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Original article

Soluble Transferrin Receptor and SFI Index– A new biomarker to identify Iron Deficiency in Drug Naïve Children with ADHD – A Case-Control Study

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Abstract

Background: Children with Attention Deficit Hyperactivity Disorder (ADHD) have been associated with iron deficiency. Among several markers, serum ferritin and serum iron have been used frequently to identify iron deficiency; however, the results are inconclusive.

Aims: To compare soluble transferrin receptor levels (sTfR), sTfR/log ferritin index (SFI), and conventional markers of iron deficiency among drug naïve children with ADHD and healthy controls.

Methods: We conducted this study in a tertiary care setting with a case-control study design. Thirty-five children with ADHD compared with age-matched 35 controls. Children were assessed on clinical aspects, hematology profile, iron biomarkers, inflammatory markers, sTfR, and SFI.

Results: We found significantly reduced mean levels of serum iron and percentage transferrin saturation and an increase in the median levels of Erythrocyte sedimentation rate (ESR) in children with ADHD compared to healthy children. However, in a clinically meaningful categorical analysis, we found that socioeconomic strata, ESR, percentage transferrin saturation, and SFI were significantly different between the groups. Finally, we found that Children with SFI ≥1.5 were approximately four times more likely to be associated with ADHD as compared to the control group. However, the percentage transferrin saturation was not associated with ADHD, after controlling for socioeconomic strata and ESR

Conclusions: SFI is a new marker, which is feasible and a better marker than serum ferritin in the identification of latent iron deficiency in children with ADHD. Serum iron and percentage transferrin saturation were also found to indicate an iron deficiency in children with ADHD. These findings must be explored in large community samples.

Keywords: Attention Deficit Hyperactivity Disorder, Serum Iron, Serum Ferritin, Iron deficiency, soluble transferrin receptor levels (sTfR), sTfR / log ferritin index (SFI)

Introduction

Children with Attention deficit hyperactivity disorder (ADHD) have been associated with iron deficiency. The gold standard to assess iron deficiency is bone marrow examination. However, it is invasive, very painful, and an uncomfortable procedure for the children and affected by recombinant human erythropoietin [1]. Hemoglobin has been used to assess significant iron deficits in the functional Iron in the later stages of iron deficiency and may be normal in pre-latent and latent stages of iron deficiency [2]. Serum ferritin identifies the residual iron stores. Hemoglobin, serum ferritin, transferrin, and percentage transferrin saturation can be affected due to inflammation, infections, or malignancy [3]. Serum iron levels have a diurnal variation and can be influenced by gender. Total iron binding capacity is dependent on the serum albumin levels and can show false values. Hence, most of the conventional markers have a limitation in their utility as sensitive and specific biomarkers of iron deficiency. Currently, there are three systematic reviews and meta-analysis and one meta-analysis which have evaluated peripheral iron using serum iron and serum ferritin levels in children with ADHD, respectively. Two studies have primarily investigated peripheral serum ferritin levels only; the review by Tan et al [4] found that SF levels were associated with susceptibility to childhood ADHD (mean difference = −23.09, 95% CI = −33.06 to −13.13, Z = 4.54, $p < 0.001$), HE: $p = 0.003$) and in the review by Scassellati et al [5], they found lower serum ferritin in ADHD than control (d = −0.86, Z = 2.52, p = 0.01), HE: p < 0.001). Ferritin is an acute-phase reactant, and levels could have been affected by infection, inflammation, and oxidative stress and hence may not give an accurate evaluation of iron storage. A systematic review and meta-analysis by Wang et al. [6] found that lower serum ferritin is associated with ADHD in children, rather than serum iron (SMD = - 0.40, 95% CI = -0.66 to - 0.14), raising doubt on the clarity on the association between serum iron and ADHD. However, employing a sensitivity analysis, another recent systematic review and meta-analysis by Tseng et al. [7] in a subgroup analysis revealed a significantly lower serum iron level in the children with ADHD than in those without ADHD (Hedges' $g = -0.186$, 95% CI = −0.323 to −0.048, $p = 0.008$). This review provided preliminary evidence that deficient iron storage in children with ADHD may be involved in the pathophysiology of ADHD. They summarised that very few studies had assessed these relationships in drug-free children with ADHD and in those who have not been on iron supplementation. There is a need to identify Iron deficiency in children with ADHD who are drug naïve, with new markers that are not influenced by inflammation or infections.

