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PREVALENCE OF IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA IN 3 TO 5 MONTHS OLD BREASTFED HEALTHY INFANTS

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Abstract:
Background: The vulnerability of infants to iron deficiency (ID) and iron deficiency anemia (IDA) is well-recognized globally, given that iron demands are elevated during this period of rapid physical and cognitive development.
Aim: To assess the prevalence of iron deficiency and iron deficiency anemia in exclusively breastfed 3 to 5 month's old infants, born at term with a birth-weight of ≥2500 grams and the Influence of Socioeconomic Status, Gender, Gestational Age, and Mode of Delivery on Infant Iron Status.
Methods: Prospective Cross-sectional study carried in the Postgraduate Department of Pediatrics at 500 Bedded Children Hospital, Associated Hospital Government Medical College, Srinagar involving 130 healthy infants, aged 3 to 5 months, who were exclusively breastfed, along with their mothers. Ethical clearance was obtained from the Ethical and Medical Review Board of the Government Medical College, Srinagar, prior to initiating the study. Written informed consent was obtained from all mothers. The study adhered to the principles outlined in the Infants were primarily recruited during routine vaccination visits or as asymptomatic siblings of children attending the nutrition clinic. Eligible infants were selected based on strict inclusion and exclusion criteria to ensure homogeneity of the study population. Blood samples were obtained to measure hemoglobin (Hb) and serum ferritin levels in both infants and mothers. Chi square test was used to analyze the relationship between two categorical variables and to compare a continuous variable at baseline and follow up. A, P-value of <0.05 was considered as statistically significant.
Results: Out of 130 infants, 28 (21.54%) were diagnosed with iron deficiency. 19 infants (14.62%) had iron deficiency anemia, characterized by low hemoglobin and serum ferritin levels. 13 infants exhibited anemia (Hb <10.5 g/dL) but had normal ferritin levels (>12 ng/mL), suggesting that their anemia may have been caused by factors such as vitamin B12 deficiency or other nutritional deficiencies, genetic disorders, hemoglobinopathies. There was a significant relationship between maternal and infant iron status. 46.43% of infants with ID had mothers who also had ID, and 47.36% of infants with IDA were born to mothers with IDA. There was a socioeconomic gradient in the

prevalence of ID and IDA. Marked increase in both ID and IDA with increasing age, particularly in the group of infants aged 151–180 days.
Conclusion: Our study reveals a significant relationship between maternal anemia and infant iron deficiency anemia with notable increase in prevalence as infants' age; these findings are consistent with existing literature that emphasizes the critical role of maternal health in influencing infant iron status and highlights the need for timely intervention.

Keywords: Iron deficiency, Iron deficiency anemia, Infants, Maternal anemia, Maternal health.

Introduction:

The vulnerability of infants to iron deficiency (ID) and iron deficiency anemia (IDA) is well-recognized globally, given that iron demands are elevated during this period of rapid physical and cognitive development. The global prevalence of ID and IDA remains a significant public health concern, particularly in low- and middle- income countries (LMICs), where dietary iron intake often falls short of the recommended levels . [1] Iron deficiency is considered the most common micronutrient deficiency worldwide, affecting approximately 30% of the global population, with infants and young children bearing a disproportionate burden of this condition. [1] Even in high-income countries, where dietary diversity and food fortification programs are prevalent, ID and IDA persist, particularly among vulnerable groups such as preterm infants, infants with low birth weight, and those who are exclusively breastfed without adequate iron supplementation. [2] Breastfeeding is universally recognized as the optimal mode of feeding for infants, providing essential nutrients, bioactive compounds, and immune protection. The World Health Organization [3] recommends exclusive breastfeeding for the first six months of life, followed by the introduction of complementary foods while continuing breastfeeding for up to two years or beyond. [4] Breast milk contains the ideal balance of nutrients to support the infant's growth and development, and its protective effects against infectious diseases have been well-documented. [5]
However, despite its numerous benefits, breast milk has a relatively low iron content, typically ranging between 0.3 to 0.5 mg/L. This is significantly lower than the iron content found in fortified infant formulas, which typically contain 4 to 12 mg/L of iron to account for the lower bioavailability of non-heme iron. [6]
The prevalence of ID and IDA in exclusively breastfed infants has raised significant concerns, particularly in populations where maternal iron deficiency is common. The fetal iron endowment largely depends on maternal iron status during pregnancy, with the majority of iron transfer to the fetus occurring in the last trimester . [7] Infants born to mothers with anemia or iron deficiency are at a higher risk of being born with lower iron stores, predisposing them to ID and IDA in early infancy . [8]
By addressing a critical gap in the literature, this study was conducted to contribute for the development of targeted interventions aimed at preventing these conditions, thereby improving the health and developmental outcomes of infants worldwide.

