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RESEARCH ARTICLE

Evaluation of Iron Status Biomarkers and Cognitive Performance Among Pediatric Patients with Iron Deficiency Anemia

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ABSTRACT

Background: Iron deficiency anemia (IDA) is the most common nutritional deficiency among children worldwide and is associated with impaired neurocognitive development. Iron plays a crucial role in neurotransmitter synthesis, myelination, and energy metabolism in the developing brain. Early identification of cognitive impairment associated with iron deficiency may facilitate timely intervention and improve developmental outcomes.

Objectives: To evaluate iron status biomarkers among pediatric patients with iron deficiency anemia and assess their association with cognitive performance.

Materials and Methods: A hospital-based cross-sectional study was conducted among 100 children aged 6–12 years attending the pediatric outpatient department of a tertiary care teaching hospital. Fifty children diagnosed with iron deficiency anemia constituted the study group, while fifty age- and sex-matched healthy children served as controls. Hemoglobin concentration, serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation were measured using standard laboratory methods. Cognitive performance was assessed using age-appropriate standardized cognitive assessment tools evaluating memory, attention, processing speed, and executive functions. Statistical analysis was performed using SPSS version 25.0. Independent t-test and Pearson correlation analysis were used to determine differences and associations. A p-value <0.05 was considered statistically significant.

Results: Children with iron deficiency anemia (IDA) had significantly lower hemoglobin (9.2 ± 1.1 vs. 12.8 ± 0.9 g/dL), serum ferritin (11.4 ± 4.2 vs. 42.8 ± 8.5 ng/mL), serum iron (38.7 ± 9.3 vs. 86.5 ± 12.1 µg/dL), and transferrin saturation (8.7 ± 2.1 vs. 27.4 ± 4.5%) compared to controls, while TIBC was significantly higher (445 ± 35 vs. 325 ± 28 µg/dL) ($p<0.001$). Cognitive performance scores, including attention, memory, processing speed, and executive function, were significantly lower in the IDA group ($p<0.001$). Overall cognitive performance showed positive correlations with hemoglobin ($r=0.52$, $p<0.001$) and serum ferritin ($r=0.48$, $p<0.001$), whereas TIBC demonstrated a negative correlation ($r=-0.41$, $p=0.004$). Cognitive scores declined progressively with increasing severity of anemia.

Conclusion: Pediatric patients with iron deficiency anemia exhibited significantly altered hematological and iron status biomarkers along with impaired cognitive performance compared to healthy controls. Lower hemoglobin and ferritin levels were significantly associated with poorer cognitive outcomes. These findings highlight the importance of early screening, diagnosis, and treatment of iron deficiency anemia to improve cognitive development and academic performance among children.

Keywords: Iron deficiency anemia; Serum ferritin; Cognitive performance; Pediatric population; Iron biomarkers; Cognitive development; Attention; Memory.

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Introduction

Iron deficiency anemia (IDA) is the most common nutritional deficiency disorder worldwide and remains a major public health problem, particularly among children in developing countries. According to the World Health Organization (WHO), iron deficiency affects nearly one-third of the global population, with children constituting one of the most vulnerable groups due to their increased iron requirements during periods of rapid growth and development [1]. Iron deficiency anemia results from inadequate dietary intake, poor absorption, increased physiological demand, or chronic blood loss and is characterized by reduced hemoglobin synthesis and impaired oxygen-carrying capacity of blood.

Beyond its hematological consequences, iron deficiency has profound effects on neurodevelopment and cognitive functioning. Iron is an essential micronutrient involved in several neurological processes, including myelination, neurotransmitter synthesis, neuronal metabolism, and synaptic plasticity. Adequate iron availability is crucial for normal brain maturation, particularly during childhood when cognitive development is at its peak [2]. Deficiency of iron during this critical period may lead to alterations in attention, memory,

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learning ability, executive functioning, and psychomotor development.

Several studies have demonstrated that children with iron deficiency anemia exhibit poorer academic achievement and lower cognitive performance compared to their healthy peers. Reduced oxygen delivery to neural tissues and impaired neurotransmitter activity have been proposed as the primary mechanisms responsible for cognitive deficits associated with iron deficiency. Furthermore, these impairments may persist even after correction of anemia if iron deficiency remains untreated for prolonged periods, emphasizing the importance of early detection and intervention [3].

