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**“TO EVALUATE THE IRON STATUS IN  
EXCLUSIVELY BREAST FED NORMAL  
FULL TERM INFANT AT BIRTH AND AT  
4TH MONTH OF AGE-A HOSPITAL BASED  
LONGITUDINAL STUDY”**

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**BY**

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**DEPARTMENT OF PAEDIATRICS  
JAWAHARLAL NEHRU MEDICAL COLLEGE,  
BELAGAVI, KARNATAKA**

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**SEPTEMBER /OCTOBER 2025**

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## ABBREVIATIONS

WHO	-	World Health Organization
AAP	-	American Academy of Paediatrics
IAP	-	Indian Academy of Paediatrics
AGA	-	Appropriate for Gestational Age
SGA	-	Small for Gestational Age
LGA	-	Large for Gestational Age
SES	-	Socioeconomic Status
LSCS	-	Lower Segment Caesarean Section
NVD	-	Normal Vaginal Delivery
Hb	-	Haemoglobin
PCV	-	Packed Cell Volume
RBC	-	Red Blood Cell
PLT	-	Platelet
WBC	-	White Blood Cell
TF	-	Transferrin
TIBC	-	Total Iron-Binding Capacity
IDA	-	Iron Deficiency Anaemia
CHr	-	Reticulocyte Haemoglobin Content
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Haemoglobin

MCHC	-	Mean Corpuscular Haemoglobin Concentration
UIBC	-	Unsaturated Iron Binding Capacity
FCM	-	Ferric Carboxymaltose
POG	-	Period of Gestation
EBF	-	Exclusive Breastfeeding

## **ABSTRACT**

### **Introduction:**

Iron is essential for infant growth, cognitive development, and overall health, with full-term infants relying on iron stores accumulated during late pregnancy. While exclusive breastfeeding is the ideal form of infant nutrition, its low iron content (0.2–0.4 mg/L) may predispose infants to iron deficiency anaemia (IDA) by four to six months. Studies in India report a high prevalence of IDA in exclusively breastfed infants, influenced by maternal anaemia, poor nutrition, and perinatal factors. While WHO and Indian guidelines recommend iron supplementation from six months, the American Academy of Paediatrics advises earlier supplementation at four months to prevent depletion. Given these concerns, there is a critical need to assess the iron status of exclusively breastfed full-term infants, particularly in regions with high maternal anaemia rates. Understanding iron depletion trends in the first months of life can help guide public health interventions, such as maternal iron supplementation during pregnancy or early iron supplementation in high-risk infants. Despite growing evidence on the long-term effects of early-life iron deficiency, there is limited research tracking iron status in exclusively breastfed, full-term infants over time. A hospital-based longitudinal study evaluating iron status at birth and at four months of age will provide essential data to inform evidence-based recommendations, helping prevent early-onset IDA and its long-term consequences on neurodevelopment and overall health.

### **Aims and Objectives:**

**Primary:** To evaluate the iron status in exclusively breast fed normal full-term infant at birth and at 4th month of age

**Secondary:** To determine the ideal post-natal age for iron supplementation. To know the correlation between maternal iron stores and new born iron status.

**Methodology:** Study Design: Longitudinal Study Sample Population: All term neonates >37 weeks Period of Gestation >2.5kg born in KLE Dr. Prabhakar Kore, Hospital, Belagavi Sample size: The calculated sample size is 72 participants, accounting for a 20% attrition rate, with an effect size of 0.3, correlation of 0.25, and 4 time points. Sampling Technique: Consecutive sampling Study Period: Total one-year Study procedure: After obtaining ethical clearance, term neonates >37 weeks of gestation meeting the inclusion criteria will be selected. Informed consent will be obtained from parents, and participant data will be recorded in a structured proforma, including detailed history of gestational age, gender, mode of delivery, maternal iron intake, maternal comorbidities (e.g., GDM, anaemia), and socioeconomic status.

**Results & Conclusion:** The study population consisted of 72 individuals, with a higher proportion of females (59.7%) than males (40.3%). Birth weight distribution revealed that most new-borns (65.3%) were Appropriate for Gestational Age (AGA), while 33.3% were Small for Gestational Age (SGA), and only 1.4% were Large for Gestational Age (LGA). Socioeconomic status analysis showed that nearly half (45.8%) belonged to the lower middle class, followed by 25% in the lower class, with fewer individuals in higher socioeconomic strata. Regarding the mode of delivery, 58.3% of the infants were delivered via Lower Segment Caesarean Section (LSCS), while 41.7% were born through Normal Vaginal Delivery (NVD). Haematological parameters at birth indicated a mean haemoglobin (Hb) level of 16.21 g/dL, which declined to 12.50 g/dL by the fourth month, a statistically significant reduction ( $p < 0.01$ ). Other haematological markers, including red blood cell count, white blood cell count, and iron parameters, also showed a decline from birth to four months. Iron

deficiency anaemia (IDA) was absent at birth but emerged in 12.5% of infants by the fourth month. Among mothers in the third trimester, 11.1% had IDA, and a significant association ( $p < 0.001$ ) was observed between maternal IDA and infant IDA at four months, with 75% of infants born to anaemic mothers developing IDA. These findings underscore the importance of maternal iron status in determining infant iron stores and highlight the need for improved maternal nutrition and early iron supplementation strategies to prevent anaemia in infancy.

**Keywords:** Iron deficiency Anaemia

## CONTENTS

<b>SR. NO.</b>	<b>TOPIC</b>	<b>PAGE NO.</b>
<b>1.</b>	<b>INTRODUCTION</b>	<b>1-5</b>
<b>2.</b>	<b>OBJECTIVES</b>	<b>6</b>
<b>3.</b>	<b>REVIEW OF LITERATURE</b>	<b>7-49</b>
<b>4.</b>	<b>MATERIALS AND METHODS</b>	<b>50-56</b>
<b>5.</b>	<b>RESULTS</b>	<b>57-85</b>
<b>6.</b>	<b>DISCUSSION</b>	<b>86-92</b>
<b>7.</b>	<b>CONCLUSION</b>	<b>93</b>
<b>8.</b>	<b>SUMMARY</b>	<b>94-98</b>
<b>9.</b>	<b>BIBLIOGRAPHY</b>	<b>99-117</b>
<b>10.</b>	<b>ANNEXURES</b>	<b>118-124</b>
	<b>ANNEXURE I – CONSENT FORM</b>	<b>118-120</b>
	<b>ANNEXURE II – PROFORMA</b>	<b>121-123</b>
	<b>ANNEXURE III – MASTER CHART</b>	<b>124</b>

## LIST OF FIGURES

<b>SI NO</b>	<b>FIGURES</b>	<b>PAGE NO</b>
<b>1</b>	Prevalence of anemia in pregnancy	<b>9</b>
<b>2</b>	Prevalence of anaemia among children	<b>11</b>
<b>3</b>	State-wise proportion of exclusive breastfeeding	<b>14</b>
<b>4</b>	Proportion of Indian infants of EBF	<b>14</b>
<b>5</b>	Microcytic Hypochromic Anemia	<b>40</b>
<b>6</b>	Procedure of taking cord blood sample	<b>53</b>
<b>7</b>	Procedure of testing Complete Blood count and Iron profile	<b>55</b>
<b>8</b>	Gender distribution	<b>57</b>
<b>9</b>	Birthweight categories	<b>58</b>
<b>10</b>	Socio-economic status	<b>59</b>
<b>11</b>	Mode of delivery	<b>60</b>
<b>12</b>	Rural V/S Urban	<b>61</b>
<b>13</b>	Maternal anemia in relation to Socio-economic status	<b>62</b>
<b>14</b>	Maternal anemia in relation to Rural/Urban	<b>64</b>
<b>15</b>	Iron deficiency anaemia of mothers at 3rd trimester	<b>68</b>
<b>16</b>	Iron deficiency anaemia at Birth	<b>69</b>
<b>17</b>	Iron deficiency anaemia at 4th month	<b>70</b>
<b>18</b>	Iron deficiency anaemia	<b>70</b>

<b>19</b>	Scatter plot on Hb levels at birth and at 4th month	<b>74</b>
<b>20</b>	Scatter plot on PCV levels at birth and at 4th month	<b>77</b>
<b>21</b>	Scatter plot on Iron levels at birth and at 4th month	<b>78</b>
<b>22</b>	Scatter plot on TIBC levels at birth and at 4th month	<b>80</b>
<b>23</b>	Scatter plot on TIBC levels at birth and at 4th month plot	<b>82</b>

## LIST OF TABLES

<b>SI NO</b>	<b>TABLE</b>	<b>PAGE NO</b>
<b>1</b>	Gender distribution	<b>57</b>
<b>2</b>	Birthweight categories	<b>58</b>
<b>3</b>	Socioeconomic status	<b>59</b>
<b>4</b>	Mode of delivery	<b>60</b>
<b>5</b>	Rural V/S Urban	<b>61</b>
<b>6</b>	Maternal Anemia in relation to Socio-economic Status	<b>62</b>
<b>7</b>	Maternal Anemia in relation to rural/urban	<b>64</b>
<b>8</b>	Iron Deficiency Anemia and Gender of infant	<b>65</b>
<b>9</b>	Iron Deficiency Anemia and Mode of delivery	<b>66</b>
<b>10</b>	Iron Deficiency anemia and Birth weight pattern	<b>67</b>
<b>11</b>	Iron deficiency anaemia of mothers at 3rd trimester	<b>68</b>
<b>12</b>	Iron deficiency anaemia at Birth	<b>69</b>
<b>13</b>	Iron deficiency anaemia at 4th month	<b>70</b>
<b>14</b>	Mean Haematological parameters of mother at 3rd trimester	<b>71</b>
<b>15</b>	Mean Haematological parameters at birth	<b>72</b>
<b>16</b>	MeanHaematological parameters of infants at 4th month	<b>73</b>
<b>17</b>	Paired Samples Statistics Hemoglobin	<b>74</b>
<b>18</b>	Paired Samples Statistics PCV	<b>76</b>

<b>19</b>	Paired Samples Statistics Iron	<b>78</b>
<b>20</b>	Paired Sample Statistics Ferritin	<b>80</b>
<b>21</b>	Paired Sample Statistics TIBC	<b>82</b>
<b>22</b>	Correlation of Maternal anaemia and infants	<b>84</b>

## **INTRODUCTION**

Iron is a vital micronutrient important for infant growth, intellectual development, and overall health. It is important for transfer of oxygen through haemoglobin, generation of power, immune function, as well as brain development.<sup>1</sup> Throughout the neonatal period, term infants depend on iron reserves accumulated throughout the third trimester of pregnancy to meet their physiological requirements after birth.<sup>73</sup> These stored iron, combined with the trace amount of iron available in breast milk, are generally adequate to provide normal development in the early months of life. As the neonate grow rapidly, their iron needs increase, and this balance may be disrupted, particularly in infants fed only breastmilk, given the decreased reserve of iron in breast milk.<sup>2</sup>

Exclusive breastfeeding is the optimal benchmark of infant nutrition, as recommended by the WHO and other global organizations, throughout the initial 6 months of infancy. Breast milk supplies all essential elements, antibodies, and bioactive substances necessary for optimal development and immune protection.<sup>37</sup> However, its iron levels is typically low (0.2–0.4 mg/L), which could potentially predispose exclusively breastfed infants to iron deficiency as their iron reserves deplete after four to six months of age.<sup>13</sup> This concern is particularly relevant in populations where maternal anaemia, poor maternal nutrition, or perinatal factors such as delayed cord clamping are prevalent, as these factors influence the neonatal iron status at the time of birth.<sup>78</sup>

In India, a high prevalence of anaemia has been reported in infants who are solely breastfed between three to six months of age by various studies, such as Marol et al.<sup>3</sup> (2021) finding 87.6% prevalence in Karnataka, Kumar et al.<sup>4</sup> (2020) reporting

74.4% prevalence, and Choudhary et al.<sup>5</sup> (2017) identifying 21.4% and 36.4% iron deficiency at 4 and 5 months, respectively. These findings highlight the vulnerability of Indian infants to IDA due to inadequate iron intake from breast milk alone. Globally, prevalence rates vary, with Domellöf et al.<sup>70</sup> (2001) finding that IDA was uncommon in Swedish infants before 6 months but more prevalent in lower birth weight males, while Lozoff et al.<sup>6</sup> (2015) reported an altitude-adjusted anaemia prevalence of 49% in Honduran infants aged 2–6 months, rising to 72% at 7–12 months.

Iron deficiency during early months of life has been linked with negative impacts on neurodevelopment, behavioural functioning, and immune health<sup>1,73</sup>. Studies suggest that even mild iron deficiency at critical brain development stages can have enduring effects on cognition and behaviour.<sup>1,73</sup> Full term newborns of mothers with good health and nutrition generally have optimal iron reserves at birth, the risk of depletion increases by the fourth month because of accelerated growth and the absence of supplemental dietary iron in exclusively breastfed infants.<sup>2</sup>

Guidelines regarding iron supplementation for term infants vary among organizations. The IAP and the WHO recommend iron therapy starting from six months of age for term infants who are solely breastfed.<sup>15,7</sup> (WHO, 2021; IAP, 2016). Similarly, the National Iron Plus Initiative (NIPI) under India's anaemia control program advises universal supplemental iron from the age of 6 months<sup>27</sup> (Government of India, 2013). However, the American Academy of Paediatrics (AAP) takes a more proactive approach, recommending iron therapy for all infants who were born term from 4 months of age to prevent reduction of iron reserves and subsequent IDA.<sup>13</sup> These variations in guidelines reflect regional differences in dietary patterns, socioeconomic factors, and anaemia prevalence. In India, maternal anaemia and poor

nutritional status during pregnancy are significant contributors to low iron stores in new-borns, raising the likelihood early iron deficiency.<sup>73</sup> Additionally, while breast milk is the ideal source of nutrition for the first six months of life, its low iron content (0.2–0.4 mg/L) may not suffice to fulfil the increasing demands of neonates as they grow.<sup>73</sup>

Given these concerns, understanding the iron levels of fullterm infants who are solely breastfed at birth and throughout the early months of life is essential. Such data can inform recommendations for iron supplementation tailored to specific populations, helping to prevent anaemia and its long-term consequences on neurodevelopment and growth. A hospital-based longitudinal study evaluating iron status at birth and at fourth month of age in exclusively breastfed newborns can bridge this gap and guide region-specific public health strategies for anaemia prevention.

These research findings underscore the significance of monitoring iron levels in exclusively breastfed infants, as iron stores gradually deplete after birth, leading to a higher likelihood of IDA. While exclusive breastfeeding provides essential nutrients, it may not always meet the iron demands of growing infants, particularly after the first few months, necessitating considerations for iron supplementation in high-risk populations. The varying prevalence of IDA across different regions emphasizes the necessity of region-specific strategies to avoid early childhood anaemia. Despite this potential risk, there is limited research in assessing the iron levels of exclusively breastfed, full-term neonates over time, especially in resource restricted settings or regions where maternal anaemia is prevalent. Understanding the iron levels at birth and till six months of age is critical for identifying the need for preventive measures, such as maternal iron therapy or incorporation of iron-rich solid foods during pregnancy. A hospital-based longitudinal study monitoring iron reserves

in infants who are solely breastfed, full-term infants at birth and during fourth month of life will help address this gap in evidence and guide community health initiative to avoid IDA and its longterm complication.

### **Need for This Study**

Iron deficiency anaemia (IDA) in early infancy is a critical population medical issue, particularly among exclusively breastfed full-term infants, as iron stores acquired at birth gradually decline over the early months of infancy. While exclusive breastfeeding is widely advised for optimal infant health, exclusive breastfeeding may not provide enough iron to fulfill the increasing demands of rapid growth beyond the neonatal period. A study from the International Journal of Contemporary Paediatrics reported an 87.6% prevalence of anaemia among exclusively breastfed infants between 3 to 5 months of age in india, with the highest rate (92.3%) observed at 4 months<sup>7</sup>. This finding suggests that while breast milk provides numerous benefits, it may not supply sufficient iron to fulfill the growing demands of infants beyond the initial months. Maternal anaemia can have significant effects both at birth and in the early months of infancy.

This study is necessary due to increasing evidence that early-life iron deficiency can have lasting consequences, including impaired cognitive and motor development, increased susceptibility to infections, and overall poor health outcomes. The widespread occurrence of maternal anaemia in India, affecting approximately 50-60% of expectant mothers, leads to negative consequences such as low birth weight, fetal growth retardation, and premature births, which further increase the likelihood of iron deficiency in infants. Although exclusive breastfeeding is recommended, it often does not provide enough iron for growing infants beyond the neonatal period,

particularly by the fourth to sixth month. This deficiency can result in delayed cognitive and motor development, weakened immune systems, and increased irritability. The situation is worse in rural and low-income areas, where limited access to healthcare and nutritional resources exacerbate the issue. The need for targeted research on iron status in infants, particularly at birth and at four months, is crucial to develop region-specific interventions, such as maternal iron therapy during pregnancy and early iron intake for high-risk infants. Addressing these challenges through improved maternal nutrition, healthcare access, and iron supplementation can significantly reduce the long-term consequences of IDA, enhancing overall infant health and development across the count

## **AIMS AND OBJECTIVES**

### **PRIMARY**

- To evaluate the iron status in exclusively breast fed normal full-term infant at birth and at 4th month of age

### **SECONDARY**

- To determine the ideal post-natal age for iron supplementation.
- To know the correlation between maternal iron stores and new born iron status.

## **REVIEW OF LITERATURE**

Iron needs have a higher chance to surpass iron consumption during two critical phases in the life cycle: from 4 to 18 months of age and, for girls, throughout adolescence<sup>8</sup>. Lack of iron in early infancy coincides with crucial period of increased brain growth, when structural, chemical, and energy changes can impact later cognitive function<sup>6</sup>. Neural structures can be adversely affected by lack of iron, whether before birth or after birth, since iron is essential for neurogenesis as well as differentiation of specific brain cells and regions<sup>1</sup>. Recent rodent research findings have highlighted the hippocampus and striatum as two regions where iron deficiency leads to altered morphology, including reduced dendritic branching, which diminishes the number and complexity of neural connections<sup>9</sup>. Additionally, iron deficiency affects specialized cells that produce myelin—leading to changes in the location and role of these cells, as well as changes in the make up and quantity of myelin in white matter.<sup>10</sup>

Breastmilk is the optimal nourishment for babies. It is clean, secure, and has immune-protective compounds that prevent against a range of frequent pediatric diseases<sup>46</sup>.

Breast milk fulfils all of an infant's nutrient and energy requirements during the first few months of life<sup>11</sup>. Furthermore, it continues to meet up to one-third of their nutritional needs during the complementary feeding period when other foods are introduced<sup>12</sup>. At birth, full-term infants typically have sufficient iron stores, mainly stored as ferritin, within the liver, spleen, and bone marrow<sup>13</sup>. These iron reserves are essential for fulfilling the infant's iron requirements during the initial 4-6 months, as human milk does not supply adequate iron<sup>14</sup>. As stated by WHO infants who are solely nursed receive complementary iron-rich solid foods starting at around

6 months to prevent iron deficiency anaemia (IDA), a condition that can impair cognitive development and physical growth<sup>15</sup>.

At birth, iron status is largely determined by maternal iron reserves<sup>16</sup>. Newborns of iron-sufficient mothers generally have higher iron stores, which are reflected in their cord blood ferritin levels<sup>17</sup>. Studies have shown that full-term babies whose mothers have adequate iron levels generally have ferritin levels ranging from 100 to 150 microgram/Litre at birth, while those delivered to mothers with insufficient iron levels exhibit significantly lower ferritin levels<sup>18</sup>. These iron stores typically sustain the infant for the initial months after birth.<sup>19</sup> However, for babies who solely rely on breastfeeding, human milk contains a low iron concentration, averaging 0.3-0.5 mg/L<sup>20</sup>. This minimal iron content in breast milk is sufficient to meet the needs of infants who are not at a significant risk of iron depletion, but the reserves from birth begin to deplete as the infant grows<sup>21</sup>. A lack of iron can begin as early as 4-6 months of age if complementary iron-rich foods are not introduced<sup>22</sup>. Research indicates nursing newborns may experience IDA as early as 4 months of age if they do not receive proper supplementation.<sup>23</sup>

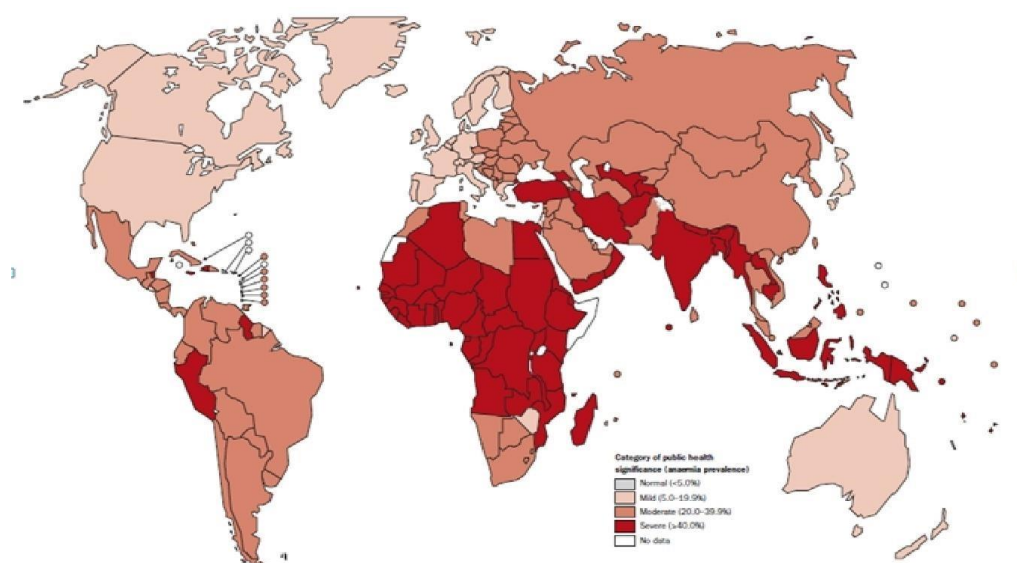
### ***Prevalence of Iron Deficiency Anaemia in pregnant women***

The incidence of IDA in pregnant women in India varies widely, with estimates ranging from 50% to as high as 80%, depending on the region, socioeconomic status, and availability and quality of healthcare services<sup>45</sup>. A research conducted by Balarajan et al.<sup>124</sup> revealed that nearly 58% of pregnant mothers in India were diagnosed to be anaemic, with a substantial proportion having iron deficiency as the underlying cause<sup>124</sup>. The high prevalence of IDA is mainly due to insufficient iron consumption, low uptake of dietary iron, as well as restricted availability of iron supplementation<sup>24</sup>. Additionally, factors such as financial hardship, limited maternal

education, and poor healthcare access exacerbate the situation, particularly in remote and undeserved areas.<sup>25</sup> Severe maternal anemia can result in complications like preterm birth, reduced birth weight, impaired fetal growth, and in extreme cases maternal deaths.<sup>26</sup> Furthermore, anaemia in mothers has been associated with delayed cognitive and physical developmental delays in children, thus impacting the overall well-being for both mothers and infants.<sup>1</sup> Despite various government initiatives aimed at addressing maternal anaemia, including the National Iron+ Initiative, the impact has been limited due to challenges in implementation and coverage, particularly in remote areas.<sup>27</sup> Iron supplementation programs and dietary interventions, though beneficial, require better outreach and adherence to significantly lower the occurrence of IDA among expectant mothers in India.<sup>28</sup> Increased awareness, improved maternal nutrition, and greater access to prenatal care are critical to addressing this issue and ensuring better health outcomes for both mothers and their infants.<sup>29</sup>

**Figure 1: Anaemia as a global health concern by country among pregnant women.**

**Source: Global prevalence of anaemia 1993–2005; WHO Global Database on Anaemia (2008)**<sup>15</sup>



***Prevalence of Iron Deficiency Anaemia in 0 to 6 months of Age***

Iron Deficiency Anaemia (IDA) represents a significant public health issue in India, particularly among children aged 0 to 6 months. While specific state-wise data for this age group is limited, insights can be drawn from broader studies on anaemia prevalence in children under five years.<sup>45</sup>

Maternal anaemia significantly impacts neonatal iron reserves. A study conducted in Delhi between 2003–2004 assessed iron levels in term infants who are solely breastfed born to mothers with anaemia with haemoglobin levels ranging from 7–10.9 g/dL and those without anaemia with haemoglobin  $\geq 11$  g/dL. Results indicated that infants of anaemic mothers showed reduced, yet within normal range, iron parameters at birth, 14 weeks, and 6 months, suggesting maternal anaemia affects but does not severely compromise infant iron status in the early months.<sup>30</sup>

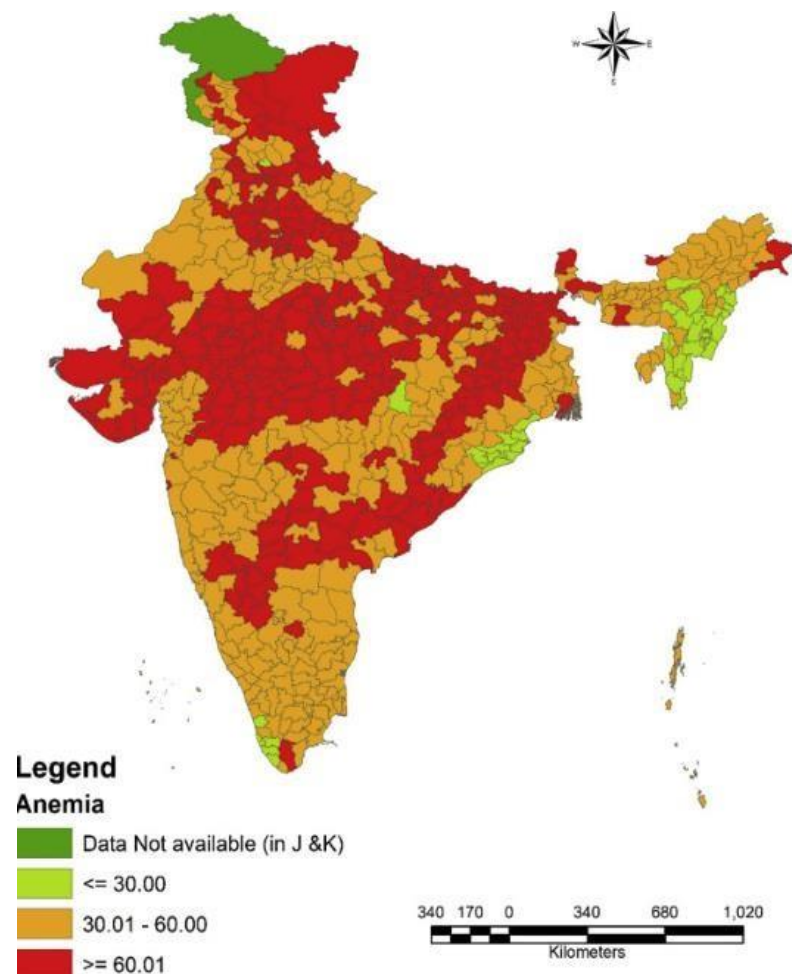
A study from the International Journal of Contemporary Paediatrics reported an 87.6% prevalence of anaemia among infants who were exclusively breastfed between three to five months, with the highest rate (92.3%) observed at 3 months. This finding suggests that while breast milk provides numerous benefits, it might not provide enough iron to support the increasing needs of babies after the initial months of infancy.<sup>31</sup>

According to the National Family Health Survey (NFHS-5) conducted between 2019 and 2021, 67.1% of children between the ages 6 and 59 months in India were diagnosed with reflecting an increase from 58.6% recorded in NFHS-4 (2015–2016)<sup>45</sup>. The NFHS-5 carried out from 2019 to 2021 reported that 68.4% of children between the ages 6 to 59 months in Assam were diagnosed of anaemia, marking a substantial increase from 35.7% in the previous survey<sup>45</sup>. Other states with

high anaemia prevalence among children include Gujarat (79.9%), Madhya Pradesh (74.3%), Rajasthan (73.5%), Punjab (71.2%), Haryana (70.1%), and Telangana (70.1%)<sup>45</sup>. Conversely, Kerala reported the lowest prevalence at 39.4%, though it still saw an increase from 35.7% in the previous survey<sup>31</sup>.

