). The pregnant women were classified into normal, over weight and obese based on WHO classification of BMI [Table-1].

Table-1 *BMI*

Classification	BMI	Risk Of Comorbidities
Underweight	<18.5	Low(but risk of other clinical problems is increased)
Normal	18.5-24.9	Average
Overweight Pre obese	25-29.9	Increased
Obese class 1	30-34.9	Moderate
Obese class 2	35-39.9	Severe
Obese class 3	>40	Very severe

The complex mechanism of placental iron transfer, the substances and factors regulating the placental iron transfer are also elucidated, for better understanding of mechanism of obesity affecting placental iron transfer [1]. Iron need is significantly higher in pregnant women compared to non-pregnant state. The absorbed iron is used to increase maternal erythrocyte mass, fulfill fetal and placental iron needs and to compensate blood loss during delivery [37]. For the usage of iron by the cell it needs to be captured, internalized and delivered into the cell in soluble form. Three proteins are involved in this function. These are ferroportin, transferrin and ferritin [53,55].

Steps of placental iron transfer:

1. Syncytiotrophoblast takes iron- transferrin complex from maternal circulation via transferrin receptor.
2. The holo-transferrin/transferrin receptor were endocytosed, iron is released from the complex in acidified endosome.
3. Iron is transported into the cytosol with the help of divalent metal transporter (DMT1).
4. Iron is exported from the basolateral side of the syncytiotrophoblast into fetal Circulation by ferroportin[30].

The amount of iron transported must be kept in optimum range. This is mainly regulated by Iron Regulatory Element (IRE) and Iron Regulatory Protein (IRP) [40]. In obesity, the inflammatory mediators like C-Reactive protein and hepcidin are increased. In obesity there is a marked inflammation which has a potent effect on iron homeostasis by reducing intestinal iron absorption. IL6 and lipopolysaccharides induced macrophages stimulates the human hepatocytes to secrete hepcidin which has an inhibitory effect on ferroportin, thus decreasing the iron transfer to the fetus [1].

In obesity the inflammatory mediators stimulate hepcidin release by following pathways:

1. BMP/SMAD Pathway
2. Janus Kinase Pathway (JAK/STAT pathway)
3. Hepcidin- Ferroportin interaction

Hepcidin-Ferroportin interaction: The hepcidin which is increased by the inflammatory mediator like CRP binds to ferroportin. Hepcidin-Ferroportin complex is internalized. The complex is phosphorylated which causes ubiquination of ferroportin causing ultimate destruction of ferroportin, thus preventing placental iron transfer.

Materials and Methods

This study is a prospective observational cohort study conducted with 90 eligible pregnant women in three groups normal, overweight and obese based on WHO criteria taking 30 in each group.

Study Period : June 2013- May 2014 (12 months)

Sample Size : 90 pregnant women

For sample size calculation, the mean serum iron level in overweight is assumed as 60. The obese people will have mean value of 1/3rd of overweight. To demonstrate the difference with 95% confidence interval and 80% power, the minimum sample size required was 30 per arm including the control group, a total of 90 pregnant women were required.

The following inclusion and exclusion criteria were followed:

Inclusion Criteria

1. Singleton pregnancy
2. Term pregnancy
3. Both normal and caesarian delivery.

Exclusion Criteria

1. Mothers with Twins/Preterm /IUGR.
2. Inflammatory conditions like chorioamnionitis, premature rupture of membranes.
3. Infections
4. Overt and gestational diabetes mellitus.
5. Preeclampsia.
6. Iron deficiency anemia.

These pregnant women were enrolled in the study after informed consent and confirmation of pregnancy. They were followed throughout the pregnancy to exclude the complications listed above.

Antenatal Investigations Performed

In the first booking visit, the pregnant mother's BMI was calculated and they were categorized into groups based on WHO guidelines. Then all the basic investigations to check her haemoglobin, sugars, renal and thyroid function test were performed.

