

Iron Status and Cognitive Function in Children Aged 1 Month to 5 Years

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ABSTRACT

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Background and Objective: To our knowledge in our country not many studies have been done regarding the iron status and cognitive function in children. Therefore, the aim of this study was to investigate the relationship between serum iron levels and cognitive function in children aged 1 month to 5 years in our institution.

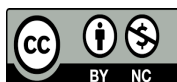
Methods: This study was conducted for 18 months in the pediatric population of a tertiary care hospital in Mysore, India, after obtaining approval from the Ethics Committee of the institution. A total of 255 subjects aged between 1 month and 5 years were selected as per the eligibility criteria. From these children, a venous blood sample was sent for determination of iron levels and total iron binding capacity. Cognitive abilities were assessed using the age-appropriate Ages and Stages Questionnaire (ASQ-3).

Findings: The mean age of the study population was 22.87±14.83 months and the male-to-female ratio was 1.7:1. Totally, 230 out of 255 children were iron deficient (90.2%), 91.3% boys and 88.3 % girls. Anemia was found in 55.7% of the children, with 94.3% of those with anemia suffering from ID. ID was found to have a negative significant effect on fine motor skills ($p < 0.0001$) and problem-solving ability ($p=0.01$). Gross motor skills were significantly impaired by ID ($p<0.0001$).

Conclusion: This study re-emphasizes that ID and IDA have a negative impact on cognitive development and performance, with more severe effects in the last stage of ID – IDA.

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Introduction

Infancy and childhood are crucial and critical periods of growth and iron is very essential for brain development in the first few years of life [1, 2]. Iron deficiency (ID) causes alterations in areas such as the hippocampus and changes in metabolic processes like mitochondrial damage, brain dopamine metabolism, and myelination culminating with a negative impact on growth and may result in poor cognitive function [3]. Cognitive disturbances have been seen in ID without anemia as well as iron-deficiency anemia (IDA) [4]. Cognitive limitations not only affect a child's physical health but also can affect language skills and behavior, leading to difficulties in the family's daily life [5]. Since many studies have not been done in our country, it was imperative to detect and understand the correlation between ID with or without anemia and cognitive impairment in children aged 1 month to 5 years. This age group was selected for this study because most of the human development happens during this period.

Methods

Design and sample

We did a prospective cross-sectional study on 255 eligible children in the age group of 1 month to 5 years visiting our hospital, for 18 months. Assuming that the percentage of Cognitive impairment in children is 21% according to the data available at our developmental clinic over the last 5 years, with an absolute precision of 5% and confidence interval of 95%, a sample of 255 children needed to be collected.

Ethical clearance was obtained from the Ethics Committee of the institution and informed consent was taken from the parents/guardians of the children selected. A convenience sampling method was used and children diagnosed with developmental delay, history of NICU admission/Perinatal insults like birth asphyxia or severe neonatal hyperbilirubinemia requiring exchange transfusions or serum bilirubin ≥ 20 mg/dl requiring intensive phototherapy; and known cases of thalassemia or other hemolytic disorders requiring frequent blood transfusions were excluded from the study.

Data collection

The parent/legal guardian of the children was interviewed with an internally validated semi-structured questionnaire to obtain details on the subjects' demographic data, obstetric history of the mother, mode of delivery and birth weight and postnatal risk factors, socioeconomic status, type of family, consanguinity and nutritional history pertaining to iron-rich food. Anthropometric data were interpreted using WHO Standard Growth charts [6, 7]. The blood sample was collected from all the children for estimating hemoglobin, RBC indices, peripheral blood smear, and iron profile which included total iron binding capacity and serum iron. Serum ferritin levels were not considered because it is also an acute phase reactant and would be falsely high in certain conditions. The diagnosis of ID and IDA were confirmed by the laboratory tests. Anemia cut-offs were based on the WHO criteria. Babies aged 2–4 months with physiological anemia were excluded from the study. All the children meeting the eligibility criteria were then administered the Ages and Stages Questionnaire (ASQ-3) to assess cognitive function and skills. The developmental score was interpreted by the developmental Pediatrician. The results were logged and displayed as frequency tables, graphs, and cross-tabulations.

