ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 10 | October - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

"A STUDY ON ROLE OF RETICULOCYTE HEMOGLOBIN-CONCENTRATION IN PREVENTION OF BEHAVIOURAL DISORDERS DUE TO IRON DEFICIENCY"

Paediatrics

Dr Chandra Deve Varma B S K* MD Pediatrics, Associate Professor, Pediatrics, Maharajah's Institute of Medical Sciences, Nellimarla *Corresponding Author

ABSTRACT

Introduction: Iron is required for electron transport, adenosine triphosphate (ATP) production, DNA synthesis, mitochondrial function, neurotransmitter synthesis, and myelination and is critical for brain growth and function. The effects of iron deficiency are anemia and most importantly neurodevelopmental impairments which are long lasting and sometimes irreversible. **Aims:** The main goal of this study was to estimate the prevalence of Iron deficiency in children with behavioural alterations and to know the correlation between these behavioural alterations and iron deficiency. **Materials And Methods:** This is a prospective cohort study done on 250 healthy children with behavioural alterations (irritability, mood swings, poor school performance, loss of appetite and pica) at a tertiary care hospital, south India. Blood samples of these children were collected and sent for red blood cell indices (Hemoglobin, mean corpuscular haemoglobin, mean corpuscular volume, reticulocyte haemoglobin concentration). We performed a statistical comparison between behavioural alterations and red blood cell indices by using a paired t-test and chi-square test for multiple groups. A probability of P<0.05 (two-sided) was used to consider the difference as significant. **Results:** In the present study, the poor school performance, poor appetite, pica are statistically significant with respect to only decreased hemoglobin but not to decreased reticulocyte hemoglobin (CHr), we can correlate these symptoms mainly to manifested iron deficiency anemia rather than latent iron deficiency. Mood swings and irritability are statistically significant with respect to both decreased hemoglobin and decreased chr so we can correlate these symptoms to the stage of latent iron deficiency without manifested anemia. **Conclusions:** Iron deficiency per se causes behavioural disorders with or without anemia. Reticulocyte hemoglobin can be used as a marker for early iron deficiency which is comparatively cheaper than other early iron deficiency markers. All infants and children should be supplemented with iron during latent phase of iron deficiency to prevent behavioural disorders.

KEYWORDS

Iron, Anaemia, Irritability, Pica, Mood swings, Reticulocyte haemoglobin concentration.

INTRODUCTION:

Anaemia is the most prevalent health problem in pregnant women and children of developing countries and the most common cause is the deficiency of micronutrient iron in the diet[1]. Iron deficiency develops in three stages, the prelatent phase where bone marrow iron stores and serum ferritin are reduced, next is latent phase where serum iron and transferrin saturations are reduced and finally the stage of iron deficiency anaemia (IDA) where haemoglobin and hematocrit levels fall[2]. Iron is a part of haemoglobin, myoglobin, cytochromes, and various enzymes. It is required for electron transport, adenosine triphosphate (ATP) production, DNA synthesis, mitochondrial function, neurotransmitter synthesis, and myelination and is critical for early brain growth and function during the foetal period and infancy[3]. IDA can lead to devastating, potentially irreversible consequences like developmental delay, generalized weakness, and learning disabilities in young children if left undiagnosed/ untreated[4]. There is evidence that iron deficiency without anemia causes fatigue, and auditory and visual disturbances, associated with poor cognitive development[5].

Diagnosis of an IDA can be established using a set of serological & biochemical tests, including ferritin, serum iron, transferrin saturation, and total iron-binding capacity[6]. However, these iron parameters can be affected by several physiological & pathological statuses, including inflammations, infections, dietary intake & circadian rhythm which limit their diagnostic value. When the erythrocyte hemoglobin (Hb) content is measured, the diagnosis is delayed as the life span of normal erythrocytes is 120 days. Reticulocyte Hemoglobin decreases earlier, as the reticulocyte life span is 24–48 hours. Reticulocyte Hemoglobin content (CHr) recently emerged as a more accurate parameter to monitor iron stores and reflect the bioavailability of iron[6]. The functional iron available for erythropoiesis over the previous three days measured by CHr reflects the second stage of ID, iron-restricted erythropoiesis, before the development of overt anemia[6].

