A study of hematological indices and bone marrow morphology in patients presenting with anemia

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Abstract

Introduction: The initial workup of anemia includes assessment of hematological indices and examination of the peripheral blood smears. Laboratory evaluation without marrow examination is usually not confirmatory. The objective of this study is to find the underlying etiopathology of anemia and correlate hematological indices, bone marrow study with clinical examination findings in differentiating causes of anemia.

Materials and Methods: The present observational study was carried out on 138 patients with anemia, in which bone marrow study (BM aspiration and biopsy) was done for evaluation.
Pediatrics population below 15 years and pregnant female and patients who did not give consent for bone marrow aspiration or biopsy were excluded from the study.

Results: Dimorphic anemia was the most common non-malignant hematological cause followed by megaloblastic anemia. In hematological malignancies acute myeloid leukemia (4.34%) was most common.

Discussion: A comprehensive clinical and hematological study of patients with anemia of various hematoloqical disorders will usually help in identification of underlying cause. However, in view of wide array of etiologies, finding of underlying etiopathology continues to be a challenge for pathologist and hematologists.

Keywords: Anemia, Hematological indices, Bone marrow study.

Introduction

Anemia is common worldwide but prevalence of anemia is disproportionately high in developing countries due to poverty, inadequate diet and poor access to health resources. Anemia is not a diagnosis, but a manifestation of an underlying disorder. Thus, even mild asymptomatic anemia should be investigated to diagnose and treat the primary cause.[1]

Anemia is pathophysiologically diverse and often multifactorial. Various hematological disorders in any age group usually presents with anemia. The WHO criterion has been accepted widely for diagnosis, but its universal application has been questioned mainly because of racial differences. According to this 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 concentration in blood may vary depending on the population analyzed, age, sex, environmental conditions and food habits.[2]

Dimorphic anemia is characterized by two distinct red cell population populations. The term is most often applied when there is one population of microcytic hypochromic cells and another of normochromic cell, the latter being either normocytic or macrocytic. A dimorphic blood film can be seen in several circumstances, it can occur when iron deficiency anemia responds to iron therapy, after the transfusion of normal blood to a patient with a hypochromic anemia, sideroblastic anemia, delayed transfusion reactions and dual iron and either vitamin B12 or folic acid deficiency which is focus of our study.[3] Hemoglobin concentration (g/dl) for diagnosis of anemia and assessment of severity according to WHO, The male and female more than 14yrs of age having Hb level 11-12.9g/dl and 11-11.9g/dl respectively are classified as mild degree of anemia while in both sexes Hb level 8-10.9g/dl defined as moderate degree of anemia and Hb level below 8g/dl defined as severe degree of anemia.[4]

The initial workup of anemia always includes a thorough history and physical examination, assessment of cell count, red cell indices and examination of the peripheral smear. Laboratory evaluation without marrow examination is usually not confirmatory. The primary goal of laboratory evaluation is to determine the nature of the underlying disorder.

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.[5] It also gives explanation for unexplained cytopenias and leukemia's.[6] Bone marrow aspiration is safe invasive procedure done in the hospitals for the diagnosis and management of hematological disorder. There is very little or no risk of bleeding and can be done in case of severe thrombocytopenia. Bone marrow examination is extremely helpful of pancytopenia.[7] The presenting symptoms of pancytopenia are attributed to the anemia or thrombocytopenia and leucopenia is often seen in subsequent course of the disorder.

The management and prognosis of patient with anemia

depends on underlying etiopathology. Hence, to find out correct etiopathology in a given case is crucial. For this purpose, the objective of this study was to find the underlying etiopathology of anemia and correlate with hematological indices, bone marrow study with clinical examination findings in differentiating causes of anemia.

Materials and Methods

The present observational study was carried out in Department of Pathology, at Government Medical College and Hospital, Latur (Maharashtra- India) after obtaining permission from Institutional Ethical Committee. A total of 138 patients with anemia in which bone marrow study (BM aspiration and biopsy) were done for evaluation of anemias.

Pediatrics population below 15 years and pregnant female and patients who did not give consent for bone marrow aspiration or biopsy was excluded from the study.

Clinical history recording and examination of all the identified cases were done along with routine hematological investigations like complete blood count, peripheral smear study and bone marrow aspiration smears examination. Other investigations such as, bone marrow trephine biopsy, serological study for HIV, HbsAg, leptospirosis and dengue were done as necessary. Whole procedure was explained and written consent of patient was taken in each case either from patient or relative before starting the procedure.

