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Original Article

Relationships Between Serum Superoxide Dismutase, Catalase, Biochemical and Hematological Parameters in Children with Iron Deficiency Anemia

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ABSTRACT

Background and Objectives: Iron deficiency anemia (IDA) is the most widespread type of anemia in children. Reduction in antioxidants can occur due to IDA. The objective of the study was to assess levels of serum superoxide dismutase (SODse), catalase (CATse), biochemical, and some hematological parameters in children with IDA and healthy controls, and to find relationships between them.

Methods: Fifty-five patients with IDA and 35 healthy controls with age ranges of 6 months to less than 6 years were enrolled. Diagnosis of IDA was made based on the low hemoglobin (Hb) and Sf as per age. As the Hb level is <11 g/dl and the Sf level is <12 µg/L in children <5 years old, the Hb level is <11.5 g/dl and the Sf level is <15 µg/L in children aged 5 to <6 years.

Results: The mean age of participants was 3.132±1.67. IDA patients had significantly decreased levels of Hb ($p<0.0001$), hematocrit (Hct) ($p<0.0001$), Sf ($p<0.0001$), Si ($p<0.0001$), and SODse ($p<0.0275$), and non-significantly decreased levels of mean cell hemoglobin concentration (MCHC) ($p=0.1721$) and CATse ($p=0.1620$) compared to healthy controls. IDA patients showed significantly higher levels of red cell distribution width (RDW) ($p<0.0001$) than healthy controls. Parameters were comparable among mild and moderate degrees of IDA; only significantly decreased levels of Sf were detected in children under the age of <5 years. Significantly positive correlations were observed between Hb and Hct ($r=0.8149$, $p<0.0001$). In addition, non-significant positive correlations were found between Hb, Hct, Sf, Si, SODse, and CATse. Significantly negative correlations were observed between RDW and Hb ($r=-0.5522$, $p<0.0001$), Hct ($r=-0.4456$, $p<0.0007$), and SODse ($r=-0.2874$, $p<0.0334$), while non-significant negative correlations were found between RDW and Sf, Si as well as CATse.

Conclusion: The study findings show changes in levels of studied parameters between the IDA and healthy control groups. They also confirm the relationship between the different values of the parameters.

Keywords: Anemia, antioxidant, iron deficiency, preschool children.

1 Introduction

Anemia is the most prevalent hematological disorder seen in children, and it occurs when Hb or Hct or the amount and size of red blood cells (RBC) fall below a specific threshold that depends on age, gender, and physical data[1,2]. Iron deficiency anemia (IDA) occurs as a medical disorder when the blood iron levels drop too low to maintain typical bodily functions; a decrease in Sf, an indicator of the body's iron storage capacity, can diagnose this illness[3]. Iron is an essential mineral for immune system function, brain development, cognitive function, and the healthy operation of several biological processes, particularly erythropoiesis in children. Inadequate iron intake, excessive blood loss, an unhealthy diet lacking iron-rich foods, problems with iron absorption, and increased iron demand, especially during periods of growth, can contribute to negative iron balance, ultimately resulting in ID. Age, gender, and socio-economic status are three factors that influence the prevalence of Iron deficiency (ID). Infants and children up to three years of age are at greatest risk of iron deficiency, which accounts for more than 50% of anemia cases[4-6]. Exclusive breastfeeding is recommended for approximately six months after the baby's birth[7]. On the other hand, prolonged breastfeeding is thought to be associated with ID because breast milk is low in iron, and iron intake from complementary foods is often not sufficient[8].

IDA can have many consequences, such as an increased risk of infections and cardiovascular diseases, as well as developmental

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delays, reduced physical activity, and depression in children[9]. ID is still a serious issue for children worldwide, and the prevalence varies greatly between different countries[10]. Anemia affects more than 25% of the global population, especially IDA, which accounts for more than 50% of cases. Pre-school children are the age group most exposed to IDA[11]. Women of childbearing age, growing fetuses, children, those with chronic and inflammatory diseases, and the elderly are most likely to develop IDA. IDA is the most common blood disorder in children; In developed countries, the average rates for 0–4 years and 5–14 years are 20.1% and 5.9%, respectively, while in developing countries, the rates vary from 39% to 48.1%. Several variables influence the diagnosis, treatment, and management of IDA, such as age, gender, and health status[12,13].

Oxidative stress (OS) occurs due to an imbalance between the generation of reactive species and the antioxidant level. During biological metabolism, both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated. The body uses both enzymatic and non-enzymatic systems to minimize the harmful effects of RONS and protect cells from damaging effects[14]. In hypoxic conditions, oxidant levels rise and the antioxidant defense system is lowered, which causes OS. The pathophysiology of IDA is thought to be heavily influenced by oxidative stress[14-16]. Furthermore, IDA leads to an imbalance between antioxidants and oxidants, which ultimately causes OS, resulting in a shortened lifespan for RBCs and increasing their susceptibility to disease[17,18]. When the amount of iron decreases, it can affect the synthesis of Hb, other iron-containing proteins, cytochromes, myoglobin, CATase, and peroxidase. Due to functional defects of enzymes in the antioxidant defense system in ID conditions, the balance shifts in favor of free radicals[19,20].

Although a few previous studies have described the relationship between iron status, antioxidants, and hematological parameters[16,19,21-23]. Scientific research on the correlation between iron status indicators, antioxidants, and hematological parameters for IDA in children is very limited, especially in those under six years of age. Therefore, the primary objective of the present study is to evaluate hematological, iron status, and enzymatic antioxidant parameters in children under the age of six years with IDA as compared to healthy controls. The study also aims to identify variations in the levels of these markers based on the degree of anemia due to ID. And also to identify correlations of hematological, iron status, and enzymatic antioxidant parameters of patients with IDA.

