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Diagnosis and Differentiation of Hypochromic Microcytic Anemia among Elementary School Children in Ranya District

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Keywords

Hypochromic Microcytic Anemia
Iron Deficiency Anemia
Thalassemia Trait
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Abstract

Hypochromic microcytic anemia (HMA) is defined as decreased hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels, the most common causes of microcytic anemia in children are iron deficiency anemia (IDA) and thalassemia trait (TT). The cross-sectional study was conducted to diagnose and differentiate HMA among elementary school children in the Ranya district. A total of 134 subjects were included in the study of which 28 participants were healthy, and 106 subjects were diagnosed with HMA. The study subjects were divided into three groups. Group 1 with 28 healthy subjects, Group 2 with 38 IDA patients and Group 3 with 68 TT patients. Complete blood count, iron status (ferritin, serum iron, UIBC, TSAT (%) and TIBC), T3, T4, TSH, Erythropoietin hormone, Creatinine and GFR were estimated in all three groups. The results demonstrated that there was a significant decrease ($P < 0.0001$) in Hb, HCT, MCV, MCH and MCHC in both IDA and TT patients. While significant increase was seen in RDW and PLT count in both IDA and TT. The result revealed a significant decrease ($P < 0.0001$) in serum ferritin, serum iron and TSAT (%), whereas a significant increase in TIBC and UIBC in IDA. Serum erythropoietin (EPO) was increased significantly in both IDA and TT. Thyroid hormones (T3 and T4), TSH, serum creatinine and GFR were non-significantly changed in both IDA and TT patients.

1 Introduction

Anemia can be defined as a reduction in hemoglobin (Hb) concentration, hematocrit (Hct), or number of red blood cells (RBCs) per cubic millimeter. Anemia is the condition in which Hb or Hct is more than two standard deviations below (-2 SD) the mean for age and sex for the normal population. Causes of anemia are classified based on morphology, mean corpuscular volume (MCV) and red cell distribution width (RDW) [1]. World Health Organization (WHO) defined anemia as a Hb concentration < 11.5 g/dl in children aged 5-11 years [2].

The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The RBC indices are used in diagnosing and differentiating various types of anemia. In deficient states, anemia may be classified by cell size as macrocytic, normocytic, or microcytic, or by cell size and color as microcytic hypochromic [3].

Iron deficiency anemia (IDA) is one of the most common causes of anemia worldwide and is typically associated with microcytosis and hypochromasia. The presence of small (low MCV) and pale (low MCH) red blood cells are typically indicative of low cellular hemoglobin and results from defects in heme or globin synthesis [4].

Defects in hemoglobin synthesis usually result from insufficient iron deficiency or decreased globin production (thalassemia) or are idiopathic [5]. HMA was defined as anemia with MCV < 77 fl and MCH < 25 pg. Iron depletion was defined as serum ferritin < 15 µg/L [6]. The most common causes of microcytic anemia are iron deficiency anemia (IDA) and thalassemia trait (TT) [7]. Distinguishing these two conditions is important to provide genetic counseling and avoid unnecessary, potentially harmful iron therapy in thalassemia carriers [8].

Iron is required by the human body to perform many vital physiological functions. If the supply of iron for physiological functions is inadequate, iron deficiency is considered present. Anemia is associated with the more

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severe phases of iron deficiency [9]. Iron deficiency impairs the cognitive development of children from childhood to adolescence. It damages immune mechanisms and is associated with increased morbidity rates [8].

Thalassemia is a group of inherited blood disorders that affect the production of hemoglobin and among the most common genetic disorders worldwide [10]. Thalassemia syndromes are caused by decreased or absent production of normal globin chains. The thalassemias can be characterized as α- or β-thalassemias depending on the defective globin chain and the underlying molecular defects. They are recessive trait. Both α- and β-thalassemia carriers (heterozygotes) present with microcytic hypochromic parameters with or without mild anemia [11].

2 Material and Methods

The present study comprised a total of 134 subjects (57 males and 77 females). Their age ranged from (9-12) years. Subjects were children from different elementary schools in Ranya district. The study was carried out from December 2018 to August 2019.

2.1 Collection of the samples

Approximately 12500 children of 3rd to 6th grade from different elementary schools in Ranya district were screened by physical examination to detect signs and symptoms of anemia. A total of 448 samples were taken and the children were asked to fill out a special questionnaire form during a direct interview. Finally 134 subjects were eligible for this study.

