

*Full Length Research Paper*

## Evaluation of leucocyte and its subgroups in iron deficiency anemia

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In this study, leukocyte, granulocyte, lymphocyte and monocyte count was evaluated in patients with iron deficiency anaemia. Complete blood count data from 84 patients (with iron deficiency anaemia) admitted to our hospital's paediatrics and general medicine outpatient clinics, and 109 age- and sex-matched healthy controls, were evaluated retrospectively. Complete blood count analyses were performed with Micros 60, and serum iron and ferritin levels analyses were performed by auto analyzers. Whilst granulocyte count was higher in the group with iron deficiency anaemia, lymphocyte count was lower. No difference in monocyte count was found between the groups. Although total leukocyte count was higher in the group with anaemia, the difference was statistically insignificant. In anaemia, iron deficiency increases granulocyte but reduces lymphocyte count. The molecular basis of this dual effect of iron on cellular defence system is still, to a great extent, unexplained.

**Key words:** Iron deficiency anaemia, leucocyte, granulocyte, lymphocyte, monocyte.

### INTRODUCTION

Iron deficiency anaemia (IDA) is one of the most common health problems and is the most frequently occurring type of anaemia. IDA develops as an outcome of iron intake or absorption defect. Iron is an important component of the oxygen transporter haemoglobin. Iron deficiency causes anaemia due to a fall in haemoglobin production. Besides its role in transporting oxygen, element iron has many other important functions. Iron is a cofactor in a large number of enzymes including catalase, ribonucleotides, acid phosphatase, myeloperoxidase, xanthine oxidase and cytochromes (Rockey and Cello, 1993).

There are several reports suggesting iron deficiency to have important effects on formation of blood elements, especially thrombocytes and leukocytes (Düzgün et al., 2005). Clinical and *in vitro* studies have shown that IDA alters thrombocyte counts frequently resulting in

thrombocytosis and also affects leukocyte phagocytic functions (Habis et al., 2010; Yıldırım et al., 2011).

This study was planned to evaluate leukocyte, granulocyte, lymphocyte and monocyte counts in patients with IDA.

### PATIENTS AND METHODS

This study was performed at Private Sema Hospital, Internal Medicine and Pediatric Clinics, Istanbul, Turkey. The study subjects were recruited between January 2009 and August 2009. Eligible female patients with IDA were enrolled in this retrospective study. The inclusion criteria were: hemoglobin level <12 g/dl, serum ferritin level <5 ng/L. Patients with acute hemorrhage and infections, neoplastic and chronic inflammatory disorders such as rheumatoid arthritis, ankylosing spondylitis and systemic lupus erythematosus were excluded. IDA with children was defined by the following criteria: haemoglobin <11g/dl, serum ferritin <7 ng/L, and no intake of haematinics in preceding one month. Healthy children of the same age group were selected from the pediatric clinic in the control group. Inclusion criteria were: serum CRP levels < 0.6 mg/dl, no history of chronic disease, absence of anaemia and iron

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**Table 1.** Age, leukocyte, granulocyte, lymphocyte and monocyte values for control and patient group (mean ± standard deviation).

	n	Age (year)	Total leukocyte ( $10^3/\mu\text{l}$ )	Granulocyte( $10^3/\mu\text{l}$ )	Lymphocyte( $10^3/\mu\text{l}$ )	Monocyte( $10^3/\mu\text{l}$ )
		Mean ± Standard deviation				
Normal	109	33.1 ± 12.1	6.48 ± 1.71	3.88 ± 1.43	2.26 ± 0.50	0.341 ± 0.144
IDA	84	34.8 ± 10.3	6.78 ± 2.51	4.44 ± 2.10	1.98 ± 0.75	0.358 ± 0.745
P value		0.316	0.332	0.037	0.003	0.458

**Table 2.** RBC, HGB, HCT, PLT, iron and ferritin values for control and patient group (mean ± standard deviation).

	n	RBC ( $10^3$ million/ $\mu\text{l}$ )	HGB (g/dl)	HCT(%)	PLT ( $10^3/\mu\text{l}$ )	Iron(mg/dl)	Ferritin (ng/ml)
		Mean ± Standard deviation					
Normal	109	4.55 ± 0.35	13.05 ± 0.76	39.15 ± 2.29	266 ± 57	86.8 ± 34.3	32.8 ± 24.9
IDA	84	4.28 ± 0.44	10.50 ± 1.87	32.77 ± 4.87	301 ± 86	22.0 ± 10.1	12.9 ± 19.7
P value		0.000	0.000	0.000	0.002	0.000	0.000

RBC, Red blood cell; HGB, haemoglobin; HCT, hemotocrit; PLT, thrombocyte.

deficiency with haemoglobin >11 g/dl, MCV>80 fl, and serum ferritin >7 ng/L, and no intake of haematinics in last one month.

