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Evaluation of Platelet-to-Lymphocyte and Neutrophil-to-Lymphocyte Ratios in Egyptian Children with Iron Deficiency Anemia: A Comparative Cross-Sectional Study

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ABSTRACT

Background: The platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR), which are calculated from complete blood count (CBC), are cheap, easy to get, and useful marker of inflammation and the immune system's reaction. **Purpose:** The authors aimed to compare children with iron deficiency anemia (IDA) to healthy groups in terms of their differential leukocyte count, platelet to lymphocyte ratio, and neutrophil to lymphocyte ratio.

Patients and Methods: This comparative cross-sectional study included 61 children (24 children with IDA and 37 healthy children as control group, with matched age and sex) aged from 2–16 years recruited from the outpatient clinic, at Helwan University Hospital, Egypt. CBC was performed for all participants. Then PLR and NLR were calculated. Serum ferritin and transferrin saturation were further withdrawn only from children with IDA.

Results: Total leukocytes count and its subsets did not vary statistically significantly between people with IDA and healthy children. IDA patients were much more likely to significantly have abnormal cell counts (leukocytosis/leukopenia) ($p < 0.05$). There was no significant difference between the healthy control group and the children with IDA in the PLR and NLR ratios ($P > 0.05$).

Conclusion: Our findings suggest that PLR, NLR, and leukocyte subgroups do not significantly differ between children with IDA and healthy controls. However, abnormal leukocyte counts were more frequent in IDA patients, highlighting potential immune alterations associated with iron deficiency.

Keywords: Iron deficiency anemia, Total leukocytes count, Neutrophil to lymphocyte ratio, Platelet lymphocyte ratio, Egyptian children

INTRODUCTION

One of the most prevalent hematological disorders in the world is anemia; it is defined as decrease in hemoglobin (Hb) concentration and/or hematocrit to levels below what is normal for age. It has a significant impact on the lifelong health, particularly mental, physical, developmental as well as social well-being of children [(1)].

Iron deficiency anemia (IDA), the most widespread micronutrient deficit globally, is acknowledged by the WHO as one of the most significant global health threats currently [(2)]. It is the most prevalent anemia in both developed and developing countries. It is estimated that about 50% of types of anemia are caused by iron deficiency (ID). In Egypt, IDA affects about 30-40% of children especially school children [(3)].

It is well known that ID and IDA have different outcomes including impairment of immune function and increased susceptibility to infection, reduced work capacity, poor growth development, and mental performance, also retardation of psychomotor development and reduced learning capacity [(4)].

Iron is a necessary for the normal development of the immune system and its defeciency impairs the body's ability to generate an effective immune response. Iron is essential for immune function, as it helps in the development of immune cells, especially lymphocytes, which are crucial for producing a specific immune response to infections. A lack of iron seems to have have a bigger effect on cellular immunity than on humoral immunity [(5,6)].

In addition to its role in the production of red blood cells, iron is essential for human defensive mechanisms in a variety of other ways. Several studies also indicate that ID may affect thrombocyte count resulting in thrombocytosis and affects white blood cell count and phagocytic functions [(7,8)].

The neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) can be calculated in a complete blood count (CBC). This ratios are quick, cheap, and easy to get. It shows how the immune system is responding to inflammation [(9,10)].

The NLR is considered to mirror a balance between the innate and adaptive immune mechanisms. Meanwhile, the PLR is a useful parameter for the systemic inflammatory response. Those combined ratios are considered to be more stable in comparison to the absolute counts, and they could be a reflection to the changes in inflammatory factors and immune cells [(11,12)].

Although IDA is known to affect immune function, few studies have evaluated PLR and NLR as potential immune markers in Egyptian children. This study sought to assess the differential leukocyte count, platelet to lymphocyte ratio, and neutrophil to lymphocyte ratio in children with iron deficiency anemia in comparison to healthy controls.