During the second trimester maternal CRP was measured as it had an exact positive correlation with hepcidin in second trimester. With 75 grams of glucose, oral glucose challenge test was performed with the sugar values taken after 2 hours. The enrolled pregnant women with gestational diabetes mellitus and preeclampsia were excluded from the study.

From the third trimester the pregnant women were followed till delivery and at the time of delivery the cord blood was collected irrespective of the type of delivery.

Method of Cord Blood Collection

Immediately after delivery of the baby, 3-5ml of cord blood was collected from the umbilical vein with the help of syringe aspiration and the blood was transferred to a vacutainer and transported to the laboratory within 30 minutes in a upright position for the red cells to settle.

Then the cord blood is centrifuged at the rate of 4000 rotations for 15 minutes. The serum sample is used to measure parameters like CRP, serum iron, serum ferritin, total iron binding capacity and iron saturation using following principles and methods.

Method of Estimation of Serum Iron

Quantitative determination of cord blood serum iron was done using calorimetric assay. Normal Cord Blood Serum Iron: 90-448g/dl.

Method of Estimation of Total Iron Binding Capacity

The total iron binding capacity (TIBC) cannot be directly estimated in the laboratory. It is estimated using unsaturated iron binding which can be measured by absorbance photometry. TIBC is calculated using the formula,

Serum iron + UIBC→TIBC.

Method used to Estimate C-Reactive Protein (CRP)

The CRP was estimated using immune turbidometric assay.

Normal Cord Blood CRP: 0.01-0.35mg/L.

Method of Estimation of Iron Saturation

It cannot be determined directly in the laboratory. It is calculated from,

$$\text{Iron saturation} = \frac{\text{Serum iron}}{\text{TIBC}} \times 100$$

Normal Value of Iron Saturation: 31.5-90.9%

Method of Measuring Cord Blood Serum Ferritin

Principle of cord blood serum ferritin: A 2step immunometric test was performed.

Normal Value of Cord Blood Serum Ferritin: 62-313ng/ml

Results

Normal Group : Women with BMI 18-24.5

Overweight Group: Women with BMI 25-29.9

Obese Group: Women with BMI greater than 30

The pregnant women were taken in all the three groups were of same age group, euglycemic with no other medical disorders. The parameters like serum hemoglobin, sugars, anaemia profile, vitamin B12, folic acid and thyroid profile were individually tested in each group. These parameters were equally distributed; there was no statistically significant difference between these groups.

The increase in the inflammatory mediators like CRP in the second trimester grossly decreases the placental iron transfer which was reflected by the cord blood serum iron profile.

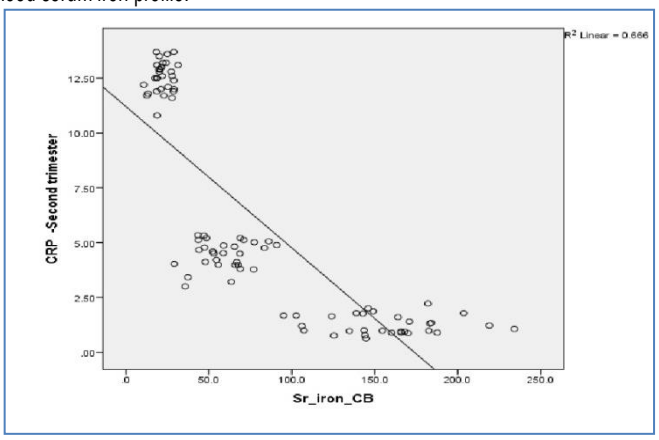

Fig-2a Scatter plot to show correlation of CRP in second trimester and cord blood serum iron

Table-2a: *Correlation between CRP in second trimester and cord blood Serum iron.*

		CRP – Second trimester	Cord blood serum iron
CRP in Second trimester	Pearson Correlation	1	-.816**
	Sig. (2 – tailed)		.0001
	N	90	90
Cord blood serum iron	Pearson Correlation	-.816**	1
	Sig. (2-tailed)	.0001	
	N	90	90
**. Correlation is significant at the 0.01 level (2-tailed).			

