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Original Article

Comparative Evaluation of Lymphocytic Count in Pre-menopausal Women with Iron Deficiency Anemia

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***ABSTRACT*:**
Background: The relationship between iron deficiency and infection susceptibility has been investigated. There is some evidence that iron is a fundamental element for normal development of the immune system and some clinical studies have emphasised the importance of iron in the integrity of the immune system. The effects of iron deficiency on cellular immune function remain controversial. **Aim:**To evaluate lymphocytic count in pre-menopausal women with Iron deficiency anemia.**Materials and method:** The study was conducted in the Department of General pathology of the medical institution. In the study group we included a total of 35 non-pregnant, pre-menopausal women between the ages of 18–40 years old presenting with IDA as the solitary pathology were included in the patient group. A total of 35 normal healthy women were also included in the study as control group. For the laboratory evaluation of blood parameters, we collected 5 mL of venous blood of subjects in sterile tube containing K3–EDTA anticoagulant. Peripheral blood smears (PBS) were prepared with Geimsa staining.**Results:** In the present study, the mean age of the patients in study group was 29.8 ± 8.32 years and in control group was 31.25± 7.88 years.The mean CD3+, CD3+/CD4+, CD3+/CD8+, and CD19+ lymphocyte counts were 1.42, 0.81, 46, and 0.31 x 109/L, respectively, in study group, and 1.93, 0.52, 0.67, and 0.27 x 10^9/L, respectively, for the control group. The absolute T lymphocytes (CD3+) and subpopulations (CD4+, CD8+) in the iron-deficient group were significantly lower than in the control group. **Conclusion:** There are significant changes in the total lymphocyte counts in women with IDA. Due to decreased lymphocyte count in pre-menopausal women with IDA, these patients may be more prone to infection.
Keywords: Anemia, Iron deficiency, pathology, lymphocytes

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INTRODUCTION

Iron is an essential element for all living cells and plays an important role in many metabolic pathways. With the binding of plasma iron to transferrin (Tf), apotransferrin is changed into a diferric form which can bind to transferrin receptors (TfR) on the cell membranes.[1,2] Virtually all cells,including reticulocytes, proliferating leukaemic cells, activatedlymphocytes and placental cells, have TfRs on theirsurfaces. In the normal adult, about 80% of them are foundin the erythroid marrow. Altered immune responses have considerable public health significance.[3,4] The relationship between iron deficiency and infection susceptibility has beeninvestigated. There is some evidence that iron is a fundamental element for normal development of the immune system and some clinical studies have emphasised the importance of iron in the integrity of the immune system. The effects of iron deficiency on cellular immune function remain controversial. Some reports indicate that iron depletion may be responsible for decreased cellular immunity while others have not reported any changes in lymphocyte subset in patients with IDA.5,6 Hence the present study was conducted to evaluate lymphocytic count in pre-menopausal women with Iron deficiency anemia.

MATERIALS AND METHOD

The study was conducted in the Department of General pathology of the medical institution. The ethical clearance for study protocol was obtained from ethical committee of the institution. The

approval of the study protocol was obtained from the ethical committee of the institute. In the study group we included a total of 35 non-pregnant, pre-menopausal women between the ages of 18–40 years old presenting with IDA as the solitary pathology were included in the patient group. The inclusion criteria were haemoglobin (HGB) level of less than 12.5 g/dL, red blood cell (RBC) count of less than 4 x 1012/L, mean corpuscular volume (MCV) of less than 80 fL, mean corpuscular haemoglobin (MCH) of less than 27 pg, serum iron level of less than 50 µg/dL, total iron binding capacity (TIBC) of more than 400 µg/dL, and serum ferritin level of less than 20 µg/dL. A total of 35 normal healthy women were also included in the study as control group. The exclusion criteria were possible thalassaemia according to the laboratory results, a history of acute or chronic infection, familial history of immunodeficiency, history of cancer, pregnancy, and a history of endocrinopathy, especially hypo- or hyperthyroidism.

For the laboratory evaluation of blood parameters, we collected 5 mL of venous blood of subjects in sterile tube containing K3–EDTA anticoagulant. Peripheral blood smears (PBS) were prepared with Geimsa staining. Cytometric analysis of peripheral blood was done by a fluorescence-activated cell sorting (FACS) count flow cytometer using monoclonal antibodies specific for CD3, CD19, CD45, CD4, and CD8 lymphocyte antigens for determining the percentage of these lymphocyte subpopulations in the samples.