PATIENTS AND METHODS

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comparative cross-sectional study was done with 61 children aged 2 to 16 years old. There were 24 children with IDA and 37 healthy children who were used as a reference group. All of the children were of the same age and gender. Some of the children who took part came from the Helwan University-funded project "Screening of Anemia, Obesity, and Undernutrition in Children aged 1–7 years at Badr City" [13]. Other children were recruited from the outpatient clinic, at Helwan University Hospital in Badr City, in Egypt. This was a convenience sample, as all children (and their parents) coming to the outpatient clinic for follow up were included in the study if they agreed to participate.

Egyptian children of both genders aged between 2–16 years, were diagnosed to have IDA according to the following: CBC showing hypochromic microcytic anemia, Low serum ferritin, Low transferrin saturation, serum C-reactive protein (CRP) levels < 0.6 mg/dl. Patients with any chronic disease, received iron therapy or blood transfusion in the last 4 months and had any signs or symptoms of infection, which would affect total leukocytes count or any of its subgroups were excluded from the study.

Iron deficiency anemia was defined when the hemoglobin level was below 11 g/dL with ferritin below 12 ng/ml up to the age of 5 years, hemoglobin level below 11.5 g/dL with ferritin below 15 ng/ml in children with ages between five and 16 years [7], or transferrin saturation below 16% [14].

Anemia severity is characterized as mild, moderate, or severe depending on the following criteria: in children with ages below 5 years classified to mild anemia when hemoglobin level between 10–10.9 g/dL, while moderate anemia when hemoglobin level between 7 – 9.9 g/dL and severe anemia when hemoglobin level below 7 g/dL. In children with ages 5 years and above classified to: mild anemia when hemoglobin level between 11 – 11.4g/dL, moderate anemia when hemoglobin level between 8 – 10.9 g/dL and severe anemia when hemoglobin level below 8 g/dL [14].

All children were subjected to:

- History taking, with emphasizing the family history, medical and nutritional history.
- Clinical examination including: general examination with special emphasis on signs of anemia e.g., pallor, nail spooning, glossitis and signs of chronic disease, e.g., jaundice, edema, clubbing, and purpura.
- Complete blood count (Sysmex KX-21N, Sysmex Corporation, Japan) with differential TLC based on blood film, where NLR and PLR were calculated. Children with IDA were subjected further to markers of iron status: serum ferritin (Beckman coulter Access 2, United States of America), serum iron, and total iron binding capacity (TIBC) (Siemens Dimension Expand plus, United States of America), and transferrin saturation was calculated.

Ethics approval:

Written permission was obtained from all subjects' parents or legal guardians after they were fully informed of the nature, methods, and goals of the study. The study was approved by the Faculty of Medicine at Helwan University's Research Ethics Committee, which made sure that patient data remained anonymous. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

SPSS version 22 was used for statistical analysis. Qualitative data were shown as percents and numbers and were compared by Chi Square test or Fisher's exact test. The Shapiro-Wilk test is used to see if numeric data were normally distributed. If the data were normally distributed, they were shown as mean, standard deviation (SD), and range. If the data were not normally distributed, they were shown as median and interquartile range (IQR). Mann whitnney u test was used for to compare between two groups of non- normally distributed variables. Spearman's correlation coefficient was calculated to assess relationship between various study variables, (+) sign indicate direct correlation & (-) sign indicate inverse correlation, also values near to 1 indicate strong correlation & values near 0 indicate weak correlation. When P was less than 0.05, the level of significance was allowed.

Sample size: Based on the study of **Hussien *et al.*** [8], which reported that the mean of TLC was 6.59 and 8.19 in patients with and without IDA respectively by OpenEpi, Version 3 program, power 90% CI 95%, The minimal required sample was calculated to be 32 (16 cases and 16 control).

RESULTS

The patient's clinical and demographic data are shown in Table 1. Patients were classified according to severity of anemia to 18 children had moderate anemia, 5 children had mild anemia and only one child had severe anemia. Comparison between studied IDA patients and the control group according to differential leukocyte count, PLT, NLR and PLR are shown in table 2. There was no statistically significant difference in PLR and NLR between IDA and healthy children ($p > 0.05$). However, abnormal leukocyte counts (leukocytosis/ leukopenia) were more frequent in the IDA group ($p = 0.04$). There was a significant inverse correlation between serum iron and ferritin with absolute eosinophil, in IDA patients. Correlation of serum iron and serum ferritin with NLR, PLR and other hematological indices in patients with IDA, are shown in table 3.