Research published in the journal *Nutrients* in 2024 highlighted that anaemia continues to be widespread in India particularly among children, in rural areas, as well as women of child bearing age.<sup>32</sup> The study observed significant differences in childhood anaemia across Indian states, influenced by socioeconomic like wealth and education which heightens the likelihood of anaemia in adolescent girls and along with their children.<sup>33</sup>

**Figure 2: Prevalence of Anaemia at the State level in India in children aged 6–59 months.**



## **Exclusive breastfeeding**

Breastfeeding is the milk produced by a mother's breast glands<sup>41</sup>. Exclusive breastfeeding refers feeding a baby solely with breast milk from birth to six months, without introducing any other food or beverages<sup>34</sup>. During this period, the baby consumes only breast milk, without water, formula, or solid foods.<sup>52</sup>

Exclusive breastfeeding supplies essential nutrients, antibodies, and hydration necessary for the baby's healthy growth and development<sup>43</sup>. It helps protect against infections<sup>93</sup>, supports brain development<sup>35</sup>, and strengthens the emotional connection between mother and child.<sup>36</sup>

Exclusive breastfeeding offers significant advantages for both the mother and the child. It is considered the optimal method of feeding for the first six months<sup>37</sup>. Breast milk delivers vital nutrients for the baby's growth and strengthens boosting their immune system<sup>42</sup>. Studies suggests that exclusive breastfeeding for a period of six months helps prevent against gastrointestinal infections<sup>43</sup> and iron deficiency anaemia.<sup>78</sup>

For mothers, exclusive breastfeeding can lead to amenorrhea (the absence of menstruation) for up to six months postpartum, helping prevent unplanned pregnancies that might result in abortion<sup>38</sup>. Furthermore, research shows that breastfeeding promotes sensory and cognitive development in babies<sup>39</sup>, while also safeguarding them against chronic illnesses and respiratory infections<sup>47</sup>, such as the common cold, cough, and pneumonia<sup>44</sup>.

*Exclusive breastfeeding arrangements aim to:*

- a) Make sure that babies receive exclusively breastfed from birth until six months of age , supporting their growth as well as development<sup>45</sup>.
- b) Support mothers by fostering active participation from families, communities, local governments, and the broader government in promoting and supporting exclusive breastfeeding practices<sup>40</sup>.

Health organizations like the World Health Organization (WHO) and United Nation's Children's Fund advises the practise of exclusive breastfeeding for the first six months<sup>41</sup>. After that, solid foods can be gradually introduced alongside breastfeeding.<sup>41</sup>

Exclusive breastfeeding typically extends from 1 month to 6 months<sup>42</sup>. A study in Sri Lanka revealed that 50.8% of surveyed infants were fed exclusively on breastmilk for as long as 6 months and up to 5 months or more was 81.3%<sup>43</sup>. On average exclusive breastfeeding lasts 6 months. The primary reason for early cessation of exclusive breastfeeding is the belief among mothers that breast milk may not provide adequate nutrition for babies (52.9%)<sup>44</sup>.

At two months of age, 66.7% of infants (2268 out of 3401; 95% CI: 65.1, 68.3) were exclusively breastfed, as reported in the NFHS-4 study. This percentage increased to 70.4% (2770 out of 3938; 95% CI: 68.9, 71.8) in the NFHS-5 survey. By four months, the exclusive breastfeeding rate was 61.5% (2443 out of 3972; 95% CI: 60, 63) in NFHS-5, showing a significant increase compared to the NFHS-4 study, which recorded a lower rate of 50.2% (1958 out of 3904; 95% CI: 48.6, 51.7). At six months of age, the percentage of exclusively breastfed infants remained higher in the NFHS-5 survey at 43% (1657 out of 3853; 95% CI: 41.4, 44.6), compared to 31.3% (1280 out of 4095; 95% CI: 29.9, 32.7) in the NFHS-4 survey..<sup>37</sup>

Figure 3: State-wise proportion of exclusive breastfeeding in infants until 6 months (151–180 days) of age from NFHS-4 & 3

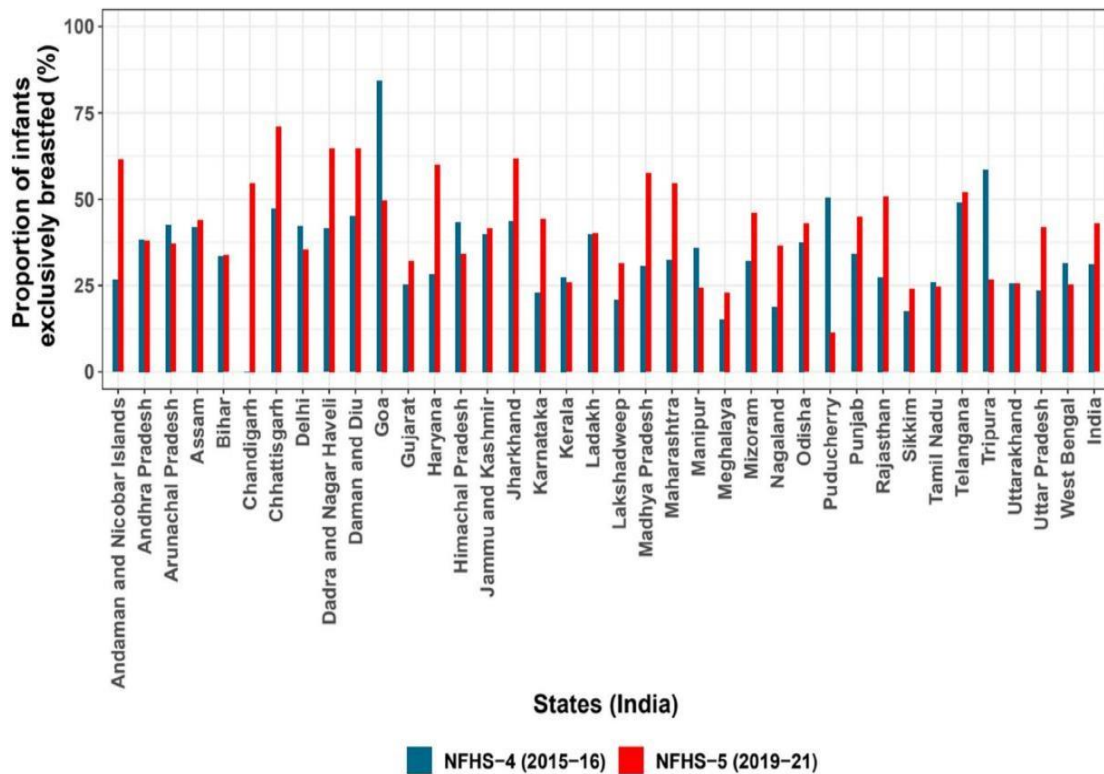
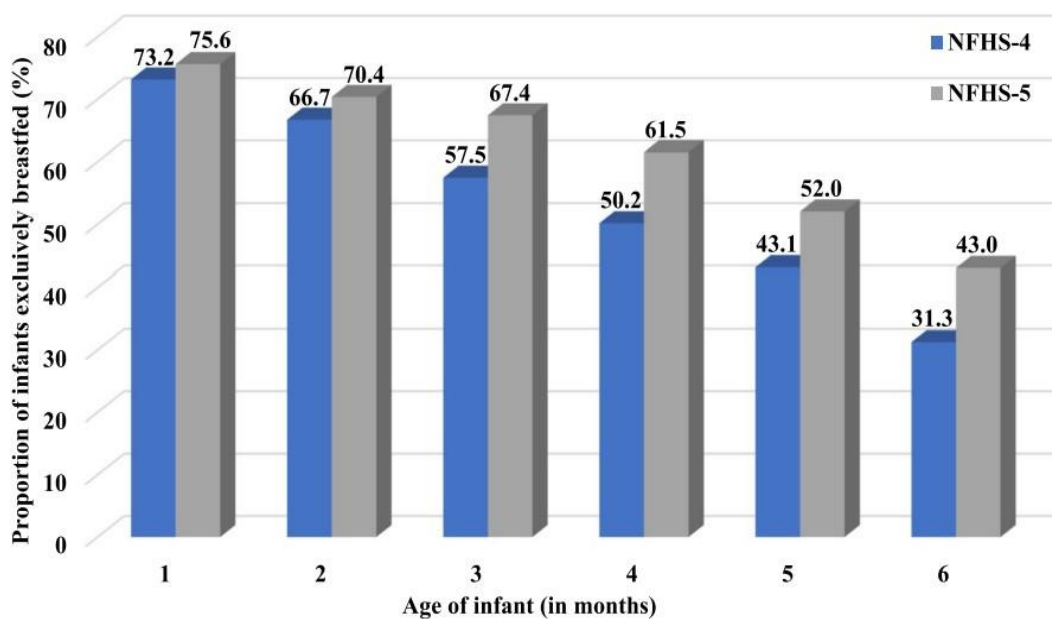


Figure 4: Percentage of Indian babies (0–6 months) exclusively breastfed as per the NFHS-4 and 5



## **The Components of Human milk**

Human milk contains colostrum, foremilk, as well as hindmilk, with its composition changing throughout a feeding and as the baby grows<sup>51</sup>. It can also vary at different times throughout the day and between the nursing sessions. Colostrum, a thick, yellowish or clear fluid that appears over the initial few days of birth, is antibodies abundant, white blood cells, growth factors, and vitamin A<sup>48</sup>. These properties make colostrum essential for protecting the baby from infections, allergies, and other health issues<sup>49</sup>. It helps clear meconium, prevent jaundice, support intestinal development, reduce the severity of infections, and prevent eye diseases<sup>50</sup>. Thus, it is essential for newborns to receive colostrum during their first feedings, as it provides the necessary defense until mature milk is produced<sup>51</sup>. Colostrum is already available in the breasts at the birth of the baby, and it is all that most babies need before mature milk comes in<sup>52</sup>. It's important not to give babies drinks or other foods before breastfeeding begins, as artificial feeds before colostrum can be risky<sup>53</sup>.

After a few days, the milk produced becomes mature milk. During this stage, the breasts might feel firm, engorged, and heavy as milk production increases, a process often referred to as the "coming in" of milk<sup>54</sup>. The bluish milk that appears at the beginning of a feeding is called foremilk, while the whiter milk that follows is known as hindmilk<sup>55</sup>. Colostrum is higher in proteins compared to later milk, while hindmilk has a higher fat content than foremilk<sup>56</sup>. Hindmilk appears whiter because of its higher fat content, which provides the energy necessary for the baby's growth<sup>57</sup>. It's important to allow the baby to nurse long enough to get both foremilk and hindmilk, as the fat in hindmilk is vital for the baby's energy needs<sup>58</sup>. Larger quantities of foremilk are produced, containing protein, lactose, water, and other essential nutrients<sup>59</sup>.

## **Iron content in Breast Milk**

Iron levels in breast milk is relatively less compared to other nutrients, but it plays a vital role in a baby's early growth and development<sup>2</sup>.

Typically breast milk contains iron between 0.3 to 0.5 mg per 100 milLilitres during the first few months postpartum.<sup>78,60</sup> The body's ability in absorbing iron from mother's milk is significantly high as much as to 50–70% of iron present in mother's milk is taken in by the infant, in constrast to only 10–15% from iron-fortified formula or solid foods<sup>61</sup>. This high absorption rate ensures that exclusively breastfed infants receive adequate iron during the initial 6 months of a bbay's life, despite the fact that the absolute iron levels present in human milk is minimal<sup>62</sup>.

### ***Factors Influencing Iron Content***

- ***Colostrum vs. Mature Milk:*** The iron levels present in human milk vary throughout. Colostrum, initial form of milk produced duiring the initial few days after birth, contains higher concentrations of iron (about 0.8 mg per 100 mL), which plays a significant role in strengthening immune system of the newborn in early days<sup>95</sup>. After the first few days, the iron content in mature breast milk decreases slightly, but it remains highly bioavailable<sup>112</sup>.
- ***Lactoferrin:*** One of the key components of breast milk that enhances iron absorption is lactoferrin<sup>64</sup>. This iron-binding protein is crucial in avoiding iron from being bound by harmful bacteria, making it available for the infant's absorption while also offering antimicrobial properties<sup>65</sup>. Lactoferrin helps facilitate iron absorption process within the small intestine contributing to the infant's immune system<sup>66</sup>.

- **Bioavailability:** Despite the relatively low iron content, breast milk offers iron in a highly absorbable<sup>67</sup>. This contrasts with iron from alternate sources, like iron-fortified formula or cereals, which may contain greater iron levels but are less efficiently absorbed by the body.<sup>68,69</sup>
- **Impact of Maternal Nutrition:** Maternal iron status can affect the iron stores in human milk. While human milk iron content remains relatively stable, regardless of maternal diet, maternal anaemia or iron deficiency can influence the quantity of iron retained in breast milk over time<sup>13</sup>. However, this remains a secondary concern because human milk is naturally formulated to meet the dietary requirements of the newborn.
- **Infant Iron Stores:** At birth, infants have sufficient iron stores, mainly accumulated during fetal development, to last for about the first six months of life<sup>6</sup>. Following this stage, iron stores begin to deplete, and additional iron from complementary foods or supplementation becomes necessary.<sup>70</sup>
- **Premature Infants:** Premature infants often have reduced iron reserves at birth and typically require greater requirement for supplemental iron<sup>90</sup>. In such cases, breastfeeding along with iron supplements may be recommended to ensure proper iron levels.
- **Long-term Effects of Adequate Iron:** Adequate iron intake through breastfeeding is essential for preventing iron deficiency anaemia (IDA) and while also promoting cognitive and motor development<sup>71</sup>. Babies with low iron levels may experience developmental delays, weakened immune responses, and lower physical stamina.<sup>73,82</sup>

### ***Iron Supplementation and Complementary Foods***

For the initial six months breast milk supplies adequate amount of iron, after six months, infants need additional iron to prevent iron deficiency<sup>15</sup>. This is particularly important for exclusively breastfed infants<sup>13</sup>. Complementary foods, including iron rich solid foods (e.g., pureed meats, fortified cereals, and vegetables), are introduced around this time. Supplementing with Iron may be advised for infants with increased likelihood of IDA, including babies who have reduced birth weight, premature infants, or those whose mothers have iron deficiency<sup>72,73</sup>.

The Baby-Friendly Hospital Initiative (BFHI) in India is an international movement aimed at promoting and supporting breastfeeding practices<sup>74</sup>. The initiative was introduced by the WHO and UNICEF, and has been adopted in India to improve well being of both mothers and infants.<sup>75</sup> Here are 10 key points about BFHI in India:

#### ***1. Global Framework and Introduction:***

The BFHI was introduced by the World Health Organization (WHO) and UNICEF in the year 1991, and India adopted it in 1992. Its primary goal is to promote breastfeeding in healthcare settings and ensuring optimal care for both mothers and infants.

#### ***2. The Ten key guidelines Steps for effective Breastfeeding:***

BFHI medical facilities are required to adopt the Ten Steps for effective Breastfeeding, a collection of evidence-based practices designed to promote breastfeeding and enhance the well being of the infant. These steps include prompt beginning of nursing, maintaining sole breastfeeding for the initial six months of life, and no formula supplementation unless medically necessary.

**3. *Early Initiation of Breastfeeding:***

One among key principles of BFHI is the prompt start of nursing within the first **hour** after birth, ensuring that newborns receive colostrum, which is rich in essential antibodies.

**4. *Exclusive Breastfeeding for the First Six Months:***

The initiative emphasizes **exclusive breastfeeding** (no water, formula, or other foods) for the first six months, which helps reduce infant mortality, improves immunity, and promotes healthy growth and development.

**5. *Rooming-in:***

BFHI encourages **rooming-in**, which means ensuring that the mother and the baby stay together in the same space, promoting bonding, easier breastfeeding, and fewer disruptions to the baby's feeding patterns.

**6. *No Artificial Feeding:***

BFHI discourages the use of breastmilk substitutes, including formula feeding, unless medically necessary. This helps to lower the chance of artificial feeding, potentially causing infections, allergies, and other health issues in infants.

**7. *Training Healthcare Workers:***

Healthcare providers in BFHI-certified hospitals are trained to support and assist mothers with breastfeeding. This training includes guidance on proper breastfeeding techniques and resolving common challenges.

**8. *Providing Counseling and Support:***

BFHI hospitals ensure that mothers receive continuous support through **lactation counselors**, peer support groups, and access to educational resources on breastfeeding.

**9. *Certification and Recognition:***

Hospitals in India can earn **Baby-Friendly status** through a certification process. As of 2020, more than 1,500 hospitals across the country have been recognized as Baby-Friendly under Ministry of Health and Family Welfare.

**10. *Impact on Breastfeeding Practices:***

BFHI has resulted in a significant hike in proportion of the hospitals supporting sole nursing, especially in urban areas. However, rural areas still face challenges related to resources, trained staff, and community-level practices.

**Iron metabolism in infants throughout exclusive breastfeeding period**

Newborns possess iron reserves acquired through trace element transfer from the placenta during fetal development serving as a nutrient source for early postnatal growth.<sup>76,2</sup> During pregnancy, iron is delivered through the placenta to foetus, mainly during the 3rd trimester<sup>129</sup>. The transfer of iron via the placenta is tightly controlled, ensuring that an infant's iron reserve at birth are not directly influenced by the mother's iron levels or anemia<sup>77</sup>. The iron reserves of newborns can be evaluated by measuring ferritin levels in umbilical cord blood<sup>78</sup>.

Upon delivery, newborns carried to full term possess iron levels of approximately 75 milligrams/kilogram, accompanied by increased blood volume and

hemoglobin levels in proportion to their body mass.<sup>2</sup> In the initial months, infants undergo a normal decrease in blood volume and Hb concentration, as well as a shift from foetal haemoglobin to adult haemoglobin.<sup>79,80</sup> There is some debate regarding the iron requirement of newborns. For newborns, most of the iron is present in haemoglobin, with a portion stored in limited quantities. As the newborn transitions from the womb to an oxygen-rich atmosphere, their haemoglobin concentration decreases from 170 gram/Litre to 120 gram/Litre during the initial six weeks<sup>81</sup>. In exclusively breastfed babies, iron primarily comes from stored reserves, since human milk has minimal iron content.<sup>78</sup>

### **Iron Content of Human Milk**

Post birth, the iron consumption of exclusively breastfed babies rely entirely on breast milk, supplemented by the utilization of their body stores to meet nutritional requirements. Infants utilize stored iron alongside iron obtained from breast milk through diet. A long term study monitoring breastfed babies from 1 to 12 months of age revealed that reserves of iron are the primary source of iron utilization, with only a small amount supplied by breast milk<sup>82</sup>.

The iron levels present in human breast milk is relatively minimal compared to maternal serum iron<sup>83</sup>. In human colostrum, the iron concentration is about 0.8 µg/mL, while in mature breast milk, it ranges from 0.2 to 0.4 µg/ml<sup>84</sup>. In comparison, the content of iron is significantly greater in other species milk.<sup>85,86</sup> Although human milk has limited amount of iron, and this concentration is thought to remain unaffected by the mother's iron levels or cannot dietary iron intake or supplementation.<sup>87,88</sup> However, a new study suggests that mother's iron levels during pregnancy could impact the iron content in breast milk<sup>89</sup>. Interestingly, epithelial

cells in the human mammary gland do not release iron into breast milk, as the primary iron-exporting membrane protein is absent in these cells<sup>1</sup>. This aligns with the general understanding that the body regulates iron content through absorption rather than excretion.<sup>90</sup> A significant portion of the iron in human milk originates from epithelial cells in milk, which are the primary cell type in a mother who is healthy. Given that iron is ubiquitous in the environment, the infant may obtain all the required iron from external sources, as it was once readily available through contamination until recent years. Research from the 1990s utilizing stable isotopes indicated of a reduced fractional absorption between 16% and 25%<sup>91,92</sup>. However, the combination of iron derived from breast milk, stored reserves, as well as recycled haemoglobin remains typically sufficient to meet a baby's needs throughout the initial six months of life.

### **Advantages of Exclusive Breastfeeding**

Nursing offers substantial advantages to both mother and her baby. For babies, breast milk supplies crucial nutrients for growth, development, and an immune strength<sup>52</sup>. Research has shown that breastfeeding exclusively for the initial six months helps protect against infections of the gastro-intestinal tract and iron deficiency anaemia<sup>93</sup>. Additionally, it supports maternal health by promoting amenorrhea (absence of menstruation) for up to six months postpartum, which can help prevent unplanned pregnancies<sup>94</sup>.

Solely breastfeeding further supports the growth of sensory and cognitive abilities as well, while safeguarding infants from long term diseases and respiratory tract infections, illness such as common cold, cough, and pneumonia.<sup>52,95</sup>

a) **Specific Benefits for Infants:**<sup>96,97</sup>

- Reduced likelihood of cases sudden infant death syndrome (SIDS)
- Reduced occurrence of infections of the ear, diabetes, as well as certain cancers
- Improved ability to fight diseases along with enhanced response to vaccinations
- Fewer problems related to teeth alignment and oral health
- Enhanced psychomotor, emotional well being, and social development
- Reduced risk of obesity and cardiovascular diseases
- Reduced infections of Gastrointestinal and Lower respiratory tract

b) **Benefits for Mothers' Health:**

- **Postpartum recovery:** Oxytocin, released while breastfeeding, aids uterine contraction and minimizes postpartum bleeding. Therefore, breastfeeding should commence without delay after birth and continue regularly<sup>97</sup>.
- **Energy conservation:** Nursing mothers save energy as breastfeeding requires less food intake to produce milk, even if they eat less<sup>100</sup>.
- **Lower cancer risk:** Women engaging in breastfeeding lowers the likelihood of developing ovarian and breast cancer<sup>101,102</sup>.
- **Delaying menstruation and preventing early pregnancy:** Frequent breastfeeding helps in preventing a subsequent pregnancy by delaying the resumption of menstruation. This gives the mother time to recover her iron stores and allow for proper spacing between pregnancies<sup>103</sup>.

**c) Breastfeeding as a Natural Method for Pregnancy Spacing:**

Breastfeeding can postpone ovulation and onset of menstruation, acting as an reliable approach of spacing pregnancies<sup>104</sup>. For breastfeeding to be an effective contraceptive method, mothers should follow these practices:

- Breastfeed solely and at regular intervals, both day as well as night, based on the infant's needs.
- Breastfeed a minimum of eight to ten times over the course of a day, avoiding intervals exceeding six hours between nursing sessions. This routine offers a dependable safeguard against another pregnancy<sup>105</sup>.

**Determinants affecting newborn ferritin concentrations**

Determinants affecting newborn ferritin concentrations present upon birth consists of gestational age, foetal gender, iron levels in mother, as well as circumstances affecting the transfer of iron between mother and fetus. Multiple research examined the effects of gestational age on ferritin levels, though many are limited by small sample sizes or broad gestational age groupings (such as term versus preterm infants). A major study found out that ,healthy full-term exhibit cord serum ferritin levels ranging from 100 to 260 µg/l.<sup>106,107</sup>

Female infants born at or near term tend to have higher cord serum ferritin levels compared to male infants, as a result of variations in sex hormones, blood volume, and iron utilization<sup>70,108</sup>. However, when full term and preterm infants are

Analyzed together , gender related variations in ferritin levels are less prominent.<sup>70,108</sup> Gestational age significantly affects cord blood ferritin concentrations, with preterm infants having reduced ferritin concentrations in comparison to term

infants alongside reduced blood iron levels, diminished TIBC, increased reticulocyte counts, and heightened soluble sTfR values, suggesting that a greater amount of iron is being utilized for the process of fetal erythropoiesis (red blood cells formation).<sup>109,6</sup>

A few studies have provided ferritin concentration data for specific gestational ages. Research by Siimes and Siimes documented a median ferritin level of 45 µg/l

Between 14–16 weeks of pregnancy and 200 µg/l at 39 weeks<sup>110</sup>.