The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistical significant.

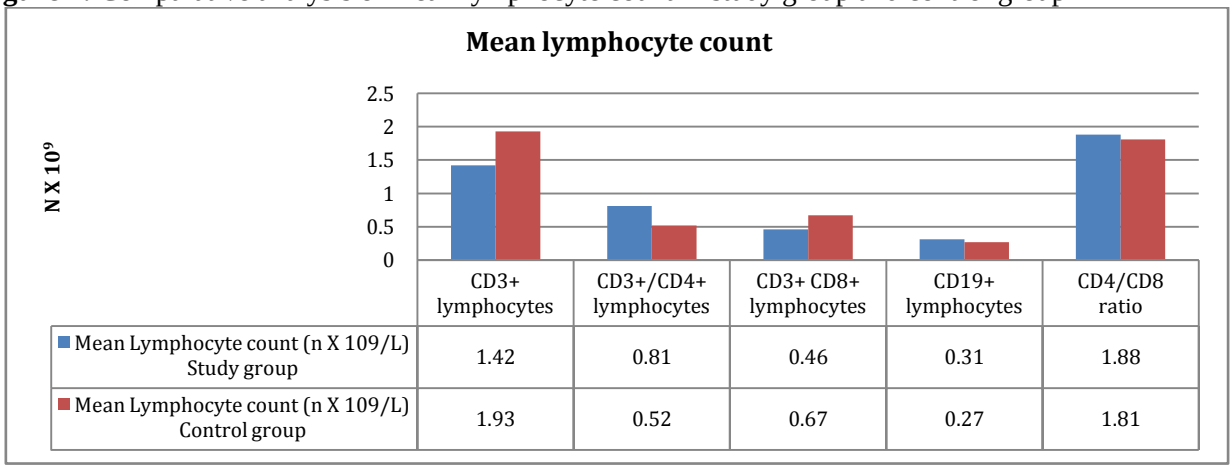
RESULTS

In the present study, the mean age of the patients in study group was 29.8 ± 8.32 years and in control group was 31.25± 7.88 years. there were 35 subjects in each group. Table 1 shows the mean lymphocyte count in pre-menopausal women with Iron deficiency anemia and normal healthy women. The mean CD3+, CD3+/CD4+, CD3+/CD8+, and CD19+ lymphocyte counts were 1.42, 0.81, 46, and 0.31 x 109/L, respectively, in study group, and 1.93, 0.52, 0.67, and 0.27 x 10^9/L, respectively, for the control group. The absolute T lymphocytes (CD3+) and subpopulations (CD4+, CD8+) in the iron-deficient group were significantly lower than in the control group. On comparing the results we observed that CD3+ lymphocyte count and CD3+/CD4+ lymphocyte count were statistically significant ($p<0.05$). The CD3+ CD8+ lymphocyte count, CD19+ lymphocyte count and CD4/CD8 ratio was statistically non-significant ($p>0.05$).[Fig 1]

Table 1: The mean lymphocyte count in pre-menopausal women with Iron deficiency anemia and normal healthy women

Lymphocytic population	Mean Lymphocyte count (n X 10^9/L)		p-value
	Study group	Control group	
CD3+ lymphocytes	1.42	1.93	0.02
CD3+/CD4+ lymphocytes	0.81	0.52	0.001
CD3+ CD8+ lymphocytes	0.46	0.67	0.09
CD19+ lymphocytes	0.31	0.27	0.88
CD4/CD8 ratio	1.88	1.81	0.22

Figure 1: Comparative analysis of mean lymphocyte count in study group and control group