In all the cases, bone marrow aspiration and biopsy was performed from Posterior superior iliac spine under all aseptic precaution. Bone marrow biopsy was performed in cases of dry tap or in cases of diluted bone marrow aspirate. The smears were studied after staining with Leishman's stain.

Observation

One hundred thirty eight patients of adult age group (15-64yrs) admitted in hospital with various hematological disorders having anemia were studied. Dimorphic anemia was the most common hematological disorder (33.34%) followed by megaloblastic anemia (26.08%). In the hematological malignancies, acute myeloid leukemia (4.35%) was most common. Metastasis to bone marrow from unknown primary were seen in 2 cases (1.45%) (Table 1).

The age range of patients in our study was 15 to 64 years. The commonest age group for presentation of anemia in all hematological disorder was between 15-24 years, a total of 52 (37.69%) cases belonged to this group. The mean age was 32.8 years. Out of 138 cases, 73 were males and 65 females. The overall male to female ratio being 1.12:1.

Generalized weakness was the most common presenting symptom, seen in 114 (82.60%) cases. Fever was the second most common presenting symptom, seen in 76 (55.07%) cases. In present study, pallor was the most common finding on physical examination as seen in 111 (80.43%) of all cases followed by splenomegaly seen in 60 (43.5%) of all cases and lymphadenopathy was least common finding seen in 9(6.52%) cases.

Out of 138 cases, 115 (83.33%) cases were presented with severe grade of anemia (Hb less than 8 gm/dl).

In present study, RDW was seen increased in most of the hematological disorders except aplastic anemia. Most of the cases of megaloblastic anemia showed increased value of MCV, while MCV was decreased in Iron deficiency anemia. Majority of cases has normal values of MCH and MCHC (Table 2).

Table 1: Etiological distribution of anemic cases in adult age group

Category	Causes	No. of cases
Non-malignant hematological disorders	Dimorphic anemia	46 (33.34%)
	Megaloblastic anemia	36 (26.08%)
	Aplastic anemia	22 (15.95%)
	Iron deficiency anemia	11 (7.97%)
	Total	**115 (83.34%)**
Malignant hematological disorders	Acute leukemia (unclassified)	3 (2.17%)
	Acute myeloid leukemia	6 (4.35%)
	Acute lymphoblastic leukemia	4 (2.90%)
	Chronic myeloid leukemia	1 (0.72%)
	Chronic lymphocytic leukemia	2 (1.45%)
	Lymphoproliferative disorder	1 (0.72%)
	Multiple myeloma	2 (1.45%)
	Myelofibrosis	2 (1.45%)
	Total	**21 (15.21%)**
Metastasis to BM	Metastasis of unknown primary	2 (1.45%)
Total no. of cases		**138 (100%)**

Table 2: Distribution of hematological indices in various hematological disorders

Type of disorders	MCV (fl) mean±SD	MCH (pg) mean±SD	MCHC (gm/dl) mean ±SD	RDW % mean ±SD	HB(g/dl) Mean± SD	
					Male	Female
Normal reference ranges[8]	92±9	29.5±2.5	33±1.5	12.8±1.2	15.0±2.0	13.5±1.5
Dimorphic anemia	86.57±16.4	25.84±7.15	28.77±5.01	17.24±2.56	5.17±1.81	4.91±2.18
Megaloblastic anemia	109±9.19	34.48±5.07	32.01±3.20	16.38±1.67	4.69±2.04	5.01±1.09
Aplastic anemia	98.11±8.78	28.86±3.43	31.17±1.97	13.98±0.72	5.03±1.36	5.17±2.34
Iron deficiency anemia	68.78±7.72	20±3.84	26.76±5.72	17.44±1.94	4.95±0.98	5.0±1.20
Malignant and metastatic disorders	88.75±10.4	27.96±3.71	31.08±2.60	15.12±2.0	5.57±1.88	6.32±2.15

A) Dimorphic anemia – Out of 46 cases of dimorphic anemia 37(80.43%) cases were having severe grade of anemia followed by 8 (17.40%) cases with moderate and 1 (2.17%) case with mild grade of anemia. Of these 46 cases 19 (41.3%) cases were found with reticulocyte count range 2-5%, 15 (32.6%) cases were found with reticulocyte count range 0.2-1%, 11 (23.9%) cases were found with reticulocyte count range 1-2%. Mean (SD) was 1.72 (±1.08). The most common peripheral smears finding in dimorphic anemia were anisopokilocytosis [35 cases (76.08%)] followed by macrocytic hypochromic [4 cases (8.70%)]. Bone marrow aspiration finding in dimorphic anemia was revealed hypercellular bone marrow showed erythroid hyperplasia with micronormoblastic and megaloblastic maturation (erythropoiesis) with giant metamyelocytes and band form (Fig. 1). In our study, assessment of bone marrow iron store by Perl's Prussian blue reaction was done in 46 cases of dimorphic anemia out of which, 19 cases (41.30%) showed 2+ grade, 10 cases (21.73%) grade 1+, 7 cases (15.2%%) grade 1+, 6 cases (13.04%) grade zero and 4 cases (8.70%) grade 4+ of iron store.

