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Original Research

Comparative Study to Analyse the Lymphocytic Count In Pre-Menopausal Women with Iron Deficiency Anemia

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ABSTRACT
Background: Iron is vital for all living organisms because it is essential for multiple metabolic processes, including DNA synthesis and oxygen and electron transport. Iron deficiency is a main nutritional deficiency disorder affecting large fractions of the world population and is a common cause of anemia. Iron deficiency anemia (IDA) is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic). **Aim of the study:** To evaluate the lymphocytic count in pre-menopausal women with iron deficiency anemia. **Materials and methods:** The study was conducted in the Department of General Pathology of the medical institution. For the study, we selected 50 pre-menopausal women between the age group of 18-35 years. It was made sure that the subjects selected for the study are anemic having hemoglobin less than 10 g/dL. For the control group, 50 pre-menopausal women with normal hemoglobin level were included. The patients with thalassemia, leukemia or any other chronic and autoimmune disease were excluded from the study. Laboratory evaluation of each subject was done. **Results:** The mean age of the patients in study group was 26.12 years and in control group was 25.22 years. There were 50 subjects in each group. The mean CD3+, CD4+, CD8+, and CD19+ lymphocyte counts were 1.56, 0.79, 0.55, and 0.39 $X10^9$/L, respectively, in study group, and 1.89, 0.49, 0.71, and 0.29 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 was statistically significant. **Conclusion**: The pre-menopausal women with iron deficiency anemia have significant change in the total lymphocyte count. Due to decreased lymphocyte count in pre-menopausal women with IDA, these patients may be more prone to infection.

Keywords: Anemia, Iron deficiency, Hemoglobin

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INTRODUCTION

Anemia, associated with iron deficiency, is most often due to increased blood loss, or impaired iron absorption.[1, 2] Iron is vital for all living organisms because it is essential for multiple metabolic processes, including DNA synthesis and oxygen and electron transport. Iron deficiency is a main nutritional deficiency disorder affecting large fractions of the world population and is a common cause of anemia. Iron deficiency anemia (IDA) is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic).[3, 4] The effects of iron deficiency on immunity remain controversial. Some reports indicate iron depletion may be responsible for decreased immunity while others did not report any change. It is important to understand the effects of IDA on the immune system due to its high prevalence in the world. Studies have provided evidence that iron deficiency anemia is more common in pre-menopausal women.[5, 6] Hence, the present study was planned to evaluate the lymphocytic count in pre-menopausal women with iron deficiency anemia.

MATERIALS AND METHODS

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. For the study, we selected 50 pre-

menopausal women between the age group of 18-35 years. It was made sure that the subjects selected for the study are anemic having hemoglobin less than 10 g/dL. For the control group, 50 pre-menopausal women with normal hemoglobin level were included. The patients with thalassemia, leukemia or any other chronic and autoimmune disease were excluded from the study. Laboratory evaluation of each subject was done. For the lab investigations, we collected 5 mL of venous blood by venipuncture from each subject and stored the blood in a sterile tube containing EDTA anticoagulant. Fluorescence-activated cell sorting (FACS) count flow cytometer using monoclonal antibodies specific for CD3, CD19, CD45, CD4, and CD8 lymphocyte antigens was used for cytometric analysis of blood 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

Table 1 shows the demographic data of patients. The mean age of the patients in study group was 26.12 years and in control group was 25.22 years. There were 50 subjects in each group. Table 2 shows the mean lymphocyte count in peripheral venous blood in pre-menopausal women with Iron deficiency anemia and normal healthy women. The mean CD3+, CD4+, CD8+, and CD19+ lymphocyte counts were 1.56, 0.79, 0.55, and 0.39 $X10^9$/L, respectively, in study group, and 1.89, 0.49, 0.71, and 0.29 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 was 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: Demographic data

Variables	Study group	Control group	p-value
No of patients	50	50	0.9
Mean age (years)	26.12	25.22	0.11
Mean Hb level	9.21	12.69	0.21

Fig 1: Comparative analysis of mean lymphocytic count in study group and control group

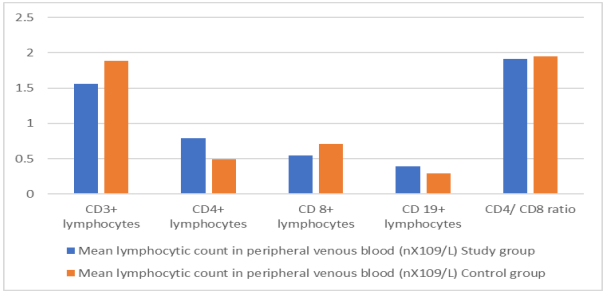

Table 2: Mean lymphocytic count in peripheral venous blood of study group and control group

Lymphocytes	Mean lymphocytic count in peripheral venous blood ($nX10^9$/L)		p-value
	Study group	Control group	
CD3+ lymphocytes	1.56	1.89	0.02*
CD4+ lymphocytes	0.79	0.49	0.005*
CD 8+ lymphocytes	0.55	0.71	0.3
CD 19+ lymphocytes	0.39	0.29	0.15
CD4/ CD8 ratio	1.91	1.95	0.51