2 Methods and Materials

2. 1 Study design and Patient selection

This was a case-control study carried out in the period between November 2024 and December 2025. The ethical approval letter was obtained from the ethics committee of the Faculty of Science and Health of Koya University (ethics form code: 13Bio, dated 23.12.2024) in the Kurdistan region of Iraq. The parents of children provided informed consent to participate in this study. A total of 90 children participated in this research; 55 children were diagnosed as IDA patients who were between the ages of 6 months and <6 years, along with 35 children who were selected as healthy controls from the same age range. Before collecting the blood samples, parents of the children were formally questioned by means of a questionnaire that collected their sociodemographic information, including age, sex, place of residence, height and weight, and any family history of anemia or other blood diseases. Children excluded from the study were those who were either under 6 months of age or over 6 years of age; those who had a history of blood transfusions or iron therapy within less than one month; and those who had other associated anemias, chronic diseases, or known hemoglobinopathies (such as thalassemia).

2. 2 Laboratory Assessments

From every child, about 4 milliliters of venous blood samples were collected. The blood was then moved into two separate tubes, one with a lavender cap and the other with a yellow cap.

2. 1. 1 Complete Blood Count

Hematological tests often make use of a lavender-cap tube that contains the anticoagulant ethylenediaminetetraacetic acid (EDTA), and the tubes are well mixed by inverting 10 times (or by a rotator mixer). After blood collection in Zanko Medical Laboratory, a complete blood count (CBC) test is immediately conducted by a Medionic M51 hematology analyzer (Boule Diagnostics, Sweden), and in Marina Medical Laboratory, by a Swelab Alfa Plus Standard hematology analyzer (Boule Diagnostics, Sweden).

2. 1. 2 Biochemical tests

After separating adequate blood for the purpose of doing a CBC test, the remaining blood was transferred into a yellow cap tube that contains a gel/clot activator. The blood was centrifuged by the LC-04L centrifuge (Zenithlab, China) or by the EBA 200 centrifuge (Hettich, Germany) at 4000 rpm for five minutes to extract serum, which will be used for the purpose of conducting biochemical testing. The obtained serum was aliquoted, transferred into a small Eppendorf tube, and stored at -86°C for further examination. Then, the serum was subsequently analyzed using the Cobas e411 immunoassay analyzer (Roche Diagnostics, Japan) to find out the concentration of Sf. The following human diagnostics ELISA kits were used to detect SODse and CATse; these were bought from Shanghai Ideal Medical Technology Co., Ltd. (Eldere, China). The analyses were performed by using the 800 TS microplate reader for the assay (Bio Tec, USA).

2. 3 Differential values

Hb levels were used for determining anemia, and Sf was used for determining ID according to WHO thresholds for children aged less than 6 years[24,25]. IDA is diagnosed as the Hb level being less than 11 g/dL and the Sf level being below 12 µg/L in children younger than 5 years old. In addition, an Hb level less than 11.5 g/dL and an Sf level below 15 µg/L were used to diagnose IDA in children aged 5 to <6 years. The severity of anemia is classified into two categories: mild and moderate[24]. Anemia in children <5

years of age, with a blood Hb level between 10 and 10.9 g/dL, is defined as mild; 7.0-9.9 g/dL is defined as moderate. For children between the ages of 5 and 6 years, a blood Hb level between 11 and 11.4 g/dL is defined as mild, and 8 to 10.9 g/dL is moderate.

2. 4 Statistical analysis

Data analysis was performed by using GraphPad Prism software version 8.0.2. Descriptive statistics were expressed as minimum and maximum, means ± standard deviation (SD), medians and interquartile ranges (IQR), frequency, percentages, and 95% confidence level of mean. The normality of the data distribution was evaluated by using the D'Agostino-Pearson Omnibus test. Depending upon the distribution of data, either the unpaired t-test or the Mann–Whitney test was selected for the comparison of the continuous variables of two unrelated groups. The unpaired t-test was employed for comparisons of means when the distribution of data was normal (parametric). While the Mann–Whitney test was employed for comparisons of the median when the distribution of data was non-normal (non-parametric). To determine the relationship among the hematological and biochemical parameters, Spearman's rho correlations were used for the non-normal data distribution of different parameters at a 95% confidence level. For all the test parameters, statistically significant differences between the IDA group and the healthy group were determined at $p<0.05$[16,19].

3 Results

The socio-demographic characteristics of the studied group are shown in Table 1. The average age of the participants was 3.132±1.67 years, and their ages varied from 6 months of age to younger than 6 years. A total of 55 children were diagnosed with IDA based on hematological and biochemical criteria. Of those individuals, there were 29 males (52.73%) and 26 females (47.27%). Among the participants, 43 (78.18%) were younger than 5 years old, while 12 (21.82%) were in the age range of 5–6 years. The study included 35 children, all diagnosed as healthy controls. Of them, 18 (51.43%) were female and 17 (48.57%) were male. Of the participants, 29 (82.86%) were less than five years old, while 6 (17.14%) were between five and six years old.

Table 1: Anthropometric measurements of the study subjects aged 6 months to <6 years.

Groups		Healthy (n=35)		IDA (n=55)	
		No.	Percentage	No.	Percentage
Age	<5y	29	82.86	43	78.18
	5 - <6y	6	17.14	12	21.82
Sex	Male	17	48.57	29	52.73
	Female	18	51.43	26	47.27

During the comparison of test parameters between healthy controls and IDA patients, indicators such as Hb, Hct, MCHC, RDW, Sf, Si, SODse, and CATse were not normally distributed ($p < 0.05$). In spite of this, most of the variables didn't follow a normal distribution, so specific statistical test approaches for parametric and non-parametric data distribution were used throughout the investigation.

The minimum-maximum, mean±SD, 95% confidence interval and median, interquartile range (IQR) level of hematological parameters are summarized in Table 2 and Figure 1. According to the findings of the study, a statistically significant difference in Hb, Hct, and RDW values was observed between healthy and IDA children ($p < 0.0001$). There was no statistically significant difference in MCHC between healthy individuals and those with IDA (p=0.1721). A significant reduction of mean/median values in Hb, Hct, and MCHC, and increase in RDW were determined in IDA than in healthy groups, that confirms the level of health problems' relationship with anemia in the IDA group, and a decrease in the average size (microcytic) of RBCs due to a decrease in concentration of Hb of RBCs that reflecting by MCHC values, along with increased variations in the cell size (anisocytosis) of RBCs that reflecting by RDW values.