Hence, we used a new marker that has been used in few studies to assess iron deficiency known as soluble transferrin receptor levels (sTfR). sTfR is a protein, which is a single polypeptide chain of 85 kDa, derived from the transferrin receptor (a transmembrane cellular protein of 190 kDa) primarily expressed in cells that require iron and can be measured in human serum. It is closely related to cellular iron demands and to erythroid proliferation rate. [8, 9] It has been found to be a specific and sensitive indicator of tissue iron stores [10], not affected by chronic inflammatory conditions, and was found to be superior to serum ferritin [11]. Further, it was suggested that sTfR/ferritin ratio could be a better estimate of body iron, but most of the studies did not find any improvement in the diagnostic efficiency compared to sTfR alone [10]. However, sTfR / log ferritin values called as sTfR/log ferritin ratio, or index (SFI) (log refers to base-10 log and not to natural log) has been found to be an outstanding indicator of iron depletion; it considers the reciprocal relationship between the two variables influenced by iron deficiency (an increase of sTfR and decrease of ferritin concentrations) [8]. This index could increase the diagnostic efficacy of sTfR alone in differentiating iron deficiency anaemia from anaemia of chronic disease (ACD), as well as mixed iron deficiency anaemia and anaemia of chronic disease from ACD alone [12]. The aim of this study is to compare the conventional markers and the new markers - sTfR and SFI in healthy children and in those with ADHD.

Methods

We used a case-control study design using children presenting to the out-patient settings of the Child Psychiatry services of St. John's Medical College Hospital, Bangalore. Boys between the age group of 5-16 years and presenting to the child psychiatry services were evaluated for ADHD, and those diagnosed to have ADHD according to DSM-IV-TR by a psychiatrist were recruited in the study. We also chose only those children who were drug naïve; those who were recently diagnosed to have ADHD and have not received any psychological or pharmacological treatments for ADHD currently or in the past. Children with epilepsy, a diagnosis of other defined psychiatric illness and known global cognitive disabilities and those receiving iron supplements within one year of our assessment were excluded. Also, those with other etiologies of anaemia, such as hemolytic anaemia or known vitamin B12/folate deficiency were excluded based on the history from the parents and children; we did not use any lab-based tests for this exclusion. Those with first or second-degree relatives diagnosed with Alcoholism, as defined by the Family Interview for Genetic Studies (FIGS) interview, were excluded from the study. Age-matched healthy children from the same locality were recruited so that the children with ADHD and the healthy children were exposed to the same environment. The parents of the children with ADHD were requested to contact their neighbors, and a visit date was given. The boys were screened for inclusion and exclusion criteria for the healthy children category, and if they fulfilled the criteria, they were enrolled into the study. We had obtained the approval of the Institutional Ethics Committee at the St. John's Medical College Hospital, Bangalore before the children were enrolled in the study. We enrolled 35 Boys with ADHD and 35 age-matched boys who were healthy controls. Informed consent was obtained from the parents and assent from children above seven years. Children were assessed on a socio-demographic questionnaire that captured age, class, height, weight, body mass index (BMI), clinical features of anaemia, cardiac examination, family history, and standard of living index. Pediatric Sleep Evaluation Questionnaire was used to assess the sleep quality, and the parents were assessed on the Adult ADHD Self-Report scale (ASRS-V 1.1) [13] symptom checklist to document possible Adult ADHD in them. Screening questionnaire: Restless Legs Syndrome - Parent Version [14] was used to assess restless legs syndrome in the children and Conners 3rd Edition™ (Conners 3™)- Parent version [15] was used to assess the severity of ADHD in the children. Anesthetic patches were used before drawing of blood so that the procedure would be painless. Then, we collected 10ml of blood for the lab parameters for assessing the hematology profile, inflammatory markers, and iron profile. They included standardized tests such as complete blood counts using automated hematology analyzer (SulpholyerHb, flow cytometry, Hydrodynamic focusing method, photometric measurement on XT 2000i Supra vital and westegren), C Reactive Protein (PETIA), Serum Iron (Photometric Ferene), Total Iron Binding Capacity (Photometric Ferene), Serum Ferritin (CLIA) and sTfR (Nephelometry). We have taken percentage Transferrin of ≤ 16%, Serum Ferritin of ≤ 16 µg/l, and normal Hb as latent iron deficiency [16] and Serum Iron ≤ 50 µg/dl [17]. We also repeated sTfR levels on a random basis in five ADHD and five Healthy Children to explore the reliability of the values. The sTfR values have been found to be lower in Indian children compared to Asian and western data. Hence, we took a higher cut off > 2.1 for this study [18]. We calculated the STfR / log ferritin index by dividing STfR levels by log of ferritin values. We took a cut of score of more than and equal to 1.50 as suggestive of Iron deficiency [19].