A note on ASQ-3: Developed in 1980 by J. Squires and D. Bricker at the University of Oregon with the first edition published in 1995 and the latest third edition, ASQ - 3 published in 2009, is a tool for developmental screening in children [8-10]. This is a system of age-specific questionnaires for children 1 to 66 months of age, with intervals of 2,4,6,8,10,12,14,16,18,20,22,24 (2 months apart) 27,30,33 and 36 months (spaced 3 months apart), and 42,48,54,60 months spaced 6 months apart. It measures 5 developmental domains: gross motor, fine motor, communication, problem-solving, and personal-social, with each domain rated, as development appears on schedule, close to the cut-off, requiring learning and play activities, and below the cut-off with the need for further assessment by a professional. The validity has been examined across different cultures and communities across the

world [11-14]. It has a test-retest reliability of 92%, sensitivity of 87.4% and specificity of 95.7% [15].

Statistical analysis

Statistical analysis was done using IBM® SPSS® version 21 software. The data were summarized using frequency and percentages and the associations of various elements were studied using Chi-square tests and Kendall Tau correlation for statistical significance to determine the correlation between serum iron levels and cognitive function in these children.

Results

Among a total of 255 children between the ages of 1 month to 5 years, the mean age was 22.87 months±14.83 months, with 63.1% boys and 36.9% girls. The antenatal characteristics of the mothers of these children showed that around 76% had an uneventful pregnancy, while 23.9% (n=61) had complications like fever with rash, anemia, antepartum hemorrhage, and gestational diabetes mellitus, and 7.8% (n=20) of them had chronic diseases like asthma, hypothyroidism or hyperthyroidism and covid-19 infection in one mother. Our analysis showed that having at least one antenatal risk factor is associated with low iron levels (p<0.05). Majority of the babies; 85.1% (n= 217) had a normal weight at birth. Only 1.6%(n=4) babies were macrosomic and 13.3%(n=34) had a low birth weight. There were no babies with a very low birth weight or extremely low birth weight. In the postnatal period, 66.2%(n=169) of children had no risk factors. The anthropometric details of these

children are represented in **Figure 1**. Of the 255 children, 87.5% (n=223) consumed cereals, 83.1% (n=212) consumed legumes, and 77.3% (n=197) consumed greens, indicating that the vast majority of our study population consumed iron-rich vegetarian sources. Direct breastfeeding, although a poor source of iron, was only considered for infants under 6 months of age. None of the infants included in the study received iron prophylaxis. The majority of children, i.e. 90.2% (n=230), were iron deficient, compared to 9.8% (n=25) with normal iron levels or no ID. The mean hemoglobin levels, RBC indices, iron, and TIBC levels in the study population are shown in Table 1(1a and 1b). Anemia was seen in more than half of the children; 55.7% (n=142) were anemic, while 44.3% (n=113) were non-anemic. Among the 142 anemic children, the majority were iron deficient 94.3% (n=134) whereas only 5.6% (n=8) had normal iron levels and among the 113 non-anemic children, 84.9% (n=96) were iron deficient and 15%(n=17) were having normal levels. The correlation between ID status and cognitive function in all ages and both genders is shown in **Table 2**. On applying Kendall's Tau correlation, it was found that a correlation exists between ID status and cognitive function suggesting that ID can lead to a decrease in cognitive function in a child. The results were fairly consistent among boys and girls. The correlation between IDA and cognitive function in all ages and both genders is depicted in **Table 3**. It was interesting to note that ID can negatively impact gross motor function too in both boys and girls, boys (p=0.002) and girls (p=0.030).

**Table 1. A: Mean Blood Cell indices among the study participants.
B: Iron and TIBC levels among the study participants. [n=255]**

A:	N.	Blood Cells Indices	Iron Deficient	Normal Iron levels	Total
			Mean [Standard Deviation]		
	1	Haemoglobin(g/dl)	10.3 [1.7]	11.1 [1.6]	10.4 [1.7]
	2	MCV (fl)	70.1 [9.0]	78.4 [7.3]	70.9 [9.2]
	3	MCH (pg)	22.7 [4.8]	26.4 [2.5]	23.1 [4.7]
	4	MCHC(g/dl)	31.8 [2.42]	33.7 [1.2]	32.0 [2.4]
	5	RDW (%)	16.5 [4.0]	14.4 [4.8]	16.3 [4.1]
	6	Platelets Levels [in Lakhs per dl]	3.9 [1.57]	5.7 [11.7]	4.1 [3.9]