B) Megaloblastic anemia-Out of 36 cases of megaloblastic anemia 32 (88.89%) cases were having severe grade of anemia followed by 4(11.11%) cases with moderate degree of anemia. Of these 36 cases of megaloblastic anemia 15 (41.66%) cases were found with reticulocyte count range 2-5%, 11(30.55%) cases were found with reticulocyte count range 1-2%, 10 (27.77%) cases were found with reticulocyte count range 0.2-1%. Mean (SD) was 1.46 (±1.07). In present study, the most common peripheral smears finding in megaloblastic anemia were anisopokilocytosis [21 cases (58.34%)] followed by macrocytic hypochromic [13 cases (36.10%)], normocytic normochromic [1 case (2.78%)] and normocytic hypochromic [1 case (2.78%)]. Bone marrow showed erythroid hyperplasia with megaloblastic change. Myelopoiesis showed giant metamyelocytes and giant band forms. Bone marrow iron study assessment was done in 36 cases of megaloblastic anemia out of which 18 cases (50%) showed 3+ grade, 12 cases (33.33%) grade 4+, 5 cases (13.88%) grade 2+, 1 case (2.77%) grade 1+ of iron store.

C) Aplastic anemia-Out of 22 cases of aplastic anemia 19(86.36%) cases were having severe grade of anemia followed by 3 (13.64%) cases with moderate degree of anemia. Of these 22 cases of aplastic anemia 16 (7.72%) cases were found with reticulocyte count below 0.2% and 6 (27.28%) cases were found with reticulocyte count range 0.2-1%. Mean (SD) was 0.37 (±0.24). In present study, the most common peripheral smears finding in aplastic anemia were normocytic hypochromic [13 cases (59.09%)] followed by normocytic normochromic [4 cases (18.18%)], anisopokilocytosis [4 case (18.18%)] and macrocytic hypochromic [1 case (4.55%)]. Bone marrow examination in aplastic anemia showed increase fat spaces and decreased cellularity. Myelopoiesis, erythropoiesis and megakaryopoiesis markedly decreased and relatively increase in lymphocytes and plasma cells are seen. Bone marrow iron study assessment was done in 22 cases of aplastic anemia out of which 9 cases (40.9%) showed 2+ grade followed by 7 cases (31.9%) grade 1+, 5 cases (22.7%) grade 3+ and 1 case (4.5%) grade zero of iron store.

D) Iron deficiency anemia-Out of 46 cases of iron deficiency anemia 10(90.90%) cases were having severe grade of anemia followed by 1(9.10%) case with moderate degree of anemia. Of these 11 cases of iron deficiency anemia, 5 (45.45%) cases were found with reticulocyte count range 1-2%, 4 (36.36%) cases were found with reticulocyte count range 0.2-1%, 2 (18.18%) cases were found with reticulocyte count range 2-5%. Mean (SD) was 1.23 (±0.65). In present study, the most common peripheral smear findings in aplastic anemia were anisopokilocytosis [6 cases (54.54%)] followed by microcytic hypochromic [4 cases (36.36%)] and normocytic normochromic [1 case (9.09%)]. Bone marrow aspiration findings in iron deficiency showed erythroid hyperplasia with micronormoblastic change while myeloid and megakaryocytes series showed normal maturation and morphology. Bone marrow iron study assessment was done in 11 cases of iron deficiency anemia out of which 7 cases (63.63%) showed zero grade and 4 cases (36.37%) grade 1+ of iron store.

E) Table 3 shows frequency of various malignant and metastatic hematological disorders in anemic cases in our study. Out of 23 cases, the most common hematological disorder was acute myeloid leukemia [6

cases (26.08%)] followed by acute lymphoblastic leukemia [4 cases (17.40%)].

