Section: Pathology

Original Research Article

DIAGNOSTIC UTILITY OF HEMATOLOGICAL INDICES, RED BLOOD CELL HISTOGRAMS AND BONE MARROW EXAMINATION IN THE DIAGNOSIS OF ANEMIA

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Abstract

Background: Anemia is one of the most common health problems in India in which the number of red blood cells (RBC) or their oxygen-carrying capacity is insufficient to meet the body's physiological needs. Iron deficiency is the major cause of anemia in India followed by vitamin B12, folate deficiency. It is only through thorough clinical history, physical examination, assessment of red cell indices, histograms along with examination of the peripheral blood smear and bone marrow that exact etiology of anemia can be found and further management of patients with anaemia can be made. The present study was carried out to know the underlying etiopathology of anemia in relation with hematological indices, red blood cell (RBC) histograms, peripheral blood smear and bone marrow study findings to differentiate causes of anemia. **Materials and Methods:** The present observational study was carried out in 109 cases at Department of Pathology from January 2023 to December 2024. Values of Haemoglobin, Red blood cell (RBC) indices were recorded from automated analyser report. Peripheral blood smear along bone marrow aspiration and biopsy were performed and examined. **Result:** Out of 109 cases majority (47.70%) of patients were from 26-35 years age group with female preponderance. Dimorphic anemia was the commonest etiology found in 63.30% cases followed by Iron deficiency anemia (22.01%), megaloblastic anemia (11.92%) and aplastic anemia (2.75%). Hemoglobin, Red blood cell indices were decreased in most cases while left shift (44.95%) was the most common histogram pattern. Assessment of iron stores on bone marrow showed decreased iron stores in most cases of dimorphic anemia and iron deficiency anemia. **Conclusion:** Dimorphic anemia was most common cause of anemia in the present study. The hematological parameters including hematological indices, RBC histograms, bone marrow examination findings were comparable to the findings of other studies. The present study concludes that comprehensive haematological study of patients with anemia will usually help in identification of underlying etiology. In a developing nation like ours it can be an easy and cost effective procedure to combat the nutritional anaemia.

INTRODUCTION

Anemia is one of the most common health problems in India. It can present as disease itself or symptom related to underlying other hematological disorders. It is defined as condition in which the number of red blood cells (RBC) or their oxygen-carrying capacity is insufficient to meet the body's physiological needs. According to WHO criterion, anemia is present if the blood concentration of hemoglobin falls below 13g/dl in men or 12g/dl in women. This rule does not apply to infants, children, pregnant women, who have their own tables of lower limits of hemoglobin concentration. The reference range of hemoglobin may vary according to the population analyzed, age, sex, environmental conditions and food habits.[1] Iron deficiency is the major cause of anemia in india followed by vitamin B12, folate deficiency. Other causes can be chronic inflammation, parasitic infections, and inherited disorders. Blood cell analysis has advanced significantly from manual procedures to automated instruments, providing more accurate and reliable results.[2] These machine provide essential information on RBC indices,

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hematocrit, and RBC distribution width (RDW) along with detailed RBC histogram which helps in the diagnosis and management of anemia cases. Normal RBC histogram is a symmetrical bell - shaped curve, providing valuable information for understanding & interpretation of various anemias. Its shift in any direction along with other parameters like red cell distribution width (RDW) and RBC indices (MCV, MCH, MCHC) and RBC count are very useful in the laboratory evaluation of RBC disorders. It is only through thorough clinical history, physical examination, assessment of cell count, red cell indices along with examination of the peripheral smear and bone marrow aspirate smear or biopsy that exact etiology of anemia can be found and further management of patients with anaemia can be made. Bone marrow examination gives more complete picture of the reaction of the hematopoietic tissue to anemia than can be gained from peripheral blood smear alone.[3] The present study was carried out to know the underlying etiopathology of anemia in relation with hematological indices, RBC histograms, peripheral blood smear and bone marrow study findings to differentiate causes of anemia.

MATERIALS AND METHODS

The present observational study was carried out in Department of Pathology at a tertiary care center from January 2023 to December 2024. A total of 109 samples of suspected anaemic patients were included in this study. Patients with with recent history of blood transfusion, those on anticoagulant medications or those with serious haemorrhagic disorders and pregnant women were excluded.

A blood sample of 3 ml was collected from patients included in the study and analysed for Complete Blood Count (CBC). The analysis of sample was done on Mindray BC-6200 automated hematology analyser and values of Haemoglobin, Red blood cell (RBC) indices along with RBC histograms were obtained. A peripheral blood smear was prepared simultaneously, air dried, stained with Leishman stain. The smears were evaluated without access to the CBC report during reporting. Bone marrow aspiration was performed from Posterior superior iliac spine in all cases under aseptic conditions. Bone marrow biopsy was performed whenever required. Written consent of patient was taken in each case either from patient or relative before starting the procedure. The smears were studied after staining with Leishman's stain. Special staining like perl's stain was done to know the iron assessment and further grading.