In general, the Hb (from six months to less than six years) levels were varied from 11.40 to 13.60 g/dL (mean =12.32±0.571, 95% CI: 12.12–12.51) and 7.20 to 11.40 g/dL (mean = 10.21±0.852, 95% CI: 9.98–10.44) in the healthy control and IDA group, respectively. The Hb levels showed variation between different age groups. The range Hb varied from 11.4 to 13.6 g/dL (mean = 12.28±0.604, 95% CI: 12.05–12.51) and 12.0 to 12.9 g/dL (mean = 12.50±0.346, 95% CI:12.14–12.86) in healthy children between age groups less than 5 years and 5–6 years, respectively. The Hb levels in IDA children ranged from 7.2 to 10.9 g/dL (mean = 10.17±0.899, 95% CI: 9.89–10.45) for those under 5 years old and from 9.5 to 11.4 g/dL (mean = 10.37±0.665, 95% CI: 9.94–10.79) for those aged 5 to 6 years.

The obtained results of the present study illustrated that the Hct levels were 32.7-39.6% (mean =37.03±2.083, 95% CI: 36.32–37.75) in healthy control children. While the observed values of Hct levels in IDA children were 24.5–36.0% (mean =31.03±2.492, 95% CI: 30.34–31.68).

The values of MCHC reflecting the average concentration of Hb within RBCs, the range of MCHC showed variation between the studied groups, ranging from 31.0 to 36.0 g/dL (mean = 33.45±1.472, 95% CI: 32.95–33.96) in healthy control children. Whereas, the MCHC levels with values ranging between 29.7 and 35.9 g/dL [mean =32.96±1.472, 95% CI: 32.56–33.35).

The opposite behavior was identified for the RDW, which was that their levels increased in the IDA group compared to the healthy control group. The RDW levels were 10.7-15.0% (12.93±1.075) in healthy controls and 11.7-23.3% (15.06±2.331) in IDA children.

Table 2: Comparison of hematologic parameters of healthy controls and IDA groups

Parameters		Healthy (n=35)				IDA (n=55)				p-value
		Min–Max	Mean	SD	95% CI	Min–Max	Mean	SD	95% CI	
Hb (g/dL) <5y – <6y		11.4–13.6	12.32	±0.571	12.12–12.51	7.2-11.4	10.21	±0.852	9.98–10.44	<0.0001
Hb (g/dL)	<5y	11.4–13.6	12.28	±0.604	12.05–12.51	7.2-10.9	10.17	±0.899	9.89–10.45	<0.0001
	5y – <6y	12.0–12.9	12.50	±0.346	12.14–12.86	9.5-11.4	10.37	±0.665	9.94–10.79	<0.0001
Hct (%)		32.7–39.6	37.03	±2.083	36.32–37.75	24.5-36.0	31.03	±2.492	30.34–31.68	<0.0001
MCHC (g/dL)		31.0–36.0	33.45	±1.472	32.95–33.96	29.7-35.9	32.96	±1.472	32.56–33.35	0.1721
RDW (%)		10.7–15.0	12.93	±1.075	12.56–13.30	11.7-23.3	15.06	±2.331	14.35–15.77	<0.0001

Hb, Hct, MCHC, and RDW values were non-normally distributed (D'Agostino-Pearson Omnibus test <0.05). Therefore, Mann-Whitney- U test was used.
SD: standard deviation, CI: confidence interval.

