Evaluation of leukocyte count and its subgroubs in iron-deficiency anemic patients

Mohammed A.B. Eldin Hussien, Fahim M. Fahim, Hekma S. Farghaly

Department of Pediatrics, Faculty of Medicine, Assiut University, Assiut, Egypt

Correspondence to Mohamed Bahaa MBBCh, M.Sc.
Assiut Unuiversity Children's Hospital, Egypt
e-mail: mostafa_bahaa2000@yahoo.com

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Background
Anemia is a common blood disease in which the hemoglobin concentration is less than the normal hemoglobin concentration for age, sex, physiological condition, and altitude. Iron-deficiency anemia (IDA) is the most common cause of anemia**.** IDA is one of the most common and prevalent, nutritional health problems in infants and young children across the world. Across the globe 1200 million people are anemic. IDA is the most common disease in the world, affecting negatively both the health and well-being of children according to the WHO report.

Objective
The aim was to evaluate the effect of IDA in anemic patients on leukocyte count and its subgroups.

Patients and methods
The study was conducted on 50 patients with IDA, 29 men and 21 women aged from 4 to 14 years selected from the Hematology Outpatient Clinic, Assuit University Children Hospital, during the period from July 1, 2017 to June 30, 2018. All cases and controls were subjected to complete blood count, serum iron, total iron binding capacity, and serum ferritin.

Results
The results of the study showed positive correlation between serum ferritin with neutrophil and monocyte counts and negative correlation between serum ferritin and lymphocyte count.

Keywords:
Iron deficiency, leukocyte count, neutrophil coun

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Introduction

Iron-deficiency anemia (IDA) is considered a public health problem requiring attention from governments, researchers, and health-care practitioners [1–4].

Iron fortification of food staples such as flour, rice, and pasta, and/or through administration of iron supplements as iron pills is important in prevention and control [5].

There are several researches that suggest that the formation of other blood elements such as platelets and white blood cells is affected by iron deficiency [6].

There are many studies that show that IDA affects thrombocyte count resulting in thrombocytosis and affects white blood cell count and phagocytic functions [7].

Aim

This study aims to evaluate the effect of IDA in anemic patients on leukocyte count and its subgroups.

Patients and methods

Patients

The study was conducted on 50 patients with IDA, 29 men and 21 women aged from 4 to 14 years from the Hematology Outpatient Clinic, Assuit University Children Hospital, during the period from July 1, 2017 to June 30, 2018. The present study was approved by the ethical committee of the Faculty of Medicine, Assuit University. Informed consents were obtained from parents of the patients and controls before their recruitment into the study after their awareness about the nature of the study.

Controls

Twenty healthy children with matchable age and sex, selected from the relatives of the patients were in the study as a control group.

Inclusion criteria
Hemoglobin level of less than 11 g/dl, ferritin of less than 20, total iron-binding capacity (TIBC) greater than 450 μg/dl.

Exclusion criteria
Cases of parasitic infestation.
Cases of allergy.
Cases of any infection.
Cases of malnutrition.

Methods
Besides history and thorough clinical examination all cases and controls were subjected to the following investigations.

Complete blood count
Complete blood count was done by automated ABX Pentra XL80 HORIBA (Horiba Medical, Koyota, Japan).

Sample collection
A measure of 2 ml of blood was drawn on K3 EDTA vacutainer for complete blood count and reticulocyte count.

Determinations of serum iron by COBAS C 311

Method
Colorimetric CAB methods.

Sample collection
A measure of 6 ml blood for separation of serum for iron, TIBC, and the rest of the serum was stored and refrigerated under – 20°C for enzyme-linked immune sorbent assay (ELISA) to estimate serum ferritin.

Specimen
Serum which is free from hemolysis.

Laboratory technique
Mix and incubate for 5 min at 25°C, 30°C, or 37°C. Read the absorbance of the standard and sample against blank.

The calculation of iron concentration (μg/dl)=a sample × 200 A standard.

TIBC

TIBC is an important indirect way to measure the amount of iron that plasma protein can bind to it which increases in cases of IDA and also decreases in cases of iron overload as hemochromatosis

TIBC with serum iron and ferritin should be done for the assessment of anemia.

Principle
First, transferrin-binding sites are saturated by reagent R1.