The continuous data was represented as means (standard deviations), median (Interquartile range), and the categorical data as frequencies. An independent sample t-test for continuous variables with parametric distribution, Mann Whitney U test for continuous variables with non-parametric distribution, and Chi-square test for categorical variables were used to compare the differences between the two groups. We also used correlation analysis between the continuous scores using Spearman's correlation to assess the relationship between important iron biomarkers and ADHD severity scores. Multivariate binary logistic regression analysis was performed to predict which biomarkers were significantly associated with ADHD. A p-value of < 0.05 was taken as statistically significant. Statistical analysis was done using SPSS for Windows (SPSS software package, version 15, SPSS Inc., Chicago, Illinois).

Results

The mean age of the children with ADHD was 9.46 ± 2.91yrs and the healthy children was 9.71 ±3.36 yrs. without any significant differences (p = 0.73). There were no significant differences in the mean scores of heights (ADHD =132.31 ±18.42 cms vs controls =133.13 ± 17.33 cms, p value = 0.85), weight (ADHD = 30.13 ±11.74 kgs. vs controls = 29.77 ± 11.58 kgs., p value = 0.89), BMI (ADHD = 16.85 ±5.25 kg/m2 vs controls = 16.22 ± 2.83 kg/m2, p value = 0.53), pulse rate (ADHD = 81.17 ±5.98 per min vs controls = 80.40 ±6.70 per min, p value = 0.225), systolic blood pressure (ADHD =107.65 ±7.99 mm of Hg vs controls =

107.42 ±6.59mm of Hg, p value = 0.895) & diastolic blood pressure (ADHD = 75.31 ±5.44 mm of Hg vs controls = 73.77 ±5.54 mm of Hg, p value = 0.244). The severity scores of the children with ADHD based on Conners' 3 subscores includes Inattention = 81.89 ±8.06, Hyperactivity-Impulsivity = 85.20 ±7.47, Global Index total = 75.46 ±11.64, Inattention subscores (based on DSM-IV-TR criteria) = 79.03±10.33 and Hyperactive-Impulsive subscores (based on DSM-IV-TR criteria) = 82.86 ±9.62. None of the children had significant sleep disturbances or restless leg syndrome in our study.

There was a significantly decreased mean level of serum iron and percentage transferrin saturation and an increase in the median levels of ESR in children with ADHD compared to healthy children. There were no significant differences in the mean values of hemoglobin, total white blood cell counts, neutrophils, lymphocytes, eosinophils, platelet count, packed cell volume, red blood cell, mean corpuscular volume, total iron-binding capacity or serum ferritin between the two groups. There were no significant differences in the median values of reticulocyte count, C reactive protein, sTfR, and SFI between the two groups (Table-1).

Table-1: Mean and median differences among children with ADHD & Healthy Children

	ADHD (n= 35) Mean ±S.D	**Control (n= 35) Mean ±S.D**	**P value**
Hb (g/dl)	12.81±1.08	13.10 ±0.95	0.238
TLC (x $10^3/\mu l$)	9.34 ±2.12	9.04 ±2.81	0.620
Neutrophil %	52.89 ±11.04	52.71 ± 8.69	0.936
Lymphocytes %	39.41 ±11.10	38.69 ±7.82	0.754
Eosinophils %	4.42 ±3.19	4.64 ±3.82	0.800
Platelet count (x $10^3/\mu l$)	3.12±0.66	3.14±0.72	0.902
PCV %	37.75 ±2.18	38.52 ±2.69	0.196
RBC (million/ µl)	4.75 ±0.37	4.82 ±0.34	0.374
MCV (fL)	77.87 ±4.47	76.58±12.94	0.579
SF (ng/ml)	32.64±21.31	34.62 ±23.82	0.715
S. Iron (µg/dl)	56.63 ±27.78	71.54 ±26.01	0.023
TIBC(µg/dl)	389.54±65.56	372.46±50.56	0.226
%TS	14.50 ±7.41	19.79 ±8.35	0.007
	Median (IQ= 25 -75)	**Median (IQ =25 - 75)**	
RC %	0.64 (0.46 - 0.92)	0.86 (0.58 – 0.94)	0.165

