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### RESEARCH ARTICLE

# Association of nutritional anemia with leukocyte and platelet counts in people of Odisha

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#### **ABSTRACT**

Background: Anemia is the most common hematological disorder in the world, due to deficiency of micronutrients such as iron, Zn, Se, Cu, folic acid, and vitamins. Iron deficiency impairs immune response and bactericidal activity of macrophages, monocytes, and neutrophils that alters the length and quality of life. Leukocyte count may be altered due to hypoxia and ischemia, which is seen in anemia. A strong association between leukocyte counts and ischemia is seen from the experimental and clinical studies. Aims and Objectives: The present study was aimed to estimate total leukocyte count (TLC) and differential leukocyte count (DLC), neutrophil-to-lymphocyte ratio (N/L ratio), and platelet count in anemia. N/L ratio has emerged as a useful index in ischemic heart disease (IHD). Materials and Methods: A total of 30 patients of nutritional anemia in both the sexes were taken as study group, whose hemoglobin level was <6 g%, and they were compared with 30 healthy subjects of same age group. The parameters studied were TLC, DLC, N/L ratio, and platelet count. Results: TLC is insignificantly higher in anemic subjects. DLC shows, significant rise in absolute neutrophil count (P < 0.005), fall in absolute lymphocyte count significantly (P < 0.005), and rise in eosinophil and basophil count which is not statistically significant. There is not much difference in monocyte count in both groups. Associated poikilocytosis and anisocytosis of red blood cells are present in anemic subjects. N/L ratio is significantly higher in anemics (P < 0.001). Platelet count is decreased significantly in patients of anemia than control subjects (P < 0.001). Conclusions: Thus, our study highlights that, immunity especially cellular immunity, is influenced by nutritional anemia. High N/L ratio in anemia is associated with IHD and severe anemia is also associated with thrombocytopenia.

KEY WORDS: Anemia; Hypoxia; Ischemia; Leukocyte; Platelet

# INTRODUCTION

Anemia is the most common hematological disorder in the world and iron deficiency is the cause in majority of these cases both in developing and developed countries.

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Micronutrient deficiency is known causes of reduced immune response and inhibitory bactericidal activity of white blood cells (WBCs) that alters the length and quality of life. Altered leukocyte count may be secondary to ischemic myocardial damage seen in anemic hypoxia. Neutrophilto-lymphocyte ratio (N/L ratio) is a useful index of inflammation in ischemic heart diseases (IHD).<sup>[1]</sup> Hypoxia induced by anemia acts as a stressor to raise leukocyte count, which is manifested as a "hematological stress syndrome."

Hence, in the current study, we evaluated the association of nutritional anemia with leukocyte and platelet counts.

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#### MATERIALS AND METHODS

The present case—control study was conducted in the Department of Physiology and Haematology of SCB Medical College, Cuttack, over 1 year from February 2014 to March 2015. The study included 30 patients of nutritional anemia within the age group of 20–40 years, with hemoglobin (Hb) level <6 g/dl, for at least 3 months duration.

Informed consent was taken from each subject, and approval of the Institution Ethical Committee was also taken. Then, detailed history and thorough medical examinations were done to exclude acute or chronic inflammatory diseases, congenital, valvular, or organic heart diseases, diabetes mellitus, respiratory diseases, obesity and using of corticosteroids, previous iron replacement therapy, and history of smoking.

A total of 30 healthy males and females of same age group were included as control subjects. Inclusion criteria were, no intake of hematinics in past 1 month and the absence of anemia or iron deficiency with, Hb >13g/dl for males and Hb >12 g/dl for females, and mean corpuscular volume (MCV) >80 fl.

A measure of 1 ml of blood sample, with ethylenediaminetetraacetic acid containing tubes, was taken by venipuncture from each patient. Using Sysmex XT-2000i Auto Analyzer, complete blood counts including total leukocyte count (TLC) and differential leukocyte count were estimated. N/L ratio was calculated.

# **Statistical Analysis**

Unpaired Student's *t*-test was done by SPSS software version 19.0. *P* < 0.05 was considered statistically significant.

#### RESULTS

Table 1 summarizes Hb concentrations in anemic patients are  $4.51 \pm 1.138$  g% compared to  $13.6 \pm 1.14$  g% in control group, which is statistically highly significant. All other parameters such as total red blood cell (TRBC), packed cell volume, MCV, mean corpuscular Hb (MCH), and MCH concentration in anemic patients are lowered compared to control group which are statistically highly significant (P < 0.001). However, platelet count is decreased significantly in patients of anemia than control subjects (P < 0.001).