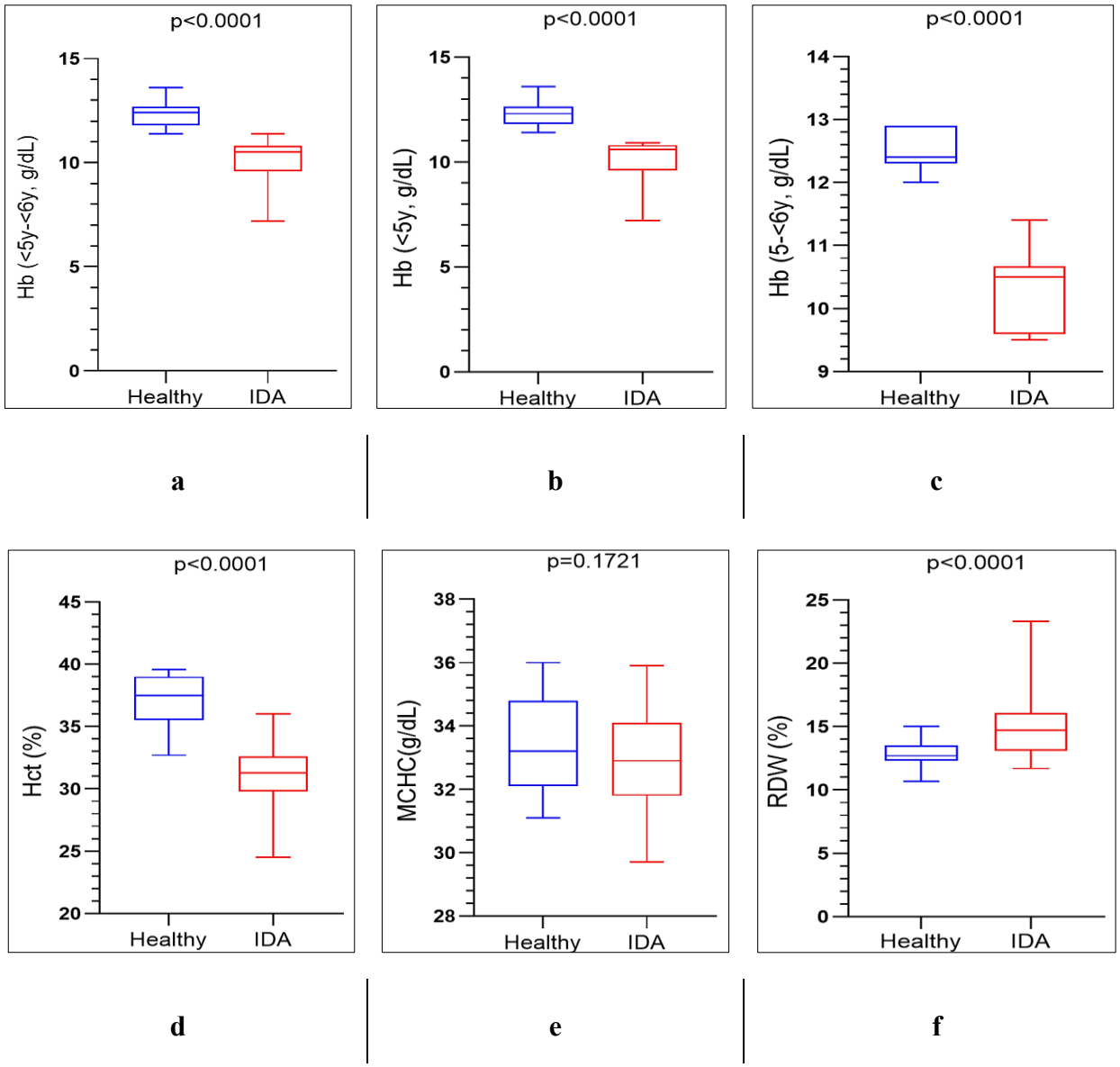


Figure 1: Box and whisker plots illustrate the levels of hemoglobin (fig. 1a, b, and c), hematocrit (fig. 1d), mean cell hemoglobin concentration (fig. 1e), and red cell distribution width (fig. 1f) in the study groups; the horizontal line in each box denotes the median, the box shows the interquartile range (25^{th}–75^{th} percentile), and the whiskers are short lines extending above and below the boxes to show the minimum and maximum values.

Table 3 and Figure 2 display the levels of Sf and Si in relation to IDA patients and healthy controls. There was significant difference in Sf, and Si values between healthy and IDA children ($p < 0.0001$) along with significantly reduction in Sf and Si levels were found in IDA group than in healthy control group, that reflecting decrease in available of iron and ID in IDA participants. According to the obtained results, the values of Sf (from six months to less than six years) varying from 33.52 to121.0 µg/dL (mean = 67.81±22.49, 95% CI: 60.09–75.54) and 3.48 to 14.43 µg/dL (mean = 9.06±2.706, 95% CI: 8.32–9.790) were in healthy control and IDA children, respectively. Sf levels were calculated regarding different age groups; the levels of Sf varied, ranging from 33.52 to 121.0 µg/dL (mean = 66.11±22.99, 95% CI: 57.37–78.86) and 48.7 to 89.8 µg/dL (mean = 76.03±19.49, 95% CI: 55.58–96.48) in healthy children between age groups less than 5 years and 5-6 years, respectively. While the Sf levels were found to range from 3.48 to 11.90 µg/dL (mean = 9.29±2.352, 95% CI: 8.56–10.01) and 4.00–14.43 µg/dL (mean = 8.23±3.724, 95% CI: 5.86–10.60) in age groups less than 5 years and 5–6 years of IDA children, respectively. Descriptive statistics showed that the Si levels ranged from 48.9-109.2 µg/dL (mean = 80.49±16.94, 95% CI: 74.67–86.31) in healthy control children. The data revealed that the Si levels ranged between 8.46 and 112.6 µg/dL (mean = 37.25±25.38, 95% CI: 30.39–44.11) in IDA children.

Table 3: Comparison of serum ferritin and serum iron levels of healthy controls and IDA groups.

Parameters		Healthy (n=35)				IDA (n=55)				p-value
		Min–Max	Mean	SD	95% CI	Min–Max	Mean	SD	95% CI	
Sf (µg/dL)[a] <5y –<6y		33.52–121.0	67.81	±22.49	60.09–75.54	3.48–14.43	9.06	±2.706	8.32–9.79	<0.0001
Sf (µg/dL)[a]	<5y	33.52–121.0	66.11	±22.99	57.37–78.86	3.48–11.90	9.29	±2.352	8.56–10.01	<0.0001
	5 –<6y	48.7–89.8	76.03	±19.49	55.58–96.48	4.00–14.43	8.23	±3.724	5.86–10.60	<0.0001
Si (µg/dL)[a]		48.9–109.2	80.49	±16.94	74.67–86.31	8.46–112.6	37.25	±25.38	30.39–44.11	<0.0001

[a] Sf, and Si values were non-normally distributed (D'Agostino-Pearson Omnibus test <0.05). Therefore, Mann-Whitney- U test was used.

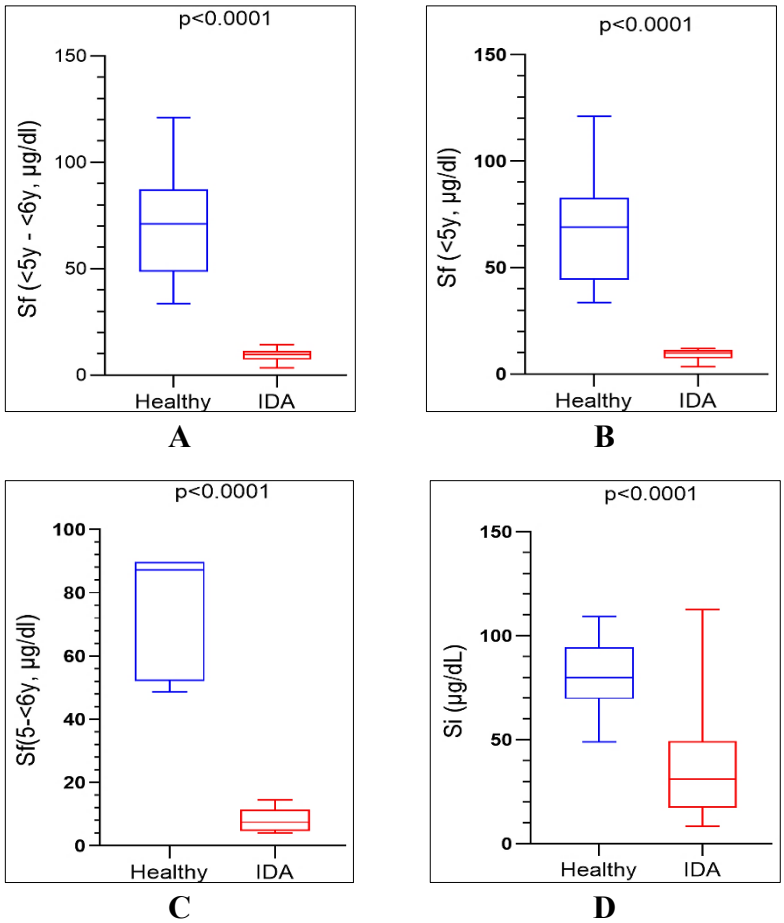


Figure 2: Box and whisker plots illustrate the levels of serum ferritin (fig. 2a, b, and c), and serum iron (fig. 2d) in the study groups; the horizontal line in each box denotes the median, the box shows the interquartile range (25th–75th percentile), and the whiskers are short lines extending above and below the boxes to show the minimum and maximum values.

