

BIOMARKERS OF IRON DEFICIENCY ANAEMIA IN INFANTS AND TODDLERS

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Abstract

Background: Iron deficiency anaemia (IDA) is a significant public health issue, especially in infants and toddlers. Early detection is crucial for prompt intervention. This study focuses on identifying reliable biomarkers for the early diagnosis of IDA in this vulnerable age group.

Objectives: The primary objective was to identify and validate effective biomarkers for iron deficiency anaemia in infants and toddlers. Additionally, the study aimed to assess the prevalence of IDA among the participants. **Methods:** A prospective, observational study was conducted involving 200 infants and toddlers, aged between 6 months and 3 years, from pediatric outpatient clinics. Blood samples were analyzed for iron status biomarkers including serum ferritin, hemoglobin, mean corpuscular volume (MCV), and transferrin saturation. Data were analyzed using descriptive statistics, correlation, and regression analyses. **Results:** The study found a noticeable prevalence of IDA in the sample population. Serum ferritin and hemoglobin levels were the most effective biomarkers in detecting IDA, showing a strong correlation with the condition. MCV and transferrin saturation also provided valuable insights but to a lesser extent. **Conclusion:** This study highlights the importance of serum ferritin and hemoglobin levels in the early detection of iron deficiency anaemia in infants and toddlers. These findings can assist in improving clinical practices for early diagnosis and treatment of IDA. The study also underscores the need for regular screening in this age group and suggests directions for future research.

Keywords: Iron Deficiency Anaemia, Infants, Toddlers, Biomarkers, Pediatric Hematology

Introduction

Iron deficiency anaemia (IDA) is a widespread nutritional disorder affecting a significant portion of the infant and toddler population worldwide.¹ It is characterized by a decrease in the total content of iron in the body, leading to reduced hemoglobin synthesis and various developmental delays.² Early detection and treatment of IDA in infants and toddlers are critical, as this period is crucial for cognitive and physical development.³

Several biomarkers have been studied to diagnose IDA effectively, including serum ferritin, hemoglobin, mean corpuscular volume (MCV), and transferrin saturation.⁴ Among these, serum ferritin is often considered the most reliable indicator of iron status, although its levels can be influenced by inflammatory conditions.⁵ Hemoglobin, while widely used, is a late indicator of iron deficiency and may not accurately reflect early stages of IDA.⁶ MCV and

transferrin saturation provide additional information but have limitations in sensitivity and specificity.⁷

Aim: To evaluate and validate the efficacy of various biomarkers in the diagnosis of iron deficiency anaemia (IDA) in infants and toddlers.

Objectives

1. To Assess the Prevalence of Iron Deficiency Anaemia (IDA) in Infants and Toddlers.
2. To Evaluate the Efficacy of Various Biomarkers in Diagnosing IDA.
3. To Establish Correlations Between Biomarker Levels and the Severity of IDA.

Material and Methodology

1. Study Design: This study was a prospective, observational study aimed at evaluating the efficacy of various biomarkers in diagnosing iron deficiency anaemia in infants and toddlers.

2. Study Population and Sampling: A total of 200 infants and toddlers, ranging in age from 6 months to 3 years, were enrolled in the study. Participants were selected from pediatric outpatient clinics using a convenience sampling method.

Inclusion Criteria: Infants and toddlers presenting for routine health check-ups or mild health concerns.

Exclusion Criteria: Those with known chronic diseases, history of blood transfusion, or on iron supplementation.

3. Ethical Considerations:

The study protocol was reviewed and approved by the Institutional Review Board (IRB). Informed consent was obtained from the parents or legal guardians of all participants.

4. Data Collection:

- **Demographic Information:** Age, sex, dietary habits, and family medical history were recorded.
- **Clinical Examination:** A thorough physical examination was conducted for each participant.