RESULTS

One hundred and nine patients having anemia were studied in this study. The Age range of patients was wide i.e. from 6 years to 78 years. Majority of patients were from 26-35 years age group (47.70%). Mean age was 33.56 years. Out of 109 cases, most of the cases were females i.e. 68 and 41 cases were males. Generalized weakness was the most common presenting symptom, seen in 95 (87.15%) cases. Fever was the second most common presenting symptom, seen in 63 (57.79%) cases. In present study, pallor was the most common finding on physical examination as seen in 106 (97.24%) of all cases followed by splenomegaly seen in 62 (56.88%) of all cases.

As per the hematology analyser reports, the cases were categorised into mild (Hb 10-12gm/dl for females and 10-13 g/dl for males), moderate (Hb 8-10 gm/dl) and severe degree of anaemia (Hb <8 gm/dl). Majority of the cases (62.38%) had severe anemia followed by moderate anemia (27.52%) and mild anemia (10.09%).

Based on the RBC indices obtained in automated analyser, maximum number of cases had decreased MCV value (<80 fl) i.e. 76 (69.72)%, decreased MCHC (<27 pg) in 70 (64.22%) cases and decreased MCHC (<32 gm/dl) in 73 (66.97%) cases. 89(81.65%) cases had increased RDW (>14) and 20 (18.34%) cases had normal RDW (<14).

RBC histograms were analysed in all cases from which majority cases showed left shift (61.46%) followed by bimodal peak (22.01%). [Table 1] shows RBC histogram patterns seen in anemia cases.

Table 1: RBC histogram patterns seen in anemia cases.

Histogram abnormality	Cases	Percentage
Left shift	49	44.95%
Normal curve	3	2.75%
Right shift	13	11.92%
Broad base	20	18.34%
Bimodal Peak	24	22.01%

After peripheral blood smear examination, the most common blood picture was anisopoikilocytosis in 70(64.22%) cases followed by dimorphic blood picure in 66(60.55%) cases. Table 2 shows peripheral blood smear findings.

Table 2: Peripheral blood picture in cases of anemia

Blood Picture	Cases	Percentage
Anisopoikilocytosis	70	64.22%
Dimorphic	66	60.55%
Microcytic Hypochromic	27	24.77%
Macrocytic Normochromic	13	11.92%

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Normocytic Normochromic	3	2.75%

Bone marrow aspiration was done in all 109 cases. Hypercellular marrow was seen in 106 cases while hypocellularity was seen in 3 cases which required bone marrow biopsy for confirmation of diagnosis. The final diagnoses after bone marrow aspiration and biopsy were dimorphic anemia in 66 (63.30%) cases followed by Iron deficiency anemia 24 (22.01%), megaloblastic anemia in 13 (11.92%) and aplastic anemia in 3 (2.75%) cases. [Table 2] shows bone marrow aspiration findings in anemia cases. Table 3 shows bone marrow aspiration findings in anemia cases.

Table 3: Bone marrow aspiration findings in anemia cases.

Bone marrow aspiration finding	Cellularity	M:E ratio	Erythroid series	Myeloid series	Megakaryocyte series
Dimorphic anaemia	Hypercellular	Decreased	Erythroid hyperplasia with micronormoblastic and megaloblastic maturation	Normal progressive maturation with giant metamyelocytes and band form	Normal in number and morphology
Megaloblastic anaemia	Hypercellular	Decreased	Erythroid hyperplasia with megaloblastic maturation	Normal progressive maturation with giant metamyelocytes and giant band form	Normal to increase in number, increase in size
Iron deficiency anaemia	Hypercellular	Decreased	Erythroid hyperplasia with micronormoblastic maturation	Normal in maturation and morphology	Normal in maturation and morphology
Aplastic anaemia	Hypocellular	Altered/ Decreased	Suppressed	Suppressed	Suppressed

Table 4: Comparison of final diagnosis on bone marrow examination, Peripheral blood smear findings and histogram abnormality.