Blood samples were collected from the brachial vein by sterile disposable syringes. Blood samples were divided into two tubes; 2 ml in an anticoagulant tube containing EDTA, using a cool box and processed for hematological analysis within 6 hours, and 3 ml of blood was allowed to clot in plain tubes for obtaining serum for iron status and hormone studying. Serum was separated after centrifugation at 3000 rpm for 10 minutes. All sera stored at freezing point (-80°C) until assay.

2.2 Control group

A total of 28 same age group (mean age: 10.54 ± 1.26 years) elementary school children (10 males and 18 females) with normal RBC indices, iron status, renal function test and hormonal parameters were selected as a control group.

2.3 Hypochromic microcytic anemia

The current study was designed to diagnose hypochromic microcytic anemia (HMA) based on hematological parameters. A total of 106 children (47 males and 59 females; mean age: 10.91 ± 1.05 years) were confirmed to have HMA. The HMA group consisted of children with Hb levels < 11.5 g/dl, MCV < 77 fl and MCH < 25 pg. Patients with HMA were differentiated according to iron status, renal function test and hormonal parameters into IDA and TT groups.

2.4 Iron deficiency anemia group

The diagnosis of IDA is confirmed by the findings of iron status and Hb level two standard deviations below normal. Iron status in IDA was assessed by serum ferritin, serum iron, transferrin saturation (TSAT), total iron binding capacity (TIBC), and unsaturated iron binding capacity (UIBC). IDA was confirmed in 38 elementary school children (15 males and 23 females; mean age: 10.87 ± 1.26 years) with serum ferritin levels < 15 ng/ml and TSAT < 16%. IDA is defined by WHO criteria as low Hb value according to age: Hb < 11.5 g/dL in 5-11 years old children with one out of the two criteria: TSAT < 16% and/or ferritin < 15 ng/ml [2,9].

2.5 Thalassemia trait group

Thalassemia trait (TT) was diagnosed in 68 children (32 males and 36 females; mean age: 10.93 ± 0.92 years). The TT group consisted of children with normal iron status (serum ferritin, serum iron, TSAT (%) and TIBC) or in the cases of beta thalassemia major ferritin level were > 1000 ng/ml.

2.6 Complete blood count measurements

Complete blood count (CBC) of all blood samples was carried out by hematology analyzer Swelab Alfa (Boule Medical AB, Sweden).

2.7 Determination of serum ferritin

Serum ferritin level was measured by Cobas e 411 immunoassay analyzer (Roche Diagnostics, Germany) which is based on the ElectroChemiLuminescence (ECL) technology.

2.8 Determination of serum iron and unsaturated iron binding capacity

Serum iron and unsaturated iron binding capacity (UIBC) were determined using Cobas Integra 400 chemistry analyzer (Roche Diagnostics, Switzerland). The assay is based on the FerroZine colorimetric method.

2.9 Calculation of total iron binding capacity

Total iron binding capacity (TIBC) is calculated based on the following equation [12]:

$$\text{TIBC} = \text{Serum iron} + \text{UIBC} \quad (1)$$

2.10 Calculation of transferrin saturation

Saturation of transferrin (TSAT) is calculated based on the following equation [12]:

$$TSAT\ (\%) = \frac{Serum\ iron}{Serum\ iron + UIBC} \times 100 \quad (2)$$

2.11 Serum Creatinine

Serum creatinine was measured using Cobas Integra 400 chemistry analyzer (Roche Diagnostics, Switzerland).

2.12 Estimated Glomerular Filtration Rate

Estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease (MDRD) equation [13]:

$$eGFR\ (ml/min/1.73m^2) = 186 \times (Creat / 88.4)^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black}) \quad (3)$$

2.13 Determination of Erythropoietin (EPO) hormone by ELISA

Serum erythropoietin levels were measured in one batch with the use of EPO ELISA kit (DRG Instruments GmbH, Germany) according to the manufacturer's protocol. This assay employs the quantitative sandwich enzyme immunoassay technique.

2.14 Determination of Thyroid Hormone

Total triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH) were measured by Cobas e 411 immunoassay analyzer (Roche Diagnostics, Germany) which is based on the ElectroChemiLuminescence (ECL) technology.

2.15 Statistical Analysis

Statistical analysis was performed using GraphPad Prism (version 8.0.2). One-way analysis of variance (ANOVA) and Student's *t*-test was used to compare the means of different groups. Data are expressed as mean ± SD and *P*-values < 0.05 were considered significant.