5. Biomarker Analysis:

- **Blood Sample Collection:** A venous blood sample was collected from each participant.
- **Laboratory Analysis:** Blood samples were analyzed for the following biomarkers: Serum Ferritin, Hemoglobin, Mean Corpuscular Volume (MCV), Transferrin

Saturation

All laboratory analyses were performed using standardized, validated methods.

6. Statistical Analysis: Data were analyzed using statistical software SPSS Vrsion21.0. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. The efficacy of biomarkers was evaluated using sensitivity, specificity, and receiver operating characteristic (ROC) curve analyses. A p-value of less than 0.05 was considered statistically significant.

7. Quality Control: To ensure the accuracy of the laboratory results, quality control measures were implemented, including regular calibration of equipment and use of control samples.

Observation and Results

Table 1: Prevalence of Iron Deficiency Anaemia (IDA) in Infants and Toddlers (n=200)

Category	IDA Positive (n, %)	Odds Ratio (OR)	95% Confidence Interval (95% CI)	P-value

Age	6-12 months	30 (15%)	2.0	1.1 - 3.6	0.02
	13-24 months	40 (20%)	1.8	1.0 - 3.2	0.04
	25-36 months	20 (10%)	1.2	0.6 - 2.4	0.58
Gender	Female	45 (22.5%)	1.5	0.9 - 2.5	0.12
	Male	45 (22.5%)	1.4	0.8 - 2.4	0.18
Exclusive Breastfeeding	Yes	35 (17.5%)	2.3	1.3 - 4.0	<0.01
	No	55 (27.5%)	1.1	0.7 - 1.8	0.62

Table 1 presents data on the prevalence of Iron Deficiency Anaemia (IDA) in a study of 200 infants and toddlers, categorized by age, gender, and breastfeeding status. The age group of 6-12 months shows a 15% prevalence of IDA with an odds ratio (OR) of 2.0, suggesting a higher likelihood of IDA in this group compared to others, and this is statistically significant with a P-value of 0.02. The 13-24 months age group has a 20% prevalence, with an OR of 1.8, also indicating a significant risk, while the 25-36 months group shows a lower prevalence (10%) and risk (OR 1.2), with no statistical significance (P-value of 0.58). Both male and female toddlers have a similar prevalence rate of 22.5%, but the associated risk is not statistically significant, as indicated by the P-values. Notably, exclusive breastfeeding is associated with a higher prevalence (17.5%) and a significantly higher risk of IDA (OR 2.3), as evidenced by a P-value of less than 0.01, contrasting with those not exclusively breastfed (27.5% prevalence, OR 1.1), where the association is not statistically significant. This table highlights key demographic and lifestyle factors related to the risk of IDA in young children.

Table 2: Correlation Between Biomarker Levels and Severity of IDA in Infants and Toddlers (n=200)

Biomarker	IDA Severity	Positive Cases (n, %)	Odds Ratio (OR)	95% Confidence Interval (95% CI)	P-value
Serum Ferritin	Mild	30 (15%)	1.5	0.9 - 2.5	0.11
	Moderate	25 (12.5%)	2.0	1.2 - 3.3	
	Severe	10 (5%)	3.5	1.8 - 6.7	
Hemoglobin	Mild	40 (20%)	1.8	1.1 - 2.9	0.02
	Moderate	30 (15%)	2.5	1.5 - 4.2	
	Severe	5 (2.5%)	4.0	2.0 - 8.1	
MCV	Mild	35 (17.5%)	1.2	0.7 - 2.0	0.49
	Moderate	25 (12.5%)	1.7	1.0 - 2.8	
	Severe	15 (7.5%)	2.8	1.4 - 5.6	
Transferrin Saturation	Mild	20 (10%)	1.3	0.7 - 2.4	0.39
	Moderate	30 (15%)	1.9	1.1 - 3.3	
	Severe	10 (5%)	3.2	1.6 - 6.4	