Final diagnosis	Peripheral blood smear	Histogram abnormality
Dimorphic anemia	Dimorphic blood picture (66) Microcytic hypochromic (3)	Left shift (25) Broad base (20) Bimodal peak (24)
Iron deficiency anemia	Microcytic hypochromic (24)	Left shift (24)
Megaloblastic anemia	Macrocytic normochromic (13)	Right shift (13)
Aplastic anemia	Normocytic normochromic (3)	Normal curve (3)

In our study, assessment of bone marrow iron store by Perl's Prussian blue reaction was done in all cases. Out of which 69 cases of dimorphic anaemia 36 (51.87%) cases showed 2+ grade, 20 (28.98%) cases grade 1+, 8 (11.59%) cases grade zero, 3 (4.34%) cases grade 3+, and 2 (2.89%) cases grade 4+ of iron store. Out of 24 cases of iron deficiency anaemia 21 cases (87.5%) showed zero grades and 3 cases (12.5%) grade 1+ of iron store. In cases of megaloblastic anemia, 6 (46.15%) cases showed 3+ grade, 5 cases (38.46%) grade 4+. Bone marrow iron study assessment in all 3 cases of aplastic anemia showed 2+ grade.

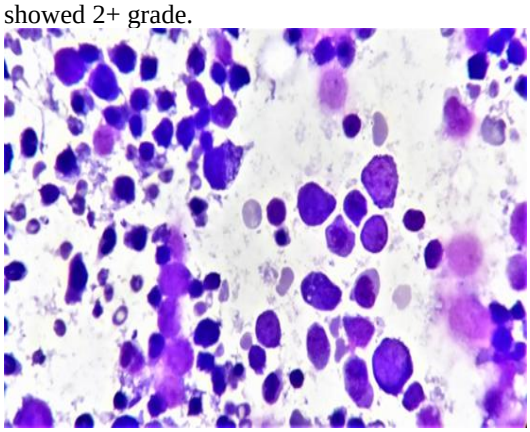

Figure 1: Bone marrow aspirate showing micronormoblastic and megaloblastic maturation- Dimorphic anaemia (Leishman's stain- 100x).

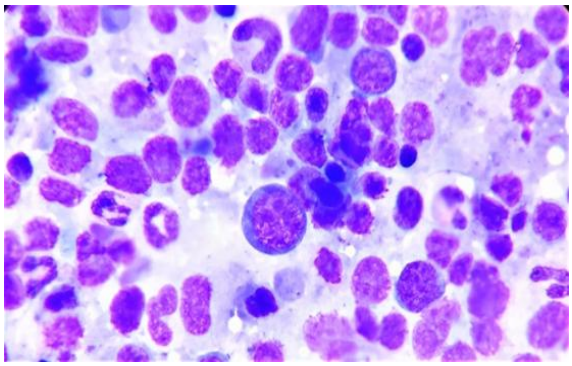

Figure 2: Bone marrow aspiration smear showing megaloblastic maturation with royal blue cytoplasm and sieve like chromatin along with Band form- Megaloblastic anaemia. (Leishman's Stain, 100x)

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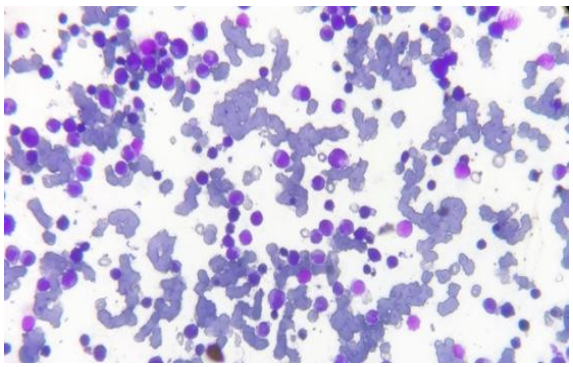

Figure 3: Bone marrow aspirate showing erythroid hyperplasia with micronormoblastic maturation- Iron Deficiency anaemia (Leishman's stain- 40x).

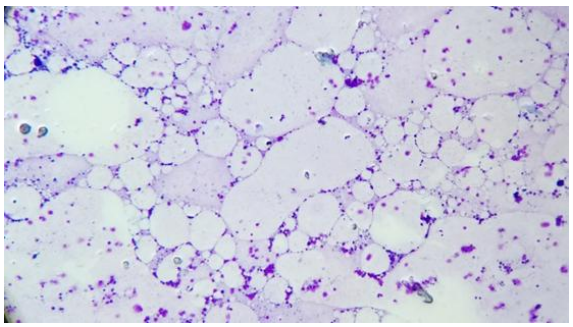

Figure 4: Bone marrow aspiration smear showing markedly hypocellular bone marrow containing fat fragments- Aplastic anaemia (Leishman's stain, 10x).