Then, the excess iron reacts with (reagent R2) to give an insoluble complex which is exposed to centrifugation and then eliminated.

Last, measuring the iron concentration by a reagent for the determination of iron in serum for estimation of TIBC.

Reagent 1: R1 iron saturating solution (250 μg/dl).
Reagent 2: R2 magnesium carbonate.

To avoid any contamination, use clean or single-use laboratory equipment free from ferrous or ferric ions. If necessary, the equipment should be washed with HCl and then rinsed with deionized water.

Reference values
Serum: 250–450 μg/day, 44.8–80.6 μmol/l.

Procedures
Iron-binding sites' saturation:

Introduce reagent 1 in a centrifugation tube, then add the sample.

Mix and incubate for 5 min, then add reagent 2.

Incubate for 20 min, shaking during this period.

Centrifugate for 10 min and then collect the supernatant.

Total iron-binding capacity determination
Measurement of iron content of the supernatant is done with iron Chromazurol or iron Ferrozine.

Calculation
Conversion factor: μmol/l × 0.179=μmol/l.

Serum ferritin using ELISA BioCheck, Catalog Number: BC-1025 (Biocheck Located in San Francisco, USA).

Principle
The ferritin quantitative test is estimated by ELISA.

The system has antiferritin antibody for solid phase from rabbit and monoclonal antiferritin antibody in the antibody-enzyme from mouse.

The sample reacts with the antibodies simultaneously resulting in the ferritin molecules between the solid-phase and the enzyme-linked antibodiesincubate for 45 min at room temperature, then washed with water to remove unbound antibodies, and then added the TMB reagent and incubated for 20 min at room temperature leading to the formation of blue color, then added stop solution, and the color is changed from blue to yellow.

The results

The study was conducted on 50 patients with IDA, 29 men and 21 women aged from 4 to 14 years recruited from the Hematology Outpatient Clinic, Assuit University Children Hospital, during the period from July 1, 2017 to June 30, 2018.

Discussion

IDA is one of the most common health problems in children because of the decrease in iron intake and malabsorption.

Iron deficiency causes a decrease in the formation of hemoglobin which leads to IDA.

Iron has a role in many important functions.

Iron plays an important role in many enzymes such as catalase, ribonucleotides, acid phosphatase, myeloperoxidase, cytochromes, and xanthine oxidase [8].

There are several researches that suggest that the formation of blood elements such as platelets and white blood cells is affected by iron deficiency [5].

There are many studies that have shown that IDA affects thrombocyte count resulting in thrombocytosis and affects white blood cell count and phagocytic functions [7].

This study aims to evaluate the leukocytes, granulocyte, lymphocyte and monocyte, eosinophil, and basophil count in iron-deficiency anemic patients.

This study is a cross-sectional study that was conducted at Assiut University Children Hospital to demonstrate effect of IDA on leukocyte count and its subgroups.

Table 1 Levels of red blood cell count, hemoglobin, hematocrit value, reticulocyte count, red blood cells parameters of patients with iron-deficiency anemia compared with control

	Patients (*n*=50)	Control (*n*=20)	*P*
RBC			
Mean±SD	4.32±0.60	5.11±0.53	0.000*
Median (range)	4.4 (2.5-5.7)	5.2 (4.2-6.1)	
Hemoglobin			
Mean±SD	8.92±1.24	12.96±0.83	0.000*
Median (range)	9.0 (5.5-10.9)	12.9 (11.7-14.6)	
HCT			
Mean±SD	28.65±3.52	36.78±1.59	0.000*
Median (range)	29.0 (17.5-38.0)	37.0 (33.0-39.5)	
Reticulocyte			
Mean±SD	0.94±0.72	0.35±0.27	0.002*
Median (range)	1.0 (0.0-2.6)	0.4 (0.0-0.8)	
MCV			
Mean±SD	67.66±8.66	81.81±4.87	0.000*
Median (range)	69.0 (51.0-83.0)	81.5 (72.8-93.0)	
MCH			
Mean±SD	22.04±3.47	29.27±2.20	0.000*
Median (range)	22.0 (15.2-31.0)	29.3 (26.0-33.5)	
MCHC			
Mean±SD	31.05±2.00	35.24±1.32	0.000*
Median (range)	31.1 (26.0-36.0)	35.0 (33.0-38.0)	