Table (1): Clinical and demographic characteristics of studied groups

	Patients Group N=24	Control Group N=37	*p*-value
Gender N (%)			
– Males – Females	11 (45.8%) 13 (54.2%)	21 (56.8%) 16 (43.2%)	0.404 c
Age (years)			
– Median (IQR)	4.6 (2.1-6.8)	4.38(3.4-6.95)	0.684u
Age category			
– < 5 years – 5 years	12 (50.0%) 12 (50.0%)	22 (59.5%) 15 (40.5%)	0.467c
Residence; **N** (%)			
Badr City	24 (100.0%)	37 (100.0%)	-
Body weight (kg)			
Median (IQR)	14.5(10.6-22.1)	16(14-20.8)	0.11u
Height (cm)			
Mean ± SD Range	104.77±21.11 80-152	107.45 ± 19.32 81 - 150	0.612t

*: Significant *P*``, N=number ,IQR(interquartile range), U:mann whitnney test,chisquare test {c}, t: t test

Table (2): Comparison between studied IDA patients and the control group according to differential leukocyte count, PLT, NLR and PLR

	Patients Group N = 24	Control Group N = 37	*P*- value
TLC (10^9/L)			
Median(IQR)	8.55(6.17-9.85)	7.6(6.6-10.5)	0.696
TLC abnormality			
Present Absent	6(25.0) 18(750)	2(5.4) 35(94.6)	<0.05*c
Neutrophil count			
Median(IQR)	3358.5(2086-5208)	3402(2219-5882.5)	0.935
Lymphocyte count Median(IQR)	3640(2446-4858)	3660(2892.5-4683)	0.965
Monocytes count			
Median(IQR)	520(394-700)	605(423.5-836)	0.388
Eosinophil's count			
Median(IQR)	0(0-179.3)	.00(0-84)	0.476
Basophils count			
Median(IQR)	0(0-0)	0(0-0)	-
PLT count ($\times 10^9$/L)			
Median(IQR)	395(274-460)	363(278.5-429.5)	0.483
NLR Median(IQR)	0.91(0.6-1.63)	.97(0.61-1.49)	.669
PLR			
Median(IQR)	94.0(66.8-145.5)	94.9(81.63-117.94)	.953

*: Significant P: N=number, NLR=Neutrophil to Lymphocyte, PLT=Platelet, PLR=Platelet to Lymphocyte Ratio, TLC=Total leukocyte count. U;Mann-Whitney test, chisquare test {c},

Table (3): Correlation of serum iron and serum ferritin with NLR, PLR, and other hematological indices in patients with IDA

Parameters	Iron (ug/dL)	
	r	P
$TLCx10^9$	-0.202	0.344
Neutrophils count	-0.044	0.838
Lymphocytes count	-0.057	0.79
Monocytes count	-0.118	0.583
Eosinophils count	**-0.607****	**0.002**
Basophils count	-0.211	0.322
NLR	0.003	0.987
PLR	0.084	0.695
Parameters	**Ferritin (ng/ml)**	
	r	**P**
$TLCx10^9$	-0.007	0.975
Neutrophils count	0.079	0.712
Lymphocytes count	0.126	0.557
Monocytes count	0.193	0.366
Eosinophil's count	**-0.452***	**0.027**
Basophils count	-0.181	0.396
NLR	0.029	0.892
PLR	-0.239	0.26

**: Correlation is significant at the 0.01 level (2-tailed). *: Correlation is significant at the 0.05 level (2-tailed), NLR=Neutrophil to Lymphocyte, PLT=Platelet, PLR=Platelet to Lymphocyte Ratio, r=correlation coefficient, TLC=Total leukocyte count.

DISCUSSION

Iron has a role in many important functions. It is considered an essential element for immune system development and integrity. There are some researches that suggested the formation of blood elements such as platelets and white blood cells is affected by iron deficiency [5,7,15].

Our data indicate that the total leukocyte count in the examined IDA patients was not substantially elevated compared to the matched control group. Likewise, many investigations indicated that the total leukocyte count in the examined IDA patients was not substantially elevated compared to the matched control group [6,16].