Assessment of iron status involves a combination of hematological and biochemical markers. Hemoglobin concentration is commonly used for screening anemia; however, it may not accurately reflect body iron stores during the early stages of deficiency. Biomarkers such as serum ferritin, serum iron, total iron-binding capacity (TIBC), transferrin saturation, and serum transferrin provide a more comprehensive evaluation of iron status and are increasingly utilized in clinical and research settings [4]. Understanding the relationship between these biomarkers and cognitive outcomes may aid in identifying children at risk of neurocognitive impairment.

Although numerous studies have investigated the prevalence and clinical manifestations of iron deficiency anemia, data regarding the association between iron status biomarkers and cognitive performance among pediatric populations remain limited, particularly in developing countries. Establishing this relationship may contribute to improved screening strategies and targeted interventions aimed at enhancing cognitive development and educational outcomes among affected children [5].

Therefore, the present study was undertaken to evaluate iron status biomarkers and assess their association with cognitive performance among pediatric patients with iron deficiency anemia.

Objectives

Primary Objective

To evaluate iron status biomarkers among pediatric patients diagnosed with iron deficiency anemia and compare them with healthy age- and sex-matched controls.

Secondary Objectives

- To assess cognitive performance among children with iron deficiency anemia and healthy controls.
- To determine the association between iron status biomarkers and cognitive performance scores.
- To evaluate the relationship between the severity of iron deficiency anemia and cognitive function.
- To identify the iron status biomarker most strongly associated with cognitive performance among pediatric patients.

Materials and Methods

Study Design and Setting

A hospital-based cross-sectional comparative study was conducted in the Department of Pediatrics in collaboration with the Department of Physiology and Central Clinical Laboratory of a tertiary care teaching hospital. The study was carried out over a period of 12 months from January 2024 to December 2024 after obtaining approval from the Institutional Ethics Committee.

Study Population

The study included children aged 6–12 years attending the Pediatric Outpatient Department (OPD) and Pediatric Inpatient Department (IPD) of the hospital during the study period.

Sample Size

A total of 100 participants were enrolled in the study. The sample comprised:

- Group I (Cases): 50 children diagnosed with iron deficiency anemia.
- Group II (Controls): 50 age- and sex-matched healthy children without anemia.

The sample size was calculated based on previous studies reporting significant differences in cognitive performance scores between children with iron deficiency anemia and healthy controls, considering a confidence level of 95%, power of 80%, and a significance level of 5%.

Inclusion Criteria

Cases

- Children aged 6–12 years.
- Diagnosed with iron deficiency anemia based on WHO criteria:
  - Hemoglobin <11.5 g/dL for children aged 6–11 years.
  - Hemoglobin <12.0 g/dL for children aged 12 years.
- Laboratory evidence of iron deficiency:
  - Serum ferritin <15 ng/mL.
  - Reduced serum iron levels.
  - Increased total iron-binding capacity (TIBC).
- Children whose parents or guardians provided written informed consent.

Controls

- Apparently healthy children aged 6–12 years.
- Normal hemoglobin levels according to age.
- Normal iron status parameters.
- Age- and sex-matched with the study group.
- Written informed consent obtained from parents or guardians.

Exclusion Criteria

- Children with anemia due to causes other than iron deficiency.
- History of hemoglobinopathies such as thalassemia or sickle cell disease.
- Chronic kidney disease, chronic liver disease, or malignancy.
- Neurological disorders affecting cognition.
- Developmental delay or intellectual disability.
- Acute infection or inflammatory disorders during the study period.
- Children receiving iron supplementation within the previous three months.
- Visual, auditory, or speech impairments interfering with cognitive assessment.
- Refusal to participate in the study.

Study Procedure

After obtaining informed consent from parents or guardians, demographic information including age, sex, socioeconomic status, dietary habits, and educational background was collected using a predesigned and pretested questionnaire.

Anthropometric measurements including height and weight were recorded using standardized procedures. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Blood Sample Collection and Laboratory Analysis

Approximately 5 mL of venous blood was collected from each participant under aseptic precautions.

Two milliliters of blood were transferred into EDTA tubes for hematological analysis and three milliliters into plain tubes for biochemical investigations.