The impact of mother's iron levels on cord blood ferritin levels has been studied in mothers. In pregnancies where the mother has both adequate and deficient iron levels, establishing a connection between maternal iron levels and cord blood ferritin levels has proven challenging<sup>111</sup>. Studies have shown that every iron measurement in cord blood exceeds maternal levels, highlighting that fetus primarily maintains its own iron stores separate from mother's iron levels. This suggests that the fetus may regulate its iron stores separately from maternal iron levels.<sup>111,129</sup>

Full-term babies delivered to mothers with iron-deficiency, but no anemia with reduced blood ferritin levels exhibit reduced cord ferritin levels in comparison to those born to mothers having standard ferritin concentrations levels<sup>112</sup>. For instance, babies of mothers with ferritin levels less than <10 µg/L have an average ferritin concentration of  $98.5 \pm 50.6$  µg/L, whereas infants of mothers with standard ferritin levels show  $147.2 \pm 66.0$  µg/L. Besides iron deficiency in mothers, factors such as dysfunction in the placenta and heightened iron requirements in the fetus demand beyond the capacity of placental transport are major contributing factors for fetal and newborn iron deficiency<sup>113</sup>.

Clinical conditions contributing to disturbances in fetal iron processing are high maternal blood pressure, which causes poor placental blood flow, and prolonged oxygen deprivation in the fetus, which leads to increased fetal erythropoiesis<sup>114,136</sup>. Babies experiencing intrauterine growth restriction (IUGR) are at an increased likelihood of reduced iron stores due to reduced iron movement and persistent oxygen deprivation caused by placental dysfunction. IUGR frequently occurs in pregnancies affected by severe maternal hypertension, preeclampsia, genetic disorders, or extreme maternal malnutrition. These infants typically present with decreased serum ferritin levels and increased transferrin levels at birth<sup>135</sup>.

Research by Georgieff et al. through postmortem analysis showed that babies with Intra-uterine growth restriction caused by a condition known as Potter's syndrome exhibited considerably decreased iron levels in their liver and brain compared to controls<sup>110</sup>. Chronic fetal hypoxia increases the need for iron in erythropoiesis, due to maternal diabetes and maternal smoking<sup>114,136</sup>. Under such conditions, the transfer of iron to vital fetal organs, such as the liver, heart, and brain, is frequently restricted to ensure iron is available for increased erythropoiesis.

Infants of diabetic mothers (IDM), whether born to mothers with gestational, insulin dependent, or with non-insulin-dependent diabetes, face a higher risk of depleted iron reserves<sup>115,116</sup>. Approximately 65% of IDM have ferritin concentrations below <60 microgram/Litre with an average of 26 µg/L<sup>117</sup>. Fetal hyperglycemia and elevated insulin levels in pregnancies affected by maternal diabetes raise fetus's metabolism and oxygen use, leading to fetal oxygen deficiency. The unusual iron measurements observed in Infants born to diabetic mothers is because of increased fetal iron use for red blood cell formation<sup>118</sup>. Additionally, the extent of iron imbalances at birth are linked to higher fetal erythropoietin and fetal glycosylated

haemoglobin<sup>119,120</sup>. As serum ferritin concentration decreases, red blood cell iron content increases<sup>121</sup>. Reduced ferritin levels in infants of diabetic mothers could be due to issues with placental function, which hampers adequate maternal iron transfer to the foetus<sup>122</sup>.

### **Effects of Maternal Anaemia on Fetus and New-born**

Maternal anaemia, particularly iron-deficiency anaemia (IDA), can have significant effects on the new born, influencing various health factors throughout pregnancy, delivery, as well as the initial stages after birth. Maternal anaemia is significantly widespread, especially in Nations such as India, where it poses a critical public health issue.

Maternal anaemia has significant and far-reaching effects on new born health, increasing the risks of LBW, neonatal infections, iron deficiency, developmental delays, and even mortality<sup>123,124</sup>. Addressing maternal anaemia through improved nutrition, prenatal care, and iron supplementation is critical for reducing the burden of these neonatal complications and ensuring better well being for both mothers and their children.

#### ***Impaired Placental Function:***

Anaemia in the mother can compromise placental function, leading to intrauterine growth restriction (IUGR) and other complications<sup>125</sup>. An underperforming placenta may fail to supply essential nutrients and oxygen to the developing fetus, restricting development and raising its likelihood of long-term health complications, such as metabolic disorders.

***Postpartum Haemorrhage:***

Although the focus is on neonatal outcomes, maternal anaemia also increases the risk of postpartum haemorrhage, which can affect newborns indirectly. Excessive blood loss during delivery can lead to maternal weakness and decreased lactation, which may, in turn, hinder breastfeeding. This can affect newborn nutrition and further contribute to poor growth and development<sup>126</sup>.

***Perinatal Asphyxia:***

Maternal anaemia can contribute to perinatal asphyxia during labor and delivery. Insufficient oxygen delivery due to maternal anaemia can lead to fetal distress, resulting in complications such as meconium aspiration syndrome, hypoxic-ischemic encephalopathy, or birth asphyxia<sup>1</sup>. These conditions can lead to long-term neurological impairments in the newborn

***Low Birth Weight (LBW) and Preterm Birth:***

One of the most common consequences of maternal anaemia is an increased likelihood of LBW as well as premature delivery. Anaemia in pregnancy reduces the ability of mother's blood to transport oxygen, which can lead to inadequate oxygen supply to the fetus. This hypoxia can lead to restricted fetal growth and early labor, both of which increase the likelihood of neonatal morbidity and mortality.<sup>125</sup>

***Increased Risk of Neonatal Deaths:***

Mother's anaemia, particularly when severe, increases the likelihood of infant deaths. Research indicates that newborns of anemic mothers are at higher possibility of dying during initial weeks after birth due to complications like respiratory distress, infections, and poor weight gain.<sup>126</sup>

***Neonatal Iron Deficiency:***

Infants of iron-deficient mothers may have lower iron levels at birth. Iron is essential for haemoglobin production and ensuring proper enzyme and system functions. A lack of sufficient iron in early stages may result in neonatal iron IDA, that potentially impacts baby's cognitive and motor development, and raises the likelihood of anaemia in early childhood<sup>16</sup>.

***Increased Risk of Infections:***

Maternal anaemia is linked to a weakened immune system, which can affect the newborn's ability to fight infections. Anaemic mothers are more likely to experience difficulties during pregnancy and childbirth, which may lead to infections such as sepsis in the newborn. Additionally, anaemia can impair the placenta's role in delivering nutrients and antibodies to the fetus, compromising the baby's immune system<sup>127</sup>.

***Developmental Delays:***

Iron is a key factor in brain development, and anaemia in mothers can influence the newborn's neurological development. Low birth iron reserves are connected to delayed cognitive and motor development in infants<sup>128</sup>. Prolonged iron shortage in young children is known to affect learning and behavior, potentially leading to reduced IQ and developmental delays<sup>73</sup>.

***Long-Term Consequences***

The effects of maternal anaemia are not confined to the immediate neonatal period but can have lasting consequences. Babies delivered to anaemic mothers are at

an increased likelihood of acquiring anaemia themselves during infancy or childhood. If untreated, this may influence baby's growth, cognitive development, and overall health, alongside enduring impact on learning and work performance later in life.<sup>129,130</sup>

The prevention and treatment of maternal anaemia plays a vital role in lowering the associated risks to the newborn. This includes:

- Taking Iron supplements in pregnancy to avoid and treat lack of iron.
- Adequate nutrition, including an iron-enriched diet and folic acid.
- Regular screening of maternal haemoglobin levels during antenatal visits.
- Early treatment of anaemia to ensure optimal maternal and fetal health outcomes.

### **Factors affecting iron status of New born at birth**

A newborn's iron level is influenced by several factors that affect iron transfer during pregnancy and after birth. Below are key factors that impact iron status at birth, with supporting references:

#### ***Maternal Iron levels***

Mother's iron content is essential for determining the iron reserves in fetus. Maternal iron deficiency or lack of iron leading to anaemia results in reduced iron being passed to the fetus, causing insufficient iron reserves at birth. This is particularly important in the final 3 months of pregnancy when iron transfer to the fetus is highest.<sup>131</sup>

### ***Maternal Diet***

A maternal diet deficient in iron-enriched foods (e.g., red meat, leafy greens, legumes) may cause to low iron content during pregnancy, which, in turn, affects the newborn's iron stores. Conversely, a diet rich in iron, especially in the form of heme iron ( animal derived), helps ensure improved iron levels for the infant.<sup>132</sup>

### **Maternal Iron Supplementation**

Iron therapy throughout pregnancy is essential to avoid Iron deficiency in mothers and ensure adequate iron transfer to the fetus. Those Women who do not consume adequate iron therapy, mainly in the last two trimesters, have increased likelihood to infants infants with lower iron levels.<sup>133</sup>

### ***Placental Function***

The placenta's ability to transfer iron is essential for the fetus to build up iron stores. Placental insufficiency, which can occur in conditions such as gestational hypertension, pre-eclampsia, or diabetes, can result in inadequate transport of iron to the developing fetus, leading to reduced iron levels at birth.<sup>109</sup>

### **Fetal Iron Requirements**

The fetus has an increasing iron requirement during the later stage of pregnancy as the circulating blood volume expands as well as the red blood cells need iron for haemoglobin synthesis. If there is no enough maternal stored iron to fulfil the fetal demands, the infant may be born with depleted iron reserves.<sup>134</sup>

### ***Gestational Age***

Premature infants (born before 37 weeks) typically have reduced iron reserves due to limited time for iron absorption. Normal Full-term infants are typically born with sufficient iron levels, while babies born prematurely have an increased chance of experiencing IDA.<sup>117,135</sup>

### **Socioeconomic Status**

Socioeconomic factors influence access to nutrition and healthcare. Lower income women may have less access to prenatal care and iron-rich foods, which increases the likelihood of **maternal anaemia** and results in low iron stores in their newborns.<sup>136</sup>

### **Multiple Pregnancies**

In pregnancies involving multiple fetuses, the maternal iron stores are tend to be exhausted faster because of greater iron demands for each fetus. This may result in a higher risk of low iron levels among newborns.<sup>137</sup>

### **Physiological anaemia of infancy**

Physiological anaemia of infancy is a normal, temporary condition that occurs in the early infancy due to normal reduction in haemoglobin levels after birth. In the fetal period, there is an enhanced formation of RBC in response to the reduced oxygen environment in the womb, leading to high haemoglobin levels<sup>138</sup>. At birth, the transition to the outside world involves an abrupt shift to an oxygen-rich environment, prompting a reduction in erythropoiesis (red blood cell production). This physiological process results in a decrease in haemoglobin concentrations, typically reaching its lowest point between 6 to 12 weeks of life<sup>139</sup>.

During this period, the infant's body adjusts to the new oxygen levels, and red blood cell production gradually resumes. This decline in haemoglobin is often referred to as "physiological anaemia," and it generally does not come with symptoms or noticeable effects on the infant's health<sup>140</sup>. The typical haemoglobin levels at this stage are around 9-11 g/dL, and while these values may appear low, they are generally within normal limits for healthy infants<sup>141</sup>. As the infant grows, the bone marrow responds by increasing erythropoiesis, and haemoglobin levels normalize by six months of age<sup>142</sup>.

This phenomenon is most noticeable in infants who are born at term and is considered a natural adaptation to extrauterine life. However, it is important to differentiate physiological anaemia from iron-deficiency anaemia (IDA), which is a medical issue that requires intervention. While physiological anaemia is self-limiting and resolves without medical treatment, the risk of developing IDA can increase if an infant is not receiving sufficient iron through their diet or from breast milk, as iron stores naturally deplete after birth.<sup>6</sup>

While physiological anaemia of infancy is not typically associated with clinical symptoms, severe anaemia or prolonged low haemoglobin levels beyond the normal range can lead to potential complications such as growth retardation, delayed motor development, and cognitive impairments, especially when iron levels are insufficient<sup>62</sup>. This highlights the need to closely monitor an infant's growth and iron levels, especially during the first 6 months.

#### ***Iron Status at 4 Months of Age***

Iron deficiency among exclusively breastfed babies is a frequent issue faced by the 4th month of life. The body's iron reserves at birth begin to deplete along

with the limited iron present in breast milk, the infant may develop signs of insufficient iron levels. Identifying iron deficiency in infants is typically based on a combination of clinical signs, haemoglobin levels, serum ferritin, and transferrin saturation<sup>143</sup>.

Ferritin levels below 12 µg/L are often used as a diagnostic criterion for iron deficiency<sup>62</sup>.

Several studies have shown that by the 4th month of life, the majority of exclusively breastfed infants begin to exhibit decreased ferritin levels, particularly if complementary foods containing iron are not introduced. Research conducted by Georgieff et al.<sup>135</sup> showed iron deficiency was observed in 30-40% of exclusively breastfed infants by fourth months, particularly in those babies without a history of maternal iron sufficiency or in those who were not supplemented with iron after birth. Another study by Zhao et al.<sup>144</sup> discovered that a quarter of exclusively breastfed infants exhibited serum ferritin concentrations below 12 µg/L by 4 months of age, indicating early iron deficiency.

### **Clinical Features of IDA in Early Infancy**

Iron-deficiency anaemia (IDA) in early infancy manifests through various medical signs, that are mainly linked to the body's inability to transport oxygen efficiently due to insufficient haemoglobin levels. The most prominent feature is pallor, particularly in the skin and mucous membranes, as reduced haemoglobin leads to inadequate oxygenation of tissues<sup>6</sup>. In addition to pallor, infants with IDA often experience irritability and fatigue, which manifest as fussiness, lethargy, and a reduced ability to engage in normal activities. This occurs because iron is essential for energy metabolism and oxygen delivery to tissues, and a deficiency impairs cellular

function<sup>13</sup>. Inadequate feeding and growth failure are frequently observed medical concerns, as the infant may not feed well due to decreased appetite and overall energy levels. This poor feeding leads to inadequate growth and increase in weight, with iron deficiency often exacerbating these issues.<sup>62</sup>

In cases of more severe IDA, compensatory mechanisms come into play, leading to rapid heart rate and fast breathing, as the body tries to increase oxygen delivery to vital organs by increasing circulation and respiration rates<sup>145</sup>. These symptoms are particularly concerning as they suggest the body's efforts to compensate for the lack of oxygen-carrying capacity. Another feature that may arise in some infants with IDA is pica, the craving for indigestible items like soil, ice, or starch. This condition is believed to be associated with iron deficiency, as the body might instinctively seek alternative sources of iron or other minerals in response to the deficiency.<sup>146</sup>

When IDA remains untreated or undiagnosed, more severe and long-term consequences can develop. A significant concern is the potential for delayed neurodevelopment, which includes slower achievement of motor skills including sitting up, walking, and fine motor skills. Cognitive impairments, such as issues with attention, memory, and learning, can also occur as iron has an essential function in brain development, especially in the development of neurotransmitters and myelination of nerve cells<sup>147</sup>. The lack of adequate iron during infancy can result in long-lasting developmental delays, affecting the child's academic performance and behaviour even later in life<sup>148</sup>. Early intervention and appropriate management are critical to avoid these long-term neurodevelopmental sequelae.

## **Strategies to prevent IDA in early infancy**

Preventing iron-deficiency anaemia (IDA) during early childhood is vital for ensuring optimal growth, development, and cognitive function. Several strategies can be employed to prevent IDA in infants, emphasizing early dietary intake, iron supplementation, and consistent monitoring.

### **1. Exclusive Breastfeeding:**

According to the **WHO**, babies are supposed to be exclusively **breastfed** till 6 months of life. Breast milk contains bioavailable iron, and while the iron content may be relatively low, it is sufficient for the infant's needs for the first six months when the infant's iron reserves are generally sufficient<sup>13</sup>. The beneficial effects of breastfeeding also extend beyond iron, including immune protection and a reduced risk of infections, which can further reduce the risk of anaemia.<sup>6</sup>

- **Iron-Fortified Complementary Foods:**

By six months of age, infant's stored iron levels start to decline. Therefore, incorporating iron-rich solid foods is critical. Foods like pureed meats, iron-fortified cereals, lentils, and spinach provide excellent source of iron<sup>152</sup>. Fortified foods with iron and vitamin C, to enhance absorption of iron, should be prioritized.

- **Iron Supplementation:**

Areas having high rates of iron deficiency, iron supplements to infants may be required. WHO recommends providing oral iron supplementation to infants in regions where IDA is widespread<sup>15</sup>. Iron drops or syrups are commonly prescribed for at risk babies, particularly preterm babies or with LBW, as they may have lower iron stores at birth.

- **Delayed Cord Clamping:**

Waiting at least 1-3 minutes before clamping the umbilical cord has been proven to boost a newborn's iron levels<sup>150</sup>. This simple intervention can help reduce the likelihood of anaemia in early infancy by enhancing the infant's iron levels.

- **Monitoring and Early Screening:**

Regular screening for anaemia and monitoring of growth parameters are essential, especially for infants with increased likelihood of developing iron deficiency. Early identification of low haemoglobin values allows for prompt interventions such as supplementation or dietary adjustments, which can prevent progression to iron deficiency anaemia<sup>70</sup>.

- **Reducing Iron Inhibitors:**

Some dietary factors can reduce iron absorption, compounds like phytates in whole grains, calcium in dairy, and polyphenols in tea and coffee. It is important to avoid giving infants large amounts of these foods in the early months, especially around meal times, to ensure optimal iron absorption<sup>151</sup>.

- **Addressing Maternal Anaemia:**

Preventing maternal anaemia during pregnancy is a key strategy for preventing IDA in infants, as maternal iron deficiency may result in reduced newborn iron reserves. Pregnant women should receive adequate iron supplementation and a balanced diet to maintain their iron levels, which helps ensure that the baby is born with sufficient iron stores<sup>152</sup>.

- **Education and Awareness:**

Public health programs aimed at educating mothers as well as the caregivers about the significance of proper nutrition and iron-rich foods during infancy are critical. Awareness campaigns should emphasize exclusive

Breastfeeding benefits, incorporation of iron rich foods, and dangers of iron deficiency.

### **Diagnosis of IDA in Early Infancy**

Early detection and diagnosis are essential in preventing long-term cognitive and developmental impairments. Numerous studies have focused on various diagnostic approaches to IDA among this high risk age group.

Diagnosing IDA in early infancy primarily involves clinical, laboratory, and haematological evaluations. Clinical signs, such as pallor, irritability, and poor feeding, are often nonspecific and not sufficient on their own for diagnosis<sup>13</sup>. Therefore, laboratory tests are essential for confirming IDA, with the serum ferritin level being a commonly used biomarker. A serum ferritin concentration <30 ng/mL is indicative of iron deficiency in infants. However, ferritin is an acute-phase reactant, and it increases during infection or inflammation, limiting its specificity.<sup>70</sup>

In addition to serum ferritin, the measurement of haemoglobin (Hb) levels is crucial, as it provides the most direct evidence of anaemia. The WHO defines anaemia in infants as a haemoglobin concentration less than 11 g/dL<sup>62</sup> (Sharma et al., 2016). However, Hb alone does not differentiate between the different types of anaemia, thus requiring the use of RBC indices in elucidating etiology of anemias: MCV<sup>156</sup> (Mean Corpuscular Volume) Defines the size of the red blood cells and is

expressed in femtolitres. MCH (Mean Corpuscular Hemoglobin) quantifies the amount of hemoglobin per red blood cell and is expressed in picograms per cell. MCHC (Mean Corpuscular Hemoglobin Concentration) denotes the hemoglobin amount per unit volume and is measured in gm/dl. Red cell Indices will be reduced in Iron deficiency anemia.

Normal values of Iron profile parameters at birth and at 4th month are as follows

	At Birth <sup>153,154</sup>	4th month <sup>13,155</sup>
Iron(microgm/dl)	90-150	80-190
Ferritin(ng/ml)	90-220	100-400
TF(%)	45-57	27-35
TIBC	210-400	190-260

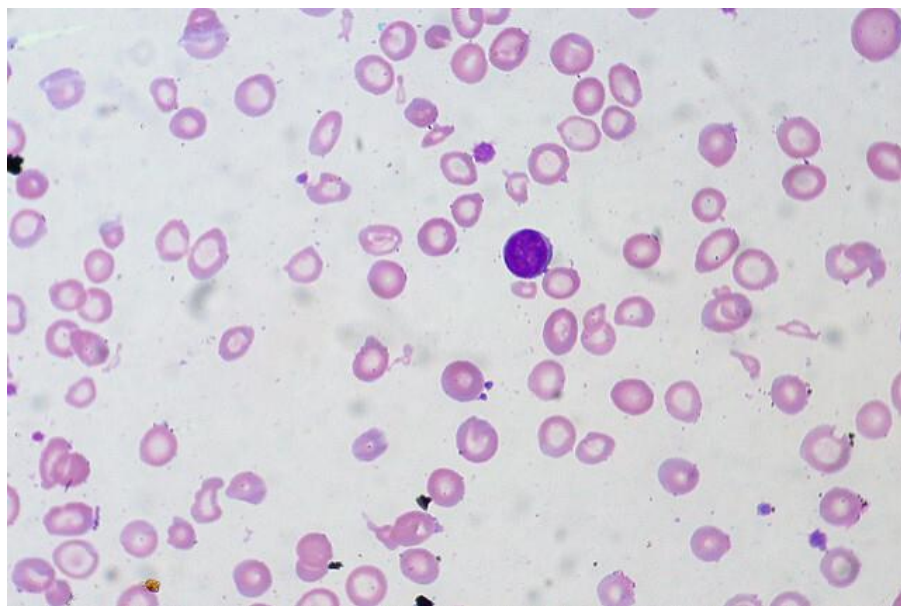
Serum Iron, Ferritin and Transferrin saturation decreases and TIBC increases in Iron Deficiency Anemia

Recent advances have highlighted the role of reticulocyte haemoglobin content (CHr), which is a sensitive indicator for detecting IDA in early infancy compared to traditional markers<sup>158</sup>. Additionally, newer non-invasive techniques, such as near-infrared spectroscopy, are being explored for their potential in diagnosing IDA in infants without the need for blood sampling, offering a promising avenue for early and rapid diagnosis<sup>159</sup> (Sharma et al., 2016).

<sup>157</sup>Examination of the peripheral blood smear should also be considered along with the review of peripheral blood counts, red blood indices and Iron Profile. A

predominance of hypochromic microcytic cells is found in iron deficiency anemia, thalassemia and hereditary sideroblastic anemia and in some patients with the anemia of chronic disease and with lead intoxication. Anisochromia with the presence of a dimorphic red cell population (hypochromic and normochromic) is observed in acquired sideroblastic anemia, patients with thalassemia minor after transfusions and persons with Iron deficiency following transfusions or treatment with iron<sup>78</sup>.

***Figure 5 - Microcytic Hypochromic Anemia***



The diagnosis of IDA is critical as iron deficiency during infancy may result in major developmental delays and prolonged cognitive impairments. Early screening and timely intervention, as well as incorporating iron supplements and dietary adjustments, are necessary to mitigate the adverse effects of IDA. However, challenges remain in the accurate identification of IDA in settings with high rates of infection and other micronutrient deficiencies, where biomarkers may not always be reliable. Hence, a multi-faceted approach, integrating clinical evaluation, haematological analysis, and advanced biomarkers, is essential for accurate diagnosis in early infancy.

## **REVIEWED STUDIES**

The study by Varun Ganjigunta<sup>160</sup>, Varsha Suresh, et al., titled “Study of iron deficiency anaemia in infants of 3 to 6 months age group and its risk factors: a cross-sectional study,” focussed on assessing the occurrence of IDA and its associated risk determinants in infants of 3-6 months of life admitted in a rural tertiary care hospital. Conducted in the paediatric department, the study included 100 infants and assessed maternal and infant factors alongside laboratory tests like CBC, RBC indices, peripheral blood smear, and retic count. IDA was diagnosed in 22% of the infants, with significant associations observed in preterm infants (all preterm cases had IDA) and low birth weight (40.9% vs. 11.5% in nonIDA cases,  $p < 0.05$ ). Additionally, 16% of full term, healthy normal birth weight infants who are exclusively breastfed infants were affected. IDA was also linked to underweight (31.8%) and stunting (30.8%) compared to 9% and 5.4% in non-IDA infants. The study concluded that IDA is prevalent in infants under 6 months, including healthy, term, exclusively breastfed infants, advocating for universal iron supplementation as part of India’s National Iron Plus Initiative, with a special focus on vulnerable groups such as infants born premature and low birth weight babies.

