AMERICAN Journal of Pediatric Medicine and Health Sciences
Volume 2, Issue 7, 2024 ISSN (E): 2993-2149

Investigating the Impact of Omega-3 Fatty Acids on Blood Cell Counts in a Rat Model of Anemia

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Abstract: This study investigates the therapeutic effects of omega-3 fatty acids on anemic rats. Forty male rats were fed a baseline diet prepared by the American Institute of Nutrition (AIN). Anemia was induced in two groups of rats using a bleed-out procedure, with hemoglobin levels at 11 g/dL or lower and hematocrit levels at 33% or lower indicating anemia. Following acclimatization, the rats were randomly assigned into four groups of ten rats each: control group (G1), omega-3 supplemented group (G2) receiving 300 mg/kg/day for two weeks, untreated anemic group (G3), and omega-3 treated anemic group (G4) receiving 300 mg/kg/day for two weeks. Blood samples were collected at the start and 28 days into the experiment using the orbital venous plexus method. Hematological parameters were analyzed using a hematology autoanalyzer, and statistical analysis was performed using SPSS version 23.

Results showed that G1, G2, and G4 had similar erythrogram and leukogram parameters with no significant differences (indicated by the letter A). In contrast, G3 exhibited significantly lower values (indicated by B) in mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), mid-sized cells (Mid), granulocytes (Gran), lymphocytes (L), and total white blood cell count (WBC). These findings suggest that the anemia had a detrimental impact on both erythrogram and leukogram parameters, while omega-3 supplementation improved these parameters, indicating its potential as a therapeutic agent for anemia.

Keywords: Omega-3 fatty acids, anemia, erythrogram, leukogram, rat model, hematological parameters, hemoglobin, packed cell volume.

Introduction:

When hemoglobin levels in the blood are below normal or the number of red blood cells (RBCs) is low, a condition known as anemia occurs. The oxygen-binding capacity of individual hemoglobin molecules may be diminished as a result of structural abnormalities or inadequate numerical development, similar to other forms of hemoglobin shortage. Organs experience hypoxia (a shortage of oxygen) when hemoglobin present within red blood cells is absent, as is the case in anemia (1).

Inadequate iron, vitamin B12, and folic acid intake—three essential elements for healthy red blood cell production—is the leading cause of anemia. Hemorrhage, genetic anomalies, chronic illness states, and pharmacological toxicity are among the many potential causes of others. A common term for anemia caused by a lack of iron, protein, certain vitamins (B12, folic acid, pyridoxine, and ascorbic acid), copper, and other minerals is nutritional anemia. Folic acid and iron deficiencies are the leading causes of nutritional anemia (2).

Among the top 20 risk factors for the distribution of disease burden globally, iron deficiency (IDA) ranks high (3). Iron deficiency anemia affects 40% of children aged 2–5 (and might reach 51% in rural areas), hence the World Health Organization is collaborating with several nations to tackle this serious problem. It was also linked to other illnesses and had a comparable incidence among pregnant women and women of childbearing age (4).

The negative health effects of iron deficiency include a higher risk of maternal and foetal morbidity and death, as well as effects on women's ability to work, their cognitive development, their resistance to infection, and their ability to conceive (5).

Many biochemical and therapeutic advantages omega-3 fatty acids provide dialysis patients (6). Despite this, poor blood levels of omega-3 fatty acids were found in chronic HD patients whose fish intake was much lower than the recommendations made by the American Heart Association (7). The poor diet of fish and fish products among HD patients is likely responsible for the reduced content of omega-3 fatty acids in erythrocyte membrane phospholipids compared to healthy controls (8). Omega-3 fatty acid supplements may help reduce inflammation in HD patients, however prior research on this topic has shown mixed results. While some research has shown that taking omega-3 supplements orally may reduce serum systemic inflammatory indicators, other studies have shown the opposite to be true (9,10). In addition, oral omega-3 fatty acids have been shown to have both a favorable (11) and no impact (12) on blood hemoglobin levels in people who are maintaining their HD.

This study aimed to investigate the Impact of Omega-3 Fatty Acids on Blood Cell Counts in a Rat Model of Anemia.

Materials and Methods:

For seven iterations, forty male rats were given a baseline meal prepared by the American Institute of Nutrition (AIN) (13). After that, two groups of rats were made anemic using the bleed-out procedure described by (14). Rats were deemed anemic if their hemoglobin levels were 11 g/dL or lower and their hematocrit levels were 33% or lower. Following the acclimatization phase, the rats were randomly assigned to one of four groups, with ten rats per group. Rats fed the main course as a control group (G1) were used for the duration of the experiment. G2: for two weeks, they were administered a pure omega-3 supplement at a dosage of 300 mg/kg/day. Group 3 consists of untreated anemic rats. G4: rats with anemia were given 300 mg/kg/day of omega-3 fatty acids for two weeks.

On the first day before to the trial's start, blood samples were taken. Later on, at 28 days into the experiment, blood samples were taken. When drawing blood, the procedure outlined by (15) was adhered to. On each day of sampling, two rats were chosen at random from each replication and given diethyl ether for anesthesia. For the hematological test, 5 ml of blood was drawn from the patient using a non-heparinized syringe via the orbital venous plexus and then placed into EDTA tubes.

Blood parameters were evaluated by using a hematology autoanalyzer.

Statistical analysis was done by using SPSS version 23.

Results:

- Groups G1, G2, and G4 show similar results across most parameters with no significant differences indicated by the same letter (A).
- Group G3 has significantly different results (indicated by B), showing lower values in MCHC, MCH, MCV, Hb, PCV, and RBC compared to the other groups.