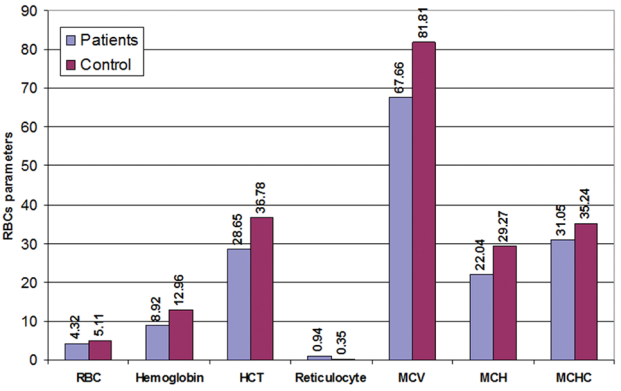
MCH, mean corpuscular hemoglobin; RBC, red blood cell.

Table 2 Iron studies in patients with iron-deficiency anemia compared with controls

	Patients (*n*=50)	Control (*n*=20)	*P*
Serum ferritin			
Mean±SD	12.90±4.94	82.90±15.13	0.000*
Median (range)	13.5 (3.6-22.0)	84.0 (54.0-110.0)	
Serum iron			
Mean±SD	29.79±10.34	94.15±16.98	0.000*
Median (range)	28.0 (13.0-55.9)	96.0 (65.0-124.0)	
TIBC			
Mean±SD	457.56±42.70	223.80±23.08	0.000*
Median (range)	459.0 (362.0-540.0)	232.5 (186.0-258.0)	

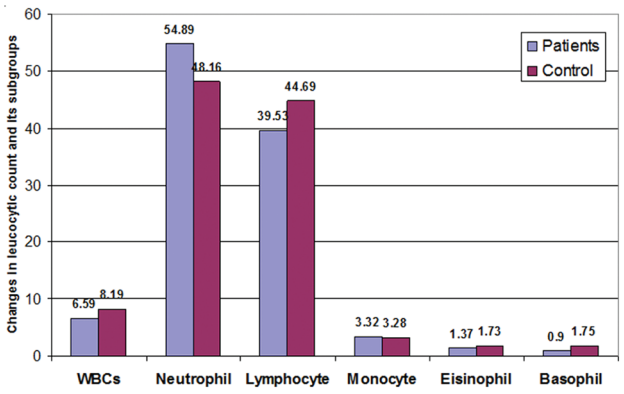
TIBC, total iron-binding capacity. *, statistically significant

Figure 1



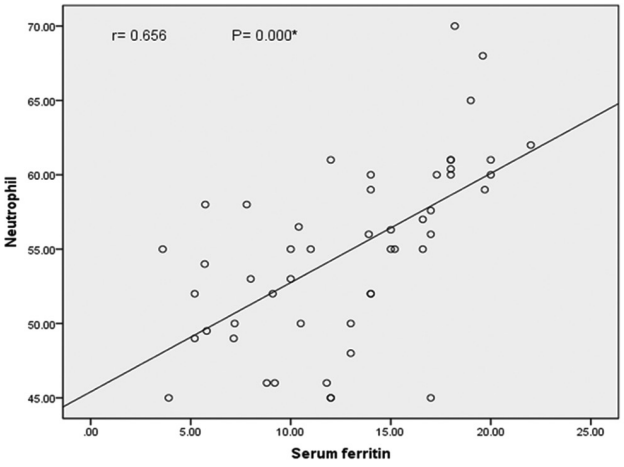
Red blood cell parameters in patients with iron-deficiency anemia compared with controls.

Figure 2



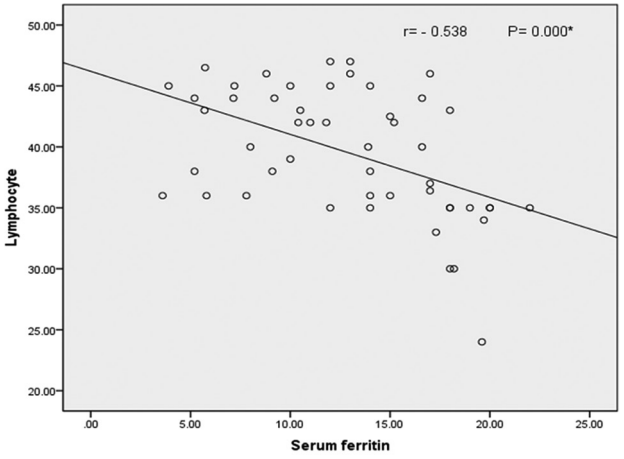
Changes in leukocyte counts and its subgroups in patients with iron-deficiency anemia compared with control.