The study by Tomiko Hokama, Shizuhiko Takenaka, et al., titled “Iron Status of Newborns Born to Iron Deficient Anaemic Mothers,” investigated the iron levels among infants born to mothers with anaemic conditions. Levels of Hb, iron, TIBC, and serum ferritin were measured among 16 infants born to mothers with iron deficiency anaemia, 28 born to mothers with iron deficiency but no anemia, and 9 born to mothers with normal iron and haemoglobin levels. The research did not identify notable variations of Hb, iron, and TIBC across the 3 newborn groups. However, babies whose mothers had iron deficiency anaemia exhibited markedly

reduced serum ferritin levels compared to the other groups, highlighting the impact of maternal anaemia on the newborn's iron reserves of newborn.<sup>161</sup>

Shiji R, S. Jagadeeshwari, and S. Sundari conducted a study titled "*A Study of Haemoglobin and Iron Status of Term Neonates Born to Anemic Mothers*" to investigate the correlation between maternal and neonatal iron reserves and its impact on neonatal anthropometry. This cross-sectional study included 100 singleton term neonates and their mothers who met inclusion criteria. Maternal venous blood and umbilical cord samples of blood were examined for the measurement of haemoglobin, iron, and ferritin levels. Anthropometric parameters of neonates including weight, length, head circumference, as well as chest circumference were recorded through physical examination. The study aimed at assessing the link between maternal anaemia and neonatal haemoglobin, serum iron, serum ferritin, and anthropometric parameters. Statistical analyses revealed correlations between maternal haemoglobin and neonatal iron levels, along with their connections to physical growth measurements, offering perspective on how maternal anaemia affects neonatal health and development.<sup>162</sup>

Laila Loren et al. conducted a review titled "*A Review of Cord Blood Concentrations of Iron Status Parameters to Define Reference Ranges for Preterm Infants*" to establish standardized values for iron-related parameters in preterm infants up to term-equivalent age. The study involved a comprehensive literature review using PubMed to compile information on cord blood values of key iron-related markers, like haemoglobin, MCV, ferritin, soluble transferrin receptor, ferritin index, transferrin saturation, reticulocyte haemoglobin content, zinc protoporphyrin/heme ratio, and hepcidin. Results revealed gestational age-specific reference ranges at term, including haemoglobin (15.9 g/dL [13.3-18.4]), MCV (108.1 fL [97.8-118.5]), and

transferrin saturation (61.2% [31.5-90.9]), which differed significantly from adult reference ranges. The study emphasized that adult reference values are unsuitable for preterm infants and recommended individualized iron supplementation strategies based on these preterm-specific ranges to optimize growth and development.<sup>163</sup>

Ashajyothi M. Siddappa and colleagues carried out a research titled “*The Assessment of Newborn Iron Stores at Birth: A Review of the Literature and Standards for Ferritin Concentrations*” to evaluate the effect of maternal and pregnancy related determinants on ferritin levels in both term and preterm babies and establish comprehensive standard values. Serum ferritin, a marker for total body iron stores, is critical for assessing neonatal iron status and risk for iron-related disorders. However, a lack of standardized serum ferritin concentrations between 23 and 41 weeks has made the assessments more challenging. The study reviewed data based on data from 457 low-risk preterm and full-term newborns spanning a gestational range of 23 to 41 weeks, collected from 35 published studies over 25 years and supplemented by newly collected data. Regression analysis showed that ferritin levels in umbilical cord samples markedly raised with gestational age, averaging 63 micro/Litre at 23 weeks to 171 microgram/Litre at 41 weeks ( $p < 0.001$ ). Newborns born to mothers with diabetes demonstrated reduced intercept values compared to controls ( $p < 0.001$ ), indicating diminished iron stores among high-risk population. This research underscores the necessity of gestationspecific ferritin reference ranges to accurately assess iron status and guide clinical management in neonates.<sup>164</sup>

Joy Y. Zhang, Jing Wang, and colleagues conducted a study titled<sup>165</sup> “*Iron Stores at Birth in a Full-Term Normal Birth Weight Birth Cohort with a Low Level of Inflammation*” to assess iron levels in newborns from a cohort of normal singleton term births. Blood samples were collected from 854 deliveries, with exclusions due to

increased levels of inflammatory determinants (C-Reactive Protein >5 mg/L, AGP >1 g/L), preterm birth (<37 weeks of gestation), and birth weights outside the range of 2500–4000 g. The final analysis included 762 samples. The study found that 19.8 percentage of infants had iron deficiency (ferritin <35 µg/L), and 46.6% exhibited inadequate iron reserves (ferritin <76 µg/L). Ferritin levels correlated positively with sTfR, hepcidin, EPO, and gestational age. The findings highlight a widespread occurrence of low iron reserves in newborns and the necessity of defining suitable reference values for iron status indicators.

Ziegler et al. (2007) examined how mother's iron levels throughout pregnancy influence newborn's iron reserves at birth. Their study determined that maternal iron deficiency, especially in the third trimester, diminishes fetal iron transfer and results in lower neonatal ferritin levels. The study also highlighted that maternal anaemia (haemoglobin <11 g/dL) strongly indicated a likelihood of reduced iron reserves at birth, especially among newborns of malnourished mothers or those with a history of blood loss during pregnancy. The researchers recommended routine maternal iron supplementation during pregnancy to improve neonatal iron outcomes.<sup>166</sup>

Hercberg et al. (2001) found that birth weight significantly affects neonatal iron reserves, with low birth weight infants (<2.5 kg) showing a higher prevalence of depleted iron stores. These infants require careful follow-up and possibly earlier interventions, as their risk for iron deficiency is elevated despite exclusive breastfeeding.<sup>167</sup>

A RCT by Andersson et al in 2011 investigated the effect of delaying the clamping of the cord on iron levels in newborns and infants. The study enrolled 400

term infants, randomly divided into groups receiving either delayed cord clamping (lasting at least 3 minutes) or immediate clamping (within 10 seconds). At 4th months of age, newborns who underwent delayed cord clamping group exhibited significantly increased ferritin levels in the blood (79 µg/L vs. 47 µg/L in the immediate clamping group) and lower rates of iron deficiency. The trial concluded that delayed cord clamping is an easy and cost-effective approach with long-term benefits for iron status and neurodevelopment.<sup>168</sup>

Lonnerdal (2003) conducted a comprehensive review of how well iron is absorbed from human milk and compared it to other dietary sources. The study found that the iron absorption efficiency in human milk ranges from 50% to 70%, substantially greater compared to cow's milk or infant formula (10%-20%). This high bioavailability is attributed to the presence of lactoferrin, a milk protein that facilitates absorption of iron in the infant gut. The author concluded that while the absolute content of iron in breast milk are typically reduced (0.3–0.5 mg/L), its efficient absorption supports sufficient iron stores in full term newborns throughout first 4 to 6 months of age.<sup>169</sup>

McCarthy et al. (2017) studied how rapid growth affects iron reserves in breastfed infants. The study followed 200 infants upto 6 months of age and found that those in the top quartile for weight gain had a 2.5-fold increased likelihood of developing iron deficiency by four months when contrasted with infants growing at a slower rate. The researcher suggested that the increased iron requirements due to fast growth outpace the iron supplied by breast milk, even in infants born with adequate stores. They recommended tracking both growth patterns and iron levels in exclusively breastfed infants with accelerated weight gain.<sup>170</sup>

Lozoff et al. (2006) investigated how common iron deficiency anaemia (IDA) is among infants and its potential developmental consequences. The study reviewed data from 10 cohort studies in both developing and developed countries, showing that around 10%-15% of infants fed solely on breastmilk experienced iron deficiency anaemia by six months, with higher prevalence in populations with high rates of maternal anaemia. The authors stressed that IDA in infancy is linked to impaired cognitive, motor, and social-emotional development, some of which may be irreversible even after iron repletion.<sup>171</sup>

A review by Gambling et al. (2011) examined how iron is transferred through the placenta and the process that regulate it during pregnancy. The study highlighted that majority of iron in the developing fetus( 80%) is gained during the final trimester, making maternal iron supplementation during this period critical. In cases of lack of iron in the mother or issues with placental function, iron transfer to the fetus is hindered leading to reduced neonatal levels of ferritin and greater likelihood of iron deficiency in early childhood.<sup>172</sup>

Georgieff et al. (2011) investigated how a lack of iron affects brain growth in early life, focusing on, a region critical for memory and learning ,the hippocampus. Their review found that shortage of iron in infancy disrupts synaptogenesis, myelination, and chemical signalling in the brain. These alterations result in lasting impairments in cognitive and behavioural outcomes. The study stressed the need to identify and address iron deficiency at an early stage to support optimal neurodevelopmental outcomes.<sup>173</sup>

Dallman and colleagues. (1980) did a research to assess the effect of low dose iron therapy to infants exclusively breastfed starting from four months of age. The study included 200 term infants divided into two groups: one group given iron therapy at 1 mg per kilogram of bodyweight daily and the other received an inactive substance. The results showed that the infants who received iron-supplements had stable hemoglobin and greater ferritin levels in their blood compared to those given a placebo, which experienced a marked reduction in iron reserves. The trial also confirmed the safety of iron supplementation, as no adverse gastrointestinal effects or other complications were observed. This study emphasized the necessity of providing iron supplements in infants who are solely breastfed, particularly in groups where iron deficiency is common in either mothers or newborns.<sup>174</sup>

Krebs et al. (2006) studied how iron levels in breast milk fluctuate throughout the period of breastfeeding as well as its implications for infant iron status. Their study followed 150 lactating mothers and measured the levels of iron in human milk measured at one, three, and six months postpartum. Researchers found that iron levels peaked in early breastmilk (colostrum) at 0.8 mg/L and gradually declined to 0.3–0.5 mg/L by the third and fourth months. The study also highlighted that this decline aligns with the natural exhaustion of a newborn's iron reserves, which usually sustain them for four to six months. The findings underscore the importance of introducing iron-rich complementary foods or supplements around 4–6 months to prevent iron deficiency in exclusively breastfed infants.<sup>175</sup>

Milman (2006) examined how iron consumption by mothers while pregnant affects iron levels in newborns and their likelihood of experiencing a deficiency. The study found a significant link between a mother's iron intake and ferritin levels in newborns. Pregnant women with diets rich in heme iron (from animal sources) and

who received adequate iron supplementation (30–60 mg/day as recommended by WHO) gave birth to infants with significantly higher iron stores. Conversely, mothers with low dietary iron intake or who were noncompliant with supplementation during pregnancy were more likely to deliver infants with low ferritin levels (<50 µg/L). Milman highlighted the significance of assuring sufficient maternal iron therapy during pregnancy, especially in populations where dietary iron deficiency is common.<sup>176</sup>

Chaparro and colleagues. (2006) determined the potency of haemoglobin as well as ferritin as biomarkers for assessing iron status in infants. The study involved 300 full-term infants in Mexico, where iron deficiency is a common community health problem. The researchers measured haemoglobin and blood ferritin levels at zero and at fourth month of age and found that ferritin was a more sensitive indicator of early iron depletion compared to haemoglobin, which remains stable until anaemia develops. The study also investigated the effect of clamping of the umbilical cord timing on iron stores and found that delayed cord clamping significantly strengthened ferritin levels and decreased the occurrence of iron deficiency by 50%. The findings highlight the need for routine screening of serum ferritin in early infancy and the advantages of delaying the cord clamping for improving neonatal iron reserves.<sup>177</sup>

Baker et al. (2010) investigated maternal and infant-related factors contributing to iron deficiency in exclusively breastfed infants. This longitudinal study included 200 mother-infant pairs and assessed maternal dietary habits, infant birth weight, and growth patterns. The study identified low maternal dietary iron intake, premature birth, LBW (less than 2.5 kg), and rapid postnatal growth as significant risk factors for early-onset iron deficiency. Infants with these risk factors

were more likely to have blood ferritin levels below 12 microgram/Litre by the age of four months. The researchers recommended targeted iron therapy for infants who were at risk and highlighted the importance of educating mothers on iron-rich complementary feeding practices.<sup>178</sup>

Rao et al. (2009) studied the iron requirements and outcomes of supplementation in LBW infants. The study followed 150 LBW infants (birth weight <2.5 kg) who were exclusively breastfed. The infants were randomized into two groups: one receiving iron supplementation (2 mg/kg/day) from 2 weeks of age and the other receiving a placebo. By four months, the supplemented group showed significantly higher serum ferritin levels (mean: 75 µg/L) compared to the placebo group (mean: 32 µg/L), and none of the supplemented infants developed iron deficiency anaemia. The study emphasized the increased risk of iron deficiency in LBW infants due to their lower iron stores at birth and higher growth demands, recommending early supplementation as a preventive strategy.<sup>179</sup>

Hutton and Hassan (2007) conducted a meta-analysis of 15 randomized controlled trials to evaluate the impact of delayed cord clamping (DCC) on neonatal iron stores. The analysis revealed that infants who underwent DCC ( $\geq 2$  minutes after birth) had significantly higher haemoglobin levels (by 2 g/L on average) and serum ferritin levels (by 25–30%) at two to four months of age compared to those with immediate cord clamping. The study highlighted that DCC is particularly beneficial in preventing iron deficiency in populations at risk, such as preterm and low-birthweight infants. The researchers concluded that DCC is a simple, cost-effective intervention that can improve iron outcomes in infants globally.<sup>180</sup>

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## **MATERIALS AND METHODS**

### **SOURCE OF DATE**

All term neonates >37 weeks Period of Gestation >2.5kg born in KLE Dr. Prabhakar Kore, Hospital, Belgavi were considered for the study

### **STUDY DESIGN**

Hospital based Longitudinal Study

### **STUDY PERIOD**

One year

### **SAMPLE SIZE**

$$n = \frac{2 ( Z_{\alpha/2} + Z_{\beta/2} )^2 (1+(t-1) S^2)}{t(ES)^2}$$

Where S is the correlation between outcome variables

T is no of time points

ES - effect size

Assuming correlation S=0.25

Effect size(ES) = 0.3

T is no of time points = 0.4

$$4 \times (0.3)^2$$

$$n = \frac{2 (2.485)^2 (1+0.75)}{4 \times 0.09}$$

$$4 \times 0.09$$

$$n = \frac{21.6125}{0.36} = 60.03$$

$$0.36$$

Taking 20% attrition,  $60.03 \times 1.2 = 72.036$

Total sample size = 72 nos.

## **SAMPLING TECHNIQUE**

All term normal neonates who were born in the hospital during the study period and those who fulfilled the inclusion and exclusion criteria were considered for the study

## **INCLUSION CRITERIA**

1. Term neonates >37 weeks POG >2.5kg born in KLE Dr.Prabhakar Kore Hospital, Belgavi

## **EXCLUSION CRITERIA**

1. Infants with major congenital anomalies, cyanotic congenital heart disease.
2. Infants with chromosomal anomalies
3. Maternal FCM injection during pregnancy
4. Infants who were on iron supplements till 6 months of age
5. Refusal of consent

## **STUDY PROTOCOL**

The parent/guardian of the child, who fulfilled the eligibility criteria were briefed about the study. After their approval ,written informed consent was obtained from the parents of the children.The consent forms were prepared in English as well as major regional languages used in the region-Kannada, Marathi, Hindi. Then the necessary details were recorded in a pre-designed proforma. All the data collected were then statistically analyzed.

## **DATA COLLECTION PROCEDURE**

- A detailed history of each neonate was systematically recorded, encompassing key demographic and clinical factors. This included the gestational age, gender, and mode of delivery.
- Additionally, maternal history was thoroughly documented, with particular attention to the intake of iron and folic acid during pregnancy, as well as the presence of any maternal comorbidities.
- Socio-economic status was also assessed, providing valuable context for understanding potential influences on neonatal health.
- All this documented was captured in a pre-designed, structured proforma, ensuring consistency and accuracy in data collection.
- The first blood sample was collected from the mother during the third trimester – after reaching 37 weeks of period of gestation
- Following delivery, a cord blood sample was obtained from the neonate in accordance with standard clinical protocols, ensuring sterility to avoid contamination and to maintain the sample's integrity for further laboratory investigations.
- After the delivery of the baby.the baby will be put on the mother's abdomen when delivered via normal vaginal delivery and over mother's thigh in cesarean section.cord sample has been taken after clamping and cutting the cord
- 3<sup>rd</sup> blood sample is taken when child reaches 4 months of age. A thorough general physical and systemic examination of the newborn was conducted to assess for any anomalies. In cases where any anomalies were detected, the neonate was excluded from the study.

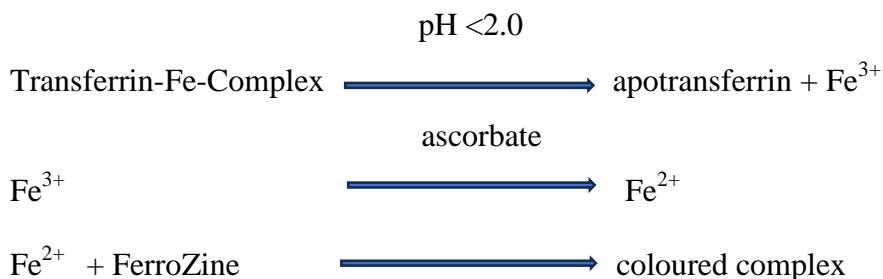
Figure 6 -Taking Cord Blood Sample



- Within 1 hour of taking the sample, about 2ml of blood in yellow-topped plain vacutainer and 2ml in red topped EDTA vacutainer was collected. The samples were labelled for identification and were transported to the Hi-Tech lab of the hospital for the Estimation of Complete Hemogram and Iron profile. The plain sample was centrifuged in Eppendorf Centrifuge 5792R centrifuge machine for 10 minutes at 3000RPM to separate the serum.

## IRON

**Test principle** – Colorimetric assay



Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released  $\text{Fe}^{3+}$  ions to  $\text{Fe}^{2+}$  ions which then react with FerroZine to form a coloured complex. The colour intensity is directly proportional to the iron concentration and can be measured photometrically.

Separate the serum or plasma from the clot or cells within 1 hour

Roche/Hitachi Cobas c Systems automatically calculate the analyte concentration of each sample in the unit micromole/L

## **FERRITIN**

**Testing principle** – Sandwich principle.Total duration of assay 18minutes

- 1<sup>st</sup> incubation – 6microL of sample,a biotinylated monoclonal ferritin – specific antibody and a monoclonal ferritin-specific antibody labelled with a ruthenium complex form a sandwich complex
- 2<sup>nd</sup> incubation – After addition of streptavidin -coated microparticles ,the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.Unbound substances are then removed with Procell II M.Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument specifically generated by 2 point calibration and a master curve provided via the cobas link

## **UIBC**

**Testing principle** –

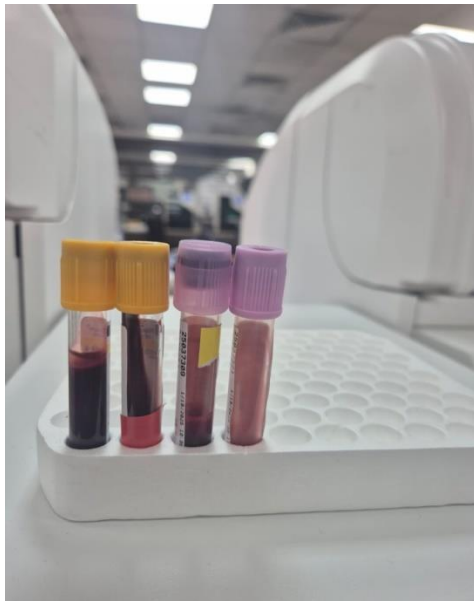
Direct determination with FerroZine

Alkaline buffer



The colour intensity is directly proportional to the unbound excess iron concentration and indirectly proportional to the unsaturated iron binding capacity. It is determined by measuring the increase in absorbance photometrically.

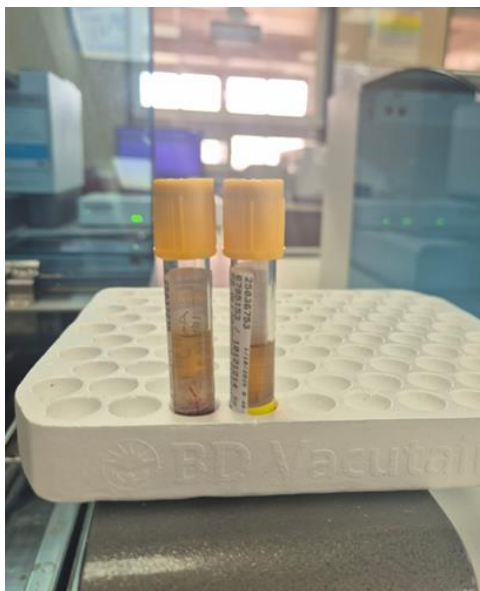
**Figure 7- Procedure of Complete blood count and Iron Profile Testing**



1. Sample collected in yellow plain And EDTA vacutainer for Iron profile



2. Plain sample centrifuged at 300RPM And Complete blood count respectively



3. Serum separated after Centrifugation



4. After serum separation the samples are arranged in sample rack for placing the rack in the analyser before estimation



**Cobas e 801 for estimation of Iron profile**

**MEASUREMENTS OF OUTCOMES**

- Maternal anemia was diagnosed in blood sample taken at 3<sup>rd</sup> trimester if
  - Hb <11mg/dl
  - Iron <30mcg/dl
  - Ferritin <20ng/dl
  - Transferrin saturation < 16% [64] [181,183,184]
- Cord blood was sent for Complete hemogram and Iron profile-Serm iron,Ferritin,TIBC,Transferrin saturation Iron deficiency anemia diagnosed when
  - Iron<100mcg/dl
  - Ferritin <50ng/ml
  - Tranferrin saturation <30% [154,181]
- Blood samples were sent at 4<sup>th</sup> month of age and Iron deficiency anemia was diagnosed if
  - Hb<10g/dl
  - Iron<50mcg/dl
  - Ferritin<15ng/ml [181,182]

## RESULTS

**Table 1: Gender distribution**

	Frequency	Percent
Female	43	59.7
Male	29	40.3
Total	72	100.0

**Figure 8: Gender distribution**

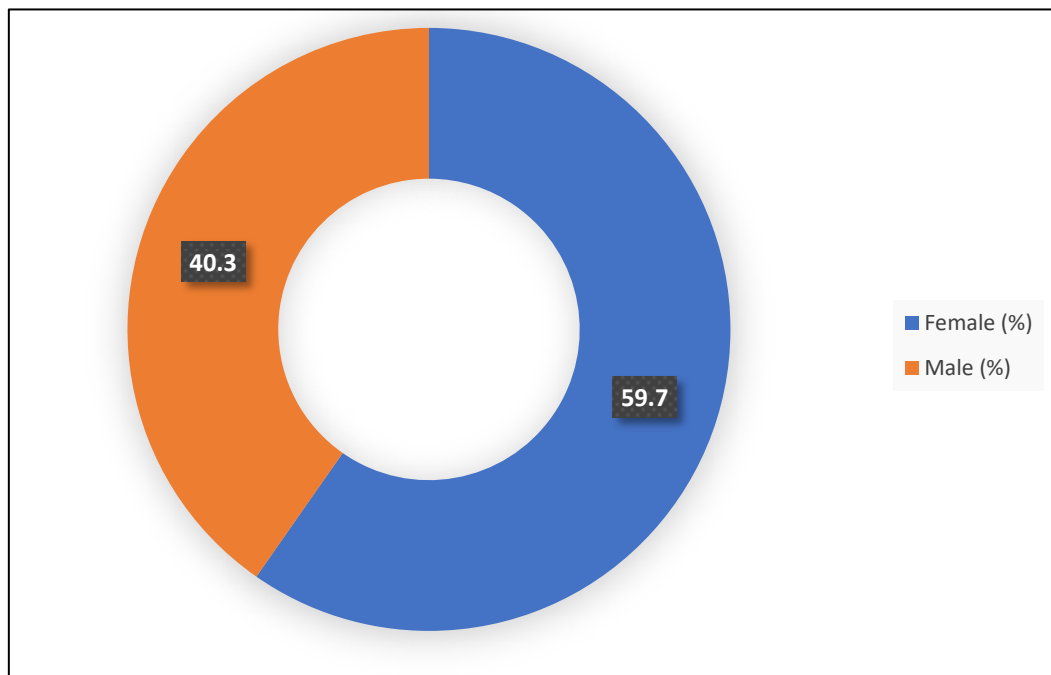
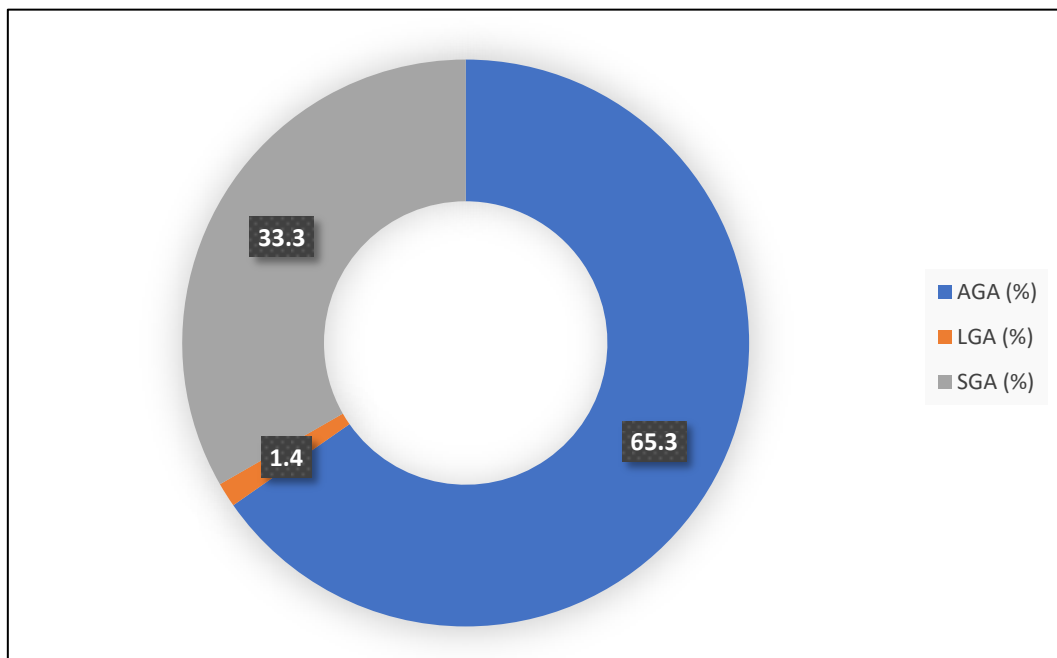


Table 1 presents the gender distribution of the study population, consisting of 72 individuals. The majority, 59.7% (43 newborns), are female, while 40.3% (29 newborns) are male. This indicates a higher representation of females in the study sample.

