

## Quantitative Impact of Maternal Antenatal Iron Stores and Iron Supplementation on Body Iron Stores of Exclusively Breast Fed Infants up to 6 Months of Age: A Cross-Sectional Study

Meenal Vaidya<sup>1</sup>, Vandana Varma<sup>2</sup>, Shiv Narayan Lahariya<sup>3</sup>, Vesti Randa<sup>4</sup>, Nidhi Agarwal<sup>5</sup>, Rajvardhan Sisodia<sup>6</sup>, Shifa Patel<sup>7</sup>, Purnima Dey Sarkar<sup>8</sup>

<sup>1</sup>Demonstrator, Department of Biochemistry, MGM Medical College, Indore, MP

<sup>2,3</sup>Associate Professor, Department of Biochemistry, MGM Medical College, Indore, MP

<sup>4</sup>Associate Professor Department of Physiology, MGM Medical College, Indore, MP

<sup>5</sup>Second year undergraduate student, MGM Medical College, Indore, MP

<sup>6</sup>Final year undergraduate student, MGM Medical College, Indore, MP

<sup>7</sup>Second year undergraduate student, MGM Medical College, Indore, MP

<sup>8</sup>Professor & Head of the Department of Biochemistry, MGM Medical College, Indore, MP

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Corresponding author: Dr. Vesti Randa

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### Abstract:

Adequate intrauterine iron status is very crucial for normal fetal brain development, postnatal brain performance and prevention of early iron deficiency, specifically for infants on exclusive breast feeding. The sole source of iron for exclusively breastfed infants up to 6 months is breast milk. Maternal iron stores (serum iron, ferritin, CBC) provide a reliable estimate to assess the antenatal iron stores of the mother. Exclusively breastfed term-infants and their mothers were observed to determine the influence of maternal antenatal iron stores and supplementation on body iron stores of infant's upto 6 months of age. Mothers supplemented with iron during pregnancy had significantly higher serum iron, hemoglobin and ferritin levels than those without any iron supplementation and infants born to iron supplemented mothers had statistically significantly higher serum iron ( $68.9 \pm 11.4 \mu\text{g/dL}$  vs.  $55.2 \pm 10.7 \mu\text{g/dL}$ ), hemoglobin ( $11.7 \pm 0.9 \text{ g/dL}$  vs.  $10.8 \pm 1.1 \text{ g/dL}$ ), and ferritin ( $28.4 \pm 6.9 \text{ ng/mL}$  vs.  $19.1 \pm 5.4 \text{ ng/mL}$ ), than those without any supplementation.

**Keywords:** Maternal iron status, Iron supplementation, Exclusive breastfeeding, Infant iron stores, Serum ferritin, Serum iron, Haemoglobin levels, Antenatal care, Iron deficiency anaemia, Third trimester pregnancy, Foetal iron transfer, Breastfed infants, Iron-folic acid adherence.

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### Introduction

Iron is an essential micronutrient for the intra-uterine growth and development of the foetus. It is vital for cellular metabolism, playing a role in various essential physiological activities like ATP production, DNA synthesis, oxygen transport among others.<sup>1</sup> The intrauterine environment plays a crucial role in shaping the baby's future health through 'programming mechanisms' according to which, the foetus adapts to adverse conditions in the womb in ways that can lead to lasting physiological changes, potentially increasing the risk of developmental delays or illnesses later in life.<sup>2</sup> During iron deficiency, cells which have high metabolic rates require more iron and are at greater risk for dysfunction. The iron demands of the maternal body increase during pregnancy, from 1 mg/day in non-pregnant females to nearly 7 mg/day in the third trimester of pregnancy (averaging at

about 4.4 mg/day).<sup>3,4</sup> Iron sufficiency is a necessity for the delivery of oxygen to the maternal-placental-fetal unit so as to support the increased oxygen consumption demand.<sup>5</sup> Since iron deficiency during pregnancy is linked with a higher risk of preterm birth, low birth weight and intrauterine growth restrictions, many countries recommend iron supplementation during the gestation period.<sup>4</sup> There is an increased risk of low birth weight and preterm births due to iron and folic acid deficiencies in the mother during pregnancy.<sup>6,7</sup> To minimise these effects, WHO recommends a daily iron supplementation of 30-40 mg and a daily folate supplement of 0.4 mg for all pregnant females across the pregnancy period.<sup>8</sup> Postnatal iron deficiency is widespread in infants and toddlers with rates nearing to around 80% in the low and middle income countries.<sup>9</sup> A study from China suggests that

postnatal iron deficiency depends largely on the foetal iron loading.<sup>10</sup> There has always been a contradiction in various studies whether or not the maternal iron store levels determine the presence or absence of low iron in the neonates. This study aims to investigate the relationship between the maternal iron status and iron supplementation with the iron stores in exclusively breastfed infants up to 6 months.

#### Methods:

This was a cross-sectional quantitative study conducted in the Department of Biochemistry and Gynaecology at a tertiary care teaching hospital in central India. The study was conducted over a period of one year. Written informed consent was obtained from all participating mothers preceding their enrolment in our study.

