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Etiological evaluation of severe anaemia on the basis of bone marrow studies in tertiary care hospital

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ABSTRACT
Background: The etiology of anaemia is multifactorial. Bone marrow examination is useful for the diagnosis of both hematological and non-hematological disorders.
Aim: To evaluate the etiology of severe anaemia on the basis of bone marrow examination.
Material and Methods:A total of 120 patients of severe anaemia attending casualty, admitted in Medicine ward/ intensive care unit (MICU) were included.Haematological investigations along with peripheral blood smear examination was done. Bone marrow aspiration procedure was performed using a standard unit protocol as adapted from ICSH guidelines.
Results: The mean hemoglobin was 5.03±1.40 gm/dl, with majority (48.33%) having hemoglobin levels less than 5 gm/dl. On bone marrow aspiration, iron deficiency anaemia (36.67%) was the most common diagnosis followed by megaloblastic anaemia (35.83%), dimorphic anaemia (19.17%), acute myeloid leukemia (4.16%), aplastic anaemia (3.33%), and pure red cell aplasia (1%).
Conclusion: In present study, based on bone marrow aspiration, iron deficiency anaemia (36.67%) was the commonest diagnosis followed by megaloblastic anaemia (35.83%), dimorphic anaemia (19.17%). Bone marrow aspiration is a safe invasive technique. Bone marrow aspiration should not be a first line investigation.
Keywords: Severe anaemia, bone marrow aspiration, iron deficiency anaemia, megaloblastic anaemia

I. INTRODUCTION

Anaemia is very commonly encountered in developing countries. The prevalence of anaemia in India is very high across all age groups. Among the adult population in rural India, anaemia is prevalent among 50% of the women, and 44.3% of the men.[1]

The etiology of anaemia is multifactorial and usually includes micronutrient deficiencies (e.g. iron, folic acid, and vitamin B12), chronic worm infections, haemoglobinopathies, malignancy and chronic non-communicable diseases, blood loss. Studies have shown that iron deficiency is the most common micro-nutrient deficiency associated with anaemia followed by folate deficiency and Vitamin B12 deficiency and other micro-nutrient deficiencies.[2,3]

Although the etiology for anaemia is truly countless, with a comprehensive history, clinical examination with routine directed laboratory investigations (biochemical and hematological), aided with bone aspiration, specific diagnosis can often be concluded. Early intervention in anemia decreases morbidity and ensures more productive life and hence correct diagnosis of anaemia is essential for management.[4]

Although, bone marrow aspiration (BMA) is an invasive test, it is crucial in the evaluation of anaemia and haematological and non haematological diseases, especially in situations where the diagnosis remains obscure even after extensive investigations.[5] Bone marrow examination is useful for the diagnosis of both hematological and non-hematological disorders.

In our tertiary care hospital, there are many cases of severe anemia which remains undiagnosed and are not fully evaluated. Therefore, there was a need to explore and establish the diagnostic utility of bone marrow examination in such cases of severe anaemia. Hence, the present study was conducted to evaluate the etiology of severe anaemia on the basis of bone marrow examination.

II. MATERIAL AND METHODS

The present observational cross-sectional study was conducted in the department of Medicine at a tertiary care teaching hospital. Approval of Ethics Committee (IEC) was obtained to carry out the study. The purpose and the details of the study

were explained to the participants who met the inclusion criteria in either of the following Marathi/Hindi/English language, after which written informed consent was taken and then enrolled in the study.

Study population

Study subjects attending casualty, admitted in Medicine ward/ intensive care unit (MICU) were included.

Sample size

In this study, 120 patients of severe anemia were included. Sample size was calculated from the following formula of observational cross – sectional study. Sample size software was used for sample size calculation with 5% level of significance, 90 % power and 10% margins of error. Sample size =Z1-α/2 2 p(1-p) /d2 Here, Z1-α/2 = standard normal variate; P=expected proportion in population; d= absolute error or precision.