3 Results and Discussion

3.1 Diagnosis of hypochromic microcytic anemia

In the current study, a total of 106 samples (47 males and 59 females) were diagnosed with hypochromic microcytic anemia. Their ages ranged between 9-12 years, with a mean of 10.91 ± 1.05 years, all having microcytic (MCV < 77 fl), hypochromic (MCH < 25 pg) and anemia (Hb < 11.5 g/dl).

There was a very highly significant decrease ($P < 0.0001$) in the mean value of Hb, HCT, MCV, MCH and MCHC in HMA patients when compared with the control group. On the other hand, the results showed that there was a very highly significant increase ($P < 0.0001$) in the mean value of RBC count and RDW in HMA patients in comparison with the control group (Table 1 and Fig. 1).

The results also demonstrated that there were significant increase (P = 0.0004, P = 0.0021, P = 0.0119) in PLT count, PCT and PDW respectively in HMA patients as compared with control group. Non-significant change was observed in WBC count among HMA patients when compared with control group.

3.2 Differentiation of hypochromic microcytic anemia

The HMA patients were differentiated into IDA and TT groups as per iron status, renal function test and hormonal parameters. A total of 106 subjects had HMA, from which 38 samples were diagnosed with IDA and 68 samples had TT. While according to the tests performed to differentiate HMA in this study, there was no patients appear to have anemia of chronic disease, chronic kidney disease and sideroblastic anemia

3.2.1 Hematological Parameters

There was a very highly significant decrease ($P < 0.001$) in mean and median of Hb, HCT, MCV, MCH and MCHC in both IDA and TT groups as compared to control group (Table 2 and Fig. 2). Hematologic disorders such as β-TT and IDA are known as common causes for microcytic anemia, and typically have similar clinical and experimental conditions [14]. The principal methods for diagnosing IDA are based on an increase in TIBC, and a decrease in serum iron, serum ferritin and transferrin saturation [15]. Our findings are in agreement with previous studies, that IDA patients had a significant difference in Hb, HCT, MCV, MCH and MCHC as compared to control group [16]. On the other hand, Karim *et al.*, (2016) demonstrated that there was a significant decrease in Hb, HCT, MCV, MCH and MCHC in β-thalassemia patients compared to controls [17].

Our results confirm that RDW was significantly increased ($P < 0.001$) in both IDA and TT patients compared to control. The results are consistent with other researches revealed that a significant increase in RDW in IDA patients [18] and a significant increase in RDW in β-thalassemia and IDA group [19].

Table 1: Comparison of hematological parameters among HMA patients and control group (Mean ± S.E).

Group	Control	HMA
RBC ($x10^6/mm^3$)	4.788 ± 0.022	5.454 ± 0.056***
Hb (g/dL)	12.82 ± 0.049	10.78 ± 0.047***
HCT (%)	38.02 ± 0.182	33.88 ± 0.254***
MCV (fl)	80.18 ± 0.218	62.51 ± 0.561***
MCH (pg)	26.87 ± 0.095	19.99 ± 0.199***
MCHC (g/dL)	33.86 ± 0.087	31.98 ± 0.164***
RDW (%)	13.82 ± 0.046	15.98 ± 0.157***
PLT ($x10^3/mm^3$)	294.2 ± 4.5	318.6 ± 6.084***
MPV (fl)	8.434 ± 0.118	8.503 ± 0.083
PCT (%)	0.234 ± 0.006	0.265 ± 0.006**
PDW (%)	10.61 ± 0.131	11.06 ± 0.095*
WBC ($x10^3/mm^3$)	6.732 ± 0.132	7.024 ± 0.176