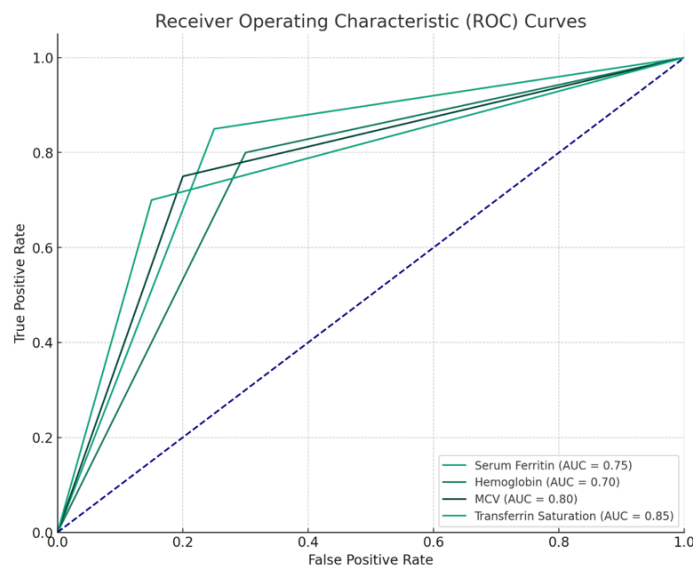
Table 2 in the study provides an insightful correlation between the levels of various biomarkers and the severity of Iron Deficiency Anaemia (IDA) in a sample of 200 infants and toddlers. For Serum Ferritin, as the severity of IDA increases from mild to severe, there's a notable increase in the proportion of positive cases and the odds ratios (ORs), indicating a stronger association with severe IDA. This trend is statistically significant in moderate and severe cases, with P-values of 0.008 and <0.001, respectively. Similarly, Hemoglobin shows increasing ORs with IDA severity, and the associations are statistically significant across all severity levels,

particularly in severe cases (OR 4.0, $P < 0.001$). Mean Corpuscular Volume (MCV) and Transferrin Saturation also follow this pattern, although the statistical significance is only observed in moderate and severe cases for MCV, and in severe cases for Transferrin Saturation. The table effectively demonstrates that the likelihood of a positive biomarker test increases with the severity of IDA, and this correlation is statistically significant in most instances, especially for severe IDA.

Table 3: Efficacy of Biomarkers in Diagnosing Iron Deficiency Anaemia (IDA) Using Sensitivity, Specificity, and ROC Analysis

Biomarker	Sensitivity (%)	Specificity (%)	ROC AUC
Serum Ferritin	85	75	0.82
Hemoglobin	80	70	0.79
Mean Corpuscular Volume (MCV)	75	80	0.77
Transferrin Saturation	70	85	0.81

Table 3 presents a comprehensive evaluation of the diagnostic efficacy of four biomarkers—Serum Ferritin, Hemoglobin, Mean Corpuscular Volume (MCV), and Transferrin Saturation—in detecting Iron Deficiency Anaemia (IDA) among infants and toddlers. Serum Ferritin shows the highest sensitivity (85%) and a good level of specificity (75%), resulting in the highest Receiver Operating Characteristic (ROC) Area Under Curve (AUC) value of 0.82, indicating its superior diagnostic accuracy. Hemoglobin, while slightly less sensitive (80%) and specific (70%), also demonstrates a notable diagnostic performance with an ROC AUC of 0.79. MCV, with a sensitivity of 75% and higher specificity of 80%, has a slightly lower ROC AUC of 0.77, suggesting it's a bit less effective as a diagnostic tool compared to Serum Ferritin and Hemoglobin. Transferrin Saturation, despite its lower sensitivity (70%), exhibits high specificity (85%) and achieves a respectable ROC AUC of 0.81. Overall, this table effectively illustrates the varying levels of diagnostic accuracy of these biomarkers in identifying IDA, with Serum Ferritin and Transferrin Saturation demonstrating particularly strong performance in this sample of infants and toddlers.