Methods:

This present study was a Prospective Cross-sectional study carried in the Postgraduate Department of Pediatrics at 500 Bedded Children Hospital, Associated Hospital Government Medical College, Srinagar. The study was conducted over a period of 18 months.
Infants were primarily recruited during routine vaccination visits or as asymptomatic siblings of children attending the nutrition clinic. Eligible infants were selected based on strict inclusion and exclusion criteria to ensure homogeneity of the study population.

- Infants with birth weight between 10th and 90th centile are considered appropriate for gestational age.
- Exclusive breastfeeding was defined as per WHO guidelines as, 'no other food or drink, not even water, except breast milk (including milk expressed or from a wet nurse), for 6 months of life, the exceptions being oral rehydration solution, drops and syrups (vitamins, minerals and

medicines). [9]

- Predominant breastfeeding indicates that the infant's predominant source of nourishment is breast milk (including milk expressed or from a wet nurse), however, the infant may have also received liquids (water and water-based drinks, fruit juice), ritual fluids and oral rehydration solution, drops or syrups (vitamins, minerals and medicines). [10]
- Iron Deficiency (ID): Defined as serum ferritin levels <12 μg/L (63). [11]
- Iron Deficiency Anemia (IDA): Defined as serum ferritin <12 μg/L along with hemoglobin (Hb) ≤10.5 g/dL (AAP guidelines).
- Iron deficiency was defined as serum ferritin < 15μg/L and Iron deficiency Anemia in mother was defined as serum ferritin <15 μg/L along with Hb ≤12 g/dl . [12]
- Wasting and Stunting: weight for length < −2 Z score and length for age < −2 Z score, respectively . [13]

A greater number of Infant-mother pairs were enrolled to account for elimination due to exclusion criteria and sample issues.
All infants underwent a detailed physical examination upon enrollment, and their anthropometric measurements (weight, length) were recorded using standardized procedures.
A thorough history was taken regarding the antenatal period, intake of any iron supplements, socioeconomic status of family and mode of delivery.

Socio-demographic data (age, gestation, date of birth, socio-economic status), and anthropometric measurement of infants, were recorded. Socioeconomic class was derived from modified Kuppuswamy's criteria. [14] Gestational age was recorded from the newborn discharge booklet, if available, or from history elicited from the mother.
Blood samples were collected from both the infants and their mothers to assess iron status. Two milliliters (2 ml) of venous blood were drawn from each participant using sterile techniques. Blood samples were stored in EDTA tubes for complete blood counts and in plain tubes for ferritin assays.

- **Infant Blood Sample:** Blood samples were obtained at one time point (during the study visit) and run through a fully automated blood cell counter for complete blood counts (CBC), including hemoglobin concentration, red blood cell indices, and ferritin levels.
- **Maternal Blood Sample:** 2 ml of venous blood was also collected from each mother. Hemoglobin and serum ferritin levels were determined using the same methodology as for the infants.