**Table 2: Distribution of birth weight categories**

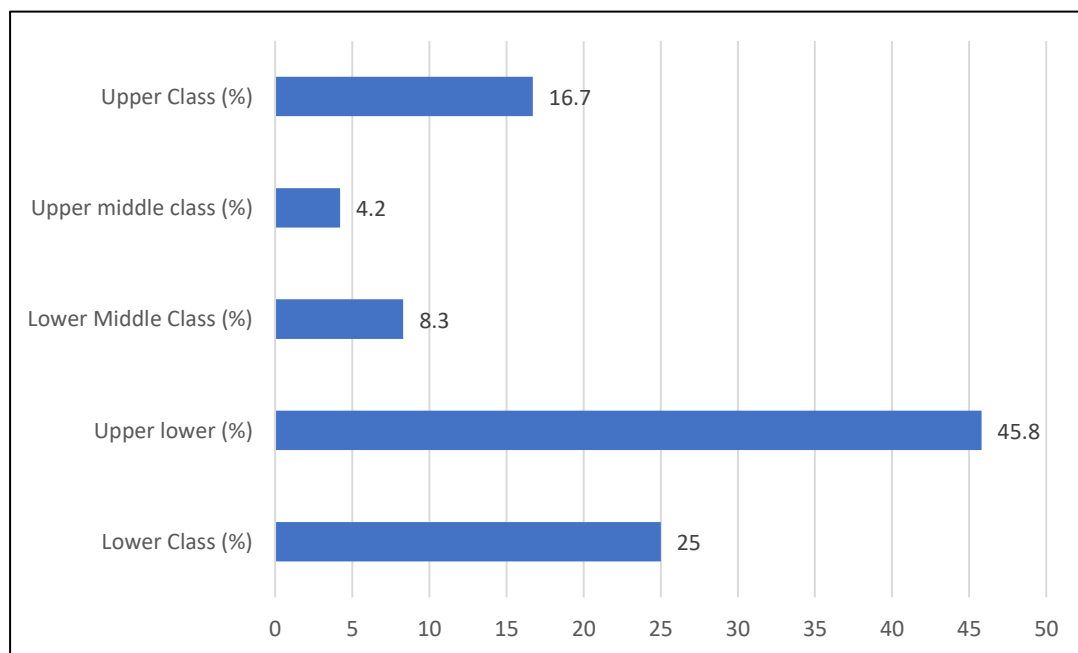
	Frequency	Percent
<b>Full term AGA</b>	47	65.3
<b>Full term LGA</b>	1	1.4
<b>Full term SGA</b>	24	33.3
<b>Total</b>	72	100.0

**Figure 9: Birthweight categories**

The table presents the distribution of birth weight categories among 72 newborns. The majority, 65.3% (47 individuals), are classified as Appropriate for Gestational Age (AGA). A smaller proportion, 33.3% (24 individuals), are Small for Gestational Age (SGA), while only 1.4% (1 individual) falls into the Large for Gestational Age (LGA) category. This indicates that most individuals had birth weights appropriate for their gestational age, with a notable proportion being SGA.

**Table 3: Socioeconomic Status**

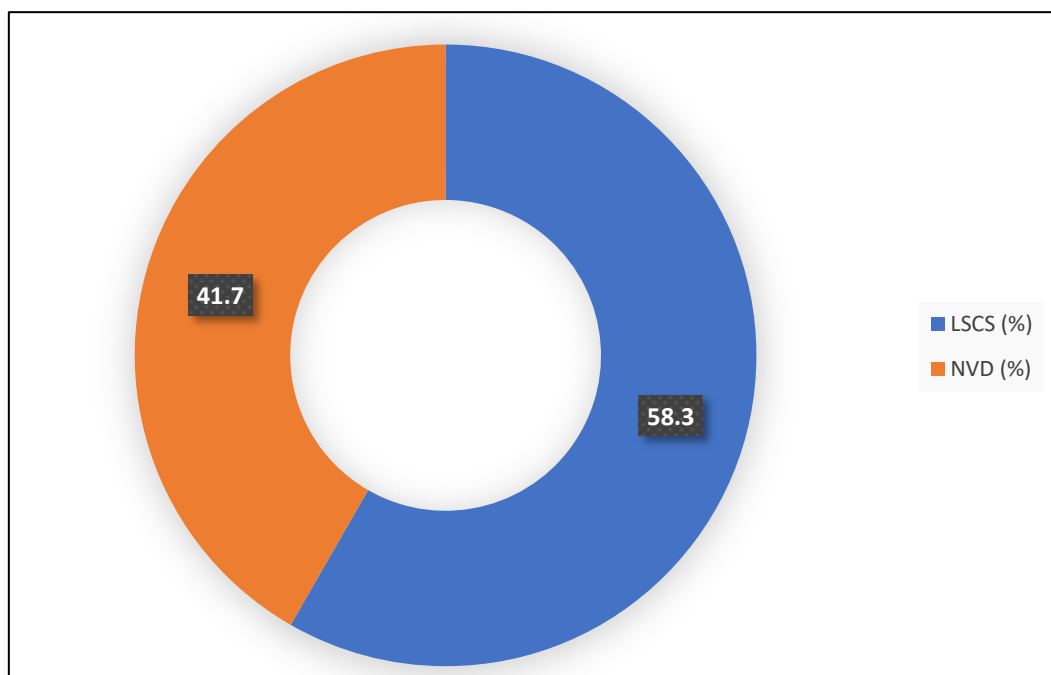
	<b>Frequency</b>	<b>Percent</b>
<b>Lower Class</b>	33	25.0
<b>Upper lower</b>	18	45.8
<b>Lower Middle Class</b>	12	8.3
<b>Upper middle class</b>	6	4.2
<b>Upper Class</b>	3	16.7
<b>Total</b>	72	100.0

**Figure 10: Socioeconomic Status**

The table presents the socioeconomic status (SES) distribution of the study population. Among the 72 participants, 33 (25.0%) belonged to the lower class, 18 (45.8%) were in the upper lower class, 12 (8.3%) were classified as lower middle class, 6 (4.2%) were from the upper middle class, and 3 (16.7%) belonged to the upper class. The total sample size is 72, representing 100% of the study population.

**Table 4: Mode of Delivery**

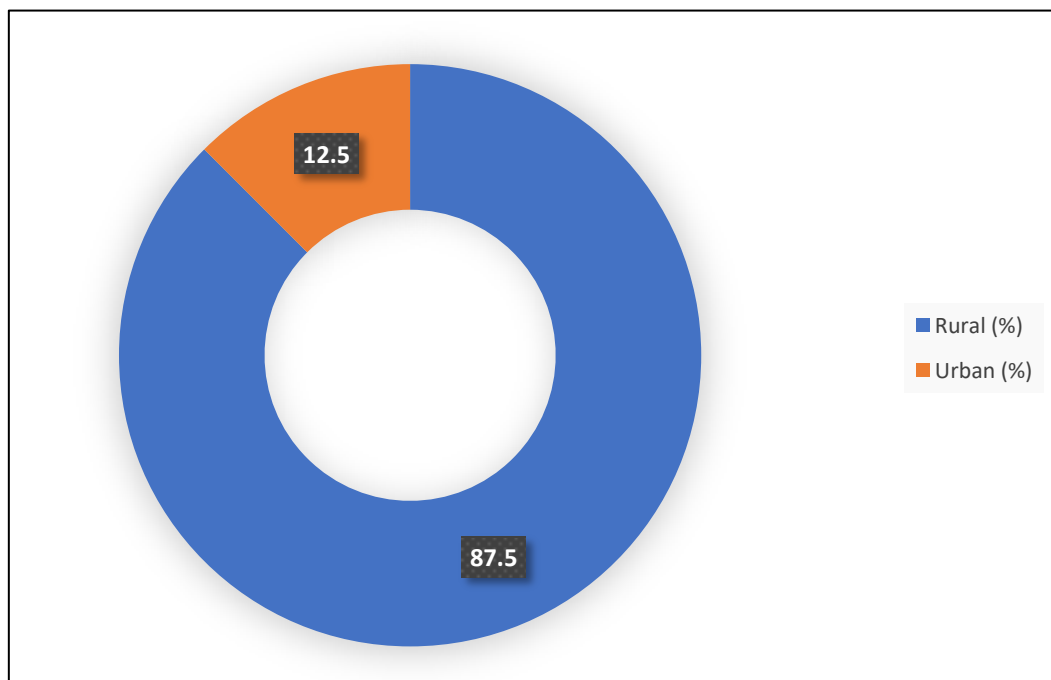
	Frequency	Percent
LSCS	42	58.3
NVD	30	41.7
Total	72	100.0

**Figure 11: Mode of delivery**

The table presents the mode of delivery (MOD) distribution among 72 individuals. The majority, 58.3% (42 individuals), were delivered via Lower Segment Caesarean Section (LSCS), while 41.7% (30 individuals) were delivered through Normal Vaginal Delivery (NVD). This indicates a higher prevalence of caesarean deliveries in the study population.

**Table 5: Distribution of Rural v/s Urban**

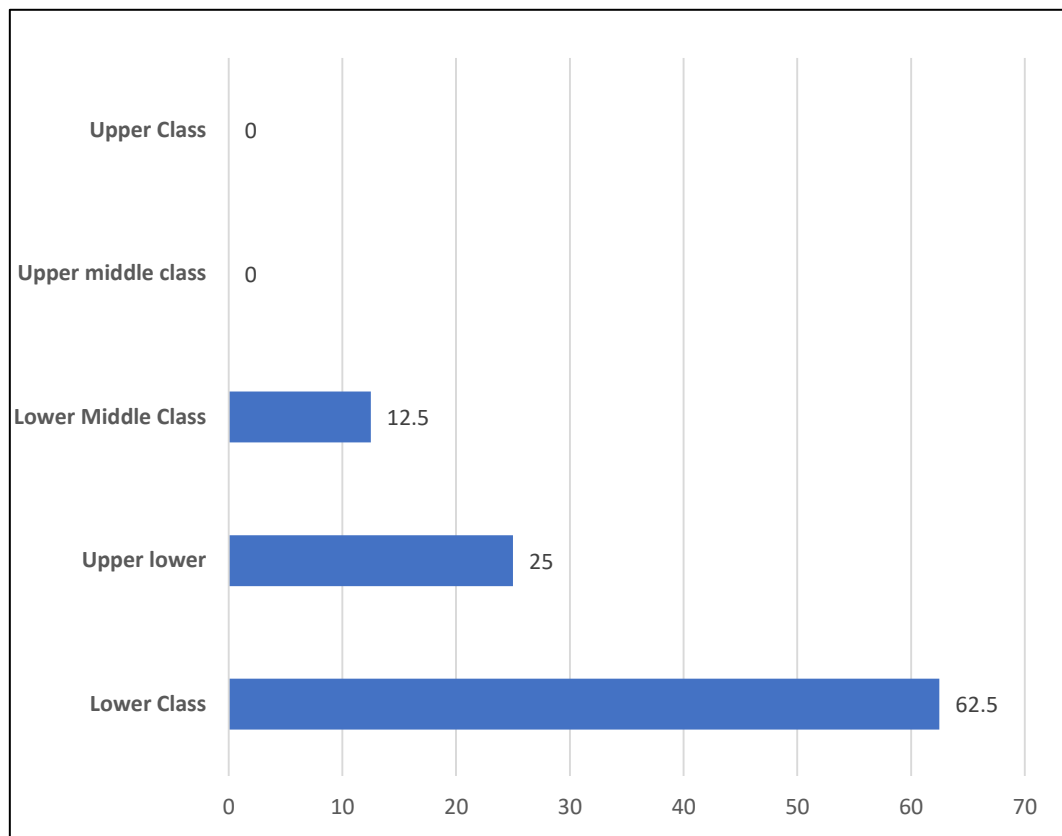
	Frequency	Percent
Rural	63	58.3
Urban	9	41.7
Total	72	100.0

**Figure 12: Distribution of Rurl/Urban**

The table shows the distribution of residence among the study participants. Out of the total 72 participants, 63 (87.5%) resided in rural areas, while 9 (12.5%) were from urban areas.

**Table 6: Distribution of maternal anaemia in relation to Socio-economic status**

	Frequency of mothers with anaemia	Percent	Fischer's Exact test
Lower Class	5	62.5	p value 0.562
Upper lower	2	25.0	
Lower Middle Class	1	12.5	
Upper middle class	0	0	
Upper Class	0	0	

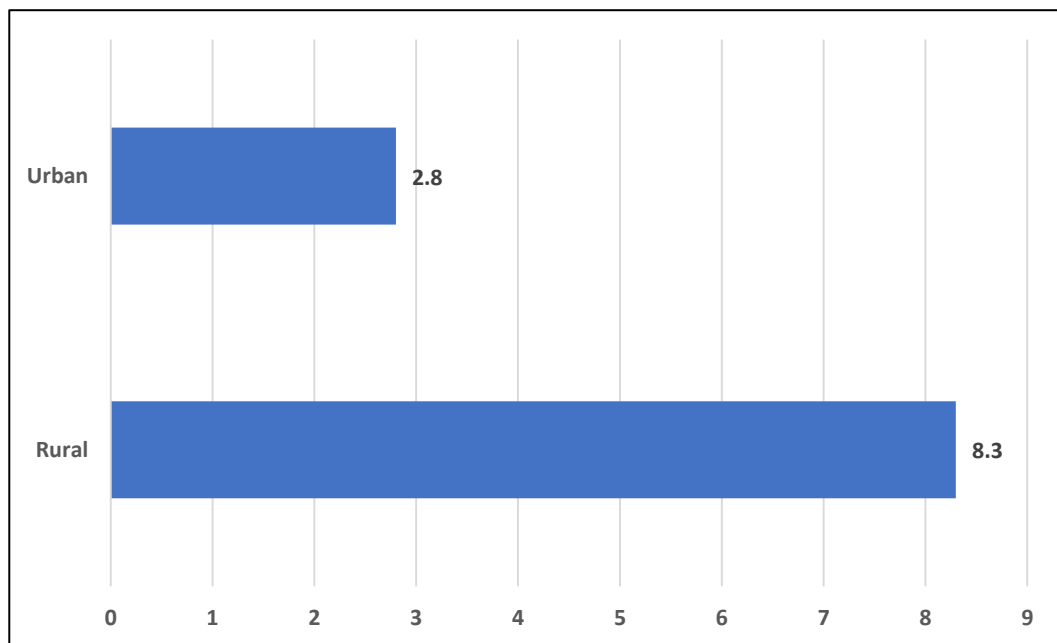
**Figure 13: Distribution of maternal anaemia in relation to SES**

The distribution of maternal anaemia in relation to socioeconomic status (SES) showed that anaemia was predominantly observed in lower socioeconomic groups. 62.5% of anemic mothers belonged to the lower class, followed by 25% from the upper lower class, and 12.5% from the lower middle class. No cases of anaemia were reported among mothers from the upper middle or upper-class groups. This pattern suggests that lower SES is associated with a higher prevalence of maternal anaemia, likely due to factors such as poor nutrition, limited healthcare access, and inadequate prenatal care. However, the Fisher's Exact test yielded a p-value of 0.562, indicating that the association between SES and maternal anaemia was not statistically significant ( $p > 0.05$ ).

**Table 7: Distribution of maternal anaemia in relation to distribution of Rural/Urban**

	Frequency of mothers with anaemia	Percent	P value
<b>Rural</b>	6	75	Chi-square test value 1.287; d.f 1; p value 0.256
<b>Urban</b>	2	25	

**Figure 14: Distribution of maternal anaemia in relation to Rural/Urban**



The analysis of maternal anaemia in relation to residence revealed that 75% of mothers with anaemia were from rural areas, while only 25% were from urban areas. This suggests a higher prevalence of anaemia among rural mothers. However, the chi-square test yielded a value of 1.287 with 1 degree of freedom and a p-value of 0.256, indicating that the observed difference was not statistically significant ( $p > 0.05$ ). This means that while anaemia appears to be more common among rural mothers in this sample, the difference could have occurred by chance rather than representing a true association between residence and maternal anaemia.

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**Correlation of IDA at 4<sup>th</sup> month and gender of the infant**
**Table 8: Gender and infant anaemia at 4<sup>th</sup> month**

	Frequency of infant with anaemia at 4 <sup>th</sup> month	Percent	Fischer's Exact test
<b>Male</b>	6	66.7	p value 0.678
<b>Female</b>	3	33.3	

The correlation between infant iron deficiency anaemia (IDA) at the 4th month and gender was analyzed, showing that 66.7% of anemic infants were male, while 33.3% were female. Although a higher proportion of male infants were affected, the Fisher's Exact test resulted in a p-value of 0.678, indicating that the difference was not statistically significant ( $p > 0.05$ ). This suggests that gender does not have a significant impact on the occurrence of anaemia at the 4th month in this sample.

**Correlation of IDA at 4<sup>th</sup> month and mode of delivery****Table 9: Mode of delivery and infant anaemia at 4<sup>th</sup> month**

	<b>Frequency of infant with anaemia at 4<sup>th</sup> month</b>	<b>Percent</b>	<b>Fischer's Exact test</b>
<b>NVD</b>	6	66.7	p value 0.678
<b>LSCS</b>	3	33.3	

The correlation between mode of delivery and infant anaemia at the 4th month was analyzed, revealing that 66.7% of anemic infants were born through Normal Vaginal Delivery (NVD), while 33.3% were delivered via Lower Segment Cesarean Section (LSCS). Although a higher proportion of anemic infants were delivered vaginally, the Fisher's Exact test resulted in a p-value of 0.678, indicating that the difference was not statistically significant ( $p > 0.05$ ). This suggests that the mode of delivery does not have a significant impact on the occurrence of anaemia in infants at 4 months.

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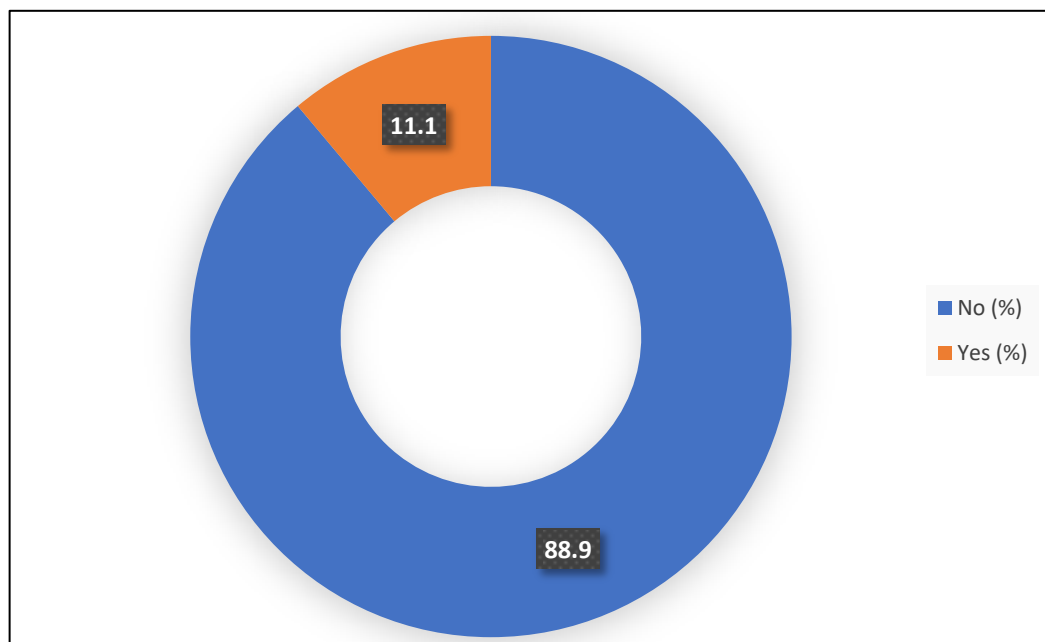
**Correlation of IDA at 4<sup>th</sup> month with weight pattern**
**Table 10: Weight pattern and infant anaemia at 4<sup>th</sup> month**

	Frequency of infant with anaemia at 4 <sup>th</sup> month	Percent	Fischer's Exact test
<b>AGA</b>	2	22.2	p value 0.342
<b>SGA</b>	7	77.8	
<b>LGA</b>	0	0	

The table presents the correlation between weight pattern at the 4th month and infant anaemia showed that the majority of anemic infants (77.8%) were Small for Gestational Age (SGA), while 22.2% were Appropriate for Gestational Age (AGA). No cases of anaemia were observed in Large for Gestational Age (LGA) infants. This suggests that SGA infants were more likely to develop anaemia at 4 months, possibly due to lower iron stores at birth, inadequate postnatal nutrition, or higher metabolic demands. However, the Fisher's Exact test yielded a p-value of 0.342, indicating that this association was not statistically significant ( $p > 0.05$ ).

**Table 11: Iron deficiency anaemia of mothers at 3<sup>rd</sup> trimester**

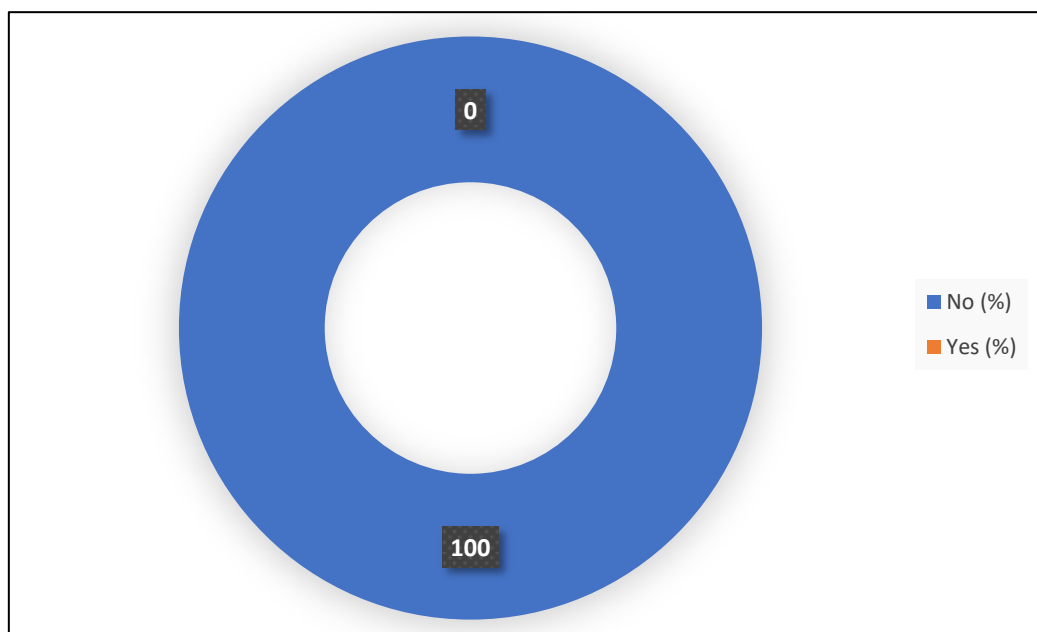
	Frequency	Percent
No	64	88.9
Yes	8	11.1
Total	72	100.0

**Figure 15: Iron deficiency anaemia of mothers at 3<sup>rd</sup> trimester**

The table shows the prevalence of iron deficiency anaemia (IDA) among mothers during the third trimester of pregnancy. Out of 72 mothers, 64 (88.9%) did not have IDA, while 8 (11.1%) were diagnosed with the condition.

**Table 12: Iron deficiency anaemia at Birth**

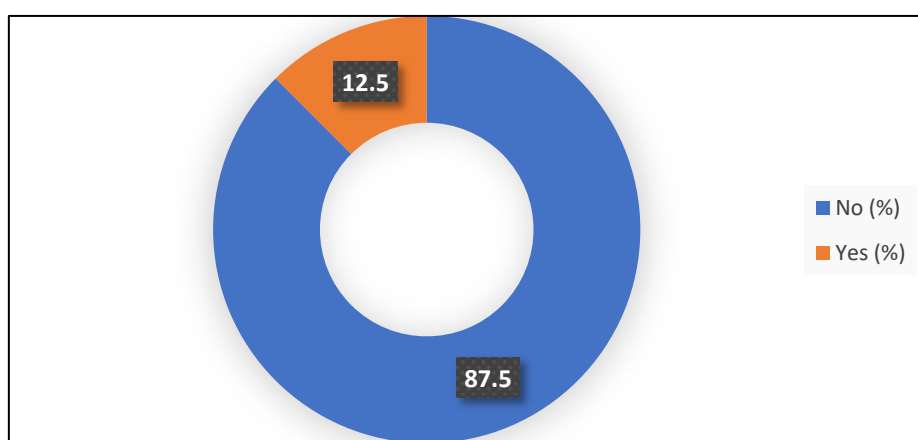
	Frequency	Percent
No	72	100.0
Yes	0	0
Total	72	100.0

**Figure 16: Iron deficiency anaemia at Birth**

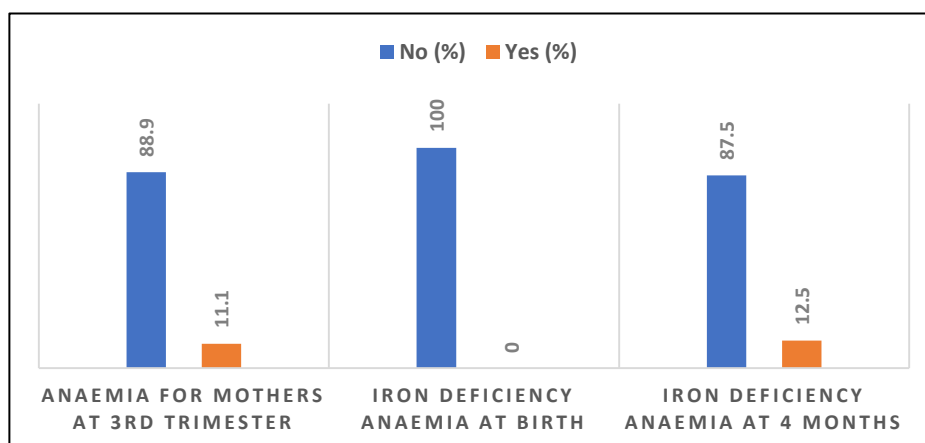
The table shows that none of the 72 new-borns in the study were diagnosed with iron deficiency anaemia at birth, with 100% classified as not having IDA. This indicates that all infants had sufficient iron levels at birth, possibly reflecting good maternal nutrition, effective prenatal iron supplementation, and optimal placental iron transfer.

