ORIGINAL ARTICLE

HEMATOLOGICAL AND BIOCHEMICAL PROFILE OF PATIENTS WITH MACROCYTIC ANEMIA AT TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND:

Anemia is a global nutritional problem, and the risk of morbidity and death is higher in all age groups. Macrocytic anemia is usually caused by abnormalities that affect the maturation of erythroid precursors in the bone marrow. Because the clinical manifestations of different types of anemia are similar, hematological parameters (such as hemoglobin, red blood cell count, and peripheral blood smear) can be used to diagnose anemia.

MATERIALS AND METHODS: This is a cross-sectional study conducted in the Department of Pathology, Chandka Medical College Hospital, Larkana for a period of 6 months (January 2020 to June 2020). A total of 54 patients with low hemoglobin levels between 15 and 65 years old were selected, and venous blood samples with an MCV>100 fL were selected as the study subjects.

RESULTS: The study included 54 patients with macrocytic anemia, with an average age of 32.88±11.38 years old, and women were dominant group. The hemoglobin level of men was slightly lower than that of women. The red blood cell count of men was slightly higher. There was a significant difference between male and female serum vitamin B12 and folic acid.

CONCLUSION:
The hemoglobin, red blood cell count, percentage of reticulocytes and peripheral blood smear are important parameters for the diagnosis of certain types of anemia. Evaluation of serum folate and vitamin B_{12} levels and other hematological parameters is important for the diagnosis of macrocytic anemia and its correlation.

KEYWORDS: Macrocytic anemia, hematological, biochemical, vitamin B_{12}, folate.

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INTRODUCTION

Anemia is a global nutritional problem that affects nearly 2 billion young people, adolescents and pregnant women at risk of morbidity and death.[1-2] In general; the disease is connected with decreased production/ increased destruction of red blood cells. The concentration of blood (RBC) or hemoglobin (Hb) affects oxygen circulation, which in turn affects maternal, and childbirth outcomes, optimal growth of children, poor learning, and decreased productivity and output during childhood.[3-4] Diet, irregular eating habits and physical conditions (such as pregnancy) are risk factors for anemia in adolescents.[5-6]

According to data from the World Health Organization (WHO), the hemoglobin concentration of men less than 13 g/dl, 12 g/dl for non-pregnant women, 11 g/dl for pregnant women, and 12 g/dl for children 14 years old, [5] 11 g/dl for children up to 11 years old and 11 g/dl for children under 5 years are the diagnostic criteria for anemia.[7] One of the basis for the classification of anemia is based on this basic mechanism including low productivity, increased destruction and massive blood loss. The second method classifies anemia based on changes in red blood cell morphology, and is usually related to the cause of red blood cell deficiency. The shape of red blood cell deficiency is classified into normal, small cell or large cell morphology.[8]

According to its size, when the average cell volume (MCV)> 100 fL, red blood cells are called ovalocytes. This is a characteristic of macrocytic anemia and is usually caused by an abnormality that alters the maturation of erythroid precursors in the bone marrow.[4,9] Megaloblastic anemia is the most common cause of macrocytic anemia. The coenzymes required for thymine and purine synthesis are almost free of vitamin B12 and folic acid, which can lead to impaired DNA synthesis, ineffective erythropoiesis and intramedullary hemolysis.[10-11]

Because the clinical symptoms of different types of anemia are similar, the differential diagnosis of macrocytic anemia can be made by physical examination, hematological parameters such as hemoglobin, red blood cell markers such as mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), and with a peripheral blood smear, the main aim of which was to assess the hematological and biochemical parameters in a patients with macrocytic anemia.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Pathology Department Chandka Medical College Hospital, Larkana between January 2020 to June 2020. Before the sample preparation, a total of 54 patients aged 15 to 65 years with low hemoglobin were examined for the diagnosis of anemia according to WHO criteria and MCV> 100 fL. Samples were collected using EDTA-containing tube and were immediately analyzed on Automated Hematology Analyzer Mindray BC-5000. The parameters assessed included hemoglobin, MCV, MCH, MCHC, reticulocyte count, and peripheral blood smear to diagnose anemia according to the WHO.[7]

The reference intervals for RBC indices were:

MCV: 80-100 fL, MCH: 27-32 pg, MCHC: 32-36 gm/dl, Reticulocytes: (Adults= 0.5%-2.5% and Infants = 0.5%-7%)

When MCV was more than 100 fL, anemia was reported as macrocytic.