Complete Blood Count (CBC): All blood samples were processed using a fully automated blood cell counter. Peripheral blood smears were examined by a hematologist in cases where abnormalities in hemoglobin, platelet count, or white blood cell count were detected.
Serum Ferritin Assay: Ferritin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit, following standard protocols. The WHO cutoff value for iron deficiency was used to define serum ferritin <12 μg/L in infants and <15 μg/L in mothers.
Any samples that were hemolysed or yielded unreliable results were excluded from the analysis. Approximately 10 hemolysed samples were discarded, and abnormal hematological profiles (e.g., leukocytosis, thrombocytopenia) were excluded from final analyses.

Ferritin was chosen as the primary biomarker for assessing iron status because it reflects iron stores and is considered a reliable early indicator of iron deficiency. Serum ferritin <12 μg/L is widely accepted as the cutoff for iron deficiency in infants. [11] Hemoglobin levels were used to assess anemia, with hemoglobin ≤10.5 g/dL serving as the diagnostic criterion for IDA based on AAP guidelines. While ferritin is an acute-phase reactant that may increase during infections or inflammation, its use in this study was justified by the relative health of the infant cohort (asymptomatic, afebrile, and apparently healthy).

Statistical Analysis:

All the data collected was entered in a Microsoft Excel Spreadsheet. The quantitative variables were calculated as frequency and percentage. Categorical variables were described as frequencies and percentages while as continuous variables as mean and standard deviation. Chi square test was used to analyze the relationshipbetweentwocategoricalvariablesandtocompareacontinuous variable at baseline and follow up. A, P-value of <0.05 was considered as statistically significant.

Results:
A total of 197 infants were initially eligible for enrollment in the study. After obtaining consent, 25 infants were excluded due to refusal, and blood samples were successfully obtained from 172 infants. Due to hemolysis (10 samples) or other abnormalities (leukocytosis, leucopenia, thrombocytopenia), an additional 42 infants were excluded, leaving a final sample size of 130 infant-mother pairs for analysis. Demographic characteristic of the study population is depicted in [Table 1].

Table 1: Demographic characteristics of the study population

Variables	Mean± SD (%)
Age (Days)	144.32±25.75
Sex M/F	56.9/43.1
Gestational Age (Weeks)	38.54±0.91
Weight (kg)	6.95±1.6
Length (cm)	64.83±8.46
Birth Route [Vaginal delivery/ Cesarean Section	63.08/36.92

The socioeconomic status was diverse with 15.38% (20 infants) belonged to upper lower class, 24.61% (32 infants) belonged to lower-middle class. 34.61% (45 infants) belonged to upper-middle and 25.38% (33 infants) belonged to upper class [Table 2].

Table 2: Socioeconomic status of families

Socioeconomic status	(%)
Upper class (I)	25.38
Upper-middle class (II)	34.62
Lower-middle class (III)	24.62
Upper lower class (IV)	15.38
Lower class (V)	0

A clear trend of decreasing haemoglobin and ferritin levels with age is noted indicating a reduction in iron stores as infants' grow. These results confirm that there are significant differences in both hemoglobin and ferritin levels across the different age groups [Table 3].

Table 3: Haemoglobin and ferritin levels with age

Age-group (in days)	Mean Hb (g/dl)	Mean Ferritin (g/dl)
90-120	11.62±1.33	74.17±40.64
121-150	11.01±1.15	35.51±24.31
151-180	10.77±18.47	25.7±17.47

P value < 0.001

Among the total of 74 male infants, 17 (22.9%) were found to have iron deficiency and among the 56 female infants, 11 (19.6%) were identified with iron deficiency. The analysis revealed that iron deficiency was slightly more prevalent in male infants (22.9%) compared to female infants (19.6%), of the 74 male infants, 12 (16.2%) were diagnosed with iron deficiency anemia. Among the 56 female infants, 7 (12.5%) were found to have iron deficiency anemia [Fig 1].