Figure 3



Correlation between serum ferritin and neutrophil in iron-deficiency anemia patients.

Figure 4



Correlation between serum ferritin and lymphocytes in patients with iron-deficiency anemia.

It included 50 children with IDA with 20 age-matched and sex-matched control children as shown in Tables 1–5 and Figs. 1–5.

Table 3 Changes in leukocyte count and its subgroups in iron-deficiency patients compared with controls

	Patients (*n*=50)	Control (*n*=20)	*P*
WBCs			
Mean±SD	6.59±1.50	8.19±1.28	0.000*
Median (range)	6.4 (4.0-9.5)	8.2 (5.4-11.0)	
Neutrophil			
Mean±SD	54.89±6.05	48.16±5.50	0.000*
Median (range)	55.0 (45.0-70.0)	48.3 (38.9-60.0)	
Lymphocyte			
Mean±SD	39.53±5.21	44.69±5.23	0.001*
Median (range)	40.0 (24.0-47.0)	44.9 (35.0-55.0)	
Monocyte			
Mean±SD	3.32±2.15	3.28±2.38	0.732
Median (range)	3.0 (0.0-8.0)	2.5 (1.0-10.0)	
Eosinophil			
Mean±SD	1.37±1.06	1.73±0.79	0.106
Median (range)	1.0 (0.0-4.0)	2.0 (0.0-3.0)	
Basophil			
Mean±SD	0.90±0.83	1.75±1.16	0.003*
Median (range)	1.0 (0.0-3.0)	2.0 (0.0-5.0)	

WBC, white blood cell

Table 4 Correlations between iron studies parameters and red blood cells parameters in patients with iron-deficiency anemia compared with controls

	Serum ferritin		Serum iron		TIBC	
	r	*P*	*r*	*P*	*r*	*P*
RBC	−0.151	0.295	0.038	0.793	0.038	0.793
Hemoglobin	−0.182	0.206	0.207	0.150	−0.230	0.108
HCT	−0.037	0.799	0.196	0.174	0.093	0.522
Reticulocyte	−0.229	0.109	−0.261	0.067	0.104	0.473
MCV	0.006	0.968	0.306	0.031*	0.026	0.859
MCH	−0.212	0.140	0.116	0.424	−0.001	0.992
MCHC	−0.152	0.292	−0.076	0.600	−0.297	0.036

MCH, mean corpuscular hemoglobin; RBC, red blood cell; TIBC, total iron-binding capacity.

The high prevalence of IDA in rural areas in our study cases represents the pattern of patient attending Assiut University Hospitals in addition to an increase in incidence of IDA in rural areas due to poverty, ignorance, and feeding problems.

The simplest and least expensive measurement is complete blood count, which gives data on hemoglobin (Hb), MCV, and RDW, a measure of variability in red cell size.

In iron deficiency, Hb synthesis is impaired and then erythrocyte protoporphyrins accumulate that leads to IDA.

The red cells become smaller and varied in size due to a decrease in hemoglobin in each cell followed by a decrease in MCV and mean corpuscular hemoglobin (MCH) [9].

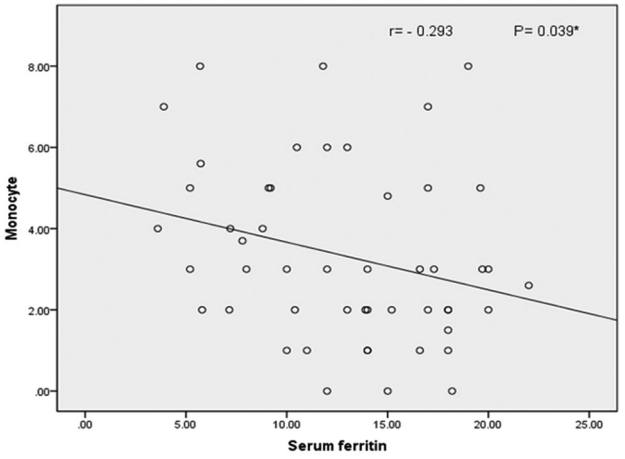
The reticulocyte count may be normal or moderately elevated, but reticulocyte counts do not show sufficient response to the degree of anemia [9].