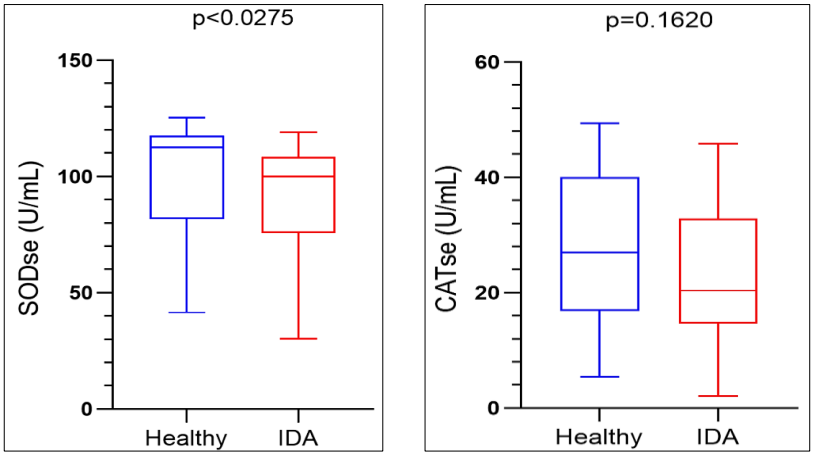
Table 4 and figure 3 showed the enzymatic antioxidant levels in the healthy control and IDA groups. The results of the current investigation of antioxidant defence system parameters also demonstrated that SODse showed a significant difference between the children with IDA and the healthy control group ($p<0.0275$). In healthy control children, the SODse levels ranged from 41.5 to 125.3 U/mL (mean = 98.52±24.20, 95% CI: 90.20–106.8). It was observed from these data that the SODse levels in IDA children varied from 30.3 to 119.0 U/mL (mean = 89.33±27.35, 95% CI: 81.96–96.69). The results showed that IDA had a more significant reduction in SODse levels.

The results exhibited that the CATse levels ranged from 5.4 to 49.4 U/mL (mean = 26.91±13.12, 95% CI: 22.40–31.42) in healthy control children. In contrast, the CATse levels in children with IDA ranged from 2.13 to 45.90 U/mL (mean = 22.92±13.06, 95% CI: 19.39–26.45). Although the CATse levels were more depressed in IDA patients, it did not show any statistically significant difference between IDA and healthy control children ($p>0.1620$).

Table 4: Comparison of Superoxide dismutase and catalase levels in the healthy control and IDA groups

Parameters	Healthy (n=35)				IDA (n=55)				p-value
	Min–Max	Mean	SD	95% CI	Min–Max	Mean	SD	95% CI	
SODse (U/mL)[a]	41.5-125.3	98.52	±24.20	90.20–106.8	30.3-119.0	89.33	±27.35	81.96–96.69	<0.0275
CATse (U/mL)[a]	5.4-49.4	26.91	±13.12	22.40–31.42	2.13-45.90	22.92	±13.06	19.39–26.45	0.1620

[a] SODse and CATse values were non-normally distributed (D'Agostino-Pearson Omnibus test <0.05). Therefore, Mann-Whitney- U test were used



a **B**

Figure 3: Box and whisker plots illustrate the levels of superoxide dismutase (fig. 3a), and catalase (fig. 3b) in the study groups; the horizontal line in each box denotes the median, the box shows the interquartile range (25th–75th percentile), and the whiskers are short lines extending above and below the boxes to show the minimum and maximum values.

Table 5 displays how the severity of anemia varies among IDA children based on age groups. The study found that out of the total number of IDA children, 33 (60%) had mild anemia, with 31 cases (56.36%) being children under the age of 5 years and 2 cases (3.64%) being children aged 5 to 6 years old. Among the total IDA children, 22 (40%) had moderate anemia, with 12 (21.82%) and 10 (18.18%) cases occurring in children under the age of 5 and those between the ages of 5 and 6, respectively.

Table 5: Distribution of severity of anemia in IDA individuals by age groups.

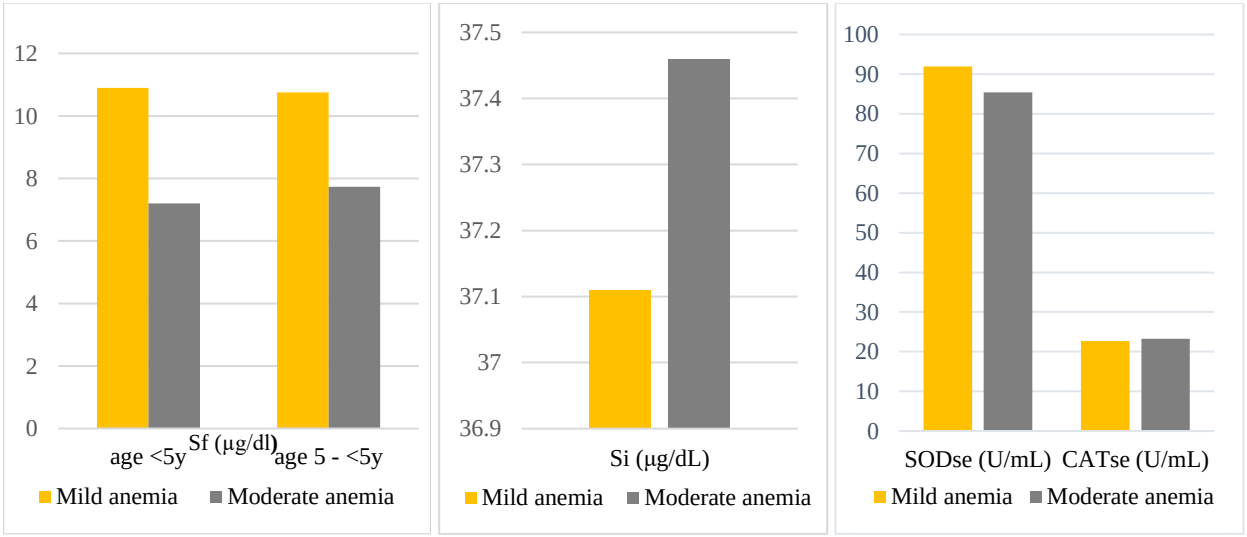
Age groups	Severity of anemia									
	Mild anemia					Moderate anemia				
	No.	%	Mean±SD	Median (IQR)	Min-Max	No.	%	Mean±SD	Median (IQR)	Min-Max
<5y	31	56.36	10.64±0.28	10.70 (10.5, 10.9)	10.0-10.9	12	21.82	8.96±0.81	9.15 (8.78, 9.5)	7.2-9.8
5 - <6y	2	3.64	11.4±0.00	11.4 (11.4, 11.4)	11.4-11.4	10	18.18	10.16±0.506	10.4 (9.5, 10.6)	9.5-10.7
Total	33	60%				22	40%			