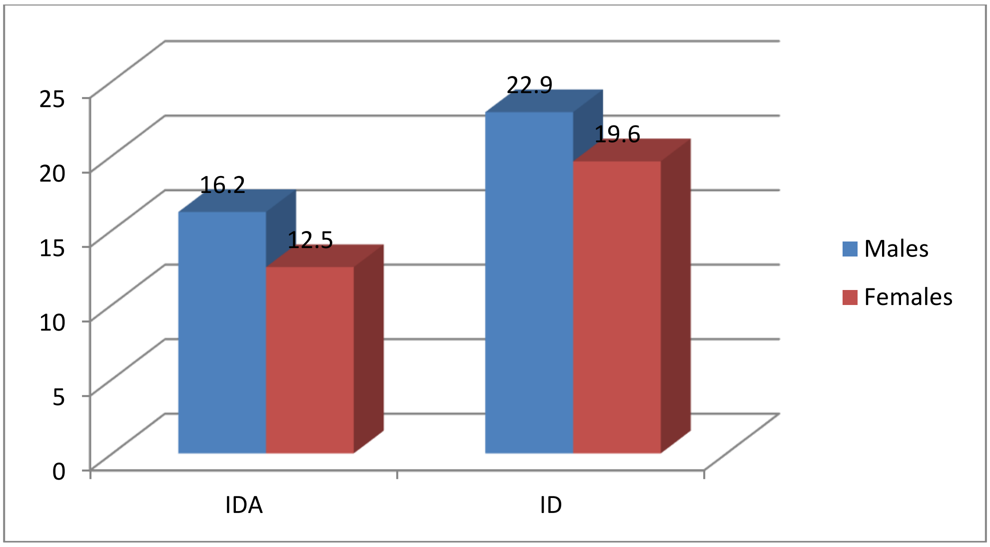

Fig 1.

The prevalence of iron deficiency showed a marked increase with decreasing socioeconomic status. Specifically, the prevalence was lowest in the upper class (12%) and highest in the upper lower class (40%). The intermediate values were observed in the upper-middle class (15.5%) and lower-middle class (28%). A similar trend was observed for iron deficiency anemia, with the highest prevalence in the upper lower class (25%) and the lowest in the upper class (6%). The prevalence in the upper-middle and lower-middle classes were 11% and 21.8%, respectively [Fig 2].

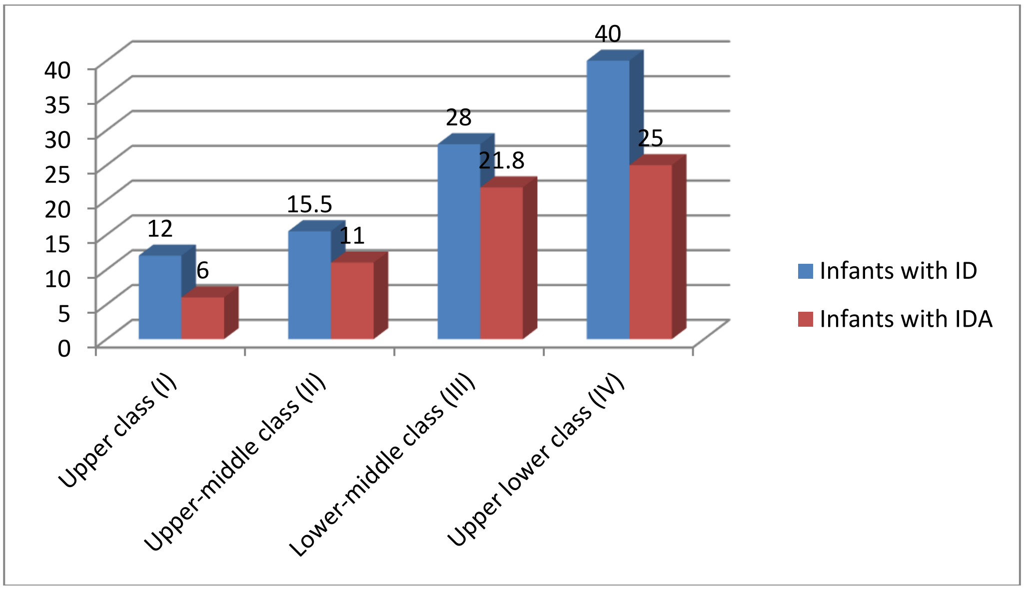

Fig 2.