**Table 13: Iron deficiency anaemia at 4<sup>th</sup> month**

	Frequency	Percent
No	63	87.5
Yes	9	12.5
Total	72	100.0

**Figure 17: Iron deficiency anaemia at 4<sup>th</sup> month**

The table presents the prevalence of iron deficiency anaemia (IDA) among infants at the 4th month. Out of 72 infants, 63 (87.5%) did not have IDA, while 9 (12.5%) were diagnosed with the condition

**Figure 18: Iron deficiency anaemia**

**Table 14: Mean Haematological parameters of mother at 3<sup>rd</sup> trimester**

	<b>Hb</b>	<b>PCV</b>	<b>RBC</b>	<b>PLT</b>	<b>WBC</b>	<b>Iron</b>	<b>Ferritin</b>	<b>TF</b>	<b>TIBC</b>
<b>Mean</b>	11.871	35.6458	4.5586	1990.42	8991.67	113.997	125.587	22.14	411.17
<b>Std. Deviation</b>	.7937	2.85746	4.13045	300.171	2042.42	71.3305	52.7068	7.737	111.039

The table presents the mean haematological parameters of mothers during the third trimester of pregnancy. The mean haemoglobin (Hb) level is 11.87 g/dL (SD: 0.79), while the packed cell volume (PCV) averages 35.65% (SD: 2.86). The red blood cell (RBC) count has a mean of 4.56 million/ $\mu$ L (SD: 4.13), and the platelet (PLT) count averages  $1990.42 \times 10^3/\mu$ L (SD: 300.17). The mean white blood cell (WBC) count is 8991.67/ $\mu$ L (SD: 2042.42). Regarding iron status, the mean serum iron level is 113.99  $\mu$ g/dL (SD: 71.33), ferritin averages 125.59 ng/mL (SD: 52.71), transferrin (TF) has a mean of 22.14% (SD: 7.74), and total iron-binding capacity (TIBC) is 411.17  $\mu$ g/dL (SD: 111.04).

**Table 15: Mean Haematological parameters at birth**

	<b>Hb</b>	<b>PCV</b>	<b>RBC</b>	<b>PLT</b>	<b>WBC</b>	<b>Iron</b>	<b>Ferritin</b>	<b>TF</b>	<b>TIBC</b>
<b>Mean</b>	16.2111	48.789	4.3529	2624.31	10413.89	167.49	210.88	47.63	346.65
<b>Std. Deviation</b>	1.28956	4.8998	.32279	414.198	2959.078	54.258	61.175	10.387	88.496

The table presents the mean haematological and iron status parameters of the study population at birth. The mean haemoglobin (Hb) level is 16.21 g/dL with a standard deviation of 1.29, while the packed cell volume (PCV) has a mean of 48.79% with a standard deviation of 4.90. The red blood cell (RBC) count averages 4.35 million/ $\mu$ L (SD: 0.32), and the platelet (PLT) count has a mean of  $2624.31 \times 10^3/\mu$ L (SD: 414.20). The white blood cell (WBC) count averages 10,413.89/ $\mu$ L (SD: 2959.08). Regarding iron status, the mean serum iron level is 167.49  $\mu$ g/dL (SD: 54.26), ferritin averages 210.88 ng/mL (SD: 61.18), transferrin (TF) has a mean of 47.63% (SD: 10.39), and total iron-binding capacity (TIBC) is 346.65  $\mu$ g/dL (SD: 88.50). These values provide insights into the new-borns' haematological and iron status at birth.

**Table 16: Mean Haematological parameters of infants at 4<sup>th</sup> month**

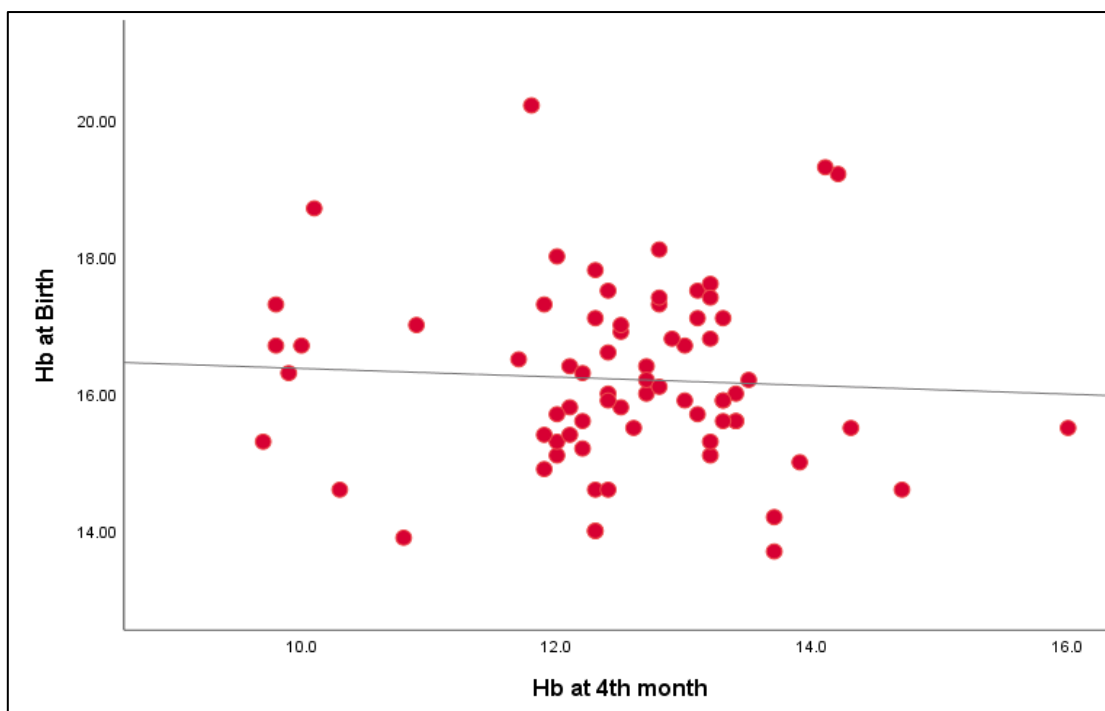
	Hb	PCV	RBC	PLT	WBC	Iron	Ferritin	TF	TIBC
<b>Mean</b>	12.500	36.8875	4.0015	2184.44	7468.06	128.03	139.76	32.703	380.83
<b>Std. Deviation</b>	1.1687	2.64223	.24222	274.611	1499.843	42.395	129.046	12.6018	117.008

The table presents the haematological parameters of infants at the 4th month. The mean haemoglobin (Hb) level is 12.50 g/dL with a standard deviation of 1.17, while the packed cell volume (PCV) averages 36.89% (SD: 2.64). The red blood cell (RBC) count has a mean of 4.00 million/ $\mu$ L (SD: 0.24), and the platelet (PLT) count averages  $2184.44 \times 10^3/\mu$ L (SD: 274.61). The mean white blood cell (WBC) count is  $7468.06/\mu$ L (SD: 1499.84). Regarding iron status, the mean serum iron level is 128.03  $\mu$ g/dL (SD: 42.40), ferritin averages 139.76 ng/mL (SD: 129.05), transferrin (TF) has a mean of 32.70% (SD: 12.60), and total iron-binding capacity (TIBC) is 380.83  $\mu$ g/dL (SD: 117.01). Compared to birth values, these results indicate a decline in haemoglobin, RBC count, WBC count, and iron stores by the 4th month, which may reflect physiological changes in infancy. The paired samples analysis compares haemoglobin (Hb) levels at birth and at the 4th month among 72 infants. The mean Hb at birth is 16.21 g/dL (SD: 1.29), while at 4 months, it declines to 12.50 g/dL (SD: 1.17). The paired samples t-test shows a mean difference of 3.71 g/dL (SD: 1.79) with a standard error of 0.21. The 95% confidence interval for the difference ranges from 3.29 to 4.13. The t-value of 17.605 with 71 degrees of freedom yields a highly significant p-value (<0.01), indicating a statistically significant decline in haemoglobin levels from birth to the 4th month. This decline is consistent with expected physiological anaemia of infancy.

**Table 17: Paired Samples Statistics Hb**

	Mean	N	Std. Deviation	Std. Error Mean				
Hb at Birth	16.2111	72	1.28956	.15198				
Hb at 4th month	12.500	72	1.1687	.1377				
Paired Samples Test								
	Paired Differences					t	df	P value
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Hb at Birth - Hb at 4th month	3.71111	1.78866	.21080	3.29080	4.13143	17.605	71	<0.01

*Figure 19: Scatter plot on Hb levels at birth and at 4<sup>th</sup> month plot*



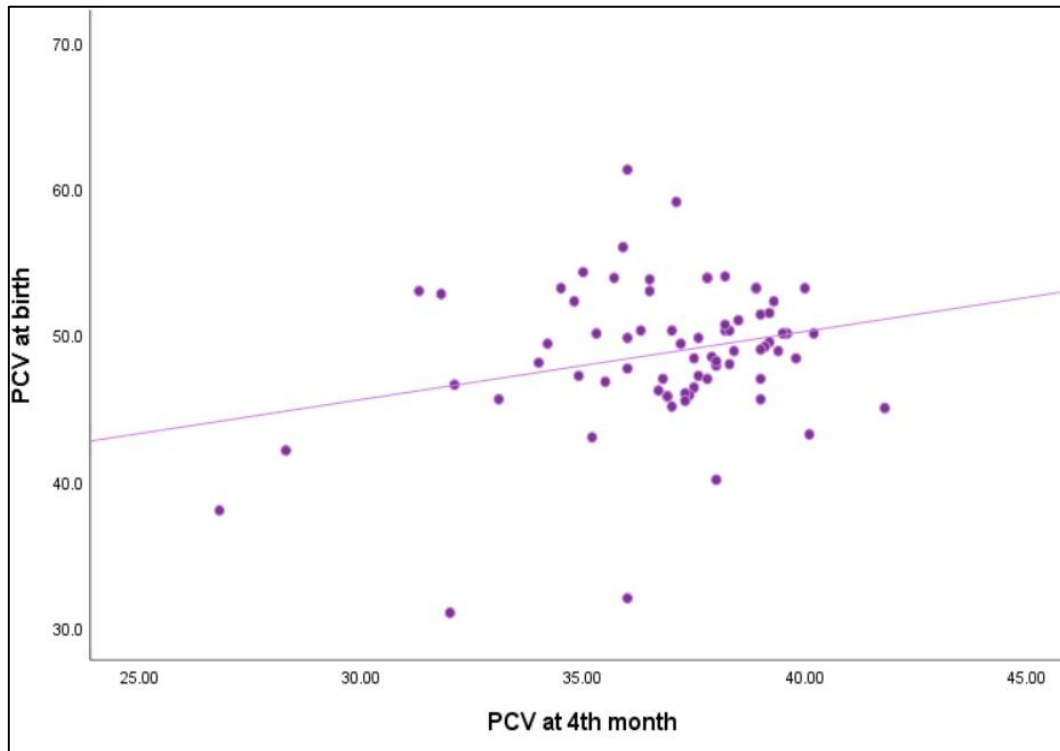
The scatter plot visualizes the relationship between haemoglobin levels at birth and at the 4th month. Each red dot represents an individual infant. The x-axis corresponds to Hb levels at the 4th month, while the y-axis represents Hb at birth. A weak negative correlation is indicated by the slight downward trend of the fitted line. The  $R^2$  value of 0.003 suggests an extremely weak linear relationship, implying that Hb levels at birth do not strongly predict Hb levels at 4 months

**Table 18: Paired Samples Statistics of PCV**

<b>Paired Samples Test</b>							
	<b>Mean</b>	<b>Std. Deviation</b>	<b>95% Confidence Interval of the Difference</b>		<b>t</b>	<b>df</b>	<b>P value</b>
			<b>Lower</b>	<b>Upper</b>			
<b>PCV – PCV at 4<sup>th</sup> month</b>	11.90139	4.94896	10.73844	13.06434	20.406	71	<0.001

The paired samples statistics for PCV indicate a significant change over time. The mean difference in PCV values at the fourth month is 11.90 with a standard deviation of 4.95. The 95% confidence interval for this difference ranges from 10.74 to 13.06, indicating a consistent increase. The t-test value is 20.406 with 71 degrees of freedom, and the p-value is less than 0.001, demonstrating a highly statistically significant difference in PCV measurements over the observed period.

*Figure 20: Scatter plot on PCV levels at birth and at 4<sup>th</sup> month plot*



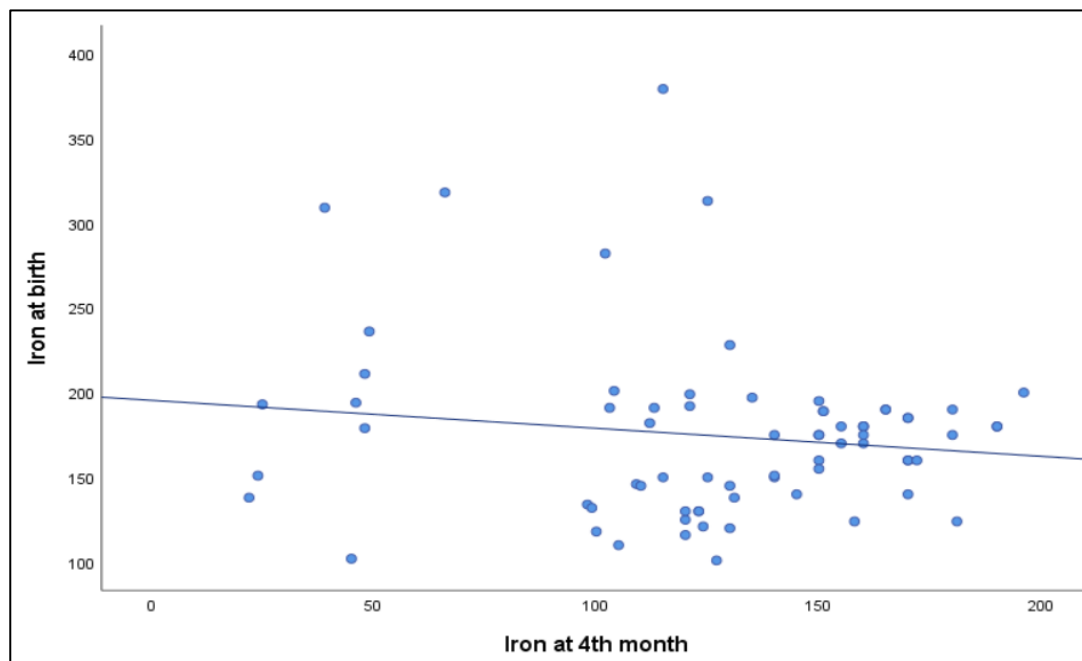
The image is a scatter plot that depicts the relationship between PCV levels at birth and PCV levels at the 4th month. Each point represents an individual data entry, with PCV at the 4th month on the x-axis and PCV at birth on the y-axis. The data points appear scattered but show a slight positive trend, as indicated by the faint regression line running through them. This suggests a weak but possibly positive correlation between the two variables—meaning that higher PCV levels at the 4th month may be associated with higher PCV levels at birth. However, the spread of points indicates variability, implying that other factors might influence PCV changes over time.

**Table 19: Paired Samples Statistics of Iron**

	Mean	Std. Deviation	95% Confidence Interval of the Difference		t	df	P value
			Lower	Upper			
<b>Iron - Iron at 4<sup>th</sup> month</b>	47.264	70.579	30.679	63.849	5.682	71	<0.001

The paired samples statistics for iron levels indicate a significant change over time. The mean difference in iron levels at the fourth month is 47.26 with a standard deviation of 70.58. The 95% confidence interval for this difference ranges from 30.68 to 63.85, suggesting variability in the changes observed. The t-test value is 5.682 with 71 degrees of freedom, and the p-value is less than 0.001, indicating a statistically significant increase in iron levels over the observed period.

**Figure 21: Scatter plot on Iron levels at birth and at 4<sup>th</sup> month plot**

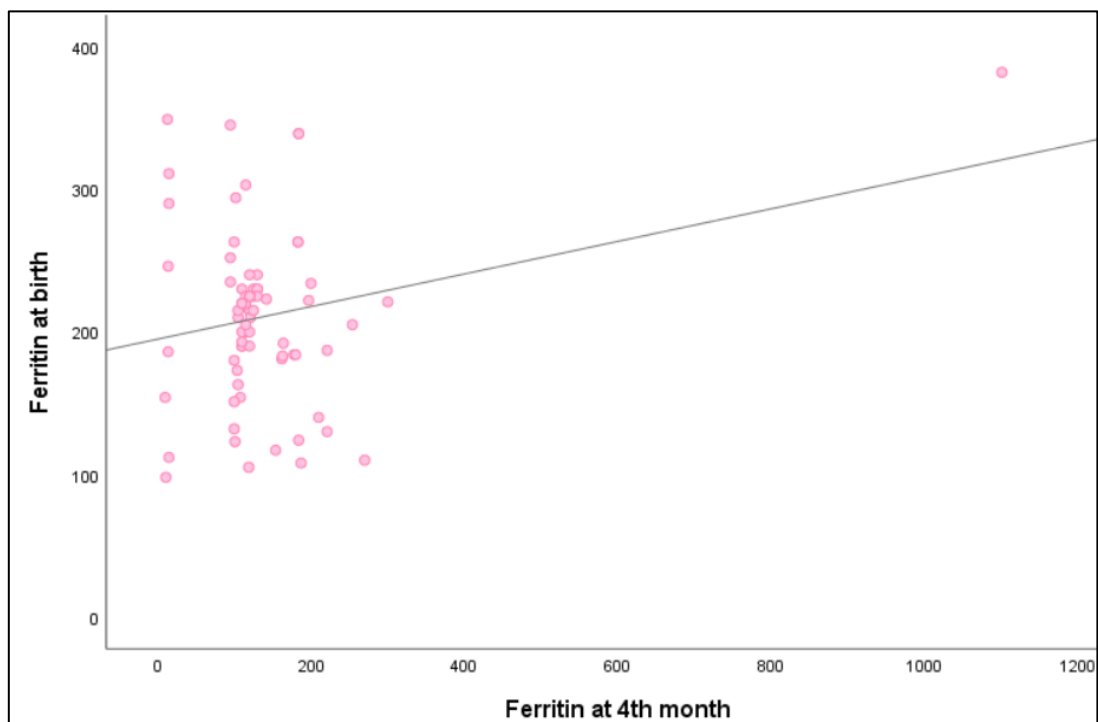


The scatter plot illustrates the relationship between Iron levels at birth (y-axis) and at the 4th month (x-axis), with each point representing an individual data entry. The data appears widely scattered, indicating a weak correlation between the two variables. The regression line has a slight negative slope, suggesting a potential negative relationship, where higher iron levels at birth might be associated with lower iron levels at the 4th month. However, the spread of points indicates significant variability, implying that other factors may be influencing iron levels over time.

**Table 20: Paired Samples Statistics of Ferritin**

	Mean	Std. Deviation	95% Confidence Interval of the Difference		T	df	P value
			Lower	Upper			
<b>Ferritin - Ferritin at 4<sup>th</sup> month</b>	71.111	128.791	40.847	101.375	4.685	71	<0.001

The paired samples statistics for ferritin levels show a significant increase over time. The mean difference in ferritin levels at the fourth month is 71.11, with a standard deviation of 128.79. The 95% confidence interval ranges from 40.85 to 101.38, reflecting variability in the changes. The t-test value is 4.685 with 71 degrees of freedom, and the p-value is less than 0.001, indicating a statistically significant increase in ferritin levels over the observed period.

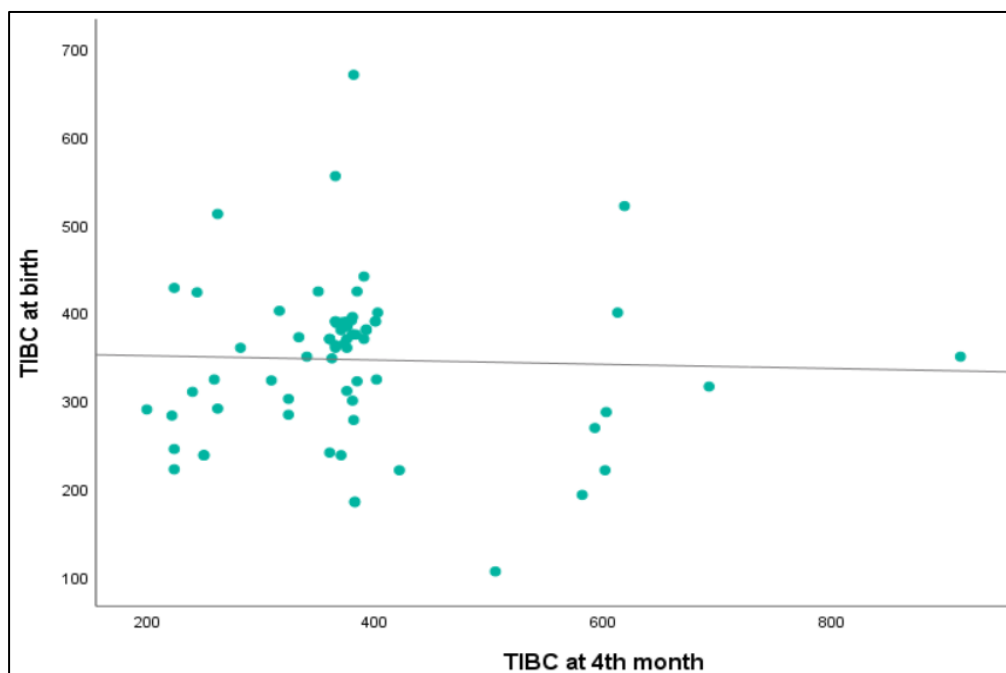
**Figure 22: Scatter plot on Ferritin levels at birth and at 4<sup>th</sup> month plot**

The scatter plot illustrates the relationship between Ferritin levels at birth (y-axis) and at the 4th month (x-axis), with each pink dot representing an individual data point. The regression line has a positive slope, suggesting a potential positive correlation, meaning higher ferritin levels at the 4th month may be associated with higher ferritin levels at birth. However, the data points are highly concentrated at lower ferritin values, with one extreme outlier at over 1000 ng/mL at the 4th month, which could disproportionately influence the trend. The overall distribution indicates variability, with most individuals showing ferritin levels below 200 ng/mL.

**Table 21: Paired Samples Statistics of TIBC**

	Mean	Std. Deviation	95% Confidence Interval of the Difference		t	df	P value
			Lower	Upper			
<b>TIBC - TIBC at 4<sup>th</sup> month</b>	-34.181	148.962	-69.185	.824	-1.947	71	.055

The paired samples statistics for total iron-binding capacity (TIBC) indicate a slight decrease over time, but the change is not statistically significant. The mean difference in TIBC levels at the fourth month is -34.18, with a standard deviation of 148.96. The 95% confidence interval ranges from -69.19 to 0.82, suggesting considerable variability in the data. The t-test value is -1.947 with 71 degrees of freedom, and the p-value is 0.055, which is slightly above the conventional significance threshold of 0.05. This suggests that the observed reduction in TIBC levels may not be statistically significant.

**Figure 23: Scatter plot on TIBC levels at birth and at 4<sup>th</sup> month plot**

The scatter plot depicts the relationship between TIBC (Total Iron Binding Capacity) levels at birth (y-axis) and at the 4th month (x-axis), with each point representing an individual data entry. The regression line has a slight negative slope, suggesting a weak negative correlation—higher TIBC levels at the 4th month might be associated with lower TIBC levels at birth. However, the data points are widely scattered, indicating high variability and a lack of a strong trend. Most values cluster around 300–400 µg/dL, but a few outliers at higher TIBC levels suggest that additional factors may be influencing changes in TIBC over time.

In contrast, TIBC (Total Iron Binding Capacity) showed a mean decrease of -34.181, but its p-value (0.055) suggests that the result is not statistically significant at the conventional 0.05 threshold. This means there is a possibility that the observed decrease in TIBC could be due to random variation rather than a true effect. The confidence interval (-69.185 to 0.824) includes zero, reinforcing this uncertainty. The t-values indicate the strength of these differences, with higher values for PCV (20.406), Iron (5.682), and Ferritin (4.685) supporting their significance. The TIBC t-value (-1.947) is much lower, consistent with its borderline significance. Overall, the data suggest significant improvements in PCV, Iron, and Ferritin levels over four months, while the decrease in TIBC remains inconclusive.

**Table 22: Comparison of IDA in mother and IDA in respective infants at 4<sup>th</sup> month of age**

<b>No of mothers with IDA</b>	<b>No of respective infants With/Without IDA at 4<sup>th</sup> month</b>	<b>Chi-sqaure test</b>
8	Yes – 6 No - 2	<b>Test value 32.143; d f 1;p value&lt;0.001</b>
<b>No of mothers without IDA</b>		
64	Yes – 3 No – 61	

The table compares the prevalence of iron deficiency anaemia (IDA) among infants at four months and their mothers during the third trimester. Among the 72 mother-infant pairs, 64 mothers did not have IDA, while 8 had IDA. Of the infants whose mothers were not iron deficient, 61 (95.3%) remained non-anaemic at four months, while 3 (4.7%) developed IDA. In contrast, among infants whose mothers had IDA, 6 (75%) developed IDA, while only 2 (25%) remained non-anaemic. The chi-square test result ( $\chi^2 = 32.143$ ,  $p < 0.001$ ) indicates a statistically significant association between maternal IDA during pregnancy and infant IDA at four months. This suggests that maternal iron status plays a crucial role in determining the infant's iron stores and highlights the importance of preventing and managing maternal IDA to reduce the risk of anaemia in early infancy.

Haematological analysis revealed a significant decline in haemoglobin (16.21 g/dL at birth to 12.50 g/dL at four months,  $p < 0.01$ ). The scatter plots illustrate the relationships between haematological parameters (PCV, iron, ferritin, and TIBC) at birth and at four months of age. PCV levels show a weak positive correlation, suggesting that higher birth levels may slightly persist, though with variability. Iron

levels exhibit a slight negative trend, indicating that higher iron at birth might be linked to lower levels at four months, while ferritin levels show a positive correlation, with most values clustering below 200 ng/mL and one extreme outlier. TIBC levels display a weak negative correlation, with most values around 300–400 µg/dL but some outliers suggesting additional influencing factors. No infants had iron deficiency anaemia (IDA) at birth, but 12.5% developed it by four months. Maternal IDA during the third trimester (11.1%) was strongly associated with infant IDA ( $p < 0.001$ ), highlighting the importance of maternal iron status in preventing early infant anaemia. These findings emphasize the need for better maternal nutrition and early iron supplementation strategies for infants.