* P< 0.05, ** P< 0.01, *** P< 0.001, relative to controls

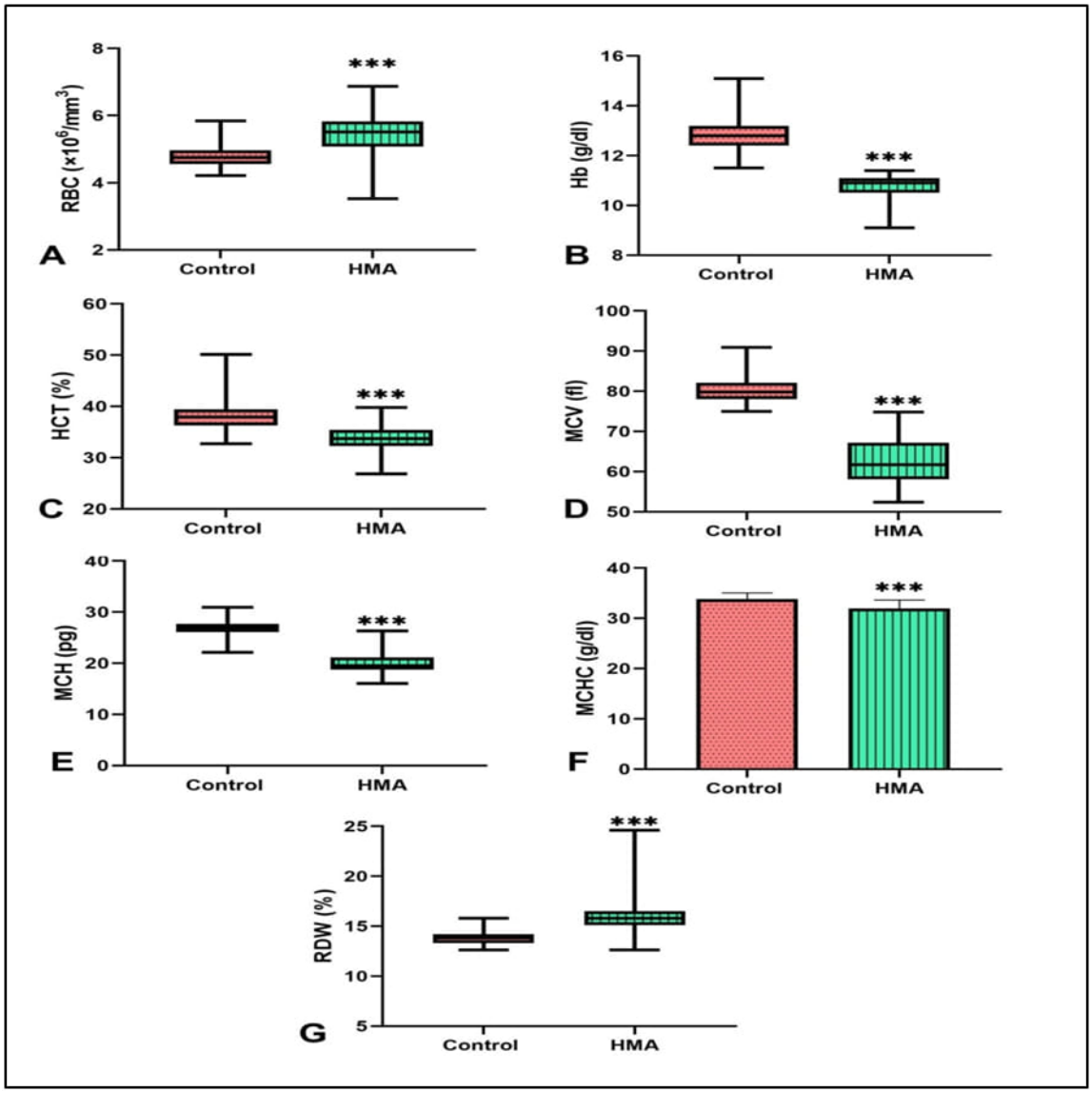

Fig. 1: Box plots of (A) RBC, (B) Hb, (C) HCT, (D) MCV, (E) MCH and (G) RDW, and bar chart of (F) MCHC shows the comparison among HMA and control groups.

Table 2: RBC count and indices in control, IDA and TT patients (Median (interquartile ranges) and Mean ± S.E).

Hematological Parameters	Groups		
	Control	IDA	Thal
RBC (x10^6/mm^3)	4.75 (4.56-4.965)	4.91 (4.57-5.33)	5.65 (5.36-5.95)***
Hb (g/dL)	12.8 (12.4-13.2)	10.8 (10.5-11)***	10.9 (10.6-11.18)***
HCT (%)	37.9 (36.3-39.4)	33.35 (31.95-34.78)***	34.5 (32.53-36.13)***
MCV (fl)	80.18 ± 0.218	65.39 ± 1.049 ***	60.91 ± 0.567***
MCH (pg)	26.9 (26.08-27.7)	21.1 (19.58-22.93)***	19.1 (18.2-20)***
MCHC (g/dL)	33.86 ± 0.087	32.43 ± 0.228***	31.72 ± 0.216 ***#
RDW (%)	13.8 (13.3-14.2)	15.8 (15.1-16.25)***	15.7 (15.3-16.48)***
PLT (x10^3/mm^3)	290 (247.8-341)	322.5 (286-372.5)**	318.5 (268-355)*
MPV (fl)	8.434 ± 0.118	8.418 ± 0.119	8.55 ± 0.11
PCT (%)	0.234 ± 0.006	0.262 ± 0.009	0.267 ± 0.008 *
PDW (%)	10.61 ± 0.131	11.01 ± 0.129	11.10 ± 0.13 *
WBC (x10^3/mm^3)	6.35 (5.4-7.8)	6.5 (5.275-7.9)	6.95 (5.925-8.075)

* P< 0.05, ** P< 0.01, *** P< 0.001, relative to controls.