Table 6 and figure 4 and show the distribution of levels of SODse, CATse, Si, and Sf according to the severity of anemia. The study's results showed that IDA biochemical markers and antioxidant defense system parameters did not differ significantly between mild and moderate anemia (p>0.05). Except that there was a significant difference between mild and moderate anemia in Sf levels in children less than 5 years old (p<0.0004). Median Sf, Si, and SODse values were much lower in IDA patients with moderate anemia as compared to those with mild anemia. Nevertheless, IDA patients with moderate anemia exhibited relatively greater mean CATse levels than IDA patients experiencing mild anemia.

Table 6: Serum ferritin, serum iron, superoxide dismutase, and catalase levels across different grades of anemia of the IDA group

Parameters		**Severity of anemia**		**p-value**
		Mild anemia	**Moderate anemia**	
		Mean±SD or Median (IQR)		
Sf (µg/dl)[a]	<5y	10.45 (9.48, 11.44)	6.78 (6.15, 9.01)	<0.0004
	5 -<6y	10.75 (7.50, 14.00)	7.36 (4.31, 11.39)	0.3636
Si (µg/dL)		33.00 (18.15, 48.75)	25.30 (15.60, 58.18)	0.8148
SODse (U/mL)[a]		100.0 (78.05, 110.40)	98.85 (45.53, 108.20)	0.4054
CATse (U/mL)[b]		22.67±12.11	23.28±14.66	0.8771

[a] Sf, Si, and SODse values were non-normally distributed across degree of anemia (D'Agostino-Pearson Omnibus test <0.05). Therefore, Mann-Whitney- U test were used. [b]CATse were normally distributed across degree of anemia (D'Agostino-Pearson Omnibus test >0.05). Therefore, Unpaired t-test were used.



a b c

Figure 4: Comparison of the mean serum ferritin (fig. 4a), serum iron (fig. 4b), superoxide dismutase and catalase levels (fig. 4c) across different grades of anemia of the IDA group.

Table 7 shows the correlation between SODse, CATse, Sf, Si, and hematological parameters. Non-parametric statistical test methods were used in this study because almost all the variables did not conform to the normal distribution. It is common to use Spearman's correlation coefficient for non-parametric distributions to examine the relationship between these variables.

The results of the study showed that there is a strong positive correlation between Hb and Hct levels, which showed a statistically significant level (r=0.8149, p<0.0001), and a moderate positive and significant correlation between Hb and MCHC (r=0.4556, p<0.0005). In addition, there was a moderately positive correlation between Hb and Sf, but this correlation was not statistically significant (0.2503, p=0.0653). A weak positive correlation was found that was not statistically significant between Hb and Si (r=0.1729, p=0.2068), SODse (r=0.1127, p=0.4125), and CATse (r=0.07042, p=0.6094), indicating that when Hb levels are elevated, there tends to be an elevation of Hct, MCHC, Sf, Si, SODse, and CATse.

It was found that the correlation between Hb and RDW shows an inverse relationship. The correlation between Hb and RDW was found to be moderately negative and statistically significant (r=-0.5522, p<0.0001). This suggests that elevated Hb levels are associated with decreased levels of RDW levels.

The Hct displayed weak positive correlations that were not statistically significant with MCHC (r=0.0454, p=0.7421), Sf

(r=0.2438, p=0.0729), Si (r=0.1426, p=0.2991), SODse (r=0.1092, p=0.4275), and CAT (r=0.1219, p=0.3752). Additionally, it is worth noting that Hct exhibits a moderately negative and statistically significant correlation with RDW (r=-0.4456, p<0.0007). This implies that when Hct levels increase, the values of Sf, Si, SODse, and CATse parameters also increase, while the levels of RDW decrease.

The MCHC exhibited the inverse relationship with RDW (r = -0.3839, p < 0.0038); as observed, it has a moderate negative and significant correlation with RDW. According to the findings of the present study, the MCHC displayed a weak positive and non-significant correlation with Sf (r=0.1203, p=0.3817), Si (r=0.1210, p=0.3788), SODse (r=0.2409, p=0.0764), and CATse (r=0.1669, p=0.2232). The findings revealed that an increase in MCHC levels means an increase in levels of Sf, Si, SODse, and CATse together, which is associated with a decrease in level of RDW.

In contrast to all the investigated parameters, the RDW showed negative correlation with all hematological and biochemical parameters. A moderate negative and significant correlation was observed in the correlation of RDW with SODse (r=-0.2874, p<0.0334). Although the RDW showed a weak negative and non-significant correlation with Sf (r = -0.1455, p = 0.2892), Si (r=-0.09917, p=0.4713), and CAT (r = -0.2291, p = 0.0925), the results of this study revealed that as the RDW increased, Sf, Si, SODse, and CATse decreased.

As illustrated in Table 7, a weak positive and non-significant correlation was found between Sf and Si (r=0.01119, p=0.9354), (SODse (r=0.2392, p=0.0786) as well as CAT (r=0.2359, p=0.0829). Demonstrating that an increase in Sf levels goes with an increase in Si., SODse, and CATse.