Peripheral Smear Examination

For the peripheral blood examination, EDTA blood smears were taken and allowed to air dry, and then 0.25% Wright stain was poured over the entire slide and allowed to stand for

2-3 minutes, followed by an equal volume of distilled water. The tiny slides were washed thoroughly, dried, and examined under a light microscope. Peripheral smear examination findings such as macrocytic or normocytic cells, hyper-segmented neutrophils, polychromatic cells, basophils, tear cells, etc. were reported.

Reticulocyte Count

Equal volumes of EDTA patient blood and the new methylene blue reagent were mixed in a tube and incubated for 10 minutes at 37°C., after which a swab was taken and analyzed by immersion oil.

Analysis of Biochemical Parameters

For vitamin B_{12} and serum folate analysis, venous blood samples were collected in gel vials, centrifuged for 10 minutes at 3500 rpm and then evaluated in a fully automated Mindray CL1000i Immunoassay.

Reference interval for Vitamin B_{12} = 211-911 pg/ml

Reference interval for Folic acid = 3.56 – 20.0 ng/ml

Data Analysis

Gender, age, hemoglobin concentration, MCV, MCH, MCHC, percentage reticulocytes and other parameters were determined, serum vitamin B_{12} and folic acid were analyzed and the data obtained were analyzed in SPSS 24.0.

RESULTS

Our study included a total of 54 patients with macrocytic anemia aged 15 to 65 years with a mean age of 32.88 ± 11.38 years, including 20 males and 34 females with male: female ratio 1:2. Figure 1 shows the age distribution of male and female patients with macrocytic anemia. In the age group> 60 years, the distribution of the hemoglobin and erythrocyte indices by age was compared, and it was found that in the age group 45-60 years, the hemoglobin level exceeds 8 g/dL, which is more than that of other patients. Levels of hemoglobin according to age groups are summarized in tables 2 and 3. In the peripheral blood smear findings, of 54 cases, 41 cases were identified as macrocytic anemia and 13 cases normocytic anemia. This macrocytic and normocytic anemia was demonstrated with additional features such as hyper-segmented neutrophils, polychromatic cells, basophilic stippling, and tear cells. Total serum levels of vitamin B12 and folic acid have been shown to be decreased in patients with macrocytic anemia. It was also found that the serum levels of these markers are significantly different in patients with male and female anemia ($p<0.05$).

DISCUSSION

First, vitamin B_{12} and folic acid deficiencies are the most likely cause of macrocytosis (MCV>100 fl), which leads to macrocytic anemia. In some cases, however, the cause remains unclear. However, anemia with normal MCV may indicate a chronic or mixed type of anemia. In this study, we used a hematological findings, peripheral blood smear examination findings and biochemical findings to identify macrocytic anemia and correlated the results with their gender and age group.

In our study, the highest incidence of macrocytic anemia was observed in females as compared to male in the maximum age group of 18 to 25 years while Banjare B et al., Deepankaret et al. and Iqbal S et al. observed the higher incidence of macrocytic anemia in men than in women. [2,13-14] A study by Rayamajhi et al. showed higher incidence in age group of 15-30 years.[5] A similar age distribution was also found by Unnikrishanan et al. and Deepankar et al. with a mean age of 35.7 ± 16.1 years and 39 years.06 ± 8.9 years respectively.[12-13]

In the distribution of hemoglobin by age,

patients aged 18 to 25 years had the lowest hemoglobin level of 7.19 ±1.81, while patients aged 46 to 60 years had the highest hemoglobin level. An increase in MCV was observed in all cases. The largest red blood cell size was found in patients under 18 years of age (109.36 ± 5.11fl) and the smallest in patients over 60 years of age (104.41 ±3.74fl). In most cases, the MCH value was increased, with a higher incidence in the age group 36-45 and less at the age of 46-60 years. Similarly, the maximum number of reticulocytes was found in the age group 18-25 while at least in people over 60.