ESR (mm/hr)	7 (5 – 7)	5 (2 – 6)	0.001
CRP (mg/dl)	0.10 (0.06 – 0.13)	0.09 (0.05 – 0.14)	0.202
STFR (mg/L)	1.72 (1.38 – 2.11)	1.67 (1.53 -1.91)	0.521
SFI	1.15 (0.86 – 1.64)	1.19 (0.92 – 1.37)	0.274

Independent sample T test used Hemoglobin (Hb), Total White blood cell counts (WBC), Neutrophils, Lymphocytes, Eosinophils, Platelet count (PC), Packed Cell Volume (PCV), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Serum Ferritin (S.Ferritin), Serum Iron (S. Iron), Total Iron Binding Capacity (TIBC), % transferrin saturation (%TS), between the two groups.
Mann Whitney U test used for Reticulocyte count (RC), Erythrocyte Sedimentation Rate (ESR), C Reactive Protein (CRP),Serum Transferrin Receptor Ferritin (sTfR) and sTfR log Ferritin Index (SFI) between the two groups. A p value of < 0.05 was taken as statistically significant.

On evaluating the clinically meaningful categorical variables, there were significantly more number of children from the higher socioeconomic strata in the ADHD group [22 (62.85%)] compared to the healthy control group [28 (80%)] who came from middle strata. Among the various markers used for iron deficiency, percentage transferrin saturation was found to be significantly lower in 20/35 children with ADHD group (57.14%) compared to 11 healthy children (31.42%). This difference was statistically significant (p = 0.030) (Table 2). Similarly, on using cut off of ≥1.5, the SFI was higher in children with ADHD [14/ 35 (40%)] compared to the healthy group (5/35 [14.28%]). This difference in the number of children above the cut-off was also statistically significant (p = 0.015). We also found that there were significantly more children who had higher levels of ESR in the ADHD group [5/35 (14.28%)] compared to none in the control group. There were no significant differences in other lab parameters between the two groups (Table-2).

Table-2: Difference in the categorical variables between the children with ADHD and healthy children

Variables	Categories	ADHD Children (n=35)	Healthy Children (n=35)	P value
Class	Kindergarten	4	2	0.607
	Primary	26	26	
	Secondary	5	7	

BMI	Normal	28	29	.314
	Overweight	3	5	
	Obese	4	1	
SLI	Low	2	2	0.01
	Middle	11	28	
	High	22	5	
Hb	Low	5	4	0.50
	Normal	30	31	
TLC	Normal	34	33	0.50
	High	1	2	
Neutrophil %	Normal	13	10	0.445
	High	22	25	
Lymphocytes %	Low	21	26	0.154
	Normal	14	9	
Eosinophils	Normal	35	35	NA
Platelets	Normal	35	35	NA
ESR	Normal	30	35	0.02
	High	5	0	
PCV	Normal	35	35	NA
RBC	Normal	35	35	NA
Reticulocyte count	Normal	35	35	NA
MCV	Low	7	5	0.376
	Normal	28	30	
CRP	Normal	32	35	0.120
	High	3	0	
TIBC	Normal	29	33	0.133
	High	6	2	
%TS	Low (≤ 16%)	20	11	0.030
	Normal (≥17%)	15	24	
S.Iron	Normal (≥51µg/dl)	22	25	0.306
	Low (≤ 50µg/dl)	13	10	
SF	Low (≤16 µg/l)	12	8	0.214
	Normal (>17 µg/l)	23	27	
sTfR	Normal (≤ 2 mg/l)	27	32	0.101
	High (≥ 2.1 mg/l)	8	3	
SFI	Normal (≤1.4)	21	30	0.015
	High (≥1.5)	14	5	