Full blood count values, serum iron and ferritin levels of 84 patients (age range: 33.1 ± 12.1) admitted to our hospital's paediatric and internal diseases outpatient clinics, and 109 age- and sex-matched healthy controls (age range: 34.8 ± 10.3) were evaluated retrospectively. Full blood count was performed using Horiba ABX Micros60 analyzer (France), serum iron level was measured with VITROS 5.1 FS analyzer (Johnson and Johnson, VITROS 5.1 FS, Biochemistry Analyser, USA) and ferritin level with IMMULITE 2500 auto analyzer (Siemens IMMULITE 2500 Immunoassay System, Germany)

#### Statistical analysis

Data analysis was performed by using SPSS for Windows (version 14.0). All data were presented in mean (±)

standard deviations (SD). The control and the IDA group were compared for haematological and immunological parameters using the unpaired t-test.

## RESULTS

Tables 1 and 2 present mean and standard deviation values from the IDA and control group test results. Whilst granulocyte count was higher ( $p=0.037$ ) in the group with iron deficiency anaemia, lymphocyte count was lower ( $p=0.003$ ). No difference in monocyte count was found ( $p=0.458$ ). Although mean total leukocyte count was higher in the group with anaemia, the difference was statistically insignificant ( $p=0.332$ ). Furthermore, whilst IDA group had significantly lower erythrocyte count, haemoglobin and hemotocrit levels, compared to the control group

( $p=0.000$ ), thrombocyte count was significantly higher ( $p=0.002$ ). By definition serum iron and ferritin levels in the IDA group were significantly lower compared to the control group ( $p=0.000$ ).

## DISCUSSION

This study shows a significant fall in lymphocyte levels in the IDA group. Available literature supports our results. Mullick et al. (2006) reported lower lymphocyte count and Lurashi et al. (1991) reported lower T lymphocyte percentage in IDA patients. There could be two reasons causing the declined lymphocyte count seen in IDA: (a) suppression of lymphocyte production, (b) increased lymphocyte destruction.

Besides a decline in the proliferation of lymphocytes isolated from the blood of IDA

patients, studies report T lymphocyte proliferation response to various stimulants not reaching levels of healthy individuals (Ekiz et al., 2005; Van Heerden et al., 1981). On the other hand, a cell culture study showed a positive effect of iron on lymphocyte proliferation (Bryan et al., 1986). Investigating the relation between serum iron and lymphocytes, Guzikowska et al. (1989) found a positive correlation between T lymphocyte count and iron. Other studies also report that iron treatments induce an increase in T lymphocyte percentage (Moraes-de-Souza et al., 1984; Krantman et al., 1982). In this context, Hoffbrand et al. (1976) suggested that reduced ribonucleotide reductase activity, that uses iron as a cofactor, could account for the fall in lymphocyte counts. Shorter lymphocyte life span could be another significant factor in reduced lymphocyte counts. Aslan et al. (2006) report increased lymphocyte DNA damage and reduced antioxidant capacities in adult female IDA patients. This could eventually cause early lymphocyte destruction or result in earlier disposal from circulation. Therefore, both suppressed lymphocyte proliferation and increased oxidative damage-induced early lymphocyte destruction can contribute to reduced lymphocyte blood count.

This study also showed that granulocyte count was significantly increased in IDA patients. Iron has important effects on both granulocyte functions and count. There are certain studies reporting increased basophil and neutrophil count in IDA patients (Tokuhira et al., 2007; Hrycek et al., 1991). Walter et al. (1986) argue that iron does not affect granulocytes in circulation but affect their development in bone marrow. Regarding increased neutrophil count several studies have shown that iron deficiency induces changes in apoptotic response (Paino et al., 2009), lower oxidative burst and oxidant product synthesis (Berrak et al., 2007) resulting in increased neutrophils life span. Furthermore, IDA patients present reduced neutrophil phagocytic activity (Banerjee et al., 1991). Thus the increased granulocyte cell count in IDA patients could compensate for the reduced phagocytic capacity.

Many authors have reported that thrombocyte count is increased in IDA patients. Iron may have an effect on the myeloid series cells – granulocytes, that is similar to its effect on thrombocyte metabolism. Heidarpour et al. (2008) study investigating the effect of iron and copper on hematologic parameters reported a rise in erythrocyte count, a significant fall in neutrophil and thrombocyte counts with no change to monocyte, lymphocyte and total leukocyte counts. Similar hematologic results can be observed in other diseases accompanied by iron deficiency. In their study on marathon athletes Fallon et al. (1999) collected blood samples from the participants at different stages of the event and observed an increase in total leukocyte, neutrophil and thrombocyte counts, a fall in serum iron levels without changes of lymphocyte and monocyte counts. The results of our study are in accordance with previous studies and suggest that iron

affects directly erythrocyte, thrombocyte, leukocyte and especially neutrophil and lymphocyte production.

## Conclusion

Iron deficiency anemia is accompanied by increased defence system in cell granulocyte count but decreased lymphocyte count. The molecular basis for this dual effect of iron on cellular defence system remains to be clarified.

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