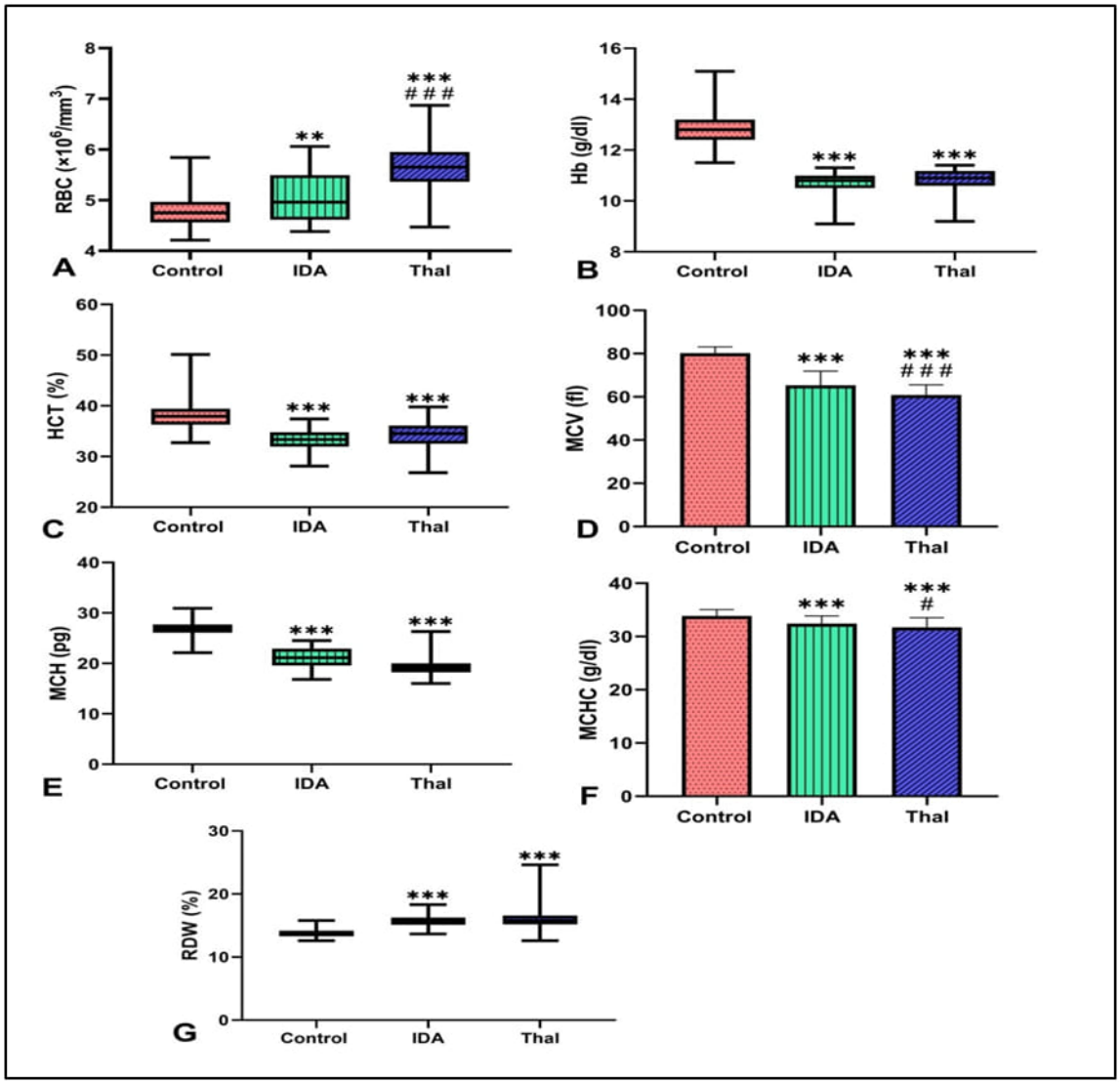

Fig. 2: Box plot of (A) RBC, (B) Hb, (C) HCT, (E) MCH, and (G) RDW and bar chart of (D) MCV and (F) MCHC shows the comparison among control, IDA and TT groups.