Hematological Parameters

The following parameters were analyzed using an automated hematology analyzer:

- Hemoglobin (Hb)
- Hematocrit (HCT)
- Red Blood Cell Count (RBC)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Concentration (MCHC)

Iron Status Biomarkers

Serum samples were analyzed for:

- Serum Ferritin (ng/mL)
- Serum Iron (µg/dL)
- Total Iron-Binding Capacity (TIBC) (µg/dL)
- Transferrin Saturation (%)
- Serum Transferrin (mg/dL)

Serum ferritin levels were estimated using enzyme-linked immunosorbent assay (ELISA). Serum iron and TIBC were measured using colorimetric methods. Transferrin saturation was calculated using the formula:

$$\text{Transferrin Saturation (\%)} = (\text{Serum Iron} / \text{TIBC}) \times 100$$

Assessment of Cognitive Performance

Cognitive performance was assessed using age-appropriate standardized neurocognitive assessment tools administered by trained investigators in a quiet environment.

The assessment evaluated the following domains:

- Attention and concentration
- Short-term memory

- Working memory
- Processing speed
- Executive functioning

Individual domain scores were recorded and combined to obtain an overall cognitive performance score. Higher scores indicated better cognitive performance.

Ethical Considerations

Ethical clearance was obtained from the Institutional Ethics Committee before commencement of the study. Participation was voluntary, and written informed consent was obtained from parents or legal guardians. Confidentiality and anonymity of participants were maintained throughout the study.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) version 25.0.

Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were expressed as frequency and percentage.

Normality of data distribution was assessed using the Shapiro–Wilk test.

- Independent Student's t-test was used to compare continuous variables between cases and controls.
- Chi-square test was applied for comparison of categorical variables.
- One-way Analysis of Variance (ANOVA) was used to compare cognitive scores across different grades of anemia severity.
- Pearson's correlation coefficient was used to determine the association between iron status biomarkers and cognitive performance scores.
- Multiple linear regression analysis was performed to identify independent predictors of cognitive performance.

A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 presents the demographic characteristics of the study participants. The mean age of children in the IDA group was 8.9 ± 1.8 years, while that of the control group was 9.1 ± 1.7 years, with no statistically significant difference between the groups (p=0.62). Similarly, the distribution of males and females was comparable between the two groups (p=0.84), indicating appropriate age and sex matching. However, children with iron deficiency anemia had significantly lower mean weight (24.6 ± 4.5 kg vs. 27.3 ± 4.2 kg), height (126.4 ± 8.9 cm vs. 129.8 ± 8.1 cm), and BMI (15.4 ± 1.8 kg/m² vs. 16.2 ± 1.5 kg/m²) compared to controls (p<0.05), suggesting poorer nutritional status among anemic children.

Table 2 compares the hematological parameters between the study groups. Children with IDA exhibited significantly lower hemoglobin levels (9.2 ± 1.1 g/dL) compared to healthy controls (12.8 ± 0.9 g/dL) (p<0.001). Hematocrit, RBC count, MCV, MCH, and MCHC were also significantly reduced in the IDA group (p<0.001 for all parameters). These findings are characteristic of microcytic hypochromic anemia and confirm the hematological diagnosis of iron deficiency anemia among the study participants.

Table 3 demonstrates significant alterations in iron status biomarkers among children with IDA. Serum ferritin and serum iron levels were markedly lower in the IDA group (11.4 ± 4.2 ng/mL and 38.7 ± 9.3 µg/dL, respectively) compared to controls (42.8 ± 8.5 ng/mL and 86.5 ± 12.1 µg/dL, respectively) (p<0.001). Transferrin saturation was also significantly reduced among anemic children (8.7 ± 2.1% vs. 27.4 ± 4.5%, p<0.001). Conversely, TIBC and serum transferrin levels were significantly elevated in the IDA group (445 ± 35 µg/dL and 392 ± 32 mg/dL, respectively) compared to controls (325 ± 28 µg/dL and 282 ± 25 mg/dL, respectively) (p<0.001). These findings indicate depleted

Table 1: Demographic Characteristics of Study Participants

Variable	*IDA Group (n=50)*	*Control Group (n=50)*	*p-value*
Age (years)	8.9 ± 1.8	9.1 ± 1.7	0.62
Male, n (%)	27 (54%)	26 (52%)	0.84
Female, n (%)	23 (46%)	24 (48%)	0.84
Weight (kg)	24.6 ± 4.5	27.3 ± 4.2	0.01
Height (cm)	126.4 ± 8.9	129.8 ± 8.1	0.04
BMI (kg/m²)	15.4 ± 1.8	16.2 ± 1.5	0.03