B:	N.	Iron Profile	Anemic	Normal or Non-Anemic	Total
			Mean [Standard Deviation]		
	1	Iron levels	24.8 [22.1]	34.4 [25.4]	29.1 [24.1]
	2	TIBC Levels	365.8 [92.3]	323.9 [87.9]	347.2 [92.6]

Table 2: Correlation between Iron deficiency status and Cognitive Function: All ages, both gender

N.	Cognitive Function	Iron Deficiency Status	P-value*
		Correlation Coefficient# [r]	
1	Communication	-0.116	0.06
2	Gross motor	-0.235	<0.0001
3	Fine motor	-0.346	<0.0001
4	Problem-solving	-0.146	0.01
5	Personal social	-0.037	0.55

Kendall's Tau Correlation

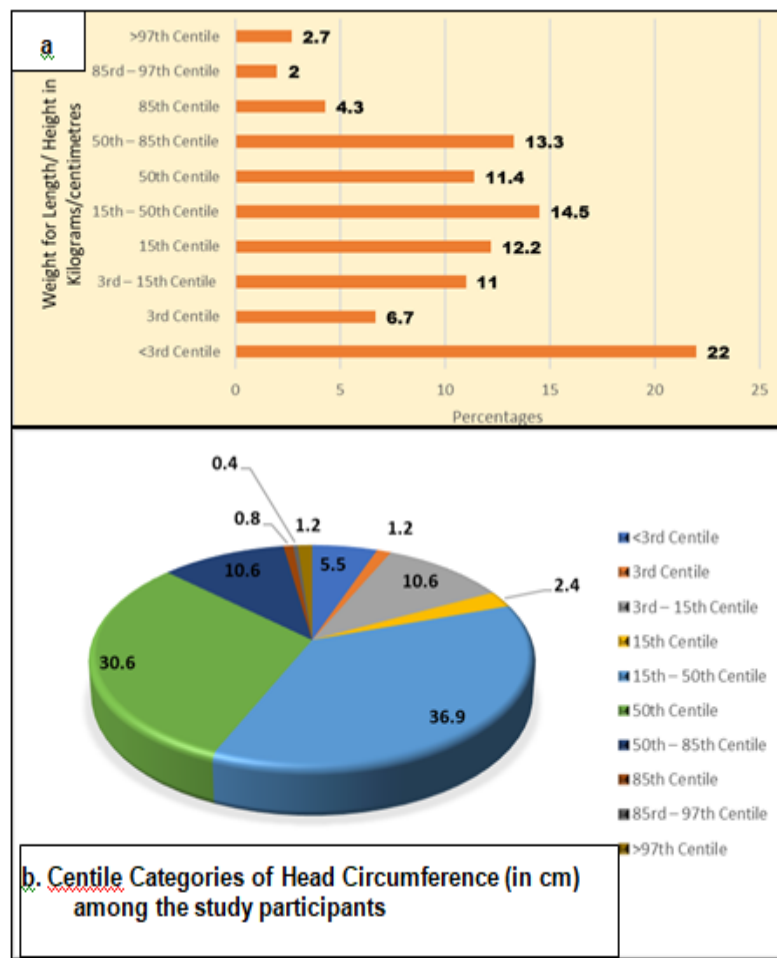
* Correlation is significant at the 0.05 level [2-tailed]

Table 3: Correlation between iron-deficiency anemia and cognitive function: All age, All Gender

N.	Cognitive Function	Iron-deficiency anemia	P-value*
		Correlation Coefficient# [r]	
1	Communication	0.14	0.019
2	Gross motor	0.18	0.002
3	Fine motor	0.41	<0.0001
4	Problem-solving	0.23	<0.0001
5	Personal social	0.12	0.036

Kendall's Tau Correlation

* Correlation is significant at the 0.05 level [2-tailed]