Out of 23 cases, 19 (82.60%) cases were found with reticulocyte count range 0.2-1%, 2 (8.70%) cases were found with reticulocyte count range 0.2-1% and 2 (8.70%%) cases with reticulocyte count below 0.2%. Mean (SD) was 0.46 (±0.26). In present study, the most common peripheral smear findings in malignant hematological and metastatic disorders were normocytic hypochromic [10 cases (43.48%)] followed by normocytic normochromic [8 cases (34.78%)], anisopokilocytosis [5 case (21.74%)].

Table 3: Distribution of malignant and metastatic disorders in anemic cases

Malignant and metastatic disorder	No. of cases	Percentage
Acute leukemia (Unclassified)	3	13.04%
Acute myeloid leukemia	6	26.08%
Acute lymphoblastic leukemia	4	17.40%
Chronic myeloid leukemia	1	4.34%
Chronic lymphocytic leukemia	2	8.70%
Lymphoproliferative disorder	1	4.34%
Myelofibrosis	2	8.70%
Multiple Myeloma	2	8.70%
Metastasis from unknown primary	2	8.70%
Total	23	100%

Table 4: Bone marrow finding in anemic cases of malignant and metastatic hematological disorder

Type of disorder	Bone marrow finding				
	Cellularity	Erythropoiesis	Myelopoiesis	Megakaryopoiesis	Others
AML	Hypercellular	Suppressed	Hyperplastic, Blast cell	Normal	Auer rods seen in AML-M3
ALL	Hypercellular	Suppressed	Blast cell	Suppressed	Cytoplasmic Vacuoles seen in ALL-L3
CML	Hypercellular	Suppressed	Hyperplastic	Hyperplastic, Dysmegakaryopoiesis	Blast cell 5%
CLL	Hypercellular	Suppressed	Hyperplastic, mature small lymphocytes population	Suppressed	5% lymphoblast
Myelofibrosis	Hypocellular	Suppressed	Suppressed	Megakaryocyte proliferation	Marked marrow fibrosis, reticulin positivity was seen on BM biopsy
Multiple Myeloma	Hypercellular	Normal	Normal	Normal	Hypercellular BM, abnormal and more than 40% of plasma cell
LPD	Hypercellular	Suppressed	Hyperplastic, mature lymphoid population	Suppressed	Lymphoblast, small mature lymphocyte
MS	Hypocellular	Suppressed	Suppressed	Suppressed	Multiple small cluster and singly scattered tumour cell

LPD-Lymphoproliferative disorders, MS-metastasis of unknown primary

Table 5: Blast cell morphology in acute leukemia

Type of Leukamia	Blast Cell						
	%	Nuclear chromatin	Nuclear membrane	Nucleoli	Cytoplasm	Granules	Auer rods
ALL-L1	55%	Coarse, homogenous	cleaved	Inconspicuous	Scanty	-	-
ALL-L2	60%	Fine	irregular	1-2 prominent	Moderate	-	-
ALL-L3	75%	--	irregular	1-2 prominent	Moderate, vacuoles	-	-
AML-M1	90%	Fine, homogenous	Regular	1-2 prominent	Scanty	+	-
AML-M3 (Hypergranular)	35%	Fine	Reniform	1-2,prominent	Moderate	+	+
AML-M6	80%	Fine	irregular	1-2 prominent	Basophilic	+	-

Total 6 cases of acute myeloid leukemia were identified in present study out of which 4 cases are AML-M3, 1 case of AML-M1 and 1 case of AML-M6. Total 4 cases of acute lymphoblastic leukemia were identified in present study out of 2 cases are ALL-L1, 1 case of ALL-2 and 1 case of ALL-L3

Table 6: Bone marrow biopsy finding in 30 cases out of 138 anemic cases with different hematological disorders

Diagnosis	Bone marrow biopsy finding
Aplastic anemia	Shows a hypocellular bone marrow with increased empty spaces between bony trabeculae, only few haematopoietic cells were seen
LPD	Hypercellular bone marrow with diffuse sheets of mature small lymphocytes population and blast cell population more than 5%. Erythroid and megakaryocytes series are markedly suppressed
MF	Shows a marked bone marrow fibrosis with Dysmegakaryopoiesis noted. Reticulin stain shows diffuse positivity
AML-M6	Shows a predominant population of erythroid precursors some with obvious dysplastic feature and more than 80% blast cell population.
CLL	Shows a hyper-cellularity with diffuse sheets of predominant small mature lymphocytes population and blast cell more than 5%, no fat cells were seen
MS	Shows a complete replacement of bone marrow by malignant tumour cells. Cells were arranged in sheets and forming glandular pattern. Individually cells are having mild to moderate eosinophilic cytoplasm and pleomorphic nuclei with prominent nucleoli.