Table 2: Hematological Parameters Among Study Participants

Parameter	*IDA group*	*Control group*	*p-value*
Hemoglobin (g/dL)	9.2 ± 1.1	12.8 ± 0.9	<0.001
Hematocrit (%)	29.8 ± 3.4	38.5 ± 2.8	<0.001
RBC Count (million/mm³)	3.9 ± 0.4	4.7 ± 0.5	<0.001
MCV (fL)	71.6 ± 4.5	84.2 ± 3.7	<0.001
MCH (pg)	22.8 ± 2.1	29.1 ± 1.8	<0.001
MCHC (g/dL)	29.7 ± 1.9	33.4 ± 1.5	<0.001

Table 3: Comparison of Iron Status Biomarkers

Biomarker	*IDA group*	*Control group*	*p-value*
Serum Ferritin (ng/mL)	11.4 ± 4.2	42.8 ± 8.5	<0.001
Serum Iron (µg/dL)	38.7 ± 9.3	86.5 ± 12.1	<0.001
TIBC (µg/dL)	445 ± 35	325 ± 28	<0.001
Transferrin Saturation (%)	8.7 ± 2.1	27.4 ± 4.5	<0.001
Serum Transferrin (mg/dL)	392 ± 32	282 ± 25	<0.001

body iron stores and compensatory increases in iron-binding capacity.

Table 4 shows the comparison of cognitive performance between children with IDA and healthy controls. The IDA group demonstrated significantly lower scores across all cognitive domains assessed. Attention scores were significantly lower in children with IDA (68.4 ± 8.2) than controls (82.5 ± 7.4) ($p<0.001$). Similar reductions were observed in memory scores (71.3 ± 7.9 vs. 85.7 ± 6.8), processing speed scores (69.8 ± 8.5 vs. 84.1 ± 7.1), and executive function scores (72.6 ± 7.3 vs. 83.8 ± 6.5), all of which were statistically significant ($p<0.001$). The overall cognitive performance score was markedly lower among children with IDA (70.5 ± 7.8) compared to controls (84.0 ± 6.9), indicating a substantial negative impact of iron deficiency anemia on cognitive functioning.

Table 5 illustrates the relationship between iron status biomarkers and cognitive performance. Hemoglobin levels demonstrated a moderate positive correlation with cognitive scores ($r=0.52$, $p<0.001$), indicating that higher hemoglobin levels were associated with better cognitive function. Serum ferritin also showed a significant positive correlation ($r=0.48$, $p<0.001$), followed by serum iron ($r=0.44$, $p=0.002$) and transferrin saturation ($r=0.39$, $p=0.006$). In contrast, TIBC exhibited a significant negative correlation with cognitive performance ($r=-0.41$, $p=0.004$), suggesting that worsening iron deficiency was associated with poorer cognitive outcomes. These findings emphasize the close relationship between iron status and neurocognitive performance in children.

Table 6 depicts the association between anemia severity and cognitive performance. Among the 50 children with IDA, 18 (36%) had mild anemia, 25 (50%) had moderate anemia, and 7 (14%) had severe anemia. The mean cognitive performance score decreased progressively with increasing severity of anemia. Children with mild anemia had the highest mean cognitive score (76.4 ± 5.2), followed by those with moderate anemia (69.8 ± 6.1), while children with severe anemia demonstrated the lowest cognitive score (61.5 ± 4.8). The difference was statistically significant ($p<0.001$), indicating that greater severity of iron deficiency anemia is associated with more pronounced cognitive impairment.

Table 4: Comparison of Cognitive Performance Scores

Cognitive domain	*IDA group*	*Control group*	*p-value*
Attention Score	68.4 ± 8.2	82.5 ± 7.4	<0.001
Memory Score	71.3 ± 7.9	85.7 ± 6.8	<0.001
Processing Speed	69.8 ± 8.5	84.1 ± 7.1	<0.001
Executive Function	72.6 ± 7.3	83.8 ± 6.5	<0.001
Overall Cognitive Score	70.5 ± 7.8	84.0 ± 6.9	<0.001