Chi square test used. Body mass index (BMI), Standard of living Index (SLI), Hemoglobin (Hb), Total White blood cell counts (WBC), Neutrophils, Lymphocytes, Eosinophils, Platelet count (PC), Packed Cell Volume (PCV), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Reticulocyte count (RC), Erythrocyte Sedimentation Rate (ESR), Serum Ferritin (S.Ferritin), Serum Iron (S. Iron), Total Iron Binding Capacity (TIBC), % transferrin saturation (%TS), C Reactive Protein (CRP), soluble Transferrin Receptor Ferritin (sTfR) and sTfR / log Ferritin Index (SFI) between the two groups. A p value of < 0.05 was taken as statistically significant.

Spearman's correlation test revealed that percentage transferrin saturation correlated significantly with the Hyperactivity subscores, Global Index, and DSM-IV-Hyperactive-Impulsive subscores of the Conners - 3 scales in children with ADHD. Also, serum iron correlated significantly with the Global Index and DSM-IV-TR Hyperactive-Impulsive subscores. However, ESR, Serum Ferritin, sTfR, or SFI did not show any significant correlation with the severity of ADHD (Table-3).

Table-3: Correlation between important Iron indices and severity scores of ADHD (Conners 3 - Parent version) in children with ADHD (n=35)

		Inattention	**Hyperactivity**	**Global Index**	**DSM-IV-TR Inattention Score**	**DSM-IV-TR Hyperactive-Impulsive scores**
ESR	ρ	- 0.004	0.134	- 0.122	- 0.053	- 0.018
	P value	0.980	0.442	0.483	0.761	0.916
%TS	*ρ*	0.098	0.341	0.370	0.222	0.463
	P value	0.574	0.045	0.029	0.199	0.015
sTfR	*ρ*	0.020	-0.087	-0.321	0.082	-0.223
	P value	0.910	0.619	0.060	0.639	0.197
S. Iron	*ρ*	0.178	0.187	0.408	0.298	0.460
	P value	0.306	0.281	0.015	0.082	0.021
SFI	*ρ*	0.137	-0.187	-0.077	0.231	-0.205
	P value	0.432	0.283	0.659	0.182	0.237

Spearman's Correlation used and correlation co-efficient represented as *ρ* (rho), 2-tailed analysis. Erythrocyte Sedimentation Rate (ESR), Percentage Transferrin Saturation (%TS), Serum Iron (S. Iron), soluble Transferrin Receptor Ferritin (sTfR) and sTfR / log Ferritin Index (SFI). A p value of < 0.05 was taken as statistically significant.

Multivariate binary logistic regression analysis was performed to assess the association between SFI and percentage transferrin saturation with ADHD in comparison with control, adjusted for socioeconomic strata (SLI), and ESR. The analysis was carried out separately for SFI and percentage transferrin saturation, based on the univariate analysis. In Model 1, we found that SFI was significantly associated with ADHD, even after adjusting for socioeconomic strata (SLI) and ESR. Children with SFI ≥1.5 were approximately four times more likely to be associated with ADHD as compared to the control group (AOR = 3.97, 95% CI: 1.03 - 15.23). However, in Model 2, the percentage transferrin saturation was not associated with ADHD, after controlling for socioeconomic strata and ESR (Table-4).

Table-4: Regression coefficients and odds ratios (and 95% confidence intervals) for iron parameters (SFI and %TS) associated with ADHD in children (n= 70 clubbing cases and controls) after adjustment for potential confounding factors

Model 1							
Variables	**B**	**S.E**	**Wald χ2**	**AOR**	**95% C.I.**		**P value**
					Lower	**Upper**	
SFI (≥1.5)	1.379	0.686	4.044	3.971	1.036	15.230	0.044
SLI	-1.649	0.598	7.595	0.192	0.060	0.621	0.006
ESR	-0.226	0.084	7.240	0.798	0.677	0.940	0.007
Constant	2.619	1.096	5.709	13.718			0.017
Model 2							
%TS (≤ 16)	0.649	0.592	1.202	1.913	0.600	6.105	0.273
SLI	-1.562	0.552	8.003	0.210	0.071	0.619	0.005
ESR	-0.206	0.084	5.985	0.814	0.690	0.960	0.014
Constant	3.029	1.058	8.197	20.678			0.004