On the other hand, there was a very highly significant increase ($P < 0.001$) in the median of RBC count in TT group in comparison with the control group. This finding is consistent with another study that revealed a significant increase in RBC count in β-thalassemia carriers compared to the control group. An increase in RBC count caused by erythropoiesis expansion indicates an attempt to compensate for decreased Hb concentration due to lower MCV and MCH [20]. Furthermore, we observed a non-significant change in RBC count in IDA as compared to the control group. This is in line with the findings of (Keramati and Maybodi, 2007) that RBC counts had a non-significant difference in IDA group compared to normal groups [21].

The results showed a highly significant increase ($P < 0.01$) in PLT count in IDA group compared to the control group. The results also showed that there was a significant increase ($P < 0.05$) in PLT count, PCT and PDW in the thalassemia group compared to the control group. This result is consistent with earlier research which showed that PLT counts were significantly higher in IDA patients compared to the normal group [22]. Also, a study conducted in Istanbul found that PLT count in β-thalassemia minor patients was higher than in the control group [23].

There was a non-significant change in the median of WBC count in both IDA and thalassemia groups when compared with the control group. This is consistent with the findings of (Kar & Altınkaynak, 2021) who observed non-significant differences in total WBC counts in both IDA and β-TT patients compared to the control group [24].

3.2.2 Iron status

The results showed a very highly significant decrease ($P < 0.001$) in the median of serum ferritin, serum iron, and transferrin saturation and a very highly significant increase ($P < 0.001$) in mean of TIBC and UIBC in IDA group as compared to the control group (Table 3 and Fig. 3). These findings are consistent with

previous studies, which suggest that statistically significant differences in iron status parameters between IDA and the control group. Serum ferritin, serum iron, and TSAT levels were significantly decreased ($P < 0.001$), whereas TIBC were significantly increased ($P < 0.01$) in IDA as compared to control [25-26]. El-Masry *et al*., (2018) also reported that there was a significant increase in UIBC in IDA patients as compared to the control group [26]. A low ferritin level is one of the most significant markers for diagnosing iron deficiency and distinguishing IDA from the anemia of chronic disorders [27].

3.2.3 Hormonal Parameters

The effects of HMA on serum Erythropoietin (EPO), Triiodothyronine (T3), Thyroxine (T4) and Thyroid-stimulating hormone (TSH) in IDA, TT and control group were showed in (Table 3 and Fig. 4).

The results of the present study showed a highly significant increase ($P < 0.01$) in serum erythropoietin (EPO) in IDA when compared to the control group. The results are consistent with the findings of (Chen *et al.*, 1998; Teke *et al.*, 2017), who found that serum EPO levels in IDA patients are significantly higher than in healthy individuals [29,30].

The results also showed that there was a significant increase ($P < 0.05$) in serum EPO in the thalassemia group as compared to the control group. This result is in line with another study that revealed a significant increase in serum EPO level in β-thalassemia carrier patients compared to the healthy group [31]. The elevated erythropoietic activity is reflected by increased serum levels of EPO [20]. Furthermore, statistical analysis of the results revealed that there was a non-significant change in serum T3, T4, and TSH in IDA as compared to the control group. This is confirmed by the study of (Tienboon and Unachak, 2003), who observed no significant difference in serum T3, T4, and TSH levels in children with iron deficiency anemia before and after iron treatment as compared to control children [32]. On the other hand, this observation differs from a previous study that found a causal relationship between IDA and thyroid function. Increased serum levels of TSH and decreased levels of serum T3 and T4 have been reported in primary school children with IDA compared to the control group [25].

The results also demonstrated that there were non-significant changes in serum T3, T4 and TSH levels in thalassemia trait (TT) compared to the control group. This is consistent with the results of (Abdulzahra *et al.*, 2011), who found no significant difference in serum T3, T4, and TSH assay between healthy subjects and thalassemia patients [33].

3.2.4 Renal Function Test

No significant changes were observed in serum creatinine and eGFR among IDA and thalassemia groups when compared with control individuals. This is supported by the study of (Şen *et al.*, 2015), who observed no significant differences in creatinine levels between children with β-thalassemia major and control subjects ($P > 0.05$) and no significant difference in eGFR values between children with β-thalassemia major and controls ($p > 0.05$) [34].