Our study included full-term, exclusively breastfed healthy infants aged  $\leq 6$  months, along with their mothers. Exclusive breastfeeding was confirmed based on data reported by the mothers and defined according to WHO criteria: infants received only breast milk, with no other liquids or solids, except for oral rehydration solutions, drops, or syrups (vitamins, minerals, or medicines).

Only those mothers who had their iron parameters documented in their antenatal records for the third-trimester were included in the study. Whereas the following were excluded: Preterm ( $< 37$  weeks of gestation) or low birth weight ( $< 2500$  grams) infants, infants with known congenital anomalies or any chronic systemic illness, infants who had received iron supplementation at any time since birth, mothers with chronic medical conditions known to affect iron metabolism (chronic kidney disease, liver disease, hemoglobinopathies, autoimmune disorders etc.)

The sample size was calculated based on previous literature reporting the correlation between maternal and infant iron status, assuming a correlation coefficient of 0.7, with an alpha of 0.05 and a power of 80%. Using these parameters, a minimum of 70 mother-infant pairs were required.

A pretested structured proforma was used to collect relevant demographic and clinical details, including maternal age, parity, gestational age at delivery, mode of delivery, and infant's birth weight and current weight. Maternal iron status was assessed using third-trimester antenatal records. Parameters included haemoglobin (Hb), serum ferritin and serum iron, depending on the availability.

Infant iron status was evaluated at the time of recruitment using venous blood samples.

The following tests were performed:

1. Haemoglobin (Hb) level using an automated haematology analyser
2. Serum ferritin using Beckman Coulter Dxi 800
3. Serum iron using Beckman Coulter AU 5800

All laboratory investigations were performed in the hospital's central laboratory following standard protocols. Ferritin values were interpreted cautiously in infants with concurrent infections or inflammation (e.g., fever, elevated CRP), and such cases were excluded from analysis.

Data entry and statistical analysis were performed using Jamovi. Data was first assessed for completeness and accuracy, and was cleaned before analysis. The distribution of continuous variables was evaluated using the Shapiro-Wilk test to determine normality. Continuous variables were expressed as mean  $\pm$  standard deviation (SD) for normally distributed data. Independent sample t-tests were used to compare means between two groups when the data followed a normal distribution.

Pearson correlation coefficients ( $r$ ) were calculated to assess the relationship between maternal iron parameters (haemoglobin, serum ferritin, serum iron) and corresponding infant iron stores. In cases where data did not meet the assumptions for Pearson correlation, Spearman's rank correlation was considered as an alternative.

All statistical tests were two-tailed, and a p-value  $< 0.05$  was considered statistically significant. The results were interpreted in the context of clinical relevance as well as statistical significance.

#### Results

**Table 1: Baseline Characteristics**

Variable	Value
	Mean $\pm$ SD
Maternal age (years)	27.4 $\pm$ 4.2
Infant age (months)	5.8 $\pm$ 0.3
Male infants (%)	51
Mothers who reported iron supplement adherence (%)	62

**Table 2: Maternal antenatal status**

Parameter	Supplemented (n=124)	Non Supplemented (n=76)	P value
	Mean $\pm$ SD	Mean $\pm$ SD	
Serum iron ( $\mu\text{g/dL}$ )	90.2 $\pm$ 12.5	72.9 $\pm$ 14.0	<0.001
Hemoglobin (g/dL)	12.1 $\pm$ 1.1	10.6 $\pm$ 1.3	<0.001
Ferritin (ng/mL)	42.6 $\pm$ 10.2	24.7 $\pm$ 8.8	<0.001

**Table 3: Infant iron status at 6 months**

Parameter	Mother Supplemented (n=124)	Mother Non Supplemented (n=76)	P value
Serum iron ( $\mu\text{g/dL}$ )	68.9 $\pm$ 11.4	55.2 $\pm$ 10.7	<0.001
Hemoglobin (g/dL)	11.7 $\pm$ 0.9	10.8 $\pm$ 1.1	<0.001
Ferritin (ng/mL)	28.4 $\pm$ 6.9	19.1 $\pm$ 5.4	<0.001

**Table 4: Correlation between maternal and infant iron stores**

Parameter	Maternal	Infant	Correlation Coefficient (r)	P value
	Mean $\pm$ SD	Mean $\pm$ SD		
Serum iron ( $\mu\text{g/dL}$ )	68.9 $\pm$ 11.4	55.2 $\pm$ 10.7	0.42	<0.001
Hemoglobin (g/dL)	11.7 $\pm$ 0.9	10.8 $\pm$ 1.1	0.38	<0.001
Ferritin (ng/mL)	28.4 $\pm$ 6.9	19.1 $\pm$ 5.4	0.47	<0.001

## Discussion

Our study aimed to analyse the influence of maternal antenatal iron stores and iron supplementation on the body iron stores of exclusively breastfed infants up to 6 months of age.