DISCUSSION

Iron is crucial for cell proliferation due to its role in DNA synthesis and maturation of T lymphocytes. It is essential for enzymes such as ribonucleotide reductase, and it is involved in DNA synthesis; therefore, the proliferative phase of lymphocyte activation is an iron-requiring step and this activity can be diminished during IDA. Some authors have Our findings showed significant decreases in total CD3+ and CD3+/CD4+. These were consistent with some earlier studies. S Kinik et al studied the effect of iron deficiency anemia (IDA) on CD71 expression by peripheral blood lymphocytes and monocytes on 43 children. Eighteen healthy age-matched children served as controls. CD71 bearing lymphocytes and monocytes were enumerated by flow cytometric analysis. At diagnosis, the mean +/- SD of CD71+ lymphocytes and monocytes was 5.9 +/- 0.76% and 23.0 +/- 4.0% in IDA patients and 12.6 +/- 0.98 % and 27.6 +/- 6.6 % in controls, respectively. Differences between CD71+ lymphocytes and monocytes were significant. In IDA patients a statistically significant correlation was seen between CD71+ lymphocytes and monocytes vs Hb, Hct, and MCV. After 30 days of oral iron therapy the number of CD71+ lymphocytes and monocytes increased significantly.Evstatiev R et al studied the mechanisms behind iron deficiency-induced thrombocytosis. Within few weeks, iron-depleted diet caused iron deficiency in young Sprague–Dawley rats, as reflected by a drop in hemoglobin, mean corpuscular volume, hepatic iron content and hepcidin mRNA in the liver. Thrombocytosis established in parallel. Moreover, platelets produced in iron deficient animals displayed a higher mean platelet volume and increased aggregation. Bone marrow studies revealed subtle alterations that are suggestive of expansion of megakaryocyte progenitors, an increase in megakaryocyte ploidy and accelerated megakaryocyte differentiation. Iron deficiency did not alter the production of hematopoietic growth factors such as thrombopoietin, interleukin 6 or interleukin 11. Megakaryocytic cell lines grown in iron-depleted conditions exhibited reduced proliferation but increased ploidy and cell size. Our data suggest that iron deficiency increases megakaryopoietic differentiation and alters platelet phenotype without changes in megakaryocyte growth factors, specifically TPO. Iron deficiency-induced thrombocytosis may have evolved to maintain or increase the coagulation capacity in conditions with chronic bleeding.[9, 10] suggested that altered levels of some interleukins (IL) and cytokines (e.g. IL-2, IL-1, IL-6, TNF-α, IL-4, IL-12p40, IFN-γ, and IL-10) might lead to immune system impairment in IDA patients. In addition, it has been suggested that altered cell marker expression may contribute to reduced T cell proliferation during iron deficiency.[7,8]

Reza Keramati M et al evaluate alteration of lymphocyte subgroups in IDA. They investigated lymphocyte subsets in pre-menopausal women with iron-deficiency anaemia; 50 normal subjects and 50 IDA (hypochromic microcytic) cases were enrolled. Experimental and control anticoagulated blood samples were evaluated using flow cytometry to determine the absolute and relative numbers of various lymphocyte subgroups. Finally, the results of the patient and control groups were compared. Mean (SD) absolute counts of lymphocytes, CD3+ cells, CD3+/CD4+ subsets (T helper) and CD3+/CD8+ subsets (T cytotoxic) in the patient group were 2.08 (0.65) x 109/L, 1.53 (0.53) x 109/L, 0.87 (0.28) x 109/L, and 0.51 (0.24) x 109/L, respectively. The results showed significant differences between case and control groups in mean absolute counts of lymphocytes, T lymphocytes, helper T cells, and cytotoxic T cells. This study showed that absolute counts of peripheral blood T lymphocytes as a marker of cell-mediated immunity may be decreased in pre-menopausal women with iron-deficiency anaemia, and that these patients may be more prone to infection.Das I et al evaluate the effects of iron deficiency anemia (IDA) and its treatment with oral iron supplementation on cell-mediated immunity (CMI) and humoral immunity (HMI) in children. A total of 40 children (<15 years) with IDA and 40 age-matched healthy children after satisfying the inclusion criteria were enrolled for this case-control study. Flow cytometric evaluation of absolute and relative numbers of cluster of differentiation 4 (CD4) and CD8 (cluster of differentiation 8) lymphocyte subgroups was carried out to assess the CMI and serum Immunoglobulin G (IgG), Immunoglobulin A (IgA), Immunoglobulin M (IgM) were measured to assess the HMI at baseline and 3 months post oral iron supplementation. Significantly lower levels of CD4+ T-cells and decreased CD4:CD8 ratios were observed in the iron deficient children. Iron supplementation significantly improved the CD4+ cell counts and CD4:CD8 ratios. However, immunoglobulin levels weren't different between the two groups. It was concluded that IDA did not influence HMI, it significantly impaired CMI, which

was improved following iron supplementation for 3 months.[11,12]

CONCLUSION

Within the limitations of the study, we conclude that there are significant changes in the total lymphocyte countsin women with IDA. Due to decreased lymphocyte count in pre-menopausal women with IDA, these patients may be more prone to infection.

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