DISCUSSION

In our study, dimorphic anaemia was the most common type of anemia followed by iron deficiency anaemia, megaloblastic anemia and aplastic anemia. The high prevalence of dimorphic maturation can be mostly due to nutritional deficiencies in rural areas. Nutritional deficiencies of both iron and vitamin B12 with folate occur together and mostly seen in pregnant women and young adults. Various studies have reported aplastic anaemia as the most common type of anemia, while megaloblastic anaemia is the commonest or second most common type of anemia in most of the sub continental studies.

Iron deficiency occurring concurrently with vitamin B12 and folate deficiency has been reported in many areas. In some cases the vitamin B12 and folate deficiency may be intermediate in degree but may become more marked after iron administration and some cases of severe dimorphic anaemia do not respond initially to iron therapy, probably owing to concomitant severe folate and occasionally Vitamin B12 deficiency. Hence, findings of the present study correlate well with other studies from India and other sub-continental studies. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects.

In the study done by Thiyagarajan P et al,[4] dimorphic anaemia (38.4%) was most common type of anemia followed by megaloblastic anaemia (33.4%). Merla J et al,[5] found Dimorphic anaemia (26.59%) as most common followed by iron deficiency anaemia (16.92%) in their study.

In this study relatively younger age group population were found to be more affected along with female preponderance. Age and sex distribution was consistent with study by Shastry SM et al,[6] Generalised weakness was the commonest presenting complaint whereas pallor and splenomegaly were the commonest clinical findings. These findings are consistent with study by Patel F et al,[7] and B N Gayathri and Kadam Satyanarayan Rao.[8] Female predominance appears to correlate with the prevalence of nutritional deficiencies in rural areas which is more common in females than males leading to dimorphic anaemia in this geographic area.

History of poor eating habits, poor quality of food, and self-avoidance of necessary foods, fasts and also increasing trend of chronic alcoholism in younger population aggravates the nutritional deficiencies of vitamin B12, folate and iron. This can be attributed to the high prevalence of combined nutritional anaemia (dimorphic anaemia) in present study. Majority of these patients can be diagnosed on serum ferritin/ iron levels, serum vitamin B12 levels, serum and red cell folate levels but primary investigations like complete blood count, red cell indices, red cell distribution width, peripheral blood smear examination and bone marrow examination are helpful in remote areas where specialised tests are not easily accessible.

The RBC histogram is generated by the automated hematology analyzer, which uses sophisticated technology to measure the size and number of red blood cells in the blood sample.[9] The normal histogram curve is typically bell-shaped and symmetrical representing mean corpuscular volume between 80-100fl.[10] The most common histogram abnormality was left shift followed by broad base and bimodal peak. In study done by Dhakar DK et al,[11] left shift was most common RBC histogram pattern observed followed by broad base which is in concordance with this study. Differences observed in other studies may be due to limited number of cases in this study.

In this study majority of the cases had severe anemia with decreased values of red cell indices, increased RDW. These findings are in consistent with the studies done by R Athar et al,[12] and Ali I et al.[13] Most common peripheral smears findings in our study were anisopoikilocytosis with dimorphic blood picture that was consistent with the study by R Athar et al.[12] In 66 cases, bone marrow aspirate smears were hypercellular, showing erythroid hyperplasia along with micronormoblastic and megaloblastic maturation, giant metamyelocytes with normal maturation of megakaryocytes. The hypocellularity was seen in 3 cases of aplastic anemia and the aspirate was mostly composed of fat cells with relative increase in plasma cells and lymphocytes. The diagnosis of aplastic anemia was confirmed on bone marrow biopsy. In study by R Athar et al,[12] bone marrow showed hypercellularity with

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micronormoblastic and megaloblastic change and trilineage dyspoiesis. In the study by Garg P et al,[14] all marrows were hypercellular, showing erythroid hyperplasia with giant metamyelocytes.

Bone marrow iron stores were decreased (grade 0 to 1+) in most cases of dimorphic anemia and iron deficiency anemia. Pujara et al,[15] found 66.6 % cases in the range of zero to 1+ grade.

CONCLUSION

Dimorphic anemia was most common cause of anemia in the present study. Most other studies have reported megaloblastic and aplastic anemia as the commonest cause. The variations in the frequency of various causes of anemia in different studies has been attributed to difference in methodology and stringency of diagnostic criteria, geographic area, period of observation, genetic differences etc. The hematological parameters including findings were comparable to the findings of other studies. In the present study hematological indices, RBC histogram patterns and final diagnosis on bone marrow examination were significantly correlated. The present study concludes that RBC parameters, histograms patterns and peripheral blood smear examination are useful screening tests while bone marrow examination is necessary to confirm the diagnosis and in identification of underlying etiology in patients with anemia before further haematological analysis. In a developing nation like ours it can be an easy and cost effective procedure to combat the nutritional anaemia.

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