Multivariate logistic regression analysis done. Variables entered on model 1: SFI, SLI, ESR and df=1. Variables entered on model 2: %TS, SLI, ESR and df=1. AOR = adjusted odds ratio, B = unstandardized regression weight , SE (B) =standard error, Wald χ2 = Wald chi-square test, AOR=adjusted odds ratio, SFI = soluble Transferrin Receptor Ferritin (sTfR) / log Ferritin Index, SLI = standard of living index, ESR=Erythrocyte Sedimentation Rate, %TS= percentage transferrin saturation

Discussion

The main aim of the study was to assess the role of a new marker sTfR and SFI in identifying iron deficiency in children with ADHD. We found that significantly more children with

ADHD [14 (40%)] were found to have a higher level of SFI compared to healthy children. This is the first clinical study reporting significantly high SFI (a new marker and an index) in children with ADHD. Even after adjusting for the effects of socioeconomic strata (SLI) and ESR, the SFI was significantly associated with ADHD. This corroborates findings by Punnonen et al. [20] and Skikne [8]. These combinations of new markers can be relatively non-invasive indices compared to bone marrow examination for identifying iron deficiency [19]. In the future, using SFI may be recommended than other individual tests.

We did not find any significant difference using the sTfR or serum ferritin per se. The only association we got with STfR was that it correlated significantly with serum iron (r = -.340, p-value = 0.046) in children with ADHD. In keeping with a previous report [9], we find that serum ferritin may not be a reliable marker of iron deficiency.

There was a significantly lower mean level of serum iron in children with ADHD compared to healthy controls. Out of 20 studies on conventional markers of iron, only nine studies have used serum iron. We found a lower value of serum iron in our study compared to other studies, including two studies done in India by Mahajan et al. [16] & Islam et al. [21]. In Mahajan et al. [16] study in children with behavioural problems, the mean levels of serum iron were 64.6 ± 29.2 µg/dl in children with behavioural problems compared to controls (99.1 ±27.5µg/dl). Also, only 4 (12.5%) of their children with behavioural problems were in the latent period of iron deficiency. In the study by Islam et al. [21], all the iron parameters such as haemoglobin, serum iron, serum ferritin, mean corpuscular volume, and mean corpuscular haemoglobin were lower among children with ADHD suggesting that most of them were already having marked iron deficiency anaemia. However, in our group, despite having lower serum iron, percentage transferrin saturation, and higher SFI, the other conventional parameters of iron were still normal, suggesting that 40% of the children with ADHD were still in the pre-latent or latent stage of iron deficiency. This finding that children with ADHD may be in pre latent or latent stages of iron deficiency is supported by a recent meta-analysis by Tseng et al. [7] who found lower serum iron levels in the children with ADHD compared to the controls after excluding a potentially confounding study. Also, they reported that children with iron deficiency were more likely to have ADHD and have more severe ADHD symptoms than those without iron deficiency. We also found that serum iron correlated significantly with the Global index and Hyperactive-Impulsive subscores (based on DSM-IV-TR criteria) of the Conners 3 - Parent version. These findings suggest that serum iron is still an important conventional marker in assessing early stages of iron deficiency as well as the marked stage of iron deficiency anaemia. However, certain precaution such as uniformity in the time of blood collection due to the diurnal effect and the effect of gender differences needs to be kept in mind.

We found a significantly lower mean value of percentage transferrin saturation in children with ADHD compared to healthy controls (Table 1). After categorizing the children into those with normal and lower levels of percentage transferrin saturation, about 20/35 children with ADHD (57.14%) had low levels compared to only 11/35 in the healthy controls (31.42%), this difference was statistically significant ($p = 0.03$). Also, percentage transferrin saturation correlated significantly with the hyperactivity subscores, Global index, and DSM-IV-TR Hyperactive-Impulsive scores of the Conners - 3 - Parent version and with Serum Iron ($\rho = 0.919$ p-value $< .0001$) in the children with ADHD. The percentage transferrin saturation is obtained by the equation that divides serum iron by total iron binding capacity, and then multiplied by 100, and it implies the percentage of how much iron is bound to the blood protein transferrin. Hence it is significantly associated with the serum iron. Very few studies have reported percentage transferrin saturation, a study by Mahajan et al. [16] in India found significantly lower mean levels of percentage transferrin saturation in children with behavioural problems [12 (47.7%) of them were ADHD children] (17.9 ±7) compared to healthy controls (26.9 ±6.7) (p-value < 0.001). The mean values were even lower in our study, and these findings suggest that percentage transferrin saturation could also remain a potential marker to identify Iron deficiency in children with ADHD, if facilities to assess sTfR are unavailable.