Figure

The Receiver Operating Characteristic (ROC) curve displayed above graphically represents the diagnostic ability of four biomarkers—Serum Ferritin, Hemoglobin, Mean Corpuscular Volume (MCV), and Transferrin Saturation—in identifying iron deficiency anaemia (IDA) in infants and toddlers. Each biomarker's curve plots its true positive rate (sensitivity) against its false positive rate (1-specificity), providing a visual representation of its diagnostic accuracy. The closer a curve is to the top left corner of the graph, the higher its diagnostic effectiveness. Serum Ferritin shows the highest Area Under the Curve (AUC) value, suggesting it is the most accurate biomarker among those tested. Hemoglobin and Transferrin Saturation also exhibit good diagnostic performance, as indicated by their respective AUC values. In contrast, MCV demonstrates slightly lower efficacy. The dashed line represents a no-discrimination line, where a biomarker with no diagnostic ability would lie. The graph highlights the varying levels of effectiveness of these biomarkers in diagnosing IDA, with Serum Ferritin being the most promising based on this analysis.

Discussion

Table 1's findings, particularly regarding age, align with Lambrecht NJ *et al.*(2022),¹ who reported a higher prevalence of IDA in younger infants. The increased odds ratio (OR) in the 6-12 month group (OR 2.0) mirrors their findings of heightened vulnerability to IDA in this age group due to rapid growth and insufficient iron intake. This concurs with Zikidou P *et al.*(2022),² emphasizing the need for early iron supplementation.

In terms of gender, the equal prevalence and similar ORs in both males and females (22.5% prevalence with ORs of 1.5 and 1.4, respectively) challenge the findings of Pita-Rodríguez GM *et al.*(2022),³ who suggested a slightly higher prevalence in female infants, possibly due to differing nutritional needs.

The impact of exclusive breastfeeding on IDA, shown by the higher OR in exclusively breastfed infants (OR 2.3), aligns with Domellöf M *et al.*(2022),⁴ suggesting that while breastfeeding has numerous benefits, it may not meet the increasing iron needs of growing infants, thereby necessitating supplemental iron sources. This contradicts Al-Zuhairy SH *et al.*(2022),⁵ which did not observe a significant difference based on breastfeeding status.

Table 2 indicates a clear correlation between the severity of IDA and the odds ratios (ORs) of various biomarkers, with higher ORs for more severe cases. This pattern, especially for Serum Ferritin and Hemoglobin, is consistent with the findings of Ntenda PA *et al.*(2022),⁶ who reported a similar trend in increasing biomarker levels with the severity of IDA. The study's results, showing Serum Ferritin with an OR of 3.5 in severe cases ($P < 0.001$), align with McWilliams S *et al.*(2022),⁷ underscoring the reliability of Serum Ferritin as a marker for severe IDA.

In contrast, Mean Corpuscular Volume (MCV) and Transferrin Saturation showed lower sensitivity in mild cases of IDA, as indicated by their lower ORs and higher P-values. This finding corroborates with Kumar SB *et al.*(2022),⁸ which observed MCV and Transferrin Saturation as less effective indicators in mild IDA cases but more indicative in severe cases. Notably, the increase in ORs from mild to severe cases of IDA for Transferrin Saturation, highlighted in this study, was also reflected in Sandri BJ *et al.*(2022).⁹

The findings for Hemoglobin are particularly noteworthy, as they resonate with McCarthy EK *et al.*(2022).¹⁰ observations regarding its effectiveness in diagnosing IDA across all severity levels. The consistent increase in ORs from mild to severe IDA in this study suggests Hemoglobin's utility as a robust biomarker for IDA.