Table 3: Iron Status, Hormonal parameters and Kidney function tests in control, IDA and TT patients (Median (interquartile ranges) and Mean ± S.E)

Biochemical Parameters	Groups		
	Control	IDA	Thal
S. Ferritin (ng/mL)	59.29 (47.83-67.3)	8.425 (6.705-9.903)***	47.12 (38.1-65.47)
S. Iron (µg/dL)	86.25 (73.98-94.5)	41.25 (33.55-47.35)***	93.55 (75.48-102.4)
TIBC (µg/dL)	355.5 ± 8.281	557.1 ± 11.13 ***	366.4 ± 5.213
UIBC (µg/dL)	272 ± 7.242	517.8 ± 11.88 ***	282.2 ± 4.467
Transferrin saturation (%)	22.8 (21.63-25.08)	7.5 (5.475-9.3)***	23.8 (20.7-27.15)
EPO (mIU/mL)	8.24 (5.715-10.62)	25.38 (8.363-51.22)**	18.95 (7.805-30.15)*
T3 (nmol/L)	2.573 ± 0.11	2.834 ± 0.09	2.658 ± 0.05
T4 (nmol/L)	118.4 ± 3.855	124.1 ± 4.199	123.1 ± 2.453
TSH (mIU/L)	2.34 (2.028 -3.093)	2.71 (1.895- 3.37)	2.59 (1.975- 3.85)
Creatinine (mg/dL)	0.37 (0.34-0.41)	0.355 (0.31-0.42)	0.35 (0.32-0.4)
GFR (ml/min/1.73m²)	294.2 ± 12.16	317.9 ± 13.65	327.9 ± 8.703

* P< 0.05, ** P< 0.01, *** P< 0.001, relative to controls.