[Table-2a] Correlation value of r=-.816**, it indicates high negative correlation at P value 0.0001. This indicates that when CRP in second trimester increases the serum iron in cord blood decreases.

Scatter Plot

Scatter plot relates correlation between two variables by the direction of plot which slopes the dots which indicates three groups. If the pattern of dots slopes from lower left to upper right (upward plot) indicates positive correlation and downward plot indicates negative correlation.

The correlation statistics showed similar results for cord blood serum ferritin and iron saturation. As the maternal CRP in the second trimester increased, the serum iron, serum ferritin and iron saturation decreased. Correlation value of r=0.563**, indicates positive correlation between CRP and TIBC at P value=0.0001. It indicates second trimester and Total Iron Binding Capacity in Cord Blood (TIBC_CB)

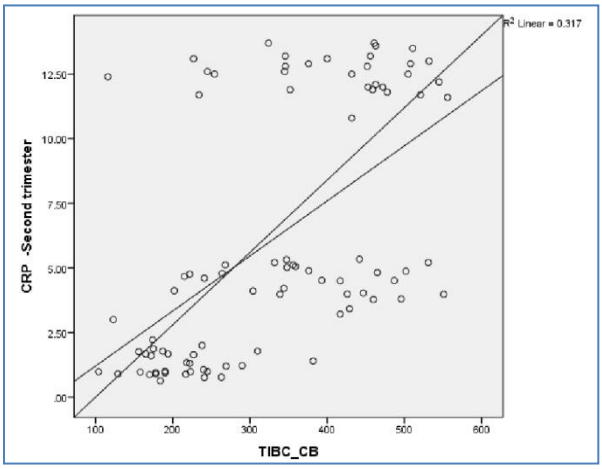

Fig-2b Scatter plot to show correlation between maternal CRP in second trimester and cord blood total iron binding capacity.

[Fig-2b], this figure indicates that total iron binding capacity of cord blood increases with increase in CRP. The similar positive correlation was found between the cord blood CRP and maternal CRP in the second trimester.

We performed one way Anova analysis which was found to be statistically significant with P value <0.0001. Thus the inflammatory mediators increase significantly in the obese than overweight and normal individuals.

The mean value of cord blood serum iron in normal group was found to be 156.6 ± 33.18, in over weight it was 59.30 ± 15.44 and in obese it was 22.05 ± 5.2. The cord blood serum iron in overweight was halved compared to normal individuals, in obese it was 1/6th than in normal group. This is evident in the graphical representation in [Fig-2c].

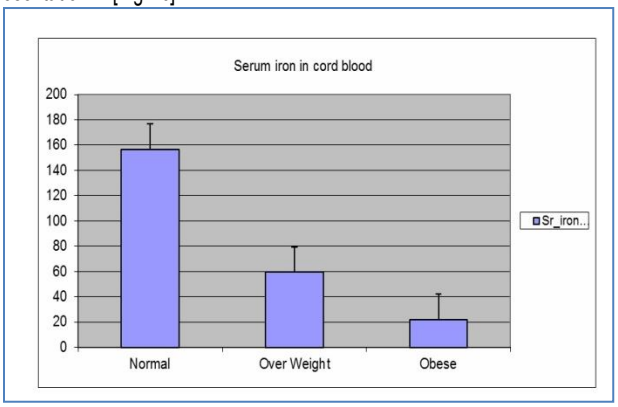

Fig-2c The error bar diagram showing serum iron in cord blood in three groups

Serum Ferritin and iron saturation showed similar relationship.

