

## Original Article

### Analysis of Lymphocytes Count in Premenopausal Women with Iron Deficiency Anemia

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#### ABSTRACT:

**Background:** Iron deficiency is one of the most common known forms of nutritional deficiency in the world. Iron-deficiency anaemia (IDA), which occurs due to nutritional deficiency, is a major health problem in developed and developing countries. **Aim of the study:** To analyze lymphocytes count in premenopausal women with iron deficiency anemia. **Materials and methods:** The study was conducted in the Department of General Pathology of the medical institution. The approval of the study protocol was obtained from the ethical committee of the institute. For the study, we selected 80 pre-menopausal women between the age group of 18-40 years who were diagnosed with iron deficiency anemia and their hemoglobin level was less than 10 g/dL. 80 pre-menopausal women with normal hemoglobin level were recruited after matching with the subjects for control group. **Results:** The mean age of the patients in study group was 30.25 years and in control group was 31.11 years. There were 80 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 1.77, 0.88, 0.69, 0.42, 1.81 X 10<sup>9</sup>/L, respectively, in study group, and 1.93, 0.52, 0.85, 0.34 and 1.90 X 10<sup>9</sup>/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:** Within the limitations of the study, this can be concluded that significant change in seen in the lymphocyte count in premenopausal women with iron deficiency anemia.

**Key words:** Anemia, iron deficiency, premenopausal women.

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#### INTRODUCTION:

Iron deficiency is one of the most common known forms of nutritional deficiency in the world. Iron-deficiency anaemia (IDA), which occurs due to nutritional deficiency, is a major health problem in developed and developing countries. IDA is characterised by a defect in haemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of haemoglobin (hypochromic). The prevalence of IDA varies according to sex, age, and geography. Young children and menstruating women are at higher risk for IDA because of higher iron needs.<sup>1, 2</sup> The prevalence of IDA has been reported to be 40% of adult males and 57% of adult females in South Asia, 2%–5% of adolescent girls and women of childbearing age in the US, 19% in France, 20% in Poland, 36% in Lebanon, 28% in India, and 21% in Turkey.<sup>3, 4</sup> Iron deficiency anaemia (IDA) is the most

prevalent deficiency disorder and the most frequent form of anaemia in pregnant women. Minor causes of anaemia are folate and vitamin B12 deficiency, haemoglobinopathy and haemolytic anaemia. Anaemia is defined as haemoglobin of <110 g/L in the first and third trimester and <105 g/L in the second trimester. The diagnosis relies on haemoglobin, a full blood count and plasma ferritin, which can be supported by plasma transferrin saturation and serum soluble transferrin receptor.<sup>5</sup> Among fertile, non-pregnant women, approximately 40% have ferritin of <or=30 microg/L, i.e. small or absent iron reserves and therefore an unfavourable iron status with respect to upcoming pregnancy. The prevalence of prepartum anaemia in the third trimester ranges 14-52% in women taking placebo and 0-25% in women taking iron supplements, dependent on the doses of iron.<sup>6</sup> Hence, the present study was

conducted to analyze lymphocytes count in premenopausal 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 80 pre-menopausal women between the age group of 18-40 years who were diagnosed with iron deficiency anemia and their hemoglobin level was less than 10 g/dL. 80 pre-menopausal women with normal hemoglobin level were recruited after matching with the subjects for control group. 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 30.25 years and in control group was 31.11 years. There were 80 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 1.77, 0.88, 0.69, 0.42, 1.81 X 10<sup>9</sup>/L, respectively, in study group, and 1.93, 0.52, 0.85, 0.34 and 1.90 X 10<sup>9</sup>/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]

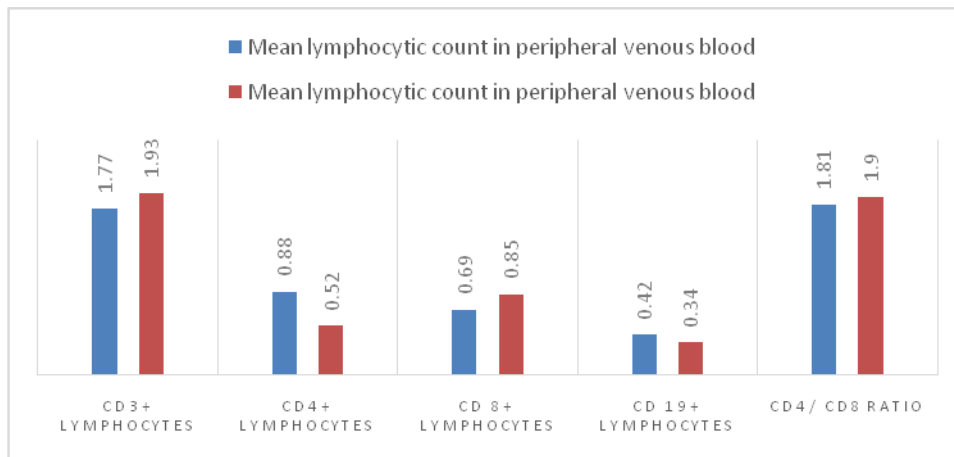
**Table 1: Demographic data**

Variables	Study group	Control group	p-value
No of patients	80	80	0.11
Mean age (years)	30.25	31.11	0.19
Mean Hb level	8.8	11.65	0.9