In comparison to our study, Pudasaini S et al. observed that people with low MCH observed low MCHC levels and low MCH and MCHC levels in children aged 12-14 years.[15-16] Anemia, low RBC counts were due to premature destruction of RBCs, and anemic patients had low PCV levels but elevated MCV, MCH, and MCHC levels, which fit well into this study. Kannan A et al. also showed other causes including leukemia, multiple myeloma and myelofibrosis etc. There was a significant difference in MCV between megaloblastic and non-megaloblastic macrocytosis.[17]

In this study, out of 54 cases, 40 showed both decreased levels of folate and vitamin B_{12} and also the distinction between males and females were found to be statistically significant ($p<0.05$). In distinction to our findings, Iqbal SP et al pointed out of 220 pernicious anemic patients, 71% of folic acid deficient patients had vitamin B_{12} deficiency in addition; however there was no any significant difference between male and female patients.[14] Similarly, Agrawal L et al ascertained that out of a hundred anemic patients, 55% of patients were diagnosed with vitamin 12 deficiency and 8% with folate deficiency.[19] Deepankar et al found 54%, 25%, and 21% of participants with vitamin B_{12} deficiency, folic acid deficiency, and combined vitamin B_{12} and folic acid deficiency respectively.[13] A study by Wyckoff KF et al reported subjects with small bodily fluid vitamin B_{12} is probably going to be while not pathology throughout the amount of post fortification and folic acid fortification led to macrocytosis; correction involving vitamin B_{12} insufficiency.[18] A review study by Batool S et al reported vitamin B_{12} and folic acid deficiency is the factor to blame for impaired DNA synthesis, ineffective erythropoiesis, and intramedullary haematolysis that ultimately breaks red blood cells.[11]

Of the 54 cases on peripheral blood smear, 41 cases had macrocytic anemia and 13 cases had normocytic anemia. These macrocytic and normocytic anemias showed characteristic features such as hyper-segmented neutrophils, polychromatic cells, basophillic stippling and teardrop-shaped cells. Similarly, in megaloblastic anemic patients in one study, macrocytes were common in 88% of patients, and hyper-segmented neutrophils were observed in 43 patients.[13] A study by Agrawal et al. also showed similar findings.[19]

Aslinia F et al. defined macrocytic anemia as a condition of anemia characterized by the presence of abnormally large red blood cells, which is generally detected by an automatic cell counter and peripheral blood smear examination. Careful examination of peripheral blood smear, including red blood cell morphology, cellular components, and the specific properties of the smear, provides important information about the possible etiology of the anemia.[20] This study found that the total number of reticulocytes in patients with macrocytic anemia was 4.11 ±2.81% with 5.01 ± 2.85% in men and 4.76 ± 3.01% in women, and a maximum value in the age group from 18 to 25 years (4.76 ±3.92%). While the minimum in the age group over 60. In contrast to our study, Rairikar SS et al. showed about 62.5% of cases of severe anemia with normal reticulocytes,

while 9.4% had low and 28.12% had increased numbe.[21]

Similarly, D'Onofrio et al. and Balci YI et al. showed that reticulocytes can be a useful marker for differentiating between iron deficiency anemia and vitamin B_{12} deficiency anemia.[23] In this study, the gender-wise distribution of hemoglobin, RBC indices, peripheral blood smear, and reticulocytes showed no vital variations between male and female patients. In contrast, blood vitamin B_{12} and folic acid levels were found to be statistically significant ($p<0.05$) in male and female anemic patients. A study by Sukla et al shows an analogous finding that is comparable our study.[24]

CONCLUSION

In this study, we determined the maximum incidence of macrocytic anemia in young women. Red blood cell markers such as MCV, MCH, Hemoglobin, reticulocyte fraction, and peripheral blood smear are important diagnostic factors for anemia to help in diagnosis of megaloblastic anemia, so clinical, hematological, and biochemical parameters are important tools for diagnosing macrocytic anemia and further help distinguish megaloblastic anemia from non - megaloblastic anemia.

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CONFLICT OF INCIDENCE

No conflict of interest declared by the authers.

AUTHORS' CONTRIBUTION

LN - Principal Investigator
MWS - Manuscript Writing