These results suggest that the treatment applied to G3 had a noticeable negative impact on the erythrogram parameters compared to the other groups G1, G2, and G4 (Table 1).

Table 1. Erythrogram parameters in studied groups

Groups	MCHC	MCH	MCV	Hb (g/dl)	PCV %	RBC (10^{12}/L)
G1	31.2±4.73 A	18.28±2.06 A	57.61±9.42 A	11.41±1.69 A	41.2±3.85 A	6.11±1.08A
G2	31.05±3.1 A	18.25±1.02 A	57.55±3.47 A	11.40±2.28 A	41.52±1.72 A	6.35±0.23 A
G3	29.0±2.84 B	15.14±0.39 B	50.1±3.58 B	9.33±2.23 B	32.28±3.73 B	4.42±1.18 B
G4	30.99±0.47 A	18.11±1.64 A	56.83±3.03 A	11.25±2.83 A	41.98±1.03 A	6.61±0.97 A

- Groups G1, G2, and G4 show similar results across most parameters with no significant differences indicated by the same letter (A).
- Group G3 has significantly different results (indicated by B), showing lower values in Mid, Gran, L, and WBC compared to the other groups.

These results suggest that the anemia in G3 had a noticeable negative impact on the white blood cell parameters compared to the other groups G1, G2, and G4.

Table 2. Effects of different treatments on WBCs

Groups	Mid(10^9/L)	Gran(10^9/L)	L(10^9/L)	WBC (10^9/L)
G1	3.93±0.97A	12.26±1.08A	10.01±1.07A	25.46±1.29A
G2	3.69±0.28A	11.98±0.88A	10.52±1.31A	25.13±1.19A
G3	2.01±0.61B	7.85±0.98B	7.25±1.74B	18.46±1.03B
G4	3.48±0.27A	10.18±0.93A	9.34±1.28A	24.12±0.75A

Discussion:

Anemia, characterized by a reduction in red blood cell (RBC) count, hemoglobin (Hb) levels, and hematocrit (PCV), is a common clinical condition that can result from various underlying causes including nutritional deficiencies, chronic diseases, and genetic disorders (16). In recent years, the potential therapeutic effects of omega-3 fatty acids have gained considerable attention for their role in modulating inflammation, lipid metabolism, and cellular functions. This discussion focuses on the efficacy of omega-3 fatty acids in treating anemia in rats, based on erythrogram and leukogram data (17).

Effects on Erythrogram Parameters

The administration of omega-3 fatty acids to anemic rats shows a promising improvement in various erythrogram parameters:

1. **Hemoglobin (Hb) Levels:** Omega-3 fatty acids may enhance erythropoiesis and improve Hb synthesis, as indicated by higher Hb levels in treated groups compared to untreated anemic controls. This could be attributed to the anti-inflammatory properties of omega-3 fatty acids, which reduce systemic inflammation and subsequently enhance iron metabolism and erythropoiesis (18).
2. **Packed Cell Volume (PCV):** An increase in PCV suggests an improvement in the overall erythrocyte mass. Omega-3 fatty acids may support the survival and proliferation of erythroid progenitor cells in the bone marrow, leading to an increase in PCV(19).
3. **Red Blood Cell (RBC) Count:** Treated rats exhibit a higher RBC count, indicating the efficacy of omega-3 fatty acids in promoting RBC production. This can be linked to the enhancement of bone marrow function and the reduction of oxidative stress, which otherwise contributes to erythrocyte destruction (20).
4. **Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV):** Consistent values of MCH and MCV in treated groups compared to controls indicate the restoration of normocytic and normochromic erythrocytes, suggesting improved erythrocyte quality and functionality (21).

Effects on Leukogram Parameters

Omega-3 fatty acids also influence leukocyte parameters, contributing to a holistic improvement in the health status of anemic rats:

1. **Total White Blood Cell (WBC) Count:** An increase in WBC count in treated rats indicates an immunomodulatory effect of omega-3 fatty acids, enhancing the body's immune response (22).
2. **Differential Leukocyte Count:** Improvements in granulocytes (Gran), lymphocytes (L), and mid-sized cells (Mid) suggest a balanced immune response. Omega-3 fatty acids may help in reducing chronic inflammation and supporting the immune system's ability to combat infections and other stressors (23).

Mechanisms of Action

The beneficial effects of omega-3 fatty acids on erythrogram and leukogram parameters can be attributed to several mechanisms:

1. **Anti-inflammatory Effects:** Omega-3 fatty acids reduce the production of pro-inflammatory cytokines, such as TNF-alpha and IL-6, which are known to inhibit erythropoiesis and contribute to anemia of chronic disease (24).
2. **Antioxidant Properties:** Omega-3 fatty acids enhance the antioxidant capacity of the body, reducing oxidative stress and protecting erythrocytes from premature hemolysis (25).
3. **Membrane Fluidity:** By incorporating into cell membranes, omega-3 fatty acids improve membrane fluidity and function, enhancing the deformability and survivability of erythrocytes (26).
4. **Iron Metabolism:** Omega-3 fatty acids may improve iron absorption and utilization, crucial for hemoglobin synthesis and red blood cell production (27).

Conclusion

The treatment of anemic rats with omega-3 fatty acids shows promising results in improving erythrogram and leukogram parameters. These improvements are likely due to the anti-inflammatory, antioxidant, and membrane-stabilizing properties of omega-3 fatty acids, which collectively enhance erythropoiesis, immune function, and overall health. While these findings are encouraging, further studies are needed to elucidate the precise molecular mechanisms and to determine the optimal dosage and duration of omega-3 fatty acid supplementation for the treatment of anemia in clinical settings.

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