Pica is habitual ingestion of non-nutritional substances. It is related to iron deficiency per se rather than anaemia[7]. The disappearance of pica after iron administration supports this hypothesis. Many studies have shown that mood disorders and simple febrile seizures may be observed in children with iron deficiency. Iron deficiency irreversibly affects motor, cognitive-behavioral domains of neurodevelopment when occurs during the critical phase of neurological maturation[8]. Studies exploring these elements of ID are very few so we tried to estimate the prevalence of irritability, mood swings, poor appetite, cognitive developmental delay (poor school performance), pica in children with iron deficiency and their correlation with the degree of iron deficiency[9].

MATERIALS AND METHODS:

This is a prospective observational study done on 328 healthy children between five to twelve years of age attending the outpatient clinic of Maharajah's Institute of Medical Sciences, Vizianagaram, and Andhra Pradesh, India from January 2022 to April 2022. The research was started after taking approval from Institutional Ethics Committee. 70 children with chronic systemic diseases, hemolytic anemia, bleeding disorders, C-reactive protein-positive , and children with the history of previous blood transfusions are excluded from the study. Enrollment is done after obtaining written informed consent from parents/guardians. Parents of eight children do not given consent to the study.

The sample size was calculated based on a previous review to detect a 25% difference with an alpha error of 5% and a power of 80%. To account for an attrition rate of 10% for sampling errors 250 children were enrolled in the study. All the children are evaluated for behavioural alterations and blood samples are collected for Red blood cell (RBC) indices haemoglobin (Hb), Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), and Reticulocyte haemoglobin concentration (CHr). The data collected and generated in the laboratory was entered in excel spreadsheets on a computer. The data was then converted to Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, USA) for analysis. The data for age, sex, clinical features, laboratory features were expressed as the geometric mean. All data were expressed as Mean±SD. We performed a statistical comparison by using a paired t-test and chi-square test for multiple groups. A probability of P<0.05 (two-sided) was used to consider the difference as significant and to reject the null hypothesis. The subject recruitment strategy was depicted in *figure 1*.

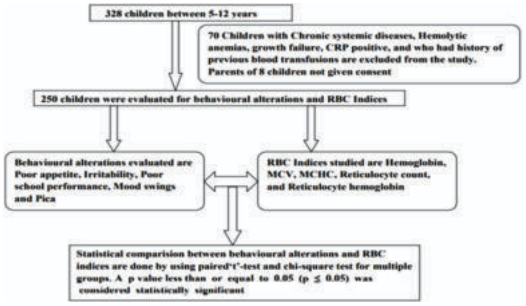

Figure 1: Subject recruitment process

RESULTS:

The mean age of the study participants was 8.96 ± 2.31 years, 28% were in the 5–7 years age group, 36% were in of 8 – 10 years age group and 36% were in of 11-12 years age group of which 28% were male and 72% were female. In the present study, the mean Hb was observed to be 11.44 ± 2.13gm%, 60% had normal Hb levels (>11.5), 12% had mild anemia (11 – 11.4), 20% had moderate anemia (8-10.9) and 8% had severe anemia. In the present study, mean MCH was 24.25 ± 4.39. 76% had MCH <27, 24% had MCV 28-32. In the present study, mean MCV was 71.21 ± 10.12, in 84% had MCV <80, 16% had MCV 81-100. In the present study, the mean reticulocyte hemoglobin (CHr) value was observed to be 22.46 ± 4.34. In 80% they had a CHr value was <27.5 and 20% had CHr>27.6. The prevalence of various behavioral disorders in our study sample are irritability 16%, mood swings 48%, poor school performance 72%, poor appetite 52%, and pica 8%.

In our study in cases with normal Hb levels, 46.6% had mood swings, 86.7% had poor performance, 46.67% had poor appetite and none had PICA and irritability. In cases of mild anemia, 33.3% had mood swings, 66.7% had poor appetite, 33.3% had PCA, 33.3% had irritability and none reported poor school performance. In cases of moderate anemia, 40% had mood swings, 60% had poor school performance, 40% had poor appetite, 40% had irritability and none reported PICA. In cases of severe anemia, all have reported mood swings and poor school performance, 50% have reported having irritability and none reported poor appetite and PICA.