Importantly, the findings of our study suggest that only 62% of mothers reported adherence (Iron–folic acid tablets for at least 90 days during pregnancy, WHO) to iron supplementation. There have been numerous studies with similar findings of low compliance of iron supplementation. Various factors may contribute to this, such as GI side effects (nausea, constipation, and metallic taste), poor awareness, late antenatal registration, forgetfulness, and a lack of counselling during the routine check-ups. Due to these reasons, we were able to find antenatal mothers not on iron supplementation which helped us draw a comparison between the two groups.

A large-scale study by Taye et al. in Ethiopia noted that only 52% of the pregnant women adhered to supplementation, primarily due to: side effects and misinformation about iron tablets.<sup>11</sup> Despite the national guidelines recommending iron and folic acid supplementation during pregnancy, a significant proportion of women either do not receive proper antenatal care or fail to adhere to the supplementation protocols.

In India, Agrawal et al. highlighted that even when iron tablets were provided cost free via government programs, a fear of side effects, inconsistent antenatal visits and poor understanding of anaemia still remained strong rationale for their loss of compliance.<sup>12</sup>

The entirety of this data suggests that there is a pressing need to raise awareness and improve the

understanding of the population, predominantly the rural and underserved population, as even providing cost free iron supplements to the mothers does not ensure a high compliance for iron supplementation. Mothers supplemented with iron during pregnancy had significantly higher serum iron (90.2  $\pm$  12.5  $\mu\text{g/dL}$  vs. 72.9  $\pm$  14.0  $\mu\text{g/dL}$ ), haemoglobin (12.1  $\pm$  1.1 g/dL vs. 10.6  $\pm$  1.3 g/dL), and ferritin levels (42.6  $\pm$  10.2 ng/mL vs. 24.7  $\pm$  8.8 ng/mL) than those without any iron supplementation. These findings are in accordance with the WHO recommendation for routine daily iron and folic acid supplementation during pregnancy for reducing maternal anaemia and adverse birth effects.<sup>13</sup>

These findings are also supported by studies such as Sangkhae et al., which focuses on the maternal–placental foetal iron transfer mechanism during the gestational period.<sup>14</sup> Such findings, however, are not common across all settings. A review by Mwangi et al. reported that even though maternal supplementation improves the body iron stores of the mother, there isn't any significant impact of the same on the body iron stores of the infants.<sup>15</sup> A similar study by Chan et al. suggests no significant impact of maternal ferritin on the infant's iron status; neither at birth, nor at 3 months of age.<sup>16</sup> These difference in findings might be due to various reasons:

As per our study, the infants born to iron supplemented mothers had significantly higher serum iron (68.9  $\pm$  11.4  $\mu\text{g/dL}$  vs. 55.2  $\pm$  10.7  $\mu\text{g/dL}$ ), haemoglobin (11.7  $\pm$  0.9 g/dL vs. 10.8  $\pm$  1.1 g/dL), and ferritin (28.4  $\pm$  6.9 ng/mL vs. 19.1  $\pm$  5.4 ng/mL), than those without any supplementation. These findings coincide with the findings of a study by Shao et al., which suggested a positive correlation

between the maternal antenatal ferritin and infant stores.<sup>17</sup>

A study by Georgieff also highlights the dependency of foetal iron acquisition on maternal iron status during the third trimester.<sup>16</sup> Unlike these findings, Zhao et al. in a randomised trial did not observe any significant difference in the iron status of 6-month-old infants independent of whether there was any maternal iron supplementation or not.<sup>18</sup> These studies demonstrate the varied nature of findings on this topic and the need for further research and reviews to establish appropriate consensus.

### Conclusion

Mothers supplemented with iron during pregnancy had significantly higher serum iron ( $90.2 \pm 12.5 \mu\text{g/dL}$  vs.  $72.9 \pm 14.0 \mu\text{g/dL}$ ), haemoglobin ( $12.1 \pm 1.1 \text{ g/dL}$  vs.  $10.6 \pm 1.3 \text{ g/dL}$ ), and ferritin levels ( $42.6 \pm 10.2 \text{ ng/mL}$  vs.  $24.7 \pm 8.8 \text{ ng/mL}$ ) than those without any iron supplementation. Infants born to iron supplemented mothers had significantly higher serum iron ( $68.9 \pm 11.4 \mu\text{g/dL}$  vs.  $55.2 \pm 10.7 \mu\text{g/dL}$ ), haemoglobin ( $11.7 \pm 0.9 \text{ g/dL}$  vs.  $10.8 \pm 1.1 \text{ g/dL}$ ), and ferritin ( $28.4 \pm 6.9 \text{ ng/mL}$  vs.  $19.1 \pm 5.4 \text{ ng/mL}$ ), than those without any supplementation. This supports the significant influence of maternal antenatal iron status and supplementation on the iron stores of exclusively breastfed infants. The positive correlations between maternal and infant iron parameters highlight the importance of ensuring optimal maternal iron levels during pregnancy. Despite clear national guidelines, poor compliance with iron supplementation remains a major barrier, often driven by side effects, lack of awareness, and inadequate antenatal counselling. These insights underscore the need for integrated public health strategies focusing not only on supplementation but also on education, awareness, early antenatal registration, and consistent follow-ups. Addressing these gaps could play a crucial role in preventing early-onset iron deficiency among infants and improving long term maternal-child health outcomes.

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