MF- Myelofibrosis, LPD- Lymphoproliferative disorders, MS- Metastasis of unknown primary

In present study, Bone marrow aspiration was performed in all 138 cases while biopsy was performed in 30 cases which include 22 cases of aplastic anemia, 2 cases of myelofibrosis, 2 cases of metastasis to bone marrow of unknown primary, 2 case of CLL, 1 case of AML-M6 and 1 case of LPD.

Discussion

In present study, most common age group for presentation of anemia was 15-24yrs which is comparable with the study by Javalgi et al,[9] Shastri et al,[10] Usman et al[11] and Dasgupta et al.[12]

Mean age in our study was 32.8 yrs which is comparable with study by Dasgupta et al[12] and Pudasaini et al.[13]

Male to female ratio in present study was 1.12:1 (slightly male predominant) that is consistent with the studies by the Javalgi et al,[9] Anjum et al[11] and Dasgupta et al.[12]

Generalized weakness (82.60%) was the commonest clinical presentation in our study which is consistent with study by Gayathri and Rao et al.[14]

In present study, pallor was most common finding on physical examination followed by splenomegaly, these finding are consistent with in study by Patel et al,[15] Gayathri and Rao et al.[14]

In the present study, causes of anemia were divided into three categories: A) Non-malignant hematological disorders B) Malignant hematological disorders C) Metastasis to bone marrow. Out of total 138 cases, 115 (83.34%) were found non-malignant hematological disorders and 21 (15.21%) were malignant hematological disorders. Metastasis to bone marrow from unknown primary was seen in 2 (1.45%) anemic cases.

Amongst non-malignant hematological disorders, dimorphic anemia 46 (33.34%) was the commonest cause followed by megaloblastic anemia 36 (26.08%). Amongst 21 cases of malignant hematological disorders, the most common cause was acute myeloid leukemia 6 (4.35%). Metastasis to bone marrow from epithelial malignancy was seen in 2 (1.45%) cases.

In Tahlan et al non-malignant hematological disorders formed the major group (82.1%) and malignant hematological disorders comprised (17.9%) cases.[16] Most common non-malignant hematological disorders were megaloblastic anemia (87%) followed by megakaryocytic

thrombocytopenia (2.9%). Most common malignant hematological disorders were acute leukemia (41%). Tariq et al in their study found aplastic anemia (36%) the most common cause followed by megaloblastic anemia (16%).[17] Gayathri and Satyanarayan Rao found megaloblastic anemia (74.04%) %) as the most common cause followed by aplastic anemia (18.26%).[14]

A) Dimorphic anemia- Dimorphic anemia showed combination of varying proportion of iron deficiency and megaloblastic anemia. Dimorphic anemia was the commonest non-malignant cause of anemia in our study and it is consistent with study by Thiyagarajan et al.[18]

In our study, 75% of cases presented with severe grade of anemia with mean Hb 5.03 (±1.94) g/dl. RDW-CV were increased in most of the cases of dimorphic anemia with mean (SD) was 17.24% (±2.56). Other red cell indices MCV, MCH, MCHC were seen normal in most of the cases with mean value (SD) 86.57(±16.46) fl, 25.84(±7.15) pg, 28.77(±5.01) g/dl. Similar findings of hematological indices were also seen in the study by Athar et al.[19]

Most common peripheral smears findings in our study were anisopoikilocytosis that was consistent with the study by Athar et al.[19]

Bone marrow aspiration study showed usually hypercellularity with micronormoblastic and megaloblastic maturation. Giant band form, giant metamyelocytes and megakaryocytes were also seen. In study by Athar et al,[19] bone marrow studied in 58 cases of dimorphic anemia which showed hypercellularity with micronormoblastic and megaloblastic change and trilineage dyspoiesis. Bone marrow iron stores were reduced (grade 0 to 1+) in 13 cases (27.3%) and were normal to increased (grade 2+ to 4+) in 33 (71.7%) cases. Pujara et al found observation close to our study and all of the cases in their study showed normal to increased (2+ to 3+) iron store in cases of dimorphic anemia.[20]

A) Megaloblastic anemia- In our study 88.89% of the cases were found with severe grade of anemia. Mean value (SD) was 4.84% (1.62) g/dl. Similar finding were also observed in the study by Jha et al[21] and Kumar et al.[22] RDW-CV and MCV were increased in most of the cases in our study. The mean (SD) values of hematological indices were close to the study by Haq et al[23] and Bilal et al.[24] The principal hematologic manifestations are varying degrees of anemia, leucopenia, thrombocytopenia, anisopoikilocytosis, macroovalocytes and hypersegmented neutrophils.