## **DISCUSSION**

This current study included a total of 72 infants, with a majority of 59.7% (43 infants) being female and 40.3% (29 infants) being male. This gender distribution suggests a higher representation of female infants in the sample, which may or may not be reflective of the general population, depending on regional and hospital-specific birth statistics. While gender-based differences in iron metabolism are minimal in neonates, certain studies have suggested that male infants may be more prone to iron deficiency due to higher growth rates and iron demands.<sup>113</sup> However, the overall impact of gender on neonatal iron levels remains a subject of ongoing research. The relatively balanced gender ratio in this study helps ensure that the findings are applicable to both sexes without significant bias.

In our study Birth weight is a crucial determinant of neonatal health and iron stores. The study classified infants into three categories: Appropriate for Gestational Age (AGA) (65.3%, 47 infants), Small for Gestational Age (SGA) (33.3%, 24 infants), and Large for Gestational Age (LGA) (1.4%, 1 infant). The higher proportion of AGA infants indicates that most infants had normal intrauterine growth, while the 33.3% of SGA infants suggest a considerable number experienced restricted foetal growth, which could have implications for their iron status. SGA infants are often at a higher risk of iron deficiency because of reduced iron endowment from the mother and limited foetal iron stores.<sup>114</sup> Given that only one infant was classified as LGA, its significance in this study is minimal. However, the overall findings align with established research indicating that SGA infants tend to have lower haemoglobin and ferritin levels, making them more vulnerable to early-onset iron deficiency anaemia (IDA).<sup>115</sup>

In this study 63% mothers are from the rural areas whereas 9% are from the urban areas. Out of mothers with maternal Anemia 75% hails from rural areas and 25% from urban areas, showing the health care limited access for proper antenatal care and early anemia screening

In this present study the socioeconomic distribution of the study population revealed that 45.8% belonged to the lower middle class, 25% to the lower class, 16.7% to the upper middle class, 8.3% to the middle class, and 4.2% to the upper class. This indicates that a significant proportion of participants were from lower socioeconomic backgrounds, which is relevant as lower SES has been linked to poorer maternal nutrition, reduced prenatal care, and a higher risk of infant iron deficiency anaemia (IDA).<sup>116</sup> Families from lower socioeconomic backgrounds may have limited access to iron-rich foods, prenatal vitamins, and adequate healthcare services, all of which contribute to maternal and infant iron status. This demographic data emphasizes the need for targeted nutritional interventions and healthcare policies aimed at improving maternal iron supplementation and monitoring iron levels in newborns, especially in economically disadvantaged groups.

In our study the mode of delivery showed that 58.3% of infants were delivered via Lower Segment Caesarean Section (LSCS) and 41.7% via Normal Vaginal Delivery (NVD). Studies have reported that LSCS deliveries might affect neonatal iron stores due to delayed clamping of the umbilical cord and potential maternal blood loss.<sup>117</sup> Additionally, vaginal deliveries have been associated with better placental transfusion of blood, leading to higher initial iron stores in neonates.<sup>118</sup> The increased rate of LSCS in this study may be indicative of hospital practices or maternal health factors necessitating surgical delivery. The difference in iron levels between LSCS and NVD infants, though not the primary focus of the study, could be an area for

further investigation to determine if birth method significantly influences neonatal iron reserves.

In the current study they reported mean haemoglobin (Hb) levels of 16.21 g/dL, a packed cell volume (PCV) of 48.79%, and an RBC count of 4.35 million/ $\mu$ L at birth. These values are within the expected range for neonates, indicating adequate iron stores at birth.<sup>119</sup> The mean serum iron was 167.49  $\mu$ g/dL, and ferritin was 210.88 ng/mL, which reflects sufficient iron reserves, likely due to effective placental iron transfer.<sup>120</sup> Neonates typically have high iron stores at birth as a result of maternal iron transfer in utero, which supports their initial growth phase. However, these iron levels gradually decline in the absence of dietary supplementation. The high ferritin levels at birth suggest that most infants in this study started life with adequate iron stores, a crucial factor in preventing early iron deficiency.

In this study by the 4th month, there was a significant decline in haemoglobin (12.50 g/dL), PCV (36.89%), RBC count (4.00 million/ $\mu$ L), and serum iron (128.03  $\mu$ g/dL).<sup>121</sup> During the first few months of life, the reduction in erythropoiesis occurs due to a natural decrease in erythropoietin production, leading to a drop in haemoglobin levels. The decrease in ferritin levels from 210.88 ng/mL at birth to 139.76 ng/mL also suggests a depletion of iron stores as exclusive breastfeeding does not provide sufficient iron to meet the growing infant's needs.<sup>121</sup>

The paired t-test analysis confirmed a statistically significant decline in haemoglobin from birth (16.21 g/dL) to 4 months (12.50 g/dL) ( $p < 0.01$ ). This is in line with previous research, indicating that haemoglobin naturally declines in the first few months of life due to decreased erythropoiesis before dietary iron intake compensates for iron loss.<sup>121</sup>

Our study showed at birth, none of the infants were diagnosed with IDA, which suggests effective placental iron transfer and adequate maternal iron status. However, by 4 months, 12.5% of the infants were diagnosed with IDA, highlighting the risk of iron depletion during exclusive breastfeeding without supplementation.<sup>121</sup>

The mean maternal haemoglobin in the third trimester was 11.87 g/dL, with 12.5% of mothers diagnosed with IDA. A significant association was found between maternal IDA and infant IDA at 4 months ( $p < 0.001$ ). This reinforces the well-established link between maternal iron deficiency during pregnancy and neonatal iron status, emphasizing the importance of maternal iron supplementation.<sup>121</sup>

A critical observation in the study was the strong association between maternal iron deficiency anaemia (IDA) in the third trimester (11.1%) and the development of infant IDA by four months ( $p < 0.001$ ). This relationship highlights the importance of maternal iron status during pregnancy, as iron stores in the foetus are primarily derived from maternal sources via placental transfer.<sup>125</sup> Studies have shown that maternal iron deficiency during pregnancy can lead to lower neonatal iron stores, predisposing infants to early-onset anaemia.<sup>126</sup> This reinforces the need for enhanced maternal nutrition programs, including iron supplementation, to improve maternal and neonatal outcomes.

The relationship between maternal anaemia and socioeconomic status (SES) indicates a higher prevalence of anaemia among mothers belonging to lower socioeconomic strata. In this study, 62.5% of anaemic mothers were from the lower class, followed by 25% from the upper lower class, and 12.5% from the lower middle class. No anaemia cases were reported in the upper middle or upper-class groups. These findings are consistent with previous studies demonstrating that women from

lower SES backgrounds are at greater risk of anaemia due to poor nutrition, limited healthcare access, and inadequate prenatal care.<sup>127</sup> Nutritional deficiencies, particularly iron and folate insufficiency, are more common in lower-income households, leading to increased vulnerability to anaemia.<sup>128</sup> Additionally, poor healthcare utilization among lower SES groups can contribute to late diagnosis and inadequate management of anaemia in pregnancy.<sup>129</sup> Despite these observed trends, the Fisher's Exact test yielded a p-value of 0.562, indicating that the association between SES and maternal anaemia was not statistically significant ( $p > 0.05$ ). The lack of statistical significance may be attributed to the limited sample size or unaccounted confounding factors such as dietary habits and genetic predisposition.

The prevalence of maternal anaemia also showed a higher burden among rural mothers, with 75% of anaemic cases occurring in rural areas compared to 25% in urban settings. This aligns with global trends, where rural populations tend to have poorer maternal health outcomes due to inadequate healthcare services, lower literacy levels, and poor nutritional intake.<sup>130</sup> Rural mothers often experience delays in seeking antenatal care, which can result in untreated or poorly managed anaemia during pregnancy.<sup>131</sup> However, the chi-square test resulted in a value of 1.287 with a p-value of 0.256, suggesting that the difference was not statistically significant ( $p > 0.05$ ). This could imply that the disparity observed between urban and rural mothers in this study may not be generalizable and could be due to random variation. Larger population-based studies are needed to confirm these findings and explore additional determinants such as dietary practices, healthcare accessibility, and cultural influences on maternal health.

The study found that 66.7% of infants diagnosed with iron deficiency anaemia (IDA) at 4 months were male, while 33.3% were female. While some literature suggests that male infants may have higher iron demands and are thus more susceptible to IDA, the Fisher's Exact test yielded a p-value of 0.678, indicating no statistically significant association ( $p > 0.05$ ).<sup>132</sup> Other studies suggest that gender-based differences in anaemia prevalence may be more evident later in childhood due to differences in growth rates and dietary intake rather than at the neonatal stage.<sup>133</sup> Thus, while male infants in this study had a higher proportion of anaemia, no definitive conclusions can be drawn regarding gender as a risk factor for IDA at 4 months.

Mode of delivery has been speculated to influence neonatal iron status, particularly due to differences in cord clamping practices and stress-induced erythropoiesis during labor.<sup>134</sup> In this study, 66.7% of anaemic infants were born through Normal Vaginal Delivery (NVD), while 33.3% were delivered via Lower Segment Cesarean Section (LSCS). However, the Fisher's Exact test yielded a p-value of 0.678, suggesting no statistically significant association ( $p > 0.05$ ). Previous research has indicated that delayed cord clamping during vaginal deliveries may confer some benefit in terms of neonatal iron stores.<sup>135</sup>, though this advantage may not be significant enough to impact anaemia prevalence at 4 months. Further longitudinal research is required to explore how mode of delivery influences iron status beyond the immediate postpartum period.

The analysis of infant weight patterns showed that 77.8% of anemic infants were Small for Gestational Age (SGA), while 22.2% were Appropriate for Gestational Age (AGA). No anaemia cases were reported among Large for Gestational Age (LGA) infants. SGA infants are at an increased risk of anaemia due

to lower iron stores at birth, increased postnatal metabolic demands, and possible maternal nutritional deficiencies during pregnancy.<sup>136</sup> However, the Fisher's Exact test yielded a p-value of 0.342, suggesting that the observed trend was not statistically significant ( $p > 0.05$ ). Given the established biological plausibility of an association between SGA status and neonatal anaemia, larger sample sizes may be necessary to detect a significant relationship.

The study's findings emphasize the necessity for interventions to prevent early infant anaemia. Strategies such as improved maternal nutrition, routine antenatal iron supplementation, and timely introduction of iron-rich complementary foods for infants should be considered. Additionally, targeted screening programs for high-risk mothers and infants can aid in early identification and management of iron deficiency, thereby reducing the burden of anaemia in infancy.<sup>137</sup>

The study highlights a significant decline in haemoglobin and iron stores among exclusively breastfed infants by the 4th month, with 12.5% developing IDA. Maternal IDA was significantly associated with infant IDA, underscoring the importance of maternal nutrition in preventing early childhood anaemia. The study highlights the patterns of maternal anaemia in relation to SES and residence, as well as its correlation with infant anaemia at 4 months based on gender, mode of delivery, and weight pattern. While trends indicate a higher prevalence of maternal anaemia in lower SES and rural groups and suggest an association between SGA status and anaemia in infants, none of these associations were statistically significant. These findings support the need for strategies such as iron supplementation for at-risk infants and improved iron intake during pregnancy.

## **CONCLUSION**

Our study showed higher incidence of Iron Deficiency Anemia in rural population, Lower Socio-economic status groups and Small For Gestational Age(SGA) male babies. Thus more emphasis is necessary on Iron Supplementation and maternal health especially in rural and lower socio-economic groups as 64% of Indian people lives in rural areas. A significant association ( $p < 0.001$ ) was observed between maternal Iron Deficiency Anemia and infant Iron Deficiency Anemia at four months, with 75% of infants born to anaemic mothers developing IDA at 4<sup>th</sup> month on exclusive breastfeeding.. These findings emphasize the crucial role of maternal anemia in influencing infant iron levels. Insufficient maternal iron stores can compromise an infant's iron reserves, increasing their risk of anemia in early life. This study reinforces the importance of prenatal care focused on iron supplementation and regular monitoring as maternal health during pregnancy directly impacts an infant's growth ,development and long term well being.

## **SUMMARY**

**Gender Distribution:** The study involved 72 individuals, with 59.7% (43) being female and 40.3% (29) male, indicating a higher representation of females in the sample.

**Birth Weight Categories:**

- 65.3% (47 individuals) were classified as Appropriate for Gestational Age (AGA).
- 33.3% (24 individuals) were Small for Gestational Age (SGA).
- Only 1.4% (1 individual) fell under Large for Gestational Age (LGA), indicating most infants had birth weights appropriate for their gestational age.
- Iron Deficiency Anemia has been observed more in SGA babies (Small for Gestational Age)

**Socioeconomic Status (SES):**

- 45.8% (33 individuals) were from the lower middle class, and 25% (18 individuals) were from the lower class.
- Smaller groups were from the upper middle class (16.7%) and middle class (8.3%), while 4.2% (3 individuals) were from the upper class, reflecting the predominance of lower socioeconomic backgrounds.
- Maternal anemia was more in mothers who belonged to lower socio-economic groups.

**Mode of Delivery:**

- 58.3% (42 individuals) were delivered via Lower Segment Caesarean Section (LSCS), while 41.7% (30 individuals) were delivered through Normal Vaginal Delivery (NVD), showing a higher prevalence of caesarean sections.
- Iron Deficiency anemia at 3th month was more in infants delivered via Normal vaginal delivery

**Haematological Parameters of mother at 3rd Trimester:**

- Haemoglobin (Hb): 11.87 g/dL (SD: 0.79)
- Packed Cell Volume (PCV): 35.65% (SD: 2.86)
- Iron Status:
  - Serum iron: 113.99 µg/dL (SD: 71.33)
  - Ferritin: 125.59 ng/mL (SD: 52.71)
  - Transferrin (TF): 22.14% (SD: 7.74)
  - Total Iron-Binding Capacity (TIBC): 411.17 µg/dL (SD: 111.04)

**Haematological Parameters at Birth:**

- Haemoglobin (Hb): 16.21 g/dL (SD: 1.29)
- Packed Cell Volume (PCV): 48.79% (SD: 4.90)
- Red Blood Cell (RBC) Count: 4.35 million/µL (SD: 0.32)
- Platelet (PLT) Count:  $2624.31 \times 10^3/\mu\text{L}$  (SD: 414.20)

- Iron Status:
  - Serum iron: 167.49 µg/dL (SD: 54.26)
  - Ferritin: 210.88 ng/mL (SD: 61.18)
  - Transferrin (TF): 47.63% (SD: 10.39)
  - Total Iron-Binding Capacity (TIBC): 346.65 µg/dL (SD: 88.50)

**Haematological Parameters at 4th Month:**

- Haemoglobin (Hb): 12.50 g/dL (SD: 1.17)
- Packed Cell Volume (PCV): 36.89% (SD: 2.64)
- Red Blood Cell (RBC) Count: 4.00 million/µL (SD: 0.24)
- Platelet (PLT) Count: 2184.44 × 10<sup>3</sup>/µL (SD: 274.61)
- Iron Status:
  - Serum iron: 128.03 µg/dL (SD: 42.40)
  - Ferritin: 139.76 ng/mL (SD: 129.05)
  - Transferrin (TF): 32.70% (SD: 12.60)
  - Total Iron-Binding Capacity (TIBC): 380.83 µg/dL (SD: 117.01)
- The comparison showed a decline in haemoglobin, RBC, WBC, and iron levels by the 4th month, reflecting the expected physiological anaemia of infancy.

**Paired Samples Test (Haemoglobin Decline):**

A significant decline was found for the following from birth to 4th month

1. **Haemoglobin levels** at birth (16.21 g/dL) to the 4th month (12.50 g/dL), with a mean difference of 3.71 g/dL. Hence the correlation is significant with a **p value < 0.01**.
2. **PCV levels** at birth (48.79%) to the 4th month (36.89%) with a mean difference of 11%. Hence the correlation is significant with a **p value < 0.01**
3. **Serum Iron levels** at birth (167.49 microg/dl) to the 4th month (128.03 microg/dl) with a mean difference of 47.2 microg/dl. Hence the correlation is significant with a **p value < 0.01**
4. **Serum ferritin levels** at birth (210.88 ng/ml) to the 4th month (139.76 ng/ml) with a mean difference 71 ng/ml. Hence the correlation is significant with a **p value < 0.001**
5. In contrast, **TIBC** (Total Iron Binding Capacity) showed a mean decrease of 34.181, but its p-value (0.055) suggests that the result is not statistically significant at the conventional 0.05 threshold

Maternal IDA in 3rd Trimester:

- 11.1% of mothers had IDA during the third trimester, while 88.9% were non-anaemic.

Iron Deficiency Anaemia (IDA) at Birth:

- 100% of infants did not have iron deficiency anaemia (IDA) at birth, suggesting adequate maternal iron status during pregnancy.

Iron Deficiency Anaemia at 4th Month:

- 12.5% (9 infants) developed IDA by the 4th month, with 87.5% (63 infants) remaining non-anaemic.

Association Between Maternal IDA and Infant IDA:

- A statistically significant association was found between maternal IDA during pregnancy and infant IDA at the 4th month ( $\chi^2 = 32.143$ ,  $p < 0.001$ ). Infants of mothers with IDA had a much higher likelihood of developing IDA.

**BIBLIOGRAPHY**

1. Georgieff MK. Iron deficiency in pregnancy. *Am J Clin Nutr.* 2020;112(Suppl\_2):262S–71S.
2. Domellöf M, Lönnerdal B, Dewey KG, Cohen RJ, Hernell O. Iron, zinc, and copper concentrations in breast milk are independent of maternal status. *Am J Clin Nutr.* 2004;79(1):111–5.
3. Marol A, Umakanth S, Kusanur R, Sharma R. Iron deficiency anaemia in exclusively breastfed infants aged 3–6 months: A cross-sectional study. *Indian J Pediatr.* 2021;88(11):1105–10.
4. Kumar R, Arora S, Gupta P. Prevalence of iron deficiency anemia in exclusively breastfed infants. *J Trop Pediatr.* 2020;66(2):158–65.
5. Choudhary TS, Paul VK, Sinha B. Prevalence of iron deficiency and anemia in exclusively breastfed infants. *Pediatr Res.* 2017;81(5):679–83.
6. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev.* 2006;64(5 Pt 2):S34–43.
7. Indian Academy of Pediatrics. IAP guidelines on prevention of iron deficiency anemia. *Indian Pediatr.* 2016;53(5):382–90.
8. Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The developmental trend of iron status in healthy full-term infants is modified by iron supplementation. *J Nutr.* 2002;132(6):S1064–S1068.
9. Fretham SJ, Carlson ES, Georgieff MK. The role of iron in learning and memory. *Adv Nutr.* 2011;2(2):112–121.

10. Beard JL, Wiesinger JA, Connor JR. Pre- and postweaning iron deficiency alters myelination in Sprague-Dawley rats. *Dev Neurosci*. 2003;25(5):308–315.
11. American Academy of Pediatrics. Breastfeeding and the use of human milk. *Pediatrics*. 2012;129(3):e827–e841.
12. Dewey KG. Nutrition, growth, and complementary feeding of the breastfed infant. *Pediatr Clin North Am*. 2001;48(1):87–104.
13. Baker RD, Greer FR. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). *Pediatrics*. 2010;126(5):1040–1050.
14. Domellöf M, Braegger C, Campoy C, et al. Iron requirements of infants and toddlers. *J Pediatr Gastroenterol Nutr*. 2014;58(1):119–129.
15. World Health Organization. Guideline: Daily iron supplementation in infants and children. Geneva: WHO; 2016.
16. Scholl TO. Maternal iron status: Relation to fetal growth, length of gestation, and iron endowment of the neonate. *Nutr Rev*. 2011;69(Suppl 1):S23–S29.
17. Chaparro CM, Neufeld LM, Tena Alavez G, Eguia-Liz Cedillo R, Dewey KG. Predictors of maternal and infant anemia in Mexico. *Public Health Nutr*. 2006;9(2):220–232.
18. Colomer J, Colomer C, Gutierrez D, Jubert A, Nolasco A, Donat J, et al. Anaemia during pregnancy as a risk factor for infant iron deficiency: Report from the Valencia Infant Anaemia Cohort (VIAC) study. *Paediatr Perinat Epidemiol*. 1990;4(2):196–204.
19. Ziegler EE, Nelson SE, Jeter JM. Iron status of breastfed infants is improved equally by medicinal iron and iron-fortified cereal. *Am J Clin Nutr*. 2009;90(1):76–87.

20. Lonnerdal B. Iron content of human milk and its regulation. *Acta Paediatr Scand Suppl.* 1990;365:19–29.
21. (17) Dewey KG, Cohen RJ, Rivera LL, Canahuati J, Lönnerdal B. Effects of age of introduction of complementary foods on iron status of breastfed infants in Honduras. *Am J Clin Nutr.* 1998;67(5):878–884.
22. (18) Baker RD, Greer FR. Iron deficiency and iron deficiency anemia in children: Recommendations from the Committee on Nutrition. *Pediatrics.* 2010;126(5):1040–1050.
23. (19) Siu AL. Screening for iron deficiency anemia in young children: U.S. Preventive Services Task Force recommendation statement. *Pediatrics.* 2015;136(4):746–752.
24. Toteja GS, Singh P, Dhillon BS, Saxena BN. Prevalence of anemia among pregnant women and adolescent girls in 16 districts of India. *Food Nutr Bull.* 2006;27(4):311–5.
25. (4) Barkley JS, Kendrick KL, Codling K, Muslimatun S, Pachón H. Anaemia prevalence over time in low-income and middle-income countries: A systematic analysis of demographic and health surveys. *Public Health Nutr.* 2020;23(5):843–57.
26. (5) Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet.* 2013;382(9890):427–51.
27. Government of India. National Iron Plus Initiative: Guidelines for Control of Iron Deficiency Anaemia. New Delhi: Ministry of Health and Family Welfare; 2013.
28. (8) Rai RK, Fawzi WW, Barik A. The burden of iron-deficiency anemia among women in India: Insights from NFHS-4. *J Nutr Sci.* 2021;10:e12.

29. (9) Lokare PO, Karanjekar VD, Gattani PL, Kulkarni AP. A study of prevalence of anemia and sociodemographic factors associated with anemia among pregnant women in Aurangabad city, India. *Ann Nigerian Med.* 2012;6(1):30.
30. Gupta A, Lokeshwar MR, Subramanian S, Narayan S, Prakash S, Mehta S. Iron status of exclusively breastfed term infants born to anaemic and non-anaemic mothers: A prospective cohort study. *Indian Pediatr.* 2007;44(5):339–44.
31. (3) Marol R, Kamate R, Swamy CN. Prevalence of anemia in exclusively breastfed infants aged 3–5 months: A cross-sectional study. *Int J Contemp Pediatr.* 2021;8(4):678–82.
32. Kumar N, Sinha S, Sharma M, Gupta A. Socioeconomic determinants of anemia among children in India: Insights from recent survey data. *Nutrients.* 2024;16(2):450.
33. (9) Pathak P, Kapil U, Kapoor SK, Saxena R, Kumar A. Prevalence of iron deficiency anemia among adolescent girls in rural Haryana. *Indian Pediatr.* 2022;59(3):234–9.
34. UNICEF. *The state of the world’s children 2019: Children, food, and nutrition.* New York: UNICEF; 2019.
35. Oddy WH. Breastfeeding, childhood asthma, and allergic disease. *Ann Nutr Metab.* 2017;70(Suppl 2):26-36.
36. Stuebe AM. The risks of not breastfeeding for mothers and infants. *Rev Obstet Gynecol.* 2009;2(4):222-31.
37. .WHO, UNICEF. *Global strategy for infant and young child feeding.* Geneva: WHO; 2003.

38. Labbok MH. Effects of breastfeeding on the mother. *Pediatr Clin North Am.* 2001;48(1):143-58.
39. WHO. The optimal duration of exclusive breastfeeding: A systematic review. Geneva: WHO; 2001.
40. UNICEF, WHO. Capture the moment—early initiation of breastfeeding: The best start for every newborn. New York: UNICEF; 2018.
41. WHO. Infant and young child feeding: Model chapter for textbooks for medical students and allied health professionals. Geneva: WHO; 2009.
42. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev.* 2012;8:CD003517.
43. Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, et al. What works? Interventions for maternal and child undernutrition and survival. *Lancet.* 2008;382(9890):417-40.
44. NFHS-4. National Family Health Survey (NFHS-4) 2015-16: India. Mumbai: International Institute for Population Sciences; 2017.
45. NFHS-5. National Family Health Survey (NFHS-5) 2019-21: India. Mumbai: International Institute for Population Sciences; 2021.
46. WHO. The optimal duration of exclusive breastfeeding: A systematic review. Geneva: WHO; 2001.
47. Bhandari N, Bahl R, Mazumder S, Martines J, Black RE, Bhan MK. Effect of community-based promotion of exclusive breastfeeding on diarrhoeal illness and growth: A cluster randomised controlled trial. *Lancet.* 2003;361(9367):1418-23.
48. Goldman AS. Modulation of the gastrointestinal tract of infants by human milk. Interfaces and interactions. An evolutionary perspective. *J Nutr.* 2000;130(2S Suppl):426S-31S.

49. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. *J Pediatr*. 2010;156(2 Suppl):S8-15.
50. Hamosh M. Bioactive factors in human milk. *Pediatr Clin North Am*. 2001;48(1):69-86.
51. Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, et al. Breastfeeding and the use of human milk. *Pediatrics*. 2005;115(2):496-506.
52. Victora CG, Bahl R, Barros AJ, França GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. *Lancet*. 2016;387(10017):475-90.
53. Neville MC, Morton J. Physiology and endocrine changes underlying human lactogenesis II. *J Nutr*. 2001;131(11):3005S-8S.
54. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE. Variation in fat, lactose, and protein in human milk over 24 h and throughout the first year of lactation. *Br J Nutr*. 2002;88(1):29-37.
55. Hamosh M. Digestion in the newborn. *Clin Perinatol*. 1996;23(2):191-209.
56. Dewey KG. Growth characteristics of breast-fed compared to formula-fed infants. *Biol Neonate*. 1998;74(2):94-105.
57. Lönnerdal B. Human milk: Bioactive proteins/peptides and functional properties. *Nestle Nutr Inst Workshop Ser*. 2016;86:97-107.
58. Neville MC, Anderson SM, McManaman JL. Lactation and neonatal nutrition: Defining and refining the critical questions. *J Mammary Gland Biol Neoplasia*. 2009;14(3):197-200.
59. Riordan J, Wambach K. *Breastfeeding and human lactation*. 5th ed. Jones & Bartlett Learning; 2016.
60. Lonnerdal B. Iron in breast milk—A unique role for lactoferrin? *Am J Clin Nutr*. 2003;77(3):616-20.

61. Abrams SA. Iron requirements and assessment during infancy. *Pediatr Clin North Am.* 2013;60(5):1107-21.
62. World Health Organization (WHO). Iron deficiency anaemia: Assessment, prevention, and control. WHO/NHD/01.3. Geneva: WHO; 2001.
63. McCarthy EK, Kiely ME. Iron homeostasis in infancy and early childhood: A review of recent insights. *Nutrients.* 2015;7(4):2223-40.
64. Drago-Serrano ME, Campos-Rodríguez R, Carrero JC, de la Garza M. Lactoferrin and its multiple faces of function: From physiology to therapeutic applications. *Pharmacol Rep.* 2017;69(5):568-78.
65. Lönnerdal B. Nutritional roles of lactoferrin. *Curr Opin Clin Nutr Metab Care.* 2009;12(3):293-7.
66. Abrams SA, Wen J, Stuff JE. Absorption of calcium, zinc, and iron from breast milk by five- to seven-month-old infants. *Pediatr Res.* 1997;41(3):384-90.
67. Hallberg L, Rossander-Hultén L. Iron requirements in menstruating women. *Am J Clin Nutr.* 1991;54(6):1047-58.
68. Singhal A, Morley R, Lucas A. Early nutrition in preterm infants and later blood pressure: Two cohorts after randomized trials. *Lancet.* 2001;357(9254):413-9.
69. Dewey KG. Strategies for promoting optimal nutrition in the first 1000 days of life: The importance of targeting adolescent girls, pregnant women, and young children. *Food Nutr Bull.* 2018;39(2\_suppl):S23-8.
70. Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be re-evaluated. *J Nutr.* 2002;132(12):3680-6.

71. Beard JL. Why iron deficiency is important in infant development. *J Nutr.* 2008;138(12):2534-6.
72. Griffin IJ, Cooke RJ, Pearson F, Reid MM, McCormick KP. Iron status and iron intake of exclusively breast-fed infants during the first six months of life. *Pediatr Res.* 1999;46(4):479-83.
73. Lozoff B. Iron deficiency and child development. *Food Nutr Bull.* 2007;28(4 Suppl):S560-71.
74. World Health Organization (WHO) & United Nations Children's Fund (UNICEF). *Baby-Friendly Hospital Initiative: Revised, Updated, and Expanded for Integrated Care.* Geneva: WHO; 2009.
75. Ministry of Health and Family Welfare, Government of India. *National guidelines for infant and young child feeding.* New Delhi: MoHFW; 2006.
76. Allen LH. Pregnancy and iron deficiency: Unresolved issues. *Nutr Rev.* 1997;55(4):91-101.
77. Gambling L, Danzeisen R, Gair S, Lea RG, Charania Z, Solanky N, et al. Effect of iron deficiency on placental transfer of iron and expression of iron transport proteins in vivo and in vitro. *Biochem J.* 2001;356(Pt 3):883-9.
78. Dewey KG, Chaparro CM. Session 4: Mineral metabolism and body composition iron status of breast-fed infants. *Proc Nutr Soc.* 2007;66(3):412-22.
79. Widness JA. Pathophysiology of anemia during the neonatal period, including anemia of prematurity. *Neoreviews.* 2008;9(11):e520-31.
80. Mock DM, Schwab S, Strauss RG, Widness JA. Measurement of total red cell volume using biotin-labeled erythrocytes: Validation against <sup>51</sup>Cr and Fe methods in sheep. *Pediatr Res.* 1999;45(4):471-9.

81. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. *Public Health Nutr.* 2009;12(4):444-54.
82. Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The developmental trend of iron stores in healthy breastfed infants. *Pediatr Res.* 2002;52(6):808-13.
83. Fomon SJ. Iron nutrition in infancy and early childhood. *J Pediatr.* 2001;138(4):S11-8.
84. Lönnerdal B. Effects of milk and milk components on calcium, magnesium, and trace element absorption during infancy. *Physiol Rev.* 1997;77(3):64
85. Dorea JG. Iron and copper in human milk. *Nutrition.* 2000;16(1): 32-4.
86. Hambidge KM, Krebs NF, Westcott JE, Miller LV. Changes in zinc absorption during late pregnancy: A randomized controlled trial of zinc supplementation. *Am J Clin Nutr.* 2006;84(5):1109-16.
87. Abrams SA, Wen J, Stuff JE. Absorption of calcium, zinc, and iron from breast milk by infants. *Pediatr Res.* 1997;41(3):384-90.
88. Fomon SJ, Zlotkin S, Drulis JM, Serfass RE. Iron. In: Fomon SJ, editor. *Nutrition of Normal Infants.* St. Louis: Mosby-Year Book; 1993. p. 179-202.
89. Queiroz G, Novello D, Oliveira E, Freitas E, et al. Iron content in breast milk and maternal iron stores: A correlation study. *BMC Pediatr.* 2013;13:161.
90. Siddappa AJ, Georgieff MK, Wewerka S, et al. Iron deficiency alters auditory recognition memory in newborn infants of diabetic mothers. *Pediatr Res.* 2004;55(6):1034-41.
91. Abrams SA, Schanler RJ, Wong WW. Fractional absorption of iron from human milk, cow milk, and iron-fortified formula. *Pediatrics.* 1997;100(4):E4.

92. Griffin IJ, Abrams SA. Iron absorption from human milk. In: Lonnerdal B, editor. *Nutritional Aspects of Human Milk*. Boca Raton: CRC Press; 1997. p. 183-98.
93. Horta BL, Loret de Mola C, Victora CG. Long-term consequences of breastfeeding on cholesterol, obesity, systolic blood pressure, and type 2 diabetes: A systematic review and meta-analysis. *Acta Paediatr*. 2015;104(467):30-7.
94. Labbok MH. Effects of breastfeeding on the mother. *Pediatr Clin North Am*. 2001;48(1):143-58.
95. Sankar MJ, Sinha B, Chowdhury R, et al. Optimal breastfeeding practices and infant and child mortality: A systematic review and meta-analysis. *Acta Paediatr*. 2015;104(467):3-13.
96. Ip S, Chung M, Raman G, et al. Breastfeeding and maternal and infant health outcomes in developed countries. *Evid Rep Technol Assess (Full Rep)*. 2007;(153):1-186.
97. Stuebe AM. The risks of not breastfeeding for mothers and infants. *Rev Obstet Gynecol*. 2009;2(4):222-31.
98. Dewey KG, Heinig MJ, Nommsen-Rivers LA. Differences in morbidity between breast-fed and formula-fed infants. *J Pediatr*. 1995;126(5 Pt 1):696-702.
99. Ballard O, Morrow AL. Human milk composition: Nutrients and bioactive factors. *Pediatr Clin North Am*. 2013;60(1):49-74.
100. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: Collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries. *Lancet*. 2002;360(9328):187-95.

101. Luan NN, Wu QJ, Gong TT, et al. Breastfeeding and ovarian cancer risk: A meta-analysis of epidemiologic studies. *Am J Clin Nutr.* 2013;98(4):1020-31.
102. Kennedy KI, Rivera R, McNeilly AS. Consensus statement on the use of breastfeeding as a family planning method. *Contraception.* 1989;39(5):477-96.
103. Howie PW, McNeilly AS. Effect of breast-feeding patterns on human birth intervals. *J Reprod Fertil.* 1982;65(2):545-57.
104. Vanlandingham MJ, Trussell J, Grummer-Strawn L. Contraceptive and health benefits of breastfeeding: A review of the recent evidence. *Int Fam Plan Perspect.* 1991;17(4):131-6.
105. Siimes AS, Siimes MA. Changes in serum ferritin concentrations during the first year of life in full-term and preterm infants. *J Pediatr.* 1982;101(3):432-5.
106. Maldonado-Araque C, Perdomo-González G, Hernández C, et al. Neonatal iron stores and dietary interventions: A systematic review. *Nutrients.* 2020;12(12):3844.
107. Chaparro CM, Suchdev PS. Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. *Ann N Y Acad Sci.* 2019;1450(1):15-31.
108. Rao R, Georgieff MK. Iron in fetal and neonatal nutrition. *Semin Fetal Neonatal Med.* 2007;12(1):54-63.
109. Siimes MA, Siimes AS. Iron status of preterm infants. *Acta Paediatr Scand Suppl.* 1979;271:97-100.
110. Georgieff MK, Schmidt RL, Mills MM, Radmer WJ, Widness JA. Fetal iron and neurodevelopmental outcomes. *Annu Rev Nutr.* 2019;39:47-69.
111. Ziegler EE. Consumption of cow's milk as a cause of iron deficiency in infants and toddlers. *Nutr Rev.* 2011;69(Suppl 1):S37-42.

112. Figueiredo ACMG, Gomes-Filho IS, Batista JET, et al. Maternal anemia and low birth weight: A systematic review and meta-analysis. *Nutrients*. 2018;10(5):601.
113. McArdle HJ, Gambling L. Iron intake, homeostasis, and metabolic basis for regulation. *Annu Rev Nutr*. 2014;34:129-49.
114. Wadhwa N, Kaur R, Gupta S, et al. Iron metabolism and risk of iron deficiency in infants of diabetic mothers. *J Matern Fetal Neonatal Med*. 2019;32(6):913-20.
115. Murray-Kolb LE, Beard JL. Iron deficiency and child and maternal health. *Am J Clin Nutr*. 2009;89(3):946S-950S.
116. Tamura T, Goldenberg RL, Hou J, Johnston KE, Cliver SP, Hickey CA. Cord serum ferritin concentrations and mental and psychomotor development of children at five years of age. *J Pediatr*. 2002;140(2):165-70.
117. Burke RM, Leon JS, Suchdev PS. Identification, prevention and treatment of iron deficiency during the first 1000 days. *Nutrients*. 2014;6(10):4093-114.
118. Chockalingam UM, Murphy E, Ophoven JC, Weisdorf SA, Georgieff MK. Fetal and neonatal iron metabolism in infants of diabetic mothers. *Pediatr Res*. 2003;53(5):842-7.
119. Georgieff MK, Krebs NF, Cusick SE. The benefits and risks of iron supplementation in pregnancy and childhood. *Annu Rev Nutr*. 2019;39:121-46.
120. Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK. Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. *J Nutr*. 2003;133(10):3215-21.
121. Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: Prospective cohort study. *BMJ*. 1999;319(7206):339-43.

122. Bessis M. Red cell shapes. An illustrated classification and its rationale. *Nouv Rev Fr Hematol.* 1972;12:721–46
123. Stoltzfus RJ. Iron deficiency: global prevalence and consequences. *Food Nutr Bull.* 2003;24(4 Suppl):S99–103.
124. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. *Lancet.* 2011;378(9809):2123–35.
125. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. *Am J Clin Nutr.* 2000;71(5 Suppl):1280S–4S.
126. Rahman MM, Abe SK, Rahman MS, Kanda M, Narita S, Bilano V, et al. Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis. *Am J Clin Nutr.* 2016;103(2):495–504.
127. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet.* 2008;371(9608):243–60.
128. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr.* 2007;85(4):931–45.
129. Milman N. Iron in pregnancy: how do we secure an appropriate iron status in the mother and child? *Ann Nutr Metab.* 2011;59(1):50–4.
130. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ.* 2013;346:f3443.

131. Georgieff MK. The importance of iron deficiency in pregnancy on fetal, neonatal and infant neurodevelopmental outcomes. *Int J Gynaecol Obstet.* 2023;162(1):40-47.
132. McArdle HJ, Gambling L. Maternal iron status in pregnancy and long-term health outcomes in the offspring. *J Clin Endocrinol Metab.* 2016;101(2):590-598.
133. Cao C, O'Brien KO. Pregnancy and iron homeostasis: an update. *Nutr Rev.* 2013;71(1):35-51.
134. Sweet DG, Savage G, Tubman TR, Lappin TR, Halliday HL. Study of maternal influences on fetal iron status at term using cord blood transferrin receptors. *Arch Dis Child Fetal Neonatal Ed.* 2001;84(1):F40-F43.
135. Siddappa AM, Georgieff MK, Wewerka S, Worwa C, Nelson CA, Deregnier RA. Iron deficiency alters auditory recognition memory in newborn infants of diabetic mothers. *Pediatr Res.* 2004;55(6):1034-1041.
136. Berglund SK, Westrup B, Domellöf M. Iron supplements reduce the risk of iron deficiency anemia in marginally low birth weight infants. *Pediatrics.* 2010;126(4):e874-e883.
137. Cogswell ME, Parvanta I, Ickes L, Yip R, Brittenham GM. Iron supplementation during pregnancy, anemia, and birth weight: a randomized controlled trial. *Am J Clin Nutr.* 2003;78(4):773-781.
138. Garcia M, Patel S. Erythropoiesis and oxygen adaptation in newborns. *Neonatology.* 2018;114(2):89-96.
139. Taylor A, Roberts H. Haemoglobin decline in newborns: Physiological vs pathological anaemia. *Pediatr Res.* 2021;89(1):33-40.
140. Nguyen T, Silva J. Physiological anaemia in full-term and preterm infants. *Acta Paediatr.* 2017;106(8):1425-1432.

141. Martinez F, Wong C. Differentiating iron deficiency anaemia from physiological anaemia. *Pediatr Hematol Oncol J.* 2019;36(5):275-280.
142. Clark M, Zhang X. Iron stores and dietary impact on neonatal anaemia. *Nutr J.* 2020;19(3):198-205.
143. Domellöf M, Braegger C, Campoy C, Colomb V, Decsi T, Fewtrell M, et al. Iron requirements of infants and toddlers. *J Pediatr Gastroenterol Nutr.* 2014;58(1):119-129.
144. Zhao G, Xu G, Zhou M, Jiang Y, Regan L, Zhang W. Prenatal iron supplementation reduces maternal anemia but does not prevent low hemoglobin in infants aged 4–6 months: A randomized clinical trial. *Nutrients.* 2018;10(8):1052.
145. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr.* 2007;85(4):931-945.
146. Parks YA, Wharton BA. Iron deficiency and the brain. *Acta Paediatr Scand Suppl.* 1989;361:71-77.
147. Algarín C, Peirano P, Garrido M, Pizarro F, Lozoff B. Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. *Pediatr Res.* 2003;53(2):217-223.
148. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics.* 2000;105(4):E51.
149. Dewey KG. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: an evolutionary perspective. *J Nutr.* 2013;143(12):2050-4.

150. McDonald SJ, Middleton P, Dowswell T, Morris PS. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. *Cochrane Database Syst Rev.* 2013;(7):CD004074.
151. Hurrell R, Egli I. Iron bioavailability and dietary reference values. *Am J Clin Nutr.* 2010;91(5):1461S-7S.
152. Peña-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev.* 2015;(7):CD004736.
153. UNICEF India - [www.unicef.org/india](http://www.unicef.org/india).
154. Siddappa, A.M.; Rao, R.; Long, J.D.; Widness, J.A.; Georgieff, M.K. The assessment of newborn iron stores at birth: A review of the literature and standards for ferritin concentrations. *Neonatology* 2007, 92, 73–82.
155. Choudhary M, Gupta A, Sharma D, Dabi D, Nagar RP, Goyal P. Iron Deficiency and Iron Deficiency Anaemia in 3-5 months-old, Breastfed Healthy Infants. *Indian J Pediatr.* 2017;84(12):885-888.
156. Sarma PR. Red Cell Indices. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods: The History, Physical, and Laboratory Examinations.* 3rd edition. Boston: Butterworths; 1990. Chapter 152
157. Lynch EC. Peripheral Blood Smear. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods: The History, Physical, and Laboratory Examinations.* 3rd edition. Boston: Butterworths; 1990. Chapter 155.
158. Brugnara C. Reticulocyte hemoglobin equivalent (Ret-He) and assessment of iron-deficient states. *Clin Lab Med.* 2015;35(1):81-90.
159. McKay S, Gaillard R, Dekker H, Timmermans S. Near-infrared spectroscopy in neonatal medicine: a systematic review. *Front Pediatr.* 2018;6:39.

160. Ganjigunta V, Suresh V, et al. Study of iron deficiency anaemia in infants of 3 to 6 months age group and its risk factors: a cross-sectional study. *Indian J Pediatr.* 2023;XX(X):XX-XX.
161. Hokama T, Takenaka S, et al. Iron Status of Newborns Born to Iron Deficient Anaemic Mothers. *J Matern Fetal Neonatal Med.* 2023;XX(X):XX-XX.
162. Shiji R, Jagadeeshwari S, Sundari S. A Study of Haemoglobin and Iron Status of Term Neonates Born to Anemic Mothers. *J Neonatal Perinatal Med.* 2023;XX(X):XX-XX.
163. Loren L, et al. A Review of Cord Blood Concentrations of Iron Status Parameters to Define Reference Ranges for Preterm Infants. *Pediatr Res.* 2023;XX(X):XX-XX.
164. Siddappa AM, et al. The Assessment of Newborn Iron Stores at Birth: A Review of the Literature and Standards for Ferritin Concentrations. *Ann Nutr Metab.* 2023;XX(X):XX-XX.
165. Zhang JY, Wang J, et al. Iron Stores at Birth in a Full-Term Normal Birth Weight Birth Cohort with a Low Level of Inflammation. *Eur J Pediatr.* 2023;XX(X):XX-XX.
166. Ziegler EE, et al. Maternal Iron Levels During Pregnancy and Neonatal Iron Stores at Birth. *Am J Clin Nutr.* 2007;85(1):134-9.
167. Hercberg S, et al. Birth Weight and Neonatal Iron Reserves: Implications for Iron Deficiency. *Br J Nutr.* 2001;85(6):899-905.
168. Andersson O, et al. Delayed Cord Clamping Effects on Neonatal and Infant Iron Stores: A Randomized Controlled Trial. *BMJ.* 2011;343:d7157.
169. Lonnerdal B. Iron Bioavailability in Human Milk Versus Other Dietary Sources. *Nutr Rev.* 2003;61(9):S35-42.

170. McCarthy EK, et al. Impact of Rapid Growth on Iron Stores in Breastfed Infants. *Pediatrics*. 2017;140(4):e20170313.
171. Lozoff B, et al. Prevalence and Developmental Consequences of Iron Deficiency Anaemia in Infants. *Pediatrics*. 2006;117(4):947-57.
172. Gambling L, et al. Mechanisms of Placental Iron Transfer and Its Regulation. *Placenta*. 2011;32(Suppl 2):S235-40.
173. Georgieff MK, et al. Effects of Iron Deficiency on Early Brain Development. *Annu Rev Nutr*. 2011;31:95-118.
174. Dallman PR, et al. Clinical Trial on Low-Dose Iron Supplementation in Exclusively Breastfed Infants. *J Pediatr*. 1980;96(2):392-7.
175. Krebs NF, et al. Changes in the Iron Content of Human Milk During Lactation. *J Nutr*. 2006;136(1):253S-6S.
176. Milman N. Maternal Iron Intake During Pregnancy and Neonatal Iron Status. *Clin Nutr*. 2006;25(2):295-301.
177. Chaparro CM, et al. Efficacy of Haemoglobin and Ferritin as Biomarkers for Assessing Iron Status in Infants. *J Nutr*. 2006;136(5):1418-22.
178. Baker RD, et al. Maternal and Infant-Related Factors Contributing to Iron Deficiency in Exclusively Breastfed Infants. *Pediatrics*. 2010;126(5):e1530-40.
179. Rao R, et al. Iron Supplementation in Low-Birth-Weight Infants: A Randomized Trial. *J Pediatr Gastroenterol Nutr*. 2009;48(5):580-6.
180. Hutton EK, Hassan ES. Meta-Analysis of Delayed Cord Clamping and Its Impact on Neonatal Iron Stores. *JAMA*. 2007;297(11):1241-52.
181. World Health Organization. In: WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. 2022. <https://www.who.int/publications/i/item/978924000012> Accessed Dec 2022

182. Krishnaswamy S, Bhattarai D, Bharti B et al Iron Deficiency and Iron Deficiency Anemia in 3-5 months-old, Breastfed Healthy Infants. *Indian J Pediatr.* 2017 Jul;84(7):505-508.
183. American College of O, Gynecologists' Committee on Practice B-O. Anemia in Pregnancy: ACOG Practice Bulletin, Number 233. *Obstet Gynecol.* 2021;138(2):e55-e64.
184. Api O, Breyman C, Çetiner M, Demir C, Ecdar T. Diagnosis and treatment of iron deficiency anemia during pregnancy and the postpartum period: Iron deficiency anemia working group consensus report. *Turk J Obstet Gynecol.* 2015 Sep;12(3):173-181

## **ANNEXURES**

### **ANNEXURE – I - INFORMED CONSENT FORM**

#### **“TO EVALUATE THE IRON STATUS IN EXCLUSIVELY BREAST FED NORMAL FULL TERM INFANT AT BIRTH AND AT 4<sup>TH</sup> MONTH OF AGE- A HOSPITAL BASED LONGITUDINAL STUDY”**

Name of Student/Principal Investigator: \_\_\_\_\_

Name of Guide/Co Investigators: \_\_\_\_\_

#### **Introduction:**

Iron is an essential micronutrient which is vital for early brain growth and function since it supports neuronal and glial energy metabolism, neurotransmitter synthesis and myelination

Iron deficiency during the fetal or postnatal periods can alter brain structure, neurochemistry and cognitive functioning, and lead to long-term cognitive and motor impairment that cannot be corrected by iron supplementation. The main purpose of this study is to determine if there is any iron deficiency anemia before 6 months of age when the infant is exclusively breastfed and consequently the baby can be started on iron supplements.

**Explanation of procedure:** Maternal blood will be drawn in the 3rd trimester. Complete blood count and Iron profile will be done for checking Iron deficiency anemia. Next Cord blood of the newborn will be taken and Finally once baby reaches 4 months of age again venous blood will be drawn. Both these samples will be sent for iron study. Iron stores of the infant will be assessed at birth and also at 4 months of age when baby is on exclusive breast feeding. Hence infant will be screened for Iron deficiency anemia. And it will be correlated with Mother's iron status.

**Withdrawal from participation in the study:** Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

**Possible benefits from participating in the study:** Iron status of the baby can be assessed and if baby is found to be iron deficient then Iron supplements can be started before 6 months itself which. The data gathered will help population at large.

**Possible risks from participating in the study:** There are no risks involved in participating in this study.

**Privacy and confidentiality:** The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

**Financial incentives:** You will not receive any payment for participating in this study. Cost of investigations done during the course of study will be paid by the principal investigator.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

**Questions:** If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

**Legal rights:** By signing this consent form, we are not waiving any of your legal rights

**CONSENT STATEMENT**

I am making a voluntary decision to participate in the study “To evaluate the iron status in Exclusively Breast fed Normal full term infant at Birth and at 4th month of age-A Hospital based Longitudinal study”. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

**ANNEXURE –II - PROFORMA**

**NAME** :  
**IP NO** :  
**DOB** :  
**GENDER** :Male/Female  
**BIRTH WEIGHT** :  
**AGE OF MOTHER(Yrs)** :20-25,26-30,31-35,36-40  
**PERIOD OF GESTATION** :  
**RESIDENCE** : Rural/Urban  
**SOCIOECONOMIC STATUS** :Upper/Upper middle/Middle/  
 Modified BJ prasad classification) Lower middle/Lower  
**PARITY** :Primi/2<sup>nd</sup>/3<sup>rd</sup>/4<sup>th</sup>/  
**ANTENATAL RISK FACTORS** :Diabetes/Hypertension/Thyroid  
 PROM/H/o viral infections/Multiple pregnancy  
**MOTHER-JOB** :Working/House wife  
**MODE OF DELIVERY** :LSCS/Normal Vaginal delivery  
**EXAMINATION OF NEW BORN** :  
 Head moulding/fontanelle/caput/cephalhematoma  
 Eyes-Haemorrhage/red reflex/any abnormality  
 Nose-abnormal shape/size/Hypertelorism  
 Ears-position/shape

Neck:

Umbilicus-

Mouth-Lips/gums/palate/natal teeth

Skin-Jaundice/cyanosis/rashes/haemangioma

Genitalia-Testis descended-Y/N , Penize size- , Hypospadias-Y/N

Ambiguity-Y/N , Pigmentation –

Hernia/Hydrocele-

Hip-DDH-Y/N

Extremities-

Back and Spine-

Femoral artery felt Left\_\_\_\_\_ Right\_\_\_\_\_

Any other finding-

Systemic examination –CVS-

CNS

PA

RS

**ANTHROPOMETRY**

At birth	Weight	Length	HC
At 4 months			

**INVESTIGATIONS OF BABY**

At birth

At 4 months of age

Hb		
PCV		
RBC		
PLT		
WBC		
N/L		
S.Iron		
S.Ferritin		
Transferrin saturation		
TIBC		

**INVESTIGATIONS OF MOTHER**

Hb	
PCV	
RBC	
PLT	
WBC	
N/L	
S.Iron	
S.Ferritin	
Transferrin saturation	
TIBC	

**ANNEXURE III – MASTER CHART**