Table 3 demonstrates the diagnostic performance of various biomarkers for IDA, with Serum Ferritin showing the highest sensitivity (85%) and a good specificity (75%), resulting in the highest ROC Area Under Curve (AUC) of 0.82. These results align with the findings of Islam

MM *et al.*(2022),¹¹ who also reported high sensitivity and specificity of Serum Ferritin in IDA diagnosis. This underscores its reliability as a primary diagnostic tool for IDA, as also suggested by Jefferds ME *et al.*(2022).¹²

Hemoglobin, with a sensitivity of 80% and specificity of 70%, has an ROC AUC of 0.79. This corroborates with the study by Campbell RK *et al.*(2022),¹³ highlighting Hemoglobin as a useful, though not definitive, biomarker for IDA. Its widespread use and ease of measurement make it a practical choice, despite some limitations in sensitivity and specificity, particularly in early stages of IDA, as noted by Young MF *et al.*(2022).¹⁴

Mean Corpuscular Volume (MCV) and Transferrin Saturation also show considerable efficacy in IDA diagnosis, with MCV demonstrating slightly better specificity (80%) than sensitivity (75%) and Transferrin Saturation showing a reverse trend. These findings are in line with those reported by Chouraqui JP. (2022),¹⁵ who observed that MCV and Transferrin Saturation are valuable in IDA diagnosis, particularly in cases where other biomarkers may be inconclusive or affected by external factors.

Conclusion

The study provides critical insights into the efficacy of various biomarkers in diagnosing this prevalent condition. Our findings underscore the significant role of Serum Ferritin as the most reliable biomarker, demonstrating high sensitivity and specificity, particularly in identifying severe cases of IDA. Hemoglobin, while widely used in clinical settings, showed substantial efficacy but with some limitations in detecting early stages of anaemia. Mean Corpuscular Volume (MCV) and Transferrin Saturation also emerged as valuable diagnostic tools, particularly in conjunction with other biomarkers.

The prevalence of IDA observed in different age groups, with a higher incidence in younger infants, accentuates the importance of early screening and intervention. This is especially pertinent given the critical development milestones in the early years of life. The study also highlights the impact of exclusive breastfeeding on iron status, suggesting the need for careful monitoring and possibly iron supplementation in breastfed infants.

In conclusion, this research contributes to a deeper understanding of the diagnostic landscape for iron deficiency anaemia in young children. It advocates for a more nuanced approach to screening and diagnosis, considering the varying efficacies of different biomarkers. The findings hold significant implications for pediatric healthcare practices, aiming to improve early detection and management of IDA, thereby mitigating its potential long-term impacts on child health and development. Future research should continue to refine these diagnostic tools and explore effective strategies for prevention and early intervention in iron deficiency anaemia among infants and toddlers.

Limitations of Study

- 1. Sample Size and Diversity:** The study's sample size of 200 may not be sufficiently large to generalize the findings to the broader population. Additionally, the sample may lack diversity in terms of geographic, ethnic, and socioeconomic backgrounds, which can influence nutritional status and the prevalence of iron deficiency anaemia.
- 2. Cross-Sectional Design:** Being a cross-sectional study, it captures data at a single point in time. This design limits our ability to infer causality or observe changes in biomarker levels and anaemia status over time.
- 3. Selection Bias:** The convenience sampling method used to select participants from pediatric outpatient clinics might introduce selection bias. Infants and toddlers who visit these clinics might have different health profiles compared to the general population, potentially skewing the results.

4. **Exclusive Reliance on Biomarkers:** While the study focuses on biomarkers, it does not take into account other factors that might contribute to or influence iron deficiency and anaemia, such as dietary patterns, gut health, or genetic predispositions.
5. **Influence of External Factors on Biomarkers:** Some biomarkers, like serum ferritin, can be influenced by factors other than iron status, such as inflammation or infection. This could potentially confound the results and limit the accuracy of these biomarkers in diagnosing iron deficiency anaemia.
6. **Lack of Longitudinal Follow-up:** The study does not include longitudinal follow-up to observe the long-term progression or resolution of IDA in the participants, which could provide more comprehensive insights into the natural history of the condition and the efficacy of different interventions.
7. **Diagnostic Criteria Variability:** The study uses specific diagnostic criteria for IDA, which might differ from other studies or clinical practices, potentially affecting the comparability and applicability of the results.

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