In our study macroovalocytes with considerable degree of anisopoikilocytosis were the main features seen in 58.21% of the cases. Hypersegmented neutrophils were seen in most of the patients.

In the study by Tilak et al, 96.22% cases showed anisocytosis, 84.90% cases showed hypersegmented neutrophils, 24.52% showed circulating erythroblasts.[25] Reticulocytosis was seen in 9.43% and relative lymphocytosis was seen in 13.20% cases. In the study by Khodke et al, 90.90% cases showed anisocytosis and 90.90% cases showed hypersegmented neutrophils.[26] In the

study Gayathri and Satyanarayan Rao hypersegmented neutrophils was noted in 51.35% of cases.[14]

Bone marrow was hypercellular with predominantly megaloblastic erythropoiesis. Giant band forms, giant metamyelocytes and megakaryocytes were also seen. Bone marrow was hypercellular with reduction of fat cells in most of the patients (83.33%). Erythroid hyperplasia with megaloblastic maturation was seen in all the cases.

Bone marrow iron stores were decreased (grade 0 to 1+) in 1 (2.78%) case and were normal to increased (grade 2 + to 4+) in 35 (97.22%) cases. In the study by Pujara et al[26] found increased (3+ to 4+) iron store in 70% cases of megaloblastic anemia, which is consistent with our study.

B) Aplastic anemia- In our study, 90.9% of aplastic anemia cases were found with severe grade of anemia. Mean value (SD) was 5.28 (1.69) g/dl. Similar finding were also observed in the study by Jha et al[21] and Kumar et al.[22] Mean (SD) of hematological indices in aplastic anemia were MCV (98.11± 8.78) fl, MCH (28.86 ± 3.43) pg, MCHC (31.17± 1.97) g/dl and RDW-CV (13.98 ± 0.72)%. Similar finding were also observed in the study by Mahendra Singh et al.[27] Daniel et al[28] found normocytic normochromic erythrocytes in 64% cases, macrocytic blood picture in 20% cases. In the study by Tilak Jain et al[25] 33.34% patients had anisocytosis and 50% patients had relative lymphocytosis. In the study by Khodke et al, 42.85% patients showed anisocytosis and 14.28% cases showed relative lymphocytosis.[26]

In the present study, on peripheral blood smear examination 59.09% had normocytic hypochromic blood picture and 18.18% patents had normocytic normochromic erythrocytes. Cellularity of bone marrow in aplastic anemia was very much reduced. Lymphocytes and plasma cells were prominent. Daniel et al[28] in their analysis of 50 cases reported 74% of patients with hypocellular marrow, 16% of patients had normocellular marrow which later became hypocellular and 10% had acellular marrow.

Bone marrow was hypocellular and the aspirate was mostly composed of fat cells in all the patients. There was relative increase in plasma cells and lymphocytes. Bone marrow trephine biopsy revealed replacement of marrow by fat cells. Bone marrow iron stores were decreased (grade 0 to 1+) in 8 (36.37%) cases and were normal to increased (grade 2 + to 3+) in 14 (63.63%) cases. Biswajit et al,[29] found increased (79.17%) iron store and decreased iron store in 21.05% cases. Pujara et al,[20] found 66.6% cases in received in the range of zero to 1+ grade.

C) Iron deficiency anemia- RDW-CV was increased in most cases of iron deficiency anemia in our study with mean (SD) value 17.44 (1.94) while MCV is decreased in most cases with mean (SD) 68.33 (14.7).In study by Javalgi et al[9] found all the cases of iron deficiency anemia had marked anisopoikilocytosis with marked microcytic red cell. In our study 54.54% of the cases had anisopoikilocytosis and 36.36% showed marked microcytic hypochromic red cell. The bone marrow was hypercellular with reduction of fat cells in most of the

patients (81.81%). Erythroid hyperplasia with micronormoblastic maturation was seen in all the cases. Myeloid and megakaryocytes series showed normal maturation and morphology. The bone marrow study by Javalgi et al[9] showed hypercellularity with altered M:E ratio and increased erythropoiesis showing micronormoblasts in most of the cases. Myelopoiesis was normal and there was slight increase in megakaryocytes. In our study, bone marrow iron were within 0 to 1+ that is decreased to completely absence of iron store was seen in all the (100%) cases. It is consistent with study by Pujara et al[20] found 92.7% cases iron store in iron deficiency anemia within range of 0 to 1+ grade.