Infants born to iron-deficient mothers were 2.67 times more likely to be iron deficient compared to infants born to mothers without iron deficiency (OR = 2.67, 95% CI: 1.14–6.37). Among the 130 mother-infant pairs, 19 infants (14.62%) were diagnosed with IDA. Among the mothers 56 (43.08%) were anemic, with 29(22.31%) specifically having iron deficiency anemia. Of the 19 infants with IDA, 9 (47.36%) were born to mothers who also had IDA, 3 (26.32%) were born to mothers with anemia but did not have iron deficiency anemia [Fig 3].

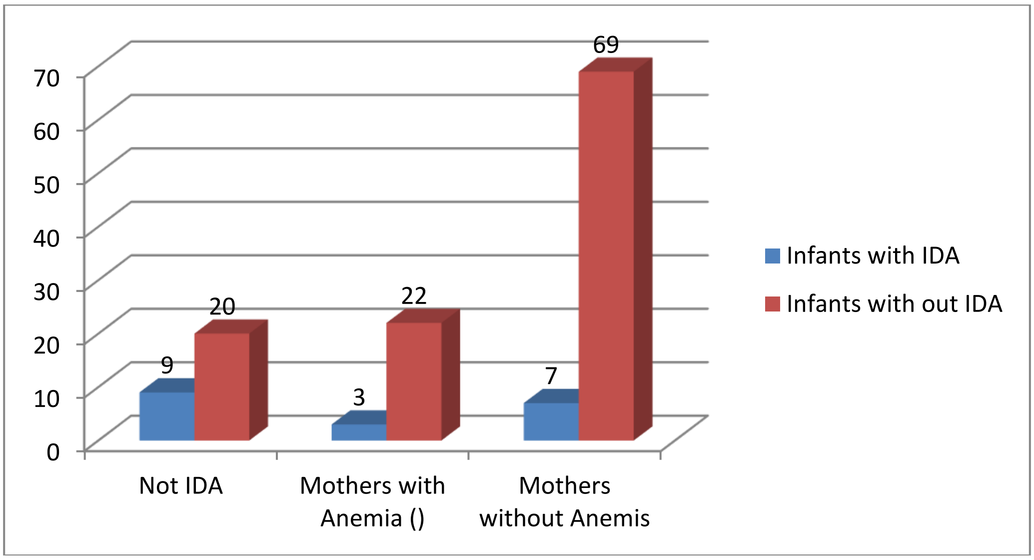

Fig 3.

Discussion:

Iron deficiency (ID) and iron deficiency anemia (IDA) are pervasive health issues that affect a significant proportion of infants globally, particularly in developing countries. These conditions are associated with a wide range of adverse outcomes, including impaired cognitive and motor development, reduced immune function, and increased susceptibility to infections. During the first few months of life, iron is essential for the rapid growth and development of the brain and other vital organs. Consequently, maintaining adequate iron levels during infancy is critical for ensuring optimal long-term health and development.

The primary aim of this study was to assess the prevalence of Iron deficiency (ID) and iron deficiency anemia (IDA) in 3 to 5-month-old healthy breastfed infants and explore its association with maternal iron status, socioeconomic factors, gestational age, delivery mode, and gender. The mean hemoglobin level in our study was 11.05 g/dL, with a range from 8.5 g/dL to 13.2 g/dL and the mean ferritin level in our study was 33.69 ng/mL, with a range from 6.08 ng/mL to 143 ng/mL. Our study revealed that 21.54% of infants (28 infants) had iron deficiency, while 14.62% were diagnosed with IDA (19 infants), with maternal anemia being a significant predictor of infant iron status.