In our study, irritability and mood swings has a statistically significant association with the Hemoglobin, MCV, MCHC, and Reticulocyte hemoglobin (CHr) values respectively. Hence Irritability has a statistically significant association with the occurrence of anemia. In our study, poor school performance has a statistically significant association with hemoglobin and MCH values but not with MCV and CHr. In our study, poor appetite and pica have a statistically significant association with Hb values but not with MCH, MCV, and CHr levels. The units in which RBC indices expressed are Hb in gm/dl, MCV in femtolitres (fl), MCH in pictograms (pg), and CHr in pictograms (pg). The statistical comparison of irritability, mood swings, poor school performance, poor appetite, and pica with RBC indices is shown in *table 1, table 2, table 3, table 4,* and *table 5* respectively.

Table 1 shows association between Irritability and RBC indices

RBC indices	Irritability (%)		Total (%)	P value
	Yes	No		
No Anemia (>11.5)	0	60	60	p<0.0001
Mild Anemia (11 – 11.4)	4	8	12	
Moderate Anemia (8 – 10.9)	8	12	20	
Severe Anemia (<8)	4	4	8	
MCH < 27	16	60	76	p<0.05
MCH < 28 – 32	0	24	24	
MCV < 80	16	68	84	p =0.06
MCV>80 - 100	0	16	16	
CHr < 27.5	16	64	80	P=0.02
CHr > 27.6	0	20	20	

Table 2 Association between Mood swings and RBC indices

RBC indices	Mood Swings (%)		Total (%)	P value
	Yes	No		
No Anemia (>11.5)	28	32	60	p<0.01
Mild Anemia (11 – 11.4)	4	8	12	
Moderate Anemia (8 – 10.9)	8	12	20	
Severe Anemia (<8)	8	0	8	
MCH < 27	44	32	76	p<0.05
MCH < 28 – 32	4	20	24	
MCV < 80	36	48	84	p =0.01
MCV>80 - 100	12	4	16	
CHr < 27.5	44	36	80	P=0.05
CHr > 27.6	4	16	20	

Table 3 shows association between poor school performance and RBC indices

RBC Indices	Poor school performance (%)		Total (%)	P value
	Yes	No		
No Anemia (>11.5)	52	8	60	p<0.001
Mild Anemia (11 – 11.4)	0	12	12	
Moderate Anemia (8 – 10.9)	12	8	20	
Severe Anemia (<8)	8	0	8	
MCH < 27	48	28	76	p<0.05
MCH < 28 – 32	24	0	24	
MCV < 80	60	24	84	p =0.77
MCV>80 - 100	12	4	16	
CHr < 27.5	56	24	80	P=0.37
CHr > 27.6	16	4	20	

Table 4 Shows association between poor appetite and RBC indices

RBC Indices	Poor appetite (%)		Total (%)	P value
	Yes	No		
No Anemia (>11.5)	28	32	60	p<0.02
Mild Anemia (11 – 11.4)	8	4	12	
Moderate Anemia (8 – 10.9)	8	12	20	
Severe Anemia (<8)	0	8	8	
MCH < 27	36	40	76	p<0.22
MCH < 28 – 32	8	16	24	
MCV < 80	36	48	84	p =0.49
MCV>80 - 100	8	8	16	
CHr < 27.5	36	44	80	P=0.68
CHr > 27.6	8	12	20	

Table 5 shows association between pica and RBC indices

RBC Indices	Pica (%)		Total (%)	P value
	Yes	No		
No Anemia (>11.5)	0	60	60	p<0.0001
Mild Anemia (11 – 11.4)	4	8	12	
Moderate Anemia (8 – 10.9)	0	20	20	
Severe Anemia (<8)	0	8	8	
MCH < 27	4	72	76	p<0.25
MCH < 28 – 32	0	24	24	
MCV < 80	4	80	84	p =0.27
MCV>80 - 100	0	16	16	
CHr < 27.5	4	76	80	P=0.16
CHr > 27.6	0	20	20	

DISCUSSION:

Two billion people are living with anemia in the world according to WHO, of which more than half are due to iron deficiency. The identified prevalence of ID is 2.5 times that of anemia as it is a late indicator of ID. As per the estimated prevalence 39% in children aged <5 years, 48% in children aged 5–14 years, 42% in women aged 15–59 years, 30% in men aged 15–59 years, and 45% in adults aged >60 years are anemic in developing countries. In low and middle-income countries, these estimated numbers have important economic and health consequences. Anemia in children has always been a varied entity across various global areas which need a constant evaluation and the present study focuses to know various nonhematological manifestations of IDA in correlation to the laboratory markers for early intervention and treatment.