Unlike our results**, Hussien *et al.* [8] and Rahmani and Demmouche [17]** reported that TLC was significantly lower in the iron-deficient group than in the healthy control group. However, **Özdemir [18]** reported that the leukocyte count is usually normal, but leukopenia may also be observed.

The findings of our investigation indicated that neutrophil levels were not substantially elevated in patients with IDA compared to the control group. Iron significantly influences both the count and functionality of granulocytes. [15] **Aly *et al.*** [6] demonstrated that there was no significant difference in neutrophil count between children with IDA and the healthy control group. **Özcan *et al.*** [15] showed that granulocyte count was significantly increased in IDA patients. However, another study done in Algeria revealed that neutrophil count was significantly lower in children with IDA than that of the control group [17].

Different theories have been proposed to explain this increase in neutrophils, and one of them is that ID alters the apoptotic response, [19] lower oxidative burst and oxidant product synthesis, leading to an increase in neutrophils life span [20]. Moreover, the increased granulocyte cell count occurred as compensation of a decrease in granulocyte phagocytic function in IDA patients [21].

From our study, no significant difference was observed between the absolute lymphocyte count in children with IDA and healthy control children. Similarly, **Mullick *et al.*** [22] reported no significant difference between the absolute lymphocytes counts in IDA patients compared to the healthy control group.

In contrast to our results, different studies showed statistically significant lower lymphocyte counts in children with IDA than those of controls [6,8]. While **Rahmani and Demmouche** [17] reported significant higher absolute lymphocyte count in children with IDA than healthy control children. This difference could be because the study group and sample size were different.

Based on what we found, there was no statistically significant difference between IDA children and healthy controls in the number of absolute eosinophils, basophils, and monocytes. In a study similar to this one, **Rahmani and Demmouche** found that there was no statistically significant difference between the basophils and eosinophils levels in children with IDA and a healthy control group [17]. **Samanta and Senapati** [16] in their study showed that TLC was elevated in anemic patients when compared to healthy controls, but with no statistical significance. In terms of differential counts, there was a significant rise in absolute neutrophil count and a significant fall in absolute lymphocyte count. Meanwhile, there was no significant rise in the eosinophil and basophil counts and little difference in monocyte counts between the two groups.

In the present study, we found that there was a significant inverse correlation between the serum iron and ferritin with the eosinophil count in IDA patients. **Hussien *et al.*** [8] reported inverse significant correlation between ferritin and both lymphocyte, and eosinophils. Eosinophilia in the CBC or peripheral smear may provide information about the underlying parasitosis [18].

From our study, the platelet count of IDA patients was not significantly higher than the control group. Similarly, some studies reported no significant increase in platelet count in children with IDA [6,23]. While **Das *et al.*** [24] in their study reported a significant higher platelet count among children with IDA than control group. It is more common for IDA patients to have thrombocytosis than thrombocytopenia [25].

Higher platelet count may result from a cross-reaction between elevated erythropoietin in IDA and thrombopoietin receptors in megakaryocytes [18].

From our findings, NLR and PLR were not significantly increased in IDA patients in comparison to the healthy control group. **Aly *et al.*** [6] also reported no significant change in NLR and PLR among children with IDA in comparison to healthy control group. **Hegde and Puranik** [3] reported that TLC count and platelet count were significantly increased. Absolute neutrophil count was increased, whereas differential lymphocyte count was decreased in anemic patients. So, NLR and PLR were increased significantly and there was a noticeable increase in the ratios among children with severe IDA. Other study showed that N/L ratio was significantly higher in the IDA anemic group than healthy control group [16].

The current study had some limitations, first of which was the small number of patients with IDA included in the study (small sample size). Second, most of our cases had moderate and mild anemia.

CONCLUSION

This study found no significant difference in PLR and NLR between IDA and healthy children. However, abnormal leukocyte counts were more prevalent in IDA patients, suggesting potential immune alterations. Larger, multicenter studies are needed to confirm these findings and determine the clinical relevance of these hematological parameters in pediatric IDA diagnosis and management.

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Conflicts of interest: The authors declare that they have no conflict of interest.

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