The mean value of cord blood total iron binding capacity in normal women was 209.63±56.37, in overweight individuals 370.12±106.30 and in obese individuals 408.79±109.5. Thus, the cord blood TIBC was 1.9 times higher in obese and it was 1.7 times higher in overweight compared to normal. We performed one way Anova analysis which showed statistically significant difference with p value <0.0001. Cord blood CRP showed similar relationship. This is shown by the [Fig-2d].

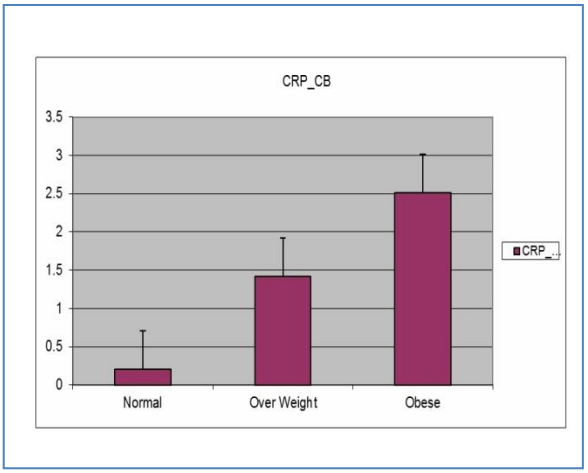

Fig-2d Showing the inflammatory mediator CRP in cord blood three groups

Thus, in the obese and overweight mothers as the maternal inflammatory mediator, CRP increases cord blood serum iron, serum ferritin and iron saturation decreases. Thus, these three parameters had negative correlation with CRP. The total iron binding capacity and cord blood CRP increased with increase in the maternal CRP. Thus, they had positive correlation with maternal CRP. This is shown in [Table-2b].

Table-2b *Cord blood iron profiles except total iron binding capacity were highest in normal group compared to overweight and obese*.

S. No	Variables	Normal	Overweight	Obese	P Value
1.	Cord blood Serum iron	156.62 ± 33.18	59.30 ± 15.44	22.05 ± 5.21	<0.0001***
2.	Cord blood Serum	142.21 ± 42.69	43.31 ± 16.65	22.38 ± 6.38	<0.0001***
3.	Cord blood Iron	73.82 ± 15.06	30.53 ± 8.09	18.99 ± 4.73	<0.0001***
4.	Cord blood total iron binding	209.63 ± 56.37	370.12 ± 106.30	408.79 ± 109.53	<0.0001***

Discussion

This study was done to prove the effect of maternal obesity on fetal iron transfer. Since Indian references were not available, the study results were compared with the western literature.

In the present study, the study group was divided into two (overweight and obese) and compared with the normal group and conclusions were drawn. [Table-3].

Mary dawn Koenig et al showed that the transfer of iron is inversely correlated with hepcidin and was directly associated with neonatal hemoglobin [30].

Mari rehu et al found that maternal CRP had positive correlation with the hepcidin at the time of delivery. Hepcidin concentration was associated with cord blood iron status but does not correlate with maternal iron status [35].

Thus in the present study, CRP was taken in the second trimester in the mother and also in the cord blood instead of hepcidin as it had positive correlation with hepcidin during the second trimester and at the time of delivery [1].

In this study over weight group has also been included in contrast to other previous studies as overweight was more prevalent than obese in Indian population.

Table-3

Dao *et al.*, 2013 [1]	Present study
Their study was conducted in two groups of obese and normal with 15 individuals in each.	The present study was conducted in three groups; normal,overweight and obese with 30 individual in each group. More subjects were included to improve the statistical significance of the study compared to the previous study and the overweight group was also included to emphasize its effect on fetal iron transfer
The pregnant women were included in the second trimester of pregnancy.	The pregnant women were recruited in the study during the initial booking visit to monitor the women even in the first trimester to exclude the complications arising in this trimester.
The maternal CRP measured in second trimester as it had a positive correlation with hepcidin.	In the present study also maternal CRP was measured in the second trimester.
The cord blood iron status was determined by serum iron and transferrin saturation.	In the present study cord blood Iron status was determined by serum iron, serum ferritin, total iron binding capacity and iron saturation to get detailed information on iron status.
The p value of cord blood iron, CRP, hepcidin and IL6 was 0.01.	In this study, the p value of CRP, serumiron and iron saturation was<0.0001 and hence found to be highly significant statistically. Hepcidin and IL6 were not done in this study because of financial constraints.