In this study, it was observed that the CHr, MCH, MCV, Hemoglobin, and irritability had a significant association where irritability is more in cases with decreased CHr, MCH, MCV, and hemoglobin levels. This reflects that irritability is both a feature of ID per se and IDA whereby early intervention and treatment at the stage of ID will prevent the development of manifested anemia and its complications. Piñero and Connor[10] study reported that ID decreases iron concentration in the brain, leading to numerous behavioral symptoms such as irritability, apathy, reduced ability to concentrate, and other cognitive-deficits which are consistent with the present study.

In this study, it was observed that that the CHr, MCH, MCH, hemoglobin, and mood swings had a significant association where as mood swings were more in cases with lesser CHr, MCH, MCV, hemoglobin levels. Mu-Hong Chen et al[11] study revealed iron deficiency increased the risk of psychiatric disorders, including mood disorders, autism spectrum disorder, attention deficit hyperactivity disorder, and developmental disorders. IDA is significantly associated with increased risks of unipolar depressive disorder, bipolar disorder, anxiety disorder, ASD (autism spectrum disorder), ADHD, delayed development, and mental retardation among children and adolescents. Wang et al. updated the evidence of the relationship between iron and

ADHD, and revealed significantly lower serum ferritin levels in children with ADHD than in controls. Benton D et al stated that iron status particularly if occurring over a long duration may adversely affect cognitive-motor development, as well as impair neuro psychological and social-emotional functioning and predispose children to anti-social behaviour in the short and long term.

In this study, it was observed that the MCH, hemoglobin levels, and poor school performance had a significant association. Poor school performance was significantly associated with the MCH, Hemoglobin levels where as poor school performance was more in cases with lesser MCH, Hb levels. There is no significant association between MCV, CHr and poor school performance. This implies that poor school performance is more common in the stage of IDA rather ID per se. S Seshadri et al[12]. Study revealed impact of the iron supplementation on cognitive function of the anemics was significantly higher than the nonanemics which are in consistent with the present study. Falkingham and colleagues[13] found no improvement in overall IQ from iron supplementation, but they reported improvements in IQ among participants with anemia and improvements in attention and concentration among adults and children older than 6 years of age which is consistent with the present study. Arcanjo et al[14] study showed that children with Deficient initial reading skills (DIRS) had lower mean levels of Hb and serum ferritin when compared to those without DIRS. They also found a positive association between DIRS and anemia; anemia was significantly associated with an increased risk of DIRS which also again consonant with the present study. Halterman et al[15]. Demonstrated that subjects with IDA had more than twice the risk of scoring below average in scholastic achievement than the children with a normal iron status by his study on 5398 children and adolescents aged 6 to 16.

J W Lawless et al[16]. Study revealed Iron supplementation improves appetite and growth in anemic Kenyan primary school children. Hanin Ghrayeb et al[17] study revealed IDA patients have a reduced appetite and treating IDA enhances appetite. A K Topaloglu et al[18] study examined the changes in plasma leptin levels in relation to expected improvement in appetite with iron treatment in children with iron deficiency. Our study reflects that poor appetite correlates more with the stage of IDA rather than ID per se.

In this study it was observed that the Hb levels and PICA had a significant association. PICA was significantly associated with the Hb levels, where PICA was more in cases with lesser Hb levels. CHr, MCV, MCH, and PICA have no statistically significant association. This implies that PICA is more common at the stage of iron deficiency anemia rather than iron deficiency per se. Diana Miao et al[19] study revealed pica is significantly associated with increased risk for anemia and low Hb, Hct. Barton et al[20] study revealed patients with pica have lower MCV, higher RDW, and higher platelet counts than patients without pica.