**Fig. 1. Anthropometric details of the study population; (a, b)**

Discussion

The aim of the current study was to find out whether there is a significant correlation between serum iron levels and cognitive function in children less than 5 years of age excluding newborns. We found that there was a significant delay in fine motor and problem-solving skills in children who were iron deficient. However, problem-solving skills were not affected in female children. Another interesting finding in our study was that both genders with ID had a delay in gross motor skills too. A prolonged state of ID can result in IDA with the global anemia prevalence being 47% in children less than 5 years of age [16]. India has more than 80% of its preschool children anemic most likely due to nutritional anemia resulting from ID [16, 17]. It is a well-known fact that the state of the continuum of ID can result in iron depletion and ultimately IDA having severe adverse effects on child health, immunity, growth and development, and maturation of cognitive skills, sometimes resulting in child mortality too [18]. Development of cognitive skills begins right from birth and is dependent on iron since it plays a very important role in brain development, myelination, and axonal development [19, 20].

We selected children aged 1 month to 5 years because this is the most sensitive period of growth with iron playing a vital role in brain development and metabolism in the first 1000 days of life [21]. Our study showed a significant correlation between ID and IDA and fine motor skills. Similar findings were observed in other studies. A study by Parkin PC et al. [22] in 2019 found that higher serum ferritin values were associated with higher cognitive function, as measured by the MSEL-ELC (Mullen Scales of Early Learning-Early Learning Composite), where 4 cognitive skills were assessed - fine motor, visual reception, receptive language, expressive language. Aishwarya V et al [23] studied the impact of ID on cognition and anthropometry from 2019 to 2020 in a medical college in India and found that children with lower hemoglobin levels had lower cognitive scores using the BKT (Binet Kamath Test). The majority of these children were also found to be underweight implying that ID is associated with poorer cognitive scores and motor and physical growth too. In 2003, Bandhu R et al [24]

studied the effect of iron therapy on cognition in anemic school-going boys among 18 anemic and 34 control subjects. Cognitive function were assessed both before and 3 months after iron supplementation using a recording of the P300 wave of AERP (Auditory Event-Related Potentials), RPMT (Raven's Progressive Matrices Test), and DSAT (Digit Span Attention Test). Although even with 3 months of iron therapy the P300 latency of anemic boys improved, they were still slower compared to the controls, but the psychometric scores using RPMT were similar to the anemic group after treatment. Most studies show that even though ID and IDA get corrected with 3 months of iron supplementation, the cognitive function are not corrected or improved post-treatment, implying that ID can be associated with long-lasting cognitive deficits even after treatment. A case-control study done by Ibrahim A et. al from 2010 to 2012 [25] in Cairo, Egypt showed that children with IDA had low height SDS (Standard deviation score), weight SDS and BMI SDS when compared to the controls, which improved significantly after treatment with oral iron. This study inferred that IDA affects both linear growth as well as weight gain which is reversible with iron therapy, thus highlighting the benefit of oral iron supplementation in preschool children.

In our study, anthropometric measurements like weight for age, height for age, and weight for length/height showed no significant association with iron levels ($p > 0.05$). But still, certain observations show that low iron levels may be associated with poorer growth among children with low iron levels as weight for age, height for age, and weight for length/height were lesser than 3rd centile in more than 50% of our children. This is in line with a study by Bhatia D et al [26] in 1993 which showed that children who were anemic had significantly lower weight, weight for age, and height for age with larger numbers in grade 2 and grade 3 malnutrition. This study signified the importance of iron in growth and anthropometric measurements. Other studies done by Ibrahim A et al [27] and Bhatia D [25] also showed that ID was associated with poorer growth in children.