Philips A.K., *et al.*, 2014 [18]	Present study
The neonatal iron status was assessed by cord blood hemoglobin, zinc protoporphyrin heme(ZnPP/H) and reticulocyte-ZnPP/H and storage iron with serum ferritin.	The placental iron status was assessed by serum iron,serum ferritin,total iron binding capacity and iron saturation.
They found in obese mothers, a Higher cord blood zinc reticulocyte protoporphyrin heme and lower serum ferritin with p value<0.05 for both.	In this study, the obesity was associated with decreased cord blood serum iron, iron saturation and serum ferritin and increased total iron binding capacity and increased CRP. The p value for all these parameters were 0.0001.Thus, proving highly significant statistically.

Andrew jones, *et al.*, 2014 [17]	Present study
The iron status in the early pregnancy was determined by soluble transferrin receptor and erythrocyte protoporphyrin.	In the present study the iron status was assessed in early pregnancy by anemia profile comprising of serum iron, total iron binding capacity, unsaturated iron binding capacity, serum ferritin, along with vitamin B12 and folic acid to exclude anemia of all causes.
Inflammation is assessed in mother in early pregnancy by CRP.	Inflammation in mother was assessed by CRP in second trimester and in cord blood. As CRP has a exact positive correlation with hepcidin at the time of delivery and second trimester. The hepcidin is a peptide that decreases placental iron transfer.
Maternal BMI was positively associated with inflammation in early pregnancy with p value<0.001 and negatively with cord blood iron status like serum ferritin and serum transferrin receptor with P value <0.01.	The obesity had negative association with serum iron, iron saturation and serum ferritin and positive association with total iron binding capacity and CRP with P value <0.0001.

Conclusion

Thus, 90 eligible pregnant women were recruited in the study in three groups. After obtaining consent their details were entered using a standardized proforma. These pregnant mothers underwent detailed clinical examination and biochemical investigations, those with complications like preeclampsia, gestational and type 2 diabetes mellitus, infections and hypothyroidism were excluded from the study.

After delivery, their cord blood was tested for iron profile to indicate the placental iron transfer to the fetus.

As per the aim of the study maternal obesity was compared with placental iron transfer. Obese mothers were categorized using BMI as a criteria and their placental iron transfer was tested using cord blood iron profile (serum iron, iron saturation, Total iron binding capacity and serum ferritin) and inflammatory markers were tested using C Reactive protein in second trimester

This study was found to be highly significant with a p value of <0.0001*** compared to previous studies. All the cord blood parameters were lower in obese and overweight individuals except TIBC and cord blood CRP. It was also found that second trimester CRP had an inverse correlation with cord blood serum iron, iron saturation and serum ferritin whereas had a positive correlation with cord blood TIBC and cord blood CRP.

It is concluded from the present study that maternal obesity decreases fetal iron transfer due to the increase in the inflammatory markers in early pregnancy especially in second trimester which decreases fetal iron transfer that was well proved in this study. Iron is an important micronutrient for fetal growth and brain development.

These obese mothers must be counseled to reduce weight before contemplating next pregnancy. Thus, life style modification and exercise for weight reduction must be advised and consequences of obesity must also be explained to these women.

Application of research: This study was done to prove the effect of maternal obesity and overweight on fetal iron transfer and to emphasize the importance of weight reduction in the women of reproductive age group to prevent this serious consequence as iron supplementation is not going to increase the placental iron transfer in these group.

Research Category: Obstetrics and Gynaecology

Abbreviations: IRE: Iron Regulatory Element , IRP: Iron Regulatory Protein

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Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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