Table 7: Spearman rho correlation coefficients of hematological, biochemical, and antioxidant parameters

Parameters		Hb (g/dL)	Hct (%)	MCHC (g/dL)	RDW (%)	Sf (µg/dl)
Hb (g/dL)	r	-	0.8149	0.4556	-0.5522	0.2503
	p	-	<0.0001	<0.0005	<0.0001	0.0653
Hct (%)	r	-	-	0.0454	-0.4456	0.2438
	p	-	-	0.7421	<0.0007	0.0729
MCHC(g/dL)	r	-	-	-	-0.3839	0.1203
	p	-	-	-	<0.0038	0.3817
RDW (%)	r	-	-	-	-	-0.1455
	p	-	-	-	-	0.2892
Si (µg/dL)	r	0.1729	0.1426	0.1210	-0.09917	0.01119
	p	0.2068	0.2991	0.3788	0.4713	0.9354
SODse (U/mL)	r	0.1127	0.1092	0.2409	-0.2874	0.2392
	p	0.4125	0.4275	0.0764	<0.0334	0.0786
CATse (U/mL)	r	0. 07042	0.1219	0.1669	-0.2291	0.2359
	p	0.6094	0.3752	0.2232	0.0925	0.0829

[a] Hb, Hct, RDW, Sf SODse and CATse values were non-normally distributed across the degree of anemia (D'Agostino-Pearson Omnibus test <0.05). Therefore, Spearman's rho correlation test was used.

4 Discussion

It has been known that IDA is the most common and widespread type of nutritional anemia in children. IDA has long-term impacts on immunity, general physical health, and mental and cognitive capacities[4,26]. Laboratory assessment of anemia usually begins with a CBC, and the diagnosis is determined based on Hb levels that are lower than the normal range for healthy children. Several indices of the CBC have the potential to provide insightful information on the underlying aetiology of anemia. Standard laboratories usually use fully automated analysers to measure these parameters. These analysers use flow cytometry to differentiate between cell types, which provides speed, accuracy, and precision[27]. Reduced Hb levels, Hct, MCHC, and high RDW are typical hematological characteristics of IDA based on the severity of anemia[1,4,28]. To diagnose anemia in children, it is helpful to collect detailed medical information, including details on the child's food, any exposures to harmful substances, and their family medical history[1].

Ferritin is a protein that stores iron and is known to control iron metabolism in a variety of species[29]. The concentration of ferritin in plasma or serum is reflective of iron stores; a low ferritin level means ID, while an increased ferritin level correlates with the hazards of iron overload. Although the majority of it is found in certain tissues, such as the liver, spleen, muscles, and bone marrow, small amount circulates in the blood. On the other hand, ferritin is an acute-phase protein exhibiting higher level during inflammation and infection. Ferritin is often used as a diagnostic test for ID in clinical settings[30,31]. Nevertheless, there are racial and ethnic differences. Kang *et al.*[32] reported that Native Africans, African Americans, and Asians often have higher serum ferritin concentrations when compared to Caucasians. This is a phenomenon that is still not fully understood, and it has the potential to result in a greater number of instances of iron deficiency being overlooked if less sensitive cut-off values are used. There has been evidence for more than a decade that it is the most accurate for determining iron status; since it reveals low levels, it is confirmed in iron IDA. While ferritin levels tend to remain normal or even rise in other

hypochromic anemias, they drop in ID. Sf and other biochemical markers of iron status, along with the patient's history and physical exam, as well as hematological markers such as Hb and red cell indices, are used to diagnose IDA[28,33]. Although Si measurement is a useful tool for determining iron status, it may not be the most effective test for ID on standalone. Furthermore, low Si levels are seen in ID, and infection. Therefore, it seems that patient characteristics and sampling time are quite important for Si. Due to food consumption and circadian rhythms, the levels of iron vary throughout the day and are usually highest in the morning[27,34].

It was observed in this study that the mean/median levels of Hb, Hct, MCHC, Si, and Sf were considerably lower in the IDA group compared to the healthy control group. Although the results of the study revealed that the RDW level was higher in the IDA group compared to the healthy control group. These findings are similar to the findings of previous studies[19,22,35-40]. Nouri *et al*.[39] compared hematological indices and biochemical parameters among healthy and IDA groups, they reported the hematological indices like RBCs, Hb, Hct, MCH, and MCHC, along with biochemical parameters such as Sf, and Si were significantly decreased in IDA group. A study in India found that a combination of RDW greater than 15% and Hb less than 10 g/dL was useful for diagnosing IDA in children aged 1 to 3 years without needing iron status indicators. This combination had a sensitivity of 99% and a specificity of 90%[41]. Different kinds of anemia may be distinguished by means of RDW. A number of factors, including anemia, sex, age, genetics, general function, and dyslipidemia, are possible to influence RDW[42,43]. These achieved results revealed that hematological parameters such as Hb, Hct, and RDW can effectively indicate underlying iron status in combination with Sf levels. Thus, Sf evaluation, when supported with CBC indices, improves diagnostic accuracy and helps demonstrate therapeutic responses in IDA. The findings exhibited that changes in Sf levels greatly impacted hematological and biochemical indicators.

The present study observed a decrease in SODse and CATse levels in IDA children compared to healthy controls. Similar observations have also been reported in different studies[16,19,37,44]. Oxidative stress is thought to be a significant factor in the development and progression of IDA. There are enzymes within erythrocytes that may combat oxidative stress, and they include CATse, glutathione peroxidase (GPse) and SODse. According to numerous studies, the level of malondialdehyde, nitric oxide, total peroxide, total oxidant status, and oxidative stress index are all elevated in children with IDA, while antioxidant concentrations (total antioxidant capacity, SODse, CATse, GPse, vitamins C and E, and others) are reduced [16,37,44,45].

Previous research has shown that RBC antioxidant capacity decreases in the presence of oxidative stress[39,46-48]. RBCs create powerful antioxidant enzymes including SODse, CATse, and GPse, which help maintain an efficient antioxidant defense system. Despite the fact that the primary factors that contribute to increased oxidative stress and impaired antioxidant defense in IDA have not been fully explained, researchers have discovered a considerable rise in lipid peroxidation, which shortens the life of RBCs and makes them more likely to cause illness when infected[17,18,47,48]. There was a decrease in the activity of the pentose phosphate pathway in the red blood cells of individuals who had IDA, according to research that was conducted by Isler *et al*.[46].