Table 5 Correlations between iron studies parameters and changes in leukocyte count and its subgroups in patients with iron-deficiency anemia

	Serum ferritin		Serum iron		TIBC	
	r	*P*	*r*	*P*	*r*	*P*
WBC	0.005	0.975	−0.044	0.762	0.105	0.467
Neutrophil	0.656	0.000*	−0.065	0.653	0.169	0.241
Lymphocyte	−0.538	0.000*	0.170	0.238	−0.040	0.781
Monocyte	−0.293	0.039*	−0.136	0.347	−0.223	0.120
Eosinophil	−0.284	0.046*	0.025	0.865	−0.054	0.710
Basophil	−0.090	0.536	0.066	0.648	−0.353	0.012*

TIBC, total iron-binding capacity; WBC, white blood cell.

Figure 5



Correlation between serum ferritin and monocytes in iron-deficiency anemia patients.

The blood smear reveals hypochromic, microcytic red cells.

Elliptocytic or cigar-shaped red cells are also seen [9].

A complete blood count is important to determine the mean corpuscular volume or red blood cell size [10].

As such, iron deficiency should be considered in all cases of microcytic anemia; also there are causes of microcytosis including chronic inflammatory states, thalassemia, sideroblastic anemia, and lead poisoning [11].

This study showed decreased Hb concentration. Haemotocrite (HCT) in IDA patients compared with controls also showed decreased mean corpuscular volume (MCV), MCH, mean corpuscular haemoglobin concentratin (MCHC) in IDA patients compared with controls.

Our study showed a decrease in the level of serum iron, serum ferritin, and TIBC in IDA patients compared with controls which are diagnostic criteria in IDA patients.

This study shows a decrease in lymphocyte count and rise in neutrophilic count in iron-deficiency anemic patients.

There is a report that show a fall in lymphocyte count and a fall in T lymphocyte percentage in iron-deficiency anemic patients [12].

There are two reasons for the fall in lymphocyte count in IDA:

(1) Decrease in lymphocyte production.
(2) Increase in lymphocyte destruction.

The study showed an important effect of iron on lymphocyte count [13].

There is a report that showed a positive correlation between T lymphocytic count and iron [14].

Some reports have suggested that iron is a cofactor in ribonucleotide reductase enzyme that causes a decrease in lymphocyte count due to iron deficiency [15].

There is a report that shows a reduction of antioxidant capacities in IDA patients leading to an increase in lymphocyte DNA damage that leads to lymphocyte destruction [16]. Reduction of lymphocyte blood count occurs due to two reasons:

(1) A decrease in lymphocyte proliferation.
(2) Increased oxidative damage and early lymphocyte destruction.

This study also showed that iron has important positive effects on granulocyte count.

There are a number of studies reporting increased basophil and neutrophil counts in IDA patients [17].

There is a report that iron affects granulocyte production in the bone marrow [18].

There are several studies that have shown that iron deficiency causes a decrease in apoptotic response that leads to an increase in granulocyte count [19].

The increase in the life span of neutrophils occur due to a decrease in oxidant product synthesis [20].

IDA patients present an increase in granulocyte cell count as compensation of a decrease in granulocyte phagocytic function [21].

There is a report that showed the effect of both iron and copper on hematologic parameters [22]. They has shown an increase in red blood cell count and a decrease in neutrophil and platelet counts.

Our study has the same result as previously mentioned studies that suggest that iron affects differential white blood cell count causing neutrophilia and lymphopenia.

The results of our study showed positive correlation between serum ferritin with neutrophil and monocyte count but showed negative correlation between serum ferritin and lymphocyte count.

Conclusion

IDA is accompanied with an increase in granulocyte count but a decrease lymphocyte count which can affect directly the health of IDA patients. The molecular basis for this effect of iron on the cellular defense system remains to be clarified.

Recommendation

(1) It is useful to do a complete blood count in all children for early detection of IDA to avoid the effect of iron deficiency on leukocyte count and subgroups.
(2) We should put into consideration the leukocyte count and its subgroups in complete blood count in IDA patients to avoid its effect on IDA patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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