The prevalence of ID and IDA observed in our study is higher than what is typically reported in high-income countries but aligns more closely with data from low- and middle-income countries (LMICs). For instance, studies from high-income countries such as the United States and Sweden report lower prevalence rates due to routine iron supplementation and fortification practices. For example, Ziegler E et al [6] found a prevalence of ID of only 6% in breastfed infants in the United States, where iron supplementation is commonly recommended starting at 4 months of age. In contrast, studies from LMICs, where routine iron supplementation is less common and socioeconomic factors may limit access to nutrient-rich diets, report higher prevalence rates. For example, Azmeraw et al. [15] in Ethiopia found a prevalence of IDA of around 20% in infants aged 6 months. The prevalence rates observed in our study align more closely with these findings, reflecting the challenges of addressing iron deficiency in resource-limited settings.

A study by Krishnaswamyet al, [16] found that prevalence of iron deficiency and iron deficiency Anemia to be 14.9% and 8.5% in 3 to 5 months old breastfed infants similar to our study. Similarly, Lambrecht et al, [17] found that iron deficiency prevalence among infants in low income settings was about 20%, similar to our results.

Rosa F.S.V. Marques et al, [18] studied a cohort of 102 healthy full term infants with B.W of ≥ 2.5 kg , found that exclusive breastfeeding protects infants from iron deficiency and iron deficiency anemia for the first 4 months of life, after this age, in accordance with literature, the findings of this study demonstrated an increase in anemia and iron deficiency rate, adding to evidence that supports the monitoring of iron levels in exclusively breastfed children , beginning at 4 months of age.

Raja Kumar Marol et al, [19] did a retrospective observational study in Karnataka India in level 2 hospital catering mainly rural children from low socioeconomic background, found that prevalence of Anemia was 87.6% in 3 – 6 months old infants, however the study population was biased towards sick children and also there is high prevalence of maternal anemia in that area.
In our study, we observed a decline in hemoglobin and ferritin levels with increasing age, particularly in the oldest age group (151-180 days), where 34% of Infants were iron deficient and 26% had Iron deficiency anemia in contrast to 8% of ID and 2.7% of IDA in 90-120 days old group. This age-related trend is consistent with existing literature, which indicates that as neonatal iron stores deplete the risk of iron deficiency increases, especially in exclusively breastfed infants. These age related increases are consistent with findings from Lozoff et al, [20] who observed rising rates of iron deficiency as infants transitioned from exclusive breastfeeding to the introduction of complementary foods. This is likely due to insufficient iron content in breast milk alone to meet the growing iron requirements of older infants. Smith et al, [21] found that iron deficiency rates rise significantly after 6 months, reflecting the increased dietary requirements and the potential inadequacy of breast milk in providing sufficient iron.
In our study The mean hemoglobin level was slightly higher in females (11.17 g/dL) compared to males (10.89 g/dL). Although this difference was not statistically significant (p value = 0.27), it may reflect differences in growth rates and metabolic demands between male and female infants. Similar to hemoglobin levels, ferritin levels were slightly higher in females (35.08 ng/mL) compared to males (30.76 ng/mL). However these differences were not statistically significant (p value = 0.19).
In our study, the prevalence of ID and IDA was slightly higher in males compared to females, although the differences were not statistically significant ($p > 0.05$). Specifically, 17 out of 74 male infants (22.9%) were diagnosed with iron deficiency compared to 11 out of 56 female infants (19.6%), while 12 male infants (16.2%) and 7 female infants (12.5%) had iron deficiency anemia. The findings of our study are in line with the literature that suggests minimal gender differences in iron deficiency during early infancy. A large cohort study by Dewey et al. [22] found no significant differences in iron status between male and female infants in their first year of life. Similarly, a study by Domellöf et al. [2] concluded that sex differences in iron metabolism are not apparent until later in childhood when puberty-related hormonal changes begin to influence erythropoiesis and iron requirements

It is well documented that pre-term infants are at a higher risk of iron deficiency and anemia due to lower iron stores at birth. Some studies suggest that even within the full term range, those born closer to 37 weeks might have slightly lower iron stores than those born at 39-40 weeks but the differences are often not clinically significant. Our study seems to align with this. While there was slightly increase in iron deficiency among infants born at 37-38 weeks (23%) compared to those born at 39-40 weeks (21%) the differences were not statistically significant, indicating that within the term age group gestational age didn't have impact on the infant iron stores.