Table 5: Correlation of Iron Status Biomarkers with Cognitive Performance Scores

Variable	*Correlation coefficient (r)*	*p-value*
Hemoglobin vs Cognitive Score	0.52	<0.001
Serum Ferritin vs Cognitive Score	0.48	<0.001
Serum Iron vs Cognitive Score	0.44	0.002
Transferrin Saturation vs Cognitive Score	0.39	0.006
TIBC vs Cognitive Score	-0.41	0.004

Table 6: Severity of Iron Deficiency Anemia and Cognitive Performance

Severity of IDA	*Number (%)*	*Mean cognitive Score*	*p-value*
Mild (Hb 10–10.9 g/dL)	18 (36%)	76.4 ± 5.2	
Moderate (Hb 7–9.9 g/dL)	25 (50%)	69.8 ± 6.1	<0.001
Severe (Hb <7 g/dL)	7 (14%)	61.5 ± 4.8	

Discussion

The present study evaluated the association between iron status biomarkers and cognitive performance among pediatric patients with iron deficiency anemia (IDA). The findings demonstrated significantly lower hematological indices, depleted iron stores, and impaired cognitive performance among children with IDA compared to healthy controls. Furthermore, significant correlations were observed between iron status biomarkers and cognitive scores, suggesting that iron deficiency adversely affects neurocognitive functioning.

The significantly lower hemoglobin, serum ferritin, and serum iron levels observed among children with IDA are consistent with previous studies that reported depleted iron stores and altered iron metabolism in pediatric anemia [6,7]. Elevated TIBC and serum transferrin levels in the present study reflect compensatory physiological responses aimed at increasing iron transport and utilization during states of iron deficiency [8].

Iron plays a vital role in brain development through its involvement in myelination, neurotransmitter synthesis, and cellular energy metabolism. Deficiency of iron during childhood may impair neuronal function and synaptic plasticity, leading to deficits in learning and cognition [9]. In the present study, children with IDA exhibited significantly lower scores in attention, memory, processing speed, and executive functioning compared to controls. Similar findings have been reported by Lozoff et al. and Falkingham et al., who observed impaired cognitive and behavioral outcomes among iron-deficient children [10,11].

The positive correlations between hemoglobin, serum ferritin, serum iron, and cognitive performance scores observed in the present study indicate that adequate iron availability is essential for optimal cognitive functioning. Serum ferritin, a sensitive indicator of body iron stores, showed a significant positive association with cognitive scores, suggesting that depletion of iron reserves may directly influence neurocognitive performance [12]. Conversely, the negative correlation between TIBC and cognitive performance further supports the adverse impact of worsening iron deficiency on brain function.

An important finding of the present study was the progressive decline in cognitive scores with increasing severity of anemia. Children with severe anemia demonstrated the poorest cognitive performance, highlighting the potential dose-dependent relationship between iron deficiency and neurocognitive impairment. Similar observations have been documented by previous investigators, who reported that severe and prolonged iron deficiency may result in more pronounced deficits in attention, memory, and academic achievement [13,14].

The findings of the present study emphasize the importance of early screening for iron deficiency anemia in school-aged children. Timely diagnosis and appropriate iron supplementation may not only improve hematological status but also enhance cognitive development and educational performance. Routine assessment of iron biomarkers, particularly serum ferritin, may help identify children at risk of cognitive impairment before severe anemia develops [15].

Limitations of the Study

The present study has certain limitations. The cross-sectional design limits the ability to establish a causal relationship between iron deficiency and cognitive impairment. The study was conducted at a single tertiary care center, which may limit the generalizability of the findings to the broader population. Cognitive performance was assessed at a single time point, and long-term follow-up after iron therapy was not performed. Additionally, factors such as socioeconomic status, parental education, dietary habits, and environmental influences that may affect cognitive development were not extensively evaluated.

Conclusion

The present study demonstrated that children with iron deficiency anemia have significantly altered iron status biomarkers and reduced cognitive performance compared to healthy controls. Hemoglobin, serum ferritin, serum iron, and transferrin saturation were positively associated with cognitive function, whereas TIBC showed a negative association. Cognitive impairment increased with the severity of anemia, underscoring the importance of early diagnosis and management of iron deficiency. Regular screening and timely intervention may contribute to improved neurocognitive development and academic performance among pediatric populations.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Ethical Clearance

Ethical clearance for the study was obtained from the Institutional Ethics Committee prior to commencement of the study. Written informed consent was obtained from the parents or legal guardians of all participating children.

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