Oxidative stress is a major cause of RBC disorders. RBCs acquire oxidants from both inside and outside the body that can interact with RBC proteins and lipids. These issues can lead to molecular damage, membrane rupture, anemia, inflammation, and hemolysis. RBCs have low-molecular-weight and enzymatic antioxidants in them to keep them safe from harm[14]. Additionally, the iron requirement for antioxidant biosynthesis may have contributed to the decrease in SODse and CATse activity in IDA participants as compared to the healthy control group[19,37,46]. SODse is a primary protective barrier against oxidants. SODse enzymes facilitate the disproportionation process that converts superoxide anion radicals into hydrogen peroxide. CATse catalysis the decomposition of hydrogen peroxide into oxygen and water. The process prevents the creation of radicals by decreasing the harmful effects of hydrogen peroxide; hence, it protects the redox balance inside cells[49-51]. It is expected that IDA patients would have lower SODse and CATse activities. This decline is likely because their CATse activity is decreased, which leads to a generation of hydrogen peroxide. As a result, SODse activity is feedback inhibited, which would eventually promote lipid peroxidation[19,52-54].

However, according to Altun *et al*.[48], SODse and CATse levels were significantly raised in IDA, even though they were not significantly different from controls. In a previous investigation, the SODse and CATse levels in the control and IDA groups were found to be similar[55]. Despite this, Madhikarmi and Murthy,[21] found that CATse levels were significantly higher in the IDA group compared to the healthy control group, while SODse levels were reduced in IDA patients. It was discovered in another study that the levels of SODse were higher in the IDA group[56]. Later studies found that IDA patients had considerably elevated CATse and SODse levels and markedly elevated malondialdehyde generation compared to control subjects[57]. Contrarily, other researchers found individuals with IDA had much reduced SODse activity[46].

The achieved results of the present study illustrated that mild and moderate kinds were detected among IDA individuals, and severe kinds of anemia were not detected. The most common kind of anemia is mild, followed by moderate anemia. These results are consistent with the findings of Li *et al*.[58], which reported that most of Chinese children aged 6 to 71 months had mild anemia, followed by moderate anemia. Adu-Amankwaah *et al*.[59] found that mild anemia affected 34% of preschool children, severe anemia affected 24%, and moderate anemia affected 42%. This result is different from what this study found. Tesema *et al*.[60] investigated the prevalence of anemia in children between the ages of 6 and 59 months and determined the degree of severity of anemia. Within the children they studied, 26.2% exhibited mild anemia, 34.9% demonstrated moderate anemia, and 3% presented

with severe anemia. Moreover, the present study identified no significant differences in the levels of SODse, CATse, and Sf between mild and moderate individuals with IDA. However, the results showed significant differences in the levels of Sf in children under the age of 5 ($p<0.05$), which indicates that oxidative stress can manifest even in mild cases of IDA, which requires immediate detection and therapy[16].

One of the primary objectives of this study was to investigate the relationship between hematological parameters and Sf, Si, SODse and CATse levels in subjects with a laboratory diagnosis of IDA. The findings of the current study show the positive relationship between different values of Hb, Hct, MCHC, Sf, Si, SODse, and CATse parameters, and negative relationship of RDW with all of these parameters. Previous researches [16,28,35,61,62] provide comparable findings with the present study. These findings align with the established pathophysiology of IDA, which is marked by a decrease in antioxidant activity, Hb content, and erythrocyte morphological properties due to low iron storage in the body[13,19]. The inverse correlation identified between RDW and biochemical and hematological parameters in this study reflects increased RBC size variation as a result of iron deficiency anemia. Anisocytosis, measured by RDW, is an early hematologic marker for the development of IDA. The increase in RDW is attributed to the fact that the bone marrow releases smaller, more differentially shaped red cells into the circulation when the iron supply is low. Similar results were reported in various studies by Yasmin *et al*.[62], and Mujib *et al*.[35]. Jamil *et al*.[28] carried out a study to find the association between SF and Hb, MCHC, RDW and other hematological parameters were compared statistically. The results showed a significant association ($p>0.05$) of low Sf with IDA in both genders. In their analysis of IDA, they found that SF positively correlated with hematological parameters, while RDW showed a significant negative correlation. CATse and SODse were shown to have a positive correlation with Sf, Si, Hb, and MCHC[16]. Altun *et al*.[48] observed no association between Hb and other antioxidant enzyme activities in their study of children; however, they found an opposite relationship between Hb and SODse levels.

Studies have also shown that iron therapy in IDA alters the oxidative state. Kurtoglu *et al*.[63] found that oxidative stress was significantly reduced after six weeks of regular iron supplementation when they compared pre- and post-supplementation levels of GPse, SODse, and CATse. In their study on oxidative stress, Gadjeva *et al*.[64] measured levels of GPse, malondialdehyde, SODse, and CATse both before and after iron therapy as well as iron in combination with vitamins. Patients whose only treatment with iron continued to have elevated levels of malondialdehyde and CATse, while their GPse and SODse activities were unaltered. However, SODse activity returned to normal and oxidative stress reduced after iron therapy with vitamins. It demonstrates that iron treatment and antioxidant supplements are more effective in IDA patients in reducing oxidative stress and increasing the defense mechanisms of antioxidants. In their study of IDA patients, Nouri *et al*.[39] found that malondialdehyde was negatively correlated with hemoglobin, and Sf. Hb, ferritin, iron, and red cell indices were positively correlated with glutathione reductase and catalase. According to the study of Mehram *et al*.[37], Sf and Si were shown to be positively correlated with Hb.

Conclusion

The results of the present study revealed that in comparison with healthy controls, children with IDA had an increase in RDW and a decrease in hematological parameters (Hb, Hct, and MCHC), biochemical parameters (Sf and Si), and enzymatic antioxidants (SODse and CATse). Additionally, in cases with mild anemia, changes in these parameters were observed. A positive correlation was found between different parameters. Whereas there was a negative correlation between RDW and all investigated parameters.

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Conflict of interest

None

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