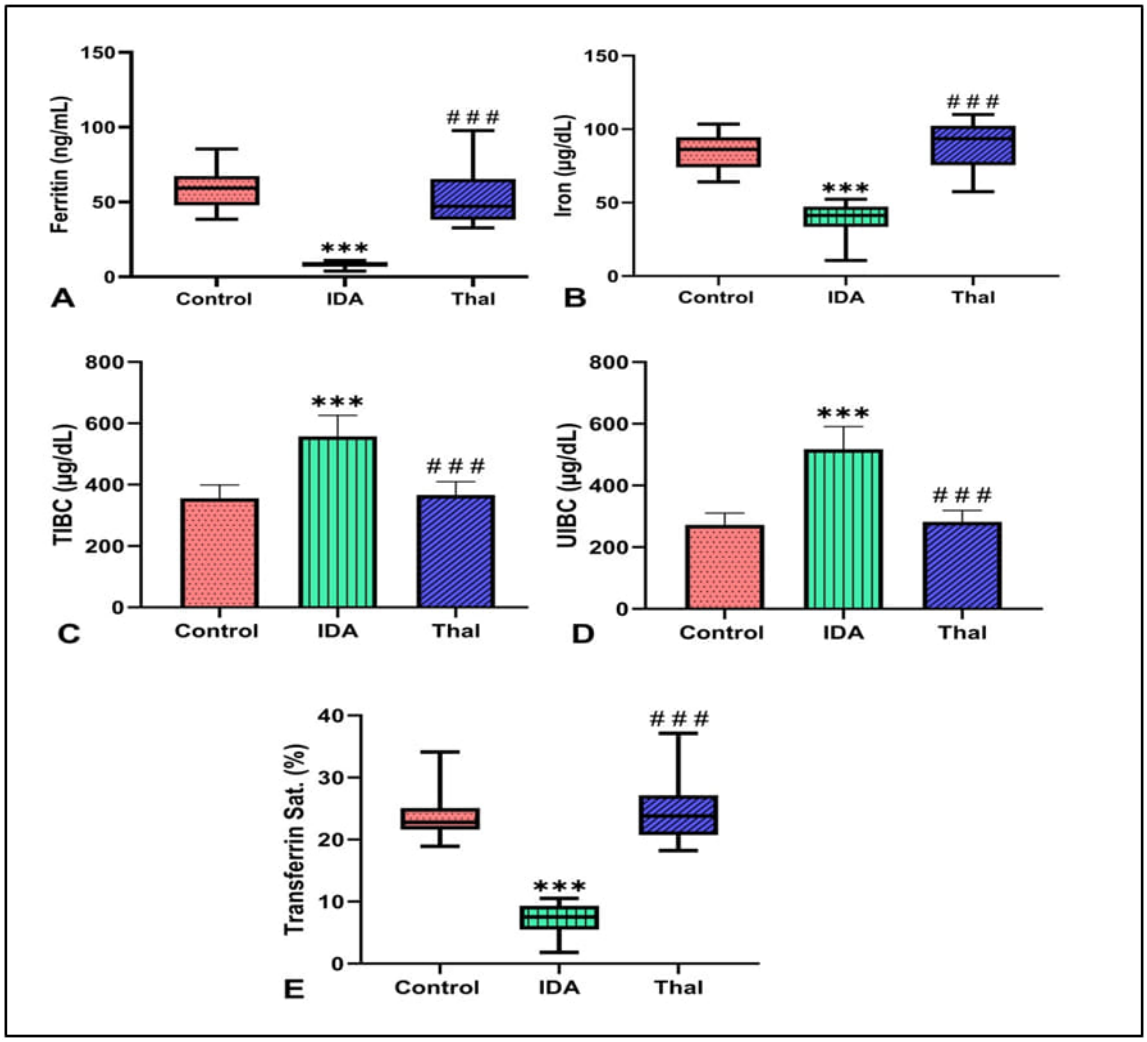

Fig. 3: Box plots of (A) Ferritin, (B) Iron and (E) Transferrin saturation and bar chart of (C) TIBC (D) and UIBC shows the comparison among control, IDA and TT groups.

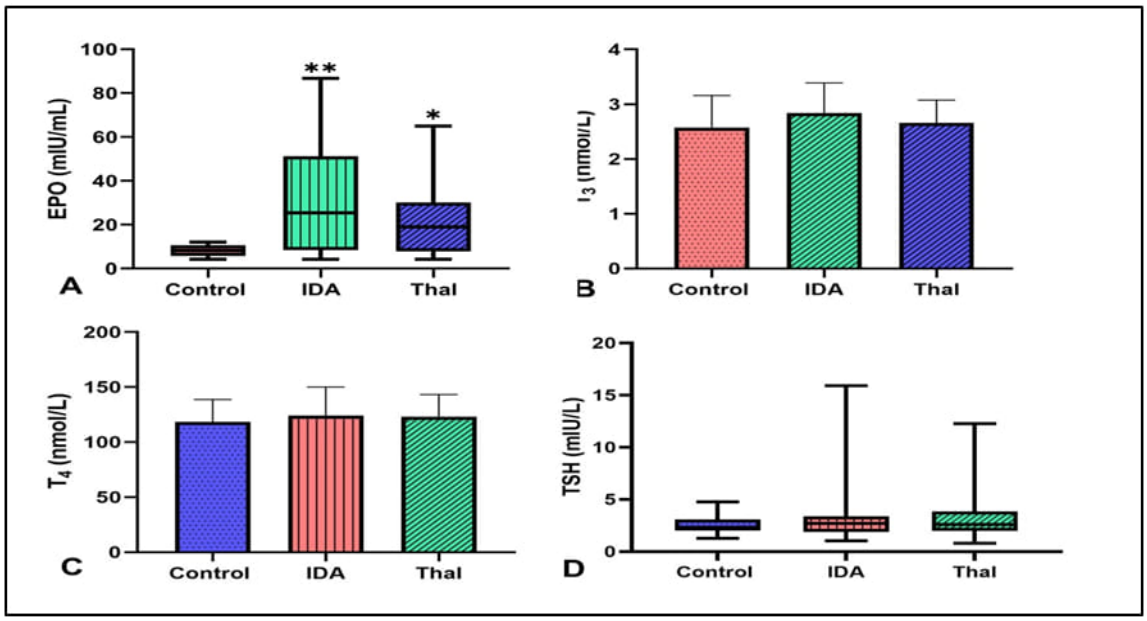

Fig. 4: Box plot of serum (A) Erythropoietin and (D) TSH and Bar charts of serum (B) T3 and (C) T4 shows the comparison among control, IDA and TT groups.

4 Conclusion

The results of the present study could draw the following conclusions:

a. Hemoglobin, HCT, MCV, MCH and MCHC were decreased, while RDW and PLT count in hypochromic microcytic anemia (HMA), IDA and TT patients were increased.

b. Serum ferritin, iron and TSAT were decreased, but TIBC and UIBC were increased in IDA, while iron status not changed in TT.

c. Serum erythropoietin level was increased in both IDA and TT subjects.

d. Thyroid hormones (T3 and T4), TSH, serum creatinine and GFR were not changed in IDA and TT patients.

The recommendations for future research and improvement are as follows:

a. Additional research is required to cover a larger population for the diagnosis of hypochromic microcytic anemia and to include all types of HMA.

b. Study the causes of IDA as well as its relationship with nutritional status and other associated factors among children.

c. The government will provide supplementary food to primary school children to reduce and prevent IDA.

d. Increase social awareness about the negative impact of consanguineous marriage to prevent thalassemia.

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