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

Lymphocytes	Mean lymphocytic count in peripheral venous blood (nX10 <sup>9</sup> /L)		p-value
	Study group	Control group	
CD3+ lymphocytes	1.77	1.93	0.02*
CD4+ lymphocytes	0.88	0.52	0.005*
CD 8+ lymphocytes	0.69	0.85	0.8
CD 19+ lymphocytes	0.42	0.34	0.35
CD4/ CD8 ratio	1.81	1.90	0.12

**Figure 1:**



**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. Reza Keramati M et al evaluated alteration of lymphocyte subgroups in IDA. 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 10<sup>9</sup>/L, 1.53 (0.53) x 10<sup>9</sup>/L, 0.87 (0.28) x 10<sup>9</sup>/L, and 0.51 (0.24) x 10<sup>9</sup>/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. Lukito W et al determined the percentage and absolute counts of the peripheral blood lymphocyte subsets, and to examine the relationship between lymphocyte subsets and nutritional status, and total mortality in an institutionalised elderly population. The sample of 115 permanent elderly residents was drawn from large geriatric institution in Melbourne, Australia. Women had higher absolute counts of various lymphocyte subsets than men. Positive correlations of serum ferritin with the number of CD8 (T-suppressor cell) and of serum iron with CD56 (natural killer, NK cells) were observed in men. In women, serum zinc was positively correlated with the absolute counts of CD3 (total T-cells), CD4 (T-helper cell) and CD19 (total B-cell). The analysis of survival data after 22 months showed that the mean number of CD4 cells of non-survivors was significantly lower than that of survivors. The biochemical indicators of iron and zinc status partly account for variations in lymphocyte subset counts, consistent with known effects of iron overload and of zinc deficiency on immunocompetence.<sup>7, 8</sup>

Khatib L et al identified the determinants of anaemia in Lebanese women of childbearing age attending health centres in Lebanon. Four hundred and seventy non-pregnant Lebanese women aged 15-45 years. Anaemia (Hb <12 g dl(-1)) and iron deficiency (ferritin <15 microg l(-1)) were prevalent in 16.0 and 27.2% of the study sample, respectively. Of the total sample, 7.7% had iron-deficiency anaemia. The percentage of women with either Hb or ferritin deficiency or both was 35.6%. Plasma folate and vitamin B12 deficiency was reported in 25.1 and 39.4%, respectively, and 12.6% of the women had both folate and vitamin B12 deficiencies. Of the anaemic group, 48.0% of the women had iron deficiency. The intake of iron was lower in iron-deficient than in non-deficient women and a positive relationship was shown between folate intake and its corresponding serum levels. Regression analysis showed that ferritin, plasma folate and family history of anaemia were significant determinants of the anaemia in the sample

of women. They concluded that anaemia not related to iron deficiency was partly explained by plasma folate deficiency. Measures to control folate and iron deficiency should be considered. Tang YM et al reviewed the changes in immune function and incidence of infectious diseases in pregnant women with iron deficiency anemia (IDA), especially marginal deficiency of iron. T lymphocyte subsets level (CD3+, CD4+ and CD8+), nature kill cells activity (CD16), interleukin-2 (IL-2) and serum IgA, IgG, IgM and complement C3 were determined in 3 different women groups, including 69 IDA pregnant women who were diagnosed by Hemoglobin, concentrations of free erythrocyteporphyrin and serum ferritin from 280 pregnant women during 30-38 weeks of gestation, 52 random sampling normal pregnant women and 50 no pregnant women examined before marriage. The prevalence of IDA for pregnant women is 24.6%. The average concentration of Hb for pregnant women of IDA is 102.00. The level of CD3+ and CD4+ cells, the ratio of CD4+/CD8+ cells, serum IL-2 as well as IgG levels in the pregnant women were significantly lower than that of those normal pregnant women. With the decreasing extent of Hb, these significant immunological indices of pregnant women will decrease. The incidence of infectious diseases in IDA pregnant women was significantly higher than that in normal pregnant women. They concluded that there are significant effects of IDA on cellular immune function and infectious disease during pregnancy.

**CONCLUSION:**

Within the limitations of the study, this can be concluded that significant change is seen in the lymphocyte count in premenopausal women with iron deficiency anemia.

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