We found a significant increase in the levels of ESR in children with ADHD compared to healthy controls, explained by about five children in the ADHD sample. However, other markers of inflammation, such as Total WBC and CRP were not significantly different between the two groups. Even after adjusting for the effect of socioeconomic strata (SLI) and ESR, it was found that the SFI was significantly associated with ADHD. Some authors have proposed an association with ADHD and inflammatory mechanisms due to positive findings related to inflammation-related genes; also, cytokines have been implicated in tryptophan metabolism and dopaminergic pathways in the brain in ADHD [22]. This is important in the context of assessing Iron deficiency since inflammation can affect some of the markers such as serum iron, total iron-binding capacity, Transferrin, percentage transferrin saturation, and serum ferritin levels. These are influenced by acute phase responses and can affect the clinical interpretation of the results [11]. However, sTfR is not affected by inflammation. Hence, future research must review this aspect and be cautious before interpreting the results in the context of significant inflammation.

We are suggesting a three-stage evaluation for iron deficiency and iron-deficiency anaemia in children with ADHD. Initially, it is advisable to do haemoglobin levels; if it is low, then there is no need to do further iron levels. However, if it is normal, then further, we can consider the percentage transferrin saturation. If both haemoglobin and percentage transferrin saturation are normal then we can consider doing sTfR and SFI (doing serum ferritin alone may not be useful due to the drawbacks), because the children with ADHD may be having early stages of iron deficiency, which may not have manifested through the conventional markers.

Identification and treating the iron deficiency in early stages can help reduce iron deficiency anaemia and improve the ADHD symptoms. Even though we have found that mean serum iron was significantly different between the groups initially, the clinically meaningful categorical analysis did not reveal any significant difference. Multivariate binary logistic regression analysis found that SFI was significantly associated with ADHD, even after adjusting for socioeconomic strata (SLI) and ESR. Children with SFI ≥1.5 were approximately four times more likely to be associated with ADHD as compared to the control group (AOR = 3.97, 95% CI: 1.03 - 15.23). Percentage transferrin saturation did not show any significant association with ADHD scores after adjusting for socioeconomic strata and ESR levels.

It is also useful to point out that newer markers such as Hepcidin have been found to be a very powerful indicator of physiological iron deficiency. Hepcidin is a recently discovered 25-amino acid peptide secreted by the liver and a key regulator of iron metabolism. It down-regulates duodenal iron absorption and macrophage iron release. It can be measured in human urine, plasma, and serum. Also, it can be used as a potential marker for the detection and differentiation of anaemia of chronic disease, anaemia of chronic disease with concomitant iron-deficiency anaemia, and pure iron-deficiency anaemia [23, 24]. Comparative studies of this new marker and the iron markers used in our study are yet to be done in children with ADHD.

The strength of our study is that we included children with ADHD who were drug-free and had strict inclusion and exclusion criteria. We also used all the conventional peripheral iron markers besides newer markers. We used only the parent version of the Conners 3; even though we attempted to contact the teachers through the parents, we could not get the teacher's report of severity of ADHD using Conners 3 – teacher's version. Some of the limitations of our study include not assessing the children's diet (Vegetarian or Non-

Vegetarian), not collecting the obstetric history of the children, and we did not do any lab-based tests to rule out other etiologies of iron deficiency.

To conclude, we have found that SFI is potentially a new marker, which is feasible and a better marker than serum ferritin in the identification of latent iron deficiency in children with ADHD. We also found that other conventional markers such as serum iron and percentage transferrin saturation can also indicate an iron deficiency in children with ADHD. However, our results suggest that early iron deficiency may be best identified using SFI. These findings need to be evaluated in a larger sample and in a community setting.

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