In our study, infants delivered vaginally had higher mean hemoglobin levels (11.12 g/dL) compared to those delivered by cesarean section (10.84 g/dL). However, the difference in hemoglobin levels was not statistically significant ($p = 0.25$). Ferritin levels were also higher in infants delivered vaginally (41.07 ng/mL) compared to those delivered by cesarean section (30.08 ng/mL), with a p-value of 0.08, indicating a trend towards statistical significance. This suggests that while there is a potential influence of delivery method on iron stores, the evidence is not definitive, warranting further investigation. These findings are consistent with existing literature, including studies by Andersson et al. [23] and Van Rheenen and Brabin. [24]
Our study observed a gradient in the prevalence of iron deficiency and iron deficiency anemia, with higher rates in infants from lower socioeconomic classes. Specifically, the prevalence of iron deficiency was 12.12% in the upper class, 15.5% in the upper-middle class, 28.57% in the lower-middle class, and 40% in the upper-lower class. Similarly, iron deficiency anemia prevalence followed this pattern, with the highest rates in the upper-lower class (25%) and the lowest in the upper class (6.06%). However, the chi-square test for iron deficiency ($p = 0.0565$) and iron deficiency anemia

(p = 0.1412) indicates that these differences are not statistically significant, suggesting that socioeconomic status alone does not significantly impact the occurrence of these conditions in this cohort. These findings are consistent with global research indicating that poverty and low socioeconomic status are risk factors for nutritional deficiencies, including iron deficiency. While socioeconomic disparities in nutritional status are evident, the lack of statistical significance in this study highlights the need for a larger sample size or additional factors to better understand the relationship between socioeconomic status and iron deficiency.

In our study, correlation between maternal iron status and infant iron status in our study is statistically significant, with a p-value of 0.024 and an odds ratio of 2.67 (95% CI: 1.14–6.37). This indicates that infants born to mothers with iron deficiency are 2.67 times more likely to be iron deficient compared to those born to non-deficient mothers. Specifically, 46.43% of iron-deficient infants had mothers who were also iron deficient. Similarly, the chi-square test for the relationship between maternal anemia and infant iron deficiency anemia (IDA) yielded a p-value of 0.017, demonstrating a significant association. The odds ratio of 4.95 (95% CI: 1.78–13.4) suggests that infants born to mothers with IDA are nearly five times more likely to develop IDA compared to those born to mothers without anemia. These findings are consistent with previous studies, such as those by Ziegler et al. [25] and Milman et al., [26] which highlight the intergenerational impact of maternal nutrition on infant health . A study by Murray-Kolb and Beard [27] found that maternal iron deficiency during pregnancy is a significant predictor of iron deficiency in infants. The study emphasized that maternal anemia not only affects fetal iron stores but also has long- term consequences for infant development. Research by Milman et al. [26] demonstrated that prenatal iron supplementation can significantly reduce the risk of neonatal iron deficiency, particularly when administered during the third trimester.

Limitations:

A major limitation of this study is its cross-sectional design, which limits the ability to establish causal relationships between maternal and infant iron status. Additionally, the relatively small sample size may reduce the generalizability of the findings to the broader population. Future studies with larger, more diverse cohorts and longitudinal follow-up are needed to validate these results.

Conclusion:

Our study reveals a significant relationship between maternal anemia and infant iron deficiency anemia with notable increase in prevalence as infants' age, these findings are consistent with existing literature that emphasizes the critical role of maternal health in influencing infant iron status and highlights the need for timely intervention.

Maternal iron status is a critical factor in preventing Iron deficiency among breastfed infants, implementing comprehensive maternal iron screening and supplementation could significantly reduce Iron deficiency and iron deficiency anemia prevalence. Future research should focus on longitudinal studies to explore casualty and evaluate the effectiveness of intervention strategies.

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