D) Hematological malignancy and metastatic disorders- In present study, values of MCV, MCH, MCHC and RDW-CV were normal in most of the cases of anemia of hematological malignancy and metastatic disorders. In the study by Jain et al[22] one case of acute myeloid leukemia showing anisocytosis, circulating erythroblasts and immature cells was reported. Khodke et al[23] found one case of acute myeloid leukemia with immature cells in the peripheral blood.

Out of 4 cases of acute lymphoblastic leukemia (ALL), 3 cases were confirmed by immunophenotyping. Peripheral blood smear study in acute lymphoblastic leukemia showed predominantly normocytic hypochromic RBCs with markedly increased number of lymphoblast. Al-Khalisi et al[27] reported 32 (30.47%) cases of acute leukemia out of which 22 (68.75%) cases were acute myeloid leukemia and 10 (31.25%) cases were acute lymphoblastic leukemia (ALL).

The most common malignant cause of anemias was AML. In present study, 2 cases of CLL were reported (8.7%) of hematological malignancy associated with anemia. Bone marrow aspiration study in CLL showed predominantly small mature lymphocytes population with more than 5% blast cell population and bone marrow biopsy showed diffuse sheets of small mature lymphocytes population with blast cell population more than 5%. Myeloid and erythroid series cells were markedly suppressed. Hamid et al[28] reported 10.9% cases of CLL in leukemias associated with anemia.

In present study, both cases of myelofibrosis were presented with fever and splenomegaly. Peripheral blood film showed marked anisopoikilocytosis. Dry tap was seen on aspiration and bone marrow biopsy was performed in both cases. Bone biopsy showed marked marrow fibrosis with dysmegakaryopoiesis. Reticulin study showed diffuse positivity. Shastry et al[10] reported 1 case (0.9%) of myelofibrosis on bone marrow biopsy.

Patients with multiple myeloma can develop anemia/pancytopenia due to replacement of bone marrow by immune proliferating cells. Tilak et al[22] reported one case of pancytopenia due to multiple myeloma in their study of 77 cases. Khodke et al[23] reported 2 cases of multiple myeloma in their study of 50 cases of pancytopenia. Peripheral blood showed anisocytosis in both of them.

There were two cases (1.45%) of multiple myeloma causing anemia. Peripheral smear findings showed normocytic normochromic anemia, low total leucocytes counts and platelet count. Bone marrow aspirate was hypercellular. Haematopoiesis was decreased with infiltration by plasma cells (30%). Many abnormal forms with uninucleate and binucleate forms were seen. Serum protein electrophoresis showed dense localised M band at gamma region. Jha et al[20] in their study of 148 cases, reported one case of plasma cell myeloma. Tariq et al[7] reported 2 (4%) cases of multiple myeloma in their study. Gayathri and Satyanarayan Rao[8] in their study reported one case of multiple myeloma. Al-Khalisi et al[27] reported 2 (1.9%) cases of multiple myeloma in their study.

In present study two patients (1.45%) presented with anemia showed deposits of epithelial malignancy on bone marrow aspiration and biopsy studied. Bone marrow biopsy was performed which showed deposits of epithelial malignancy, Erythroid hyperplasia, Leucopoiesis and megakaryocytes were reduced. Metikurke et al[29] reported 1 (1.7%) case of metastasis of adenocarcinoma on bone marrow biopsy. Shastry et al[10] reported 1 (0.9%) case of metastatic deposits of unknown primary on bone marrow biopsy.

Dimorphic anemia was commonest non-malignant cause of anemia in the present study. Most other studies have reported megaloblastic and aplastic anemia as the commonest cause. This seems to reflect higher prevalence of nutritional anemia in Indian subjects. The hematological parameters including hematological indices in patients with dimorphic anemia, megaloblastic anemia, aplastic anemia, iron deficiency anemia and malignant disorders were comparable to the findings of other studies. Uncommon etiologies like lymphoproliferative disorders, chronic myeloid leukemia, Myelofibrosis, deposits of epithelial malignancy, multiple myeloma, and chronic lymphocytic leukemia were identified in our study.