Table 2 summarizes TLC is higher in anemic subjects compared to controls which is not statistically significant. On differential count, there is significant rise in absolute neutrophil count (P < 0.005), significant fall in absolute lymphocyte count (P < 0.005), and rise in eosinophil and basophil count which is not statistically significant, not much difference in monocyte count in both groups, associated poikilocytosis and

**Table 1:** Comparison of basic parameters in control and anemia

| anemia       |  |  |
|--------------|--|--|
| Control      | Anemia   |  |
| 30           | 30   |  |
| 30.93±5.6    | 31.1±6.97  |  |
|              |  |  |
| 19           | 15   |  |
| 11           | 15   |  |
| 13.6±1.14    | 4.51±1.138★★★  |  |
| 4.838±0.526  | 2.345±0.84★★★  |  |
| 41.54±2.94   | 18.01±3.12★★★  |  |
| 85.96±4.21   | 68.12±7.43 ★ ★   |  |
| 27.53±1.77   | 19.32±3.75★★★  |  |
| 31.94±1.20   | 27.03±3.71★★★  |  |
| 326.37±91.67 | 211.4± 82.19★★★  |  |
|              | Control 30 30.93±5.6 19 11 13.6±1.14 4.838±0.526 41.54±2.94 85.96±4.21 27.53±1.77 31.94±1.20 |  |

Data presented as mean±SD, \*\*\*P<0.001. Hb: Hemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, SD: Standard deviation

**Table 2:** Comparison of TLC and DLC in control and anemia groups

| Parameters                | Control           | Anemia            |
|---------------------------|-------------------|-------------------|
| TLC (10 <sup>3</sup> /μl) | 7.185±1.684       | 7.995±2.887       |
| DLC $(10^3/\mu l)$        |                   |                   |
| N-(2.0-8.0)               | $3.801\pm1.436$   | 5.013±1.777★★     |
| L-(1.5-4.0)               | $2.43 \pm 0.637$  | 1.89±0.734★★      |
| M-(0.2-0.8)               | $0.457 \pm 0.650$ | 0.443±0.153       |
| E-(0.04-0.4)              | $0.289 \pm 0.118$ | $0.375 \pm 0.129$ |
| B-(0.0-0.1)               | $0.035 \pm 0.007$ | $0.058 \pm 0.009$ |
| NLR                       | 1.57±0.39         | 2.88±1.27★★       |

Data presented as mean $\pm$ SD,  $\star\star P$ <0.005,  $\star\star\star P$ <0.001. TLC: Total leukocyte count, DLC: Differential leukocyte count, SD: Standard deviation

anisocytosis of red blood cells in anemic subjects, and N/L ratio is significantly higher in the anemics (P < 0.001).

# **DISCUSSION**

The present study shows there is difference between all hematological parameters in two groups which is statistically significant. There is rise of leukocyte count in anemia compared to control, which is statistically not significant, which may be involved in pathogenesis of cardiovascular risk. Increased leukocyte count may be due to an action mediated through glucocorticoids. The neutrophils when activated may release proteolytic and hydrolytic enzymes leading to injury of blood vessels.<sup>[1]</sup>

The significantly increased absolute neutrophil count in anemic patients in the present study could possibly be due to compensatory activity due to inhibition in the capability to phagocytose.<sup>[2-6]</sup>

In the present study, significant lymphopenia in anemic group is well correlated with some studies done in the past. The lymphopenia could be due to reduction in the lifespan of lymphocytes secondary to oxidative damage.<sup>[7,8]</sup> As iron has an important role in development and maturation of lymphocytes, there may be lymphopenia in iron deficiency, which is supported by earlier reports.<sup>[9-11]</sup>

N/L ratio is an independent predictor and marker of mortality, which is superior to WBC count. In current study, the N/L ratio is significantly high (P < 0.001) in anemic subjects compared to controls and is consistent with earlier study.<sup>[1]</sup> This N/L ratio is highly useful in IHD, MI, etc.<sup>[12,13]</sup>

In this study, platelet count is decreased significantly in patients of anemia than control subjects which is in accordance with earlier studies.[14-17] They reported thrombocytopenia in patients with severe anemia, when the Hb level is <7 g/dl. The cause of thrombocytopenia in severe anemia could be due to high endogenous erythropoietin response.[16,18,19] In severe IDA, as megakaryocyte numbers decrease, megakarvocyte sizes increase. This may be due to the shortening of megakaryocyte maturation and the reduction in influx of precursors.[20,21] Another study reported that "moderate anemia is associated with reactive thrombocytosis."[18,19,22] In moderate IDA, the causes of thrombocytosis may be (a) increased rate of influx of precursor cells into the megakaryocyte compartment with an increased rate of efflux; (b) shortening of megakaryocyte maturation; (c) stem-cell shunt due to inhibition of erythropoiesis, resulting in increased production of other pluripotent (hemostatic compensatory mechanism); stimulator effect of transferrin on megakaryopoiesis; and (e) inhibition of iron on megakaryocyte maturation. [20,21,23]

# **Limitations and Future Scope of the Study**

As this is a cross-sectional study, longitudinal and multicentric studies are strongly recommended. Because the patients in this study are severely anemic, more sample size could not be taken.

#### **CONCLUSION**

Thus, our study highlights that, immunity especially cellular immunity, is influenced by nutritional anemia. High N/L ratio is associated with ischemic myocardial diseases, which occurs in anemia and severe anemia is also associated with thrombocytopenia.

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