In present study, prevalence of poor-school performance, mood-swings, poor-appetite, and irritability, and pica in children with ID with or without anemia were 70%, 55%, 45%, 20%, and 5% respectively. As the poor school performance, poor-appetite, picas are statistically significant with respect to only decreased Hemoglobin but not to decreased CHr, we can correlate these symptoms mainly to manifested iron deficiency anemia rather than latent iron deficiency. As the mood swings, irritability are statistically significant with respect to both decreased hemoglobin and decreased CHr we can correlate these symptoms to the stage of latent iron deficiency without manifested anemia, iron deficiency per se causes mood disorders with or without anemia, severity increases with increase in the degree of iron deficiency, symptoms being more severe in anemic children. N.Albouradi et al[21] study revealed a high prevalence of LID (latent iron deficiency) and IDA(iron deficiency anemia) among apparently healthy children who visited outpatient department. According to his study ID without anemia (i.e., LID) is diagnosed when hemoglobin (Hb)≥11 g/dl and reticulocyte-hemoglobin(CHr)<26 pg, whereas IDA is diagnosed when Hb<11g/dl and CHr<26 pg, after adjustment for age of children. His study showed prevalence of irritability, mood-swings, loss of appetite, and Pica syndrome in children with ID (with or without anemia) was 37%, 36%, 24%, and 12.5% respectively. In Powers JM et al[22], study, they have observed iron deficiency have lead to many learning abnormalities and constitutional symptoms which is in consistent with the present study. In Burner AB et al[23]., study, out of the 81 enrolled girls, Girls who received iron performed better on a test of verbal learning and memory than girls in the control group ($p<0.02$) according to a regression analysis. Iron supplementation improved verbal learning and memory, in this urban population of non-anemic iron-deficient adolescent girls.

CONCLUSION:

The behavioral alterations like mood swings and irritability are associated with iron deficiency without manifested anemia and some abnormalities like poor school performance, poor appetite and pica are associated with manifested iron deficiency. Reticulocyte hemoglobin can be used as a marker for early iron deficiency which is comparatively cheaper than other early iron deficiency markers limitation being availability of specific analyzers. The early diagnosis of iron deficiency using reticulocyte-hemoglobin concentration (CHr) and supplementation of iron prevents many behavioural disorders in children.

REFERENCES:

1. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009 Apr;12(4):444-54. doi: 10.1017/S1368980008002401. Epub 2008 May 23. PMID: 18498676
2. Cook JD. Clinical evaluation of iron deficiency. Semin Hematol. 1982 Jan;19(1):6-18. PMID: 6763340.
3. Dallman PR. Biochemical basis for the manifestations of iron deficiency. Annual Review of Nutrition. 1986 ;6:13-40. DOI: 10.1146/annurev.nu.06.070186.000305. PMID: 3524613.
4. Youdim MB, Yehuda S. The neurochemical basis of cognitive deficits induced by brain iron deficiency: involvement of dopamine-opiate system. Cell Mol Biol (Noisy-le-grand). 2000 May;46(3):491-500. PMID: 10872737
5. 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 May;64(5 Pt 2):S34-43; discussion S72-91. doi: 10.1301/nr.2006.may.s34-s43. PMID: 16770951; PMCID: PMC1540447.
6. Parodi E, Giraudo MT, Davitto M, Ansaldi G, Mondino A, Garbarini L, Franzil A, Mazzone R, Russo G, Ramenghi U. Reticulocyte parameters: markers of early response to oral treatment in children with severe iron-deficiency anemia. J Pediatr Hematol Oncol. 2012 Aug;34(6):e249-52. doi: 10.1097/MPH.0b013e3182588996. PMID: 22810756.
7. Kettaneh A, Eclache V, Fain O, Sontag C, Uzan M, Carbillon L, Stirnemann J, Thomas M. Pica and food craving in patients with iron-deficiency anemia: a case-control study in France. Am J Med. 2005 Feb;118(2):185-8. doi: 10.1016/j.amjmed.2004.07.050. PMID: 15694906.
8. Lozoff B. Early iron deficiency has brain and behavior effects consistent with dopaminergic dysfunction. J Nutr. 2011 Apr 1;141(4):740S-746S. doi: 10.3945/jn.110.131169. Epub 2011 Feb 23. PMID: 21346104; PMCID: PMC3056585.
9. Kim J, Wessling-Resnick M. Iron and mechanisms of emotional behavior. J Nutr Biochem. 2014 Nov;25(11):1101-1107. doi: 10.1016/j.jnutbio.2014.07.003. Epub 2014 Aug 2. PMID: 25154570; PMCID: PMC4253901.
10. Pinero D. J., Connor J. R. Iron in the brain: an important contributor in normal and diseased states. Neuroscientist. 2000;6(6):435–453. https://doi.org/10.1177/107385840000600607
11. Chen MH, Su TP, Chen YS, Hsu JW, Huang KL, Chang WH, Chen TJ, Bai YM. Association between psychiatric disorders and iron deficiency anemia among children and adolescents: a nationwide population-based study. BMC Psychiatry. 2013 Jun 4;13:161. doi: 10.1186/1471-244X-13-161. PMID: 23735056; PMCID: PMC3680022.
12. Seshadri S, Hirode K, Naik P, Malhotra S. Behavioural responses of young anaemic Indian children to iron-folic acid supplements. The British Journal of Nutrition. 1982 Sep;48(2):233-240. DOI: 10.1079/bjn19820109. PMID: 7115656.
13. Falkingham M, Abdelhamid A, Curtis P, Fairweather-Tait S, Dye L, Hooper L. The effects of oral iron supplementation on cognition in older children and adults: a systematic review and meta-analysis. Nutr J. 2010 Jan 25;9:4. doi: 10.1186/1475-2891-9-4. PMID: 20100340; PMCID: PMC2831810.
14. Arcanjo FP, Arcanjo CP, Santos PR. Schoolchildren with Learning Difficulties Have Low Iron Status and High Anemia Prevalence. J Nutr Metab. 2016;2016:7357136. doi: 10.1155/2016/7357136. Epub 2016 Sep 15. PMID: 27703806; PMCID: PMC5040806.
15. Halterman JS, Kaczorowski JM, Aligne CA, Auinger P, Szilagyi PG. Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. Pediatrics. 2001 Jun;107(6):1381-6. doi: 10.1542/peds.107.6.1381. PMID: 11389261.
16. Lawless JW, Latham MC, Stephenson LS, Kinoti SN, Pertet AM. Iron supplementation improves appetite and growth in anemic Kenyan primary school children. The Journal of Nutrition. 1994 May;124(5):645-654. DOI: 10.1093/jn/124.5.645. PMID: 8169656.
17. Ghrayeb H, Elias M, Nashashibi J, Youssef A, Manal M, Mahagna L, Refaat M, Schwartz N, Elias A. Appetite and ghrelin levels in iron deficiency anemia and the effect of parenteral iron therapy: A longitudinal study. PLoS One. 2020 Jun 4;15(6):e0234209. doi: 10.1371/journal.pone.0234209. PMID: 32497136; PMCID: PMC7272047.
18. Topaloglu AK, Hallioglu O, Canim A, Duzovali O, Yilgor E. Lack of association between plasma leptin levels and appetite in children with iron deficiency. Nutrition. 2001 Jul-Aug;17(7-8):657-9. doi: 10.1016/s0899-9007(01)00570-6. PMID: 11448591.
19. Miao, D., Young, S.L. and Golden, C.D. (2015), A meta-analysis of pica and micronutrient status. Am. J. Hum. Biol., 27: 84-93. https://doi.org/10.1002/ajhb.22598
20. Barton, J. C., Barton, J. C., & Bertoli, L. F. (2010). Pica associated with iron deficiency or depletion: clinical and laboratory correlates in 262 non-pregnant adult outpatients. *BMC blood disorders*, *10*, 9. https://doi.org/10.1186/1471-2326-10-9
21. Albaroudi IN, Khodder M, Al Saadi T, Turk T, Youssef LA. Prevalence, diagnosis, and management of iron deficiency and iron deficiency anemia among Syrian children in a major outpatient center in Damascus, Syria. Avicenna J Med. 2018 Jul-Sep;8(3):92-103. doi: 10.4103/ajm.AJM_169_17. PMID: 30090748; PMCID: PMC6057158.
22. Powers JM, Mahoney DH. Iron deficiency in infants and children< 12 years: Screening, prevention, clinical manifestations, and diagnosis. UpToDate. 2020.
23. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet. 1996 Oct 12;348(9033):992-6. doi: 10.1016/S0140-6736(96)02341-0. PMID: 8855856.