A comprehensive clinical and hematological study of patients with anemia of various hematological disorders will usually help in identification of underlying cause. However, in view of wide array of etiologies, finding of underlying etiopathology continues to be a challenge for pathologist and hematologists.
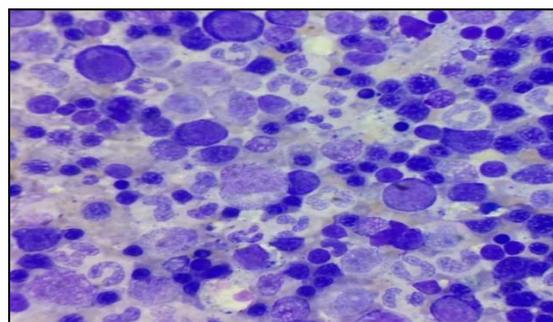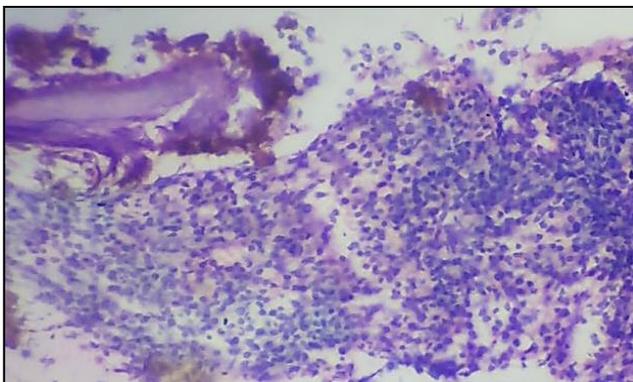

Fig. 1: Bone marrow aspiration smear showing micronormoblastic and megaloblastic maturation (Leishman's stain 100X)- Dimorphic anemia
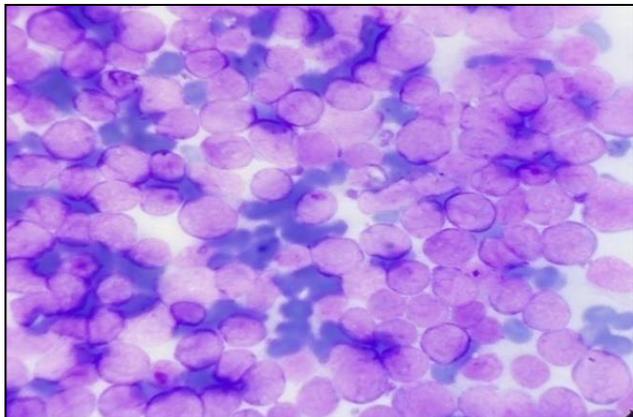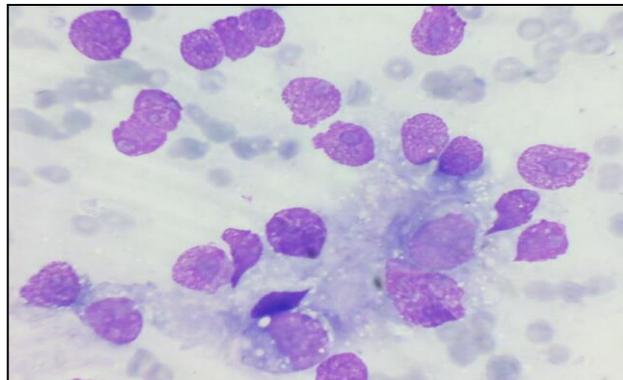
Fig. 2: Bone marrow aspiration smear showing hyperplastic bone marrow with blast cells population more than 90% having condensed chromatin with scanty cytoplasm and 1-2 prominent nucleoli. (Leishman's stain, 100X)- AML-M1
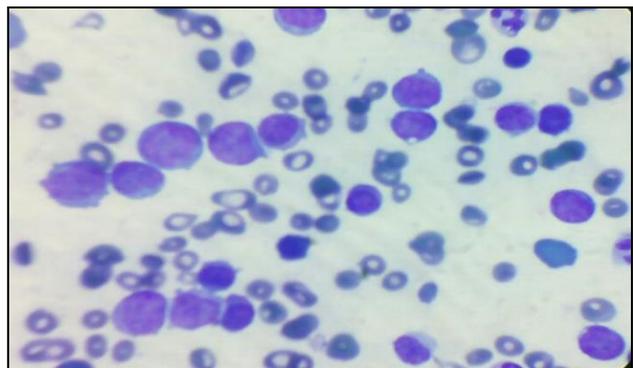
Fig. 3: Peripheral blood smear showing premature cells of erythroid series (Leishman's stain, 100X)- AML-M6

Fig. 4: Bone marrow biopsy showing hyperplastic bone marrow with erythroid series hyperplasia and diffuse sheets of erythroid series cells (H&E, 100X)- AML-M6

Fig. 5: Bone marrow aspiration smears showing tumour cells which are having hyperchromic nuclei with moderate degree of pleomorphism and prominent nucleoli (Leishman's stain, 100X)- Metastasis to bone marrow of unknown primary

Conflict of Interest: None.

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