DISCUSSION

In the present study, we observed significant decrease in total CD3+ and CD3+/CD4+ lymphocytic population in peripheral blood. These results were consistent with some earlier studies. Aly SS et al assessed the lymphocyte subsets in childhood iron deficiency anemia (IDA) with their laboratory correlations. Fifty IDA (< 18 years) and 25 age and sex-matched healthy children were enrolled and a complete history was obtained and clinical examination was performed. Complete blood count, serum iron, total iron binding capacity and serum ferritin, were performed. Flow cytometric determination of peripheral blood CD3+, CD4+, CD8+ T-lymphocytes and CD19+ B-lymphocytes and CD4/CD8 ratio were done. Patients had significantly lower hemoglobin, Serum iron, ferritin levels and higher lymphocytic count in patients compared with controls. CD3 count and percentage were significantly lower in IDA patients compared to controls. There was a Significant reduction in the CD4 count, percentage and CD4/CD8 ratio in patients compared with controls while there was no significant difference regarding CD8 count and percentage. No significant difference between the two studied groups regarding either CD19 count or percentage were found. It was concluded that IDA is associated with impaired cell-mediated immune response specifically T-cell mediated immunity. Hagag AA studied serum immunoglobulin levels and T lymphocyte subsets in children with beta- thalassemia in relation to iron overload. This study was conducted on 40 children with beta thalassemia major including 24 males and 16 females with mean age of 9.22 ± 3.9 and 20 healthy children of matched age and sex as a control. All children were subjected to assessment of infection episodes, complete blood picture, Hb electrophoresis, serum iron status, T cell subsets including CD3, CD4 and CD8 using Becton Dickinson FAC Scan flow cytometer and serum immunoglobulin levels including IgM, IgA and IgG by a commercial nephelometry assay using a BN-II device. Serum ferritin and iron were significantly higher but total iron binding capacity was significantly lower in patients than controls. CD3 and CD4 were significantly lower but CD8 was significantly higher in patients than controls. The count for CD3+, CD4+ and CD8+ T cells in patients was 1733.25 ± 381.87, 889.67 ± 282.86 and 779.72 ± 390.63 respectively as compared to 1887 ±

390.56, 1003 ± 250.96 and 663 ± 116.71 in the control group respectively.. There were significant negative correlations between CD3, CD4, IgM and ferritin and significant positive correlations between CD8, IgG, IgA and ferritin. In conclusion; Iron overload affects humeral and cell mediated immunity in thalassemic patients.[7, 8]

Valiathan R et al performed dual platform flow cytometry to determine reference ranges for lymphocyte subsets in 50 adolescents and 100 adults (age range: 21-67) along with T cell maturation, activation and co-stimulatory molecules in healthy multiracial adult population of South Florida. The lymphocyte reference ranges percentages; CD4: 27-53; CD8: 16-40; CD19+ B cells: 8-31 and CD16+CD56+ NK cells: 3-30 and for adults are: CD3: 65-88; CD4: 26-62; CD8: 14-44; CD19+ B cells: 2-27 and CD16+CD56+ NK cells: 2-27. The ranges for CD4:CD8 ratio for adolescents and adults are 0.7-2.6 and 0.6-4.4, respectively. Gender based analysis of relative percentages of lymphocyte subsets showed no significant differences between adult and adolescent males and females. The mean CD4:CD8 ratio was significantly higher in adult females than males and in adolescents this difference was not significant between genders. The mean CD3 and CD4 T cell percentages were higher and CD19 cell percentages were lower in adults compared to adolescents. Absolute lymphocyte counts showed a positive correlation with the absolute counts of CD3+, CD4+, CD8+, CD19+, CD16+CD56+, CD45RO+ and CD45RA+ cells. It was concluded that there is need for periodic evaluation and establishment of lymphocyte reference ranges for patient population served based on gender and age since these could influence immune status and treatment outcome. Mullick S et al documented the changes in T cell subsets in children in the age group of 1 to 5 yr with iron deficiency. The levels of T lymphocytes, their CD4+ and CD8+ subsets and the CD4 : CD8 ratio were evaluated in 40 iron deficient and 30 healthy children. The impact of oral iron supplementation for three months on the same parameters was also noted in 30 children. Significantly lower levels of T lymphocytes as well as CD4+ cells was observed in the iron deficient children. The CD4 : CD8 ratio was also significantly lower in this group. Iron supplementation improved the CD4 counts significantly. Their study demonstrated quantitatively altered T cell subsets in iron deficiency in children, and a relationship between the severity of haematological and immunological compromise. The clinical and epidemiological implications of this relationship have topical relevance since ID is the most common micronutrient deficiency worldwide.[9, 10]

CONCLUSION

Within the limitations of the study, we conclude that the pre-menopausal women with iron deficiency anemia have significant change in the total lymphocyte count. Due to decreased lymphocyte count in pre-menopausal women with IDA, these patients may be more prone to infection.

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