According to the Comprehensive National Nutrition Survey (CNNS) 2016-2018 [28], the

prevalence of ID in Karnataka in children 1 to 4 years of age is 52.9% and 37% in those aged 5 to 9 years. However, in our study, we had 90.2% of children who were iron deficient, with 91.3% of boys and 88.3% of girls being iron deficient. This is slightly higher when compared to WHO [16] estimates of anemia affecting 47% of children less than 5 years. These findings may not be representative of the situation in our area due to our small sample size. The correlation between ID status and cognitive function assessed using ASQ-3 was analyzed using Kendall Tau relation. Our analysis showed that as the ID worsened, poorer cognitive skills were found in the domains of fine motor ($p < 0.0001$) and problem-solving ($p = 0.01$). Although gross motor development is not considered part of cognitive skills, it was also found to be significantly poorer in those with ID ($p < 0.0001$). Various studies have shown almost all domains to be affected by ID and IDA and are comparable with our study. Pala E et al [29] from 2008 to 2009 assessed psychomotor development in ID and IDA children using DDST - II (Denver Developmental Screening Test - II) showing that 67.3% had abnormal DDST -II scores among the IDA group while 21.6% had abnormal DDST-II scores among those with ID with significant impairment in fine motor skills, language, and personal/social function. Nampija M et al [30] in 2022 showed that lower maternal and child Hemoglobin (Hb) levels were associated with reduced psychomotor (fine motor and gross motor) scores at 15 months on 933 Ugandan children, while only lower Hb levels in infancy were associated with reduced language scores at 15 months of age. A study published by Shafir T et al [31] in 2008 showed that infants with ID both with and without anemia had poorer motor function. This is similar to the observation made in our study. Shafir T et al [31] and Kariger PK [32] also showed through their studies that motor function are affected in children who are iron deficient with anemia as well as without anemia. While the study done by Nampija M et al [30] showed that low Hb in infancy is associated with reduced language scores at 15 months, our study showed no correlation between ID and communication skills ($p = 0.06$); however, children with IDA had poorer communication, showing that a correlation exists

between IDA with communication function ($p = 0.019$). The effect of ID without anemia is particularly concerning since ID without anemia is not detected by common screening procedures and is more widespread than IDA. Our analysis further revealed that children between 1 month and 1 year of age had poorer cognitive function only in fine motor ($p < 0.0001$) while in children between 1 year and 5 years of age, poorer cognitive skills were seen in domains of fine motor ($p < 0.0001$), problem-solving ($p = 0.012$) and gross motor ($p = 0.003$) too. An explanation for this would be that, in children less than one year of age, complex cognition is less developed, and hence only the fine motor domain has shown a significant relation. However, as the child grows older between 1 year and 5 years of age, complex cognitive processes dependent on the cerebral cortex like fine motor skills, problem-solving, and gross motor too are affected. Our study finding is similar to a study published in 2022 by East P et al [33] who measured iron status at 6 months, 12 months, and 18 months of age, and verbal intelligence, inattention, and SCT symptoms were assessed at age 10, while memory, processing speed, executive function, and educational attainment was assessed at age 21 years.

Even though we found in our study that certain cognitive skills are significantly affected in children below 5 years of age with ID, there were a few limitations. The samples chosen were not representative of the community since the size was small. Responses given by the parents about their child's development and skill pattern may be subject to recall bias. The sampling technique used was convenient sampling, leading to a scope for selection bias. ASQ-3 is only a screening tool to assess cognitive skills, and we did not conduct a further professional assessment for those children whose scores were below the cut-off. We did not assess the role of other micronutrients that might play a role in cognitive development like Zinc, iodine, and Omega-3 fatty acids.

Conclusions

ID and IDA correlated with poorer cognitive function in children aged 1 month to 5 years. While ID correlated with poorer cognitive skills in the

areas of fine motor skills and problem-solving abilities, IDA was found to correlate with poorer cognitive skills in all 4 areas of cognition-fine motor skills, problem-solving abilities, communication, and personal social skills. Both ID and IDA were also significantly correlated with poorer gross motor skills. Therefore, we recommend that during routine follow-up in the first 5 years of life, and especially in the first two years of life, clinicians should pay attention to determine the risk for ID and IDA through clinical examination and take measures to evaluate and treat these conditions. If the child is not provided with iron at an early age, we recommend universal iron testing at least once before the age of 2 years to start iron supplementation, as anthropometric growth may not indicate ID.

Authors` contribution: DBY: Collection of data, literature review, manuscript preparation, critical review of the manuscript; RN: Literature review, final editing, and critical review of the manuscript.

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Ethical approval

Institutional Ethics Committee approval taken from the Institutional Ethical Committee (ID ethic No. JSSMC/PG/5156/2020-21).

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Conflict of interest

There is no conflict of interest.

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