Inclusion criteria

- Study subjects with severe anaemia (hemoglobin less than 7g/dl) presented for first time.
- Study subjects of both sex and age > 12 years

Exclusion criteria

- Study subjects with hemoglobin more than 7g/dl.
- Study subjects with bleeding tendencies.
- Study subjects on cytotoxic drugs or drugs proven to cause bone marrow suppression or radiotherapy.
- Known cases of hemolytic anaemia.
- History of blood transfusion in last 6 months.
- Study subjects not wiling for informed consent.

III. METHODOLOGY

Data was collected prospectively from study subjects presenting with signs and symptoms of severe anaemia, attending Outpatient/ Casualty and admitted in Medicine ward/Intensive care unit. A pre-designed Case record form (CRF) was prepared to record the patients characteristics, clinical examination findings, Investigation details and diagnosis from each patient in a systematic manner. A detailed meticulous physical and clinical examination of every patient was carried out. Hematological investigations including complete hemogram, red blood cell indices, peripheral blood smear and bone marrow aspiration finding values was recorded. A 5 ml of venous blood was collected in Ethylene Diamine Tetra Acetate (EDTA) coated bulb from each participant under aseptic precautions for laboratory procedures. Standard procedures were followed during blood collections transportation, storage and disposal to protect the participants as well as the researchers.

Hematological analysis (Complete Blood Count (CBC) with absolute values) was conducted on an automated blood analyzer. All hematological parameters were obtained. Peripheral blood smear examination was done systematically under low, high and oil immersion microscope for RBC morphology. Differential leucocyte count was done.

Bone marrow aspiration procedure was performed using a standard unit protocol as adapted from ICSH guidelines. Bone marrow was aspirated from posterior superior iliac crest under local anesthesia using lignocaine solution under all aseptic precautions after obtaining written consent from the patient or guardian. In obese patients, sternum was used for aspiration. Salah bone marrow aspiration needle was used for the procedure, and to avoid dilution of the yield by peripheral blood, only approximately 0.3 ml bone marrow was aspirated. At the same time, peripheral blood was also obtained to complement information from the bone marrow aspiration. Few smears were air dried and stained with Leishman stain. Others were fixed in methanol for 20 minutes. The fixed smears were stained with Wright Giemsa stain. All the patients, thus selected were investigated in a systematic manner and the cause of anaemia was ascertained.

IV. STATISTICAL ANALYSIS

Data was entered in Microsoft office Excel 2010 and analyzed in SPSS software version 21. Descriptive statistics was used to express the results. Data was expressed in actual number, mean with standard deviation, percentage and proportion, wherever applicable.

V. RESULTS

The mean age of the study subjects with severe anaemia was 32.85±17.74 years., majority (37.5%) of the study subjects enrolled were in the age group of 12-20 years, followed by 29.16% in the age group of 21-30 years and 18.33% in the age group of 31 – 40 years. 8.33 % were in the age group of 41-50 years and 4.17 % in the age group of 51-60 years. Around 2.5% were in the age group of 61 years and above. Minimum age was 12 years and maximum 68 years. Out of the 120 study subjects enrolled, 71 (59.17%) were females and 49 were males (40.83%). The female to male sex ratio was 1.4:1.

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Table 1: Distribution of study subjects according to Hematological profile

Parameters	Total	Minimum	Maximum	Mean±SD
Hemoglobin (gm/dl)	120	2.30	7.0	4.6±1.40
RBC (lac)	120	0.30	7.0	3.6±9.59
MCV (fl)	120	47.40	131.00	87.06±16.80
MCHC (%)	120	19.90	41.40	29.03±4.51
RDW (%)	120	12.70	36.60	20.45±5.51
WBC (/mm3)	120	500.00	255000.00	11745.95±38879.64
Hematocrit (%)	120	8.50	36.30	19.851±6.981
Platelet Count (/mm3)	120	2690.00	7,70000.00	155834.08±675202.34
Reticulocyte Count (%)	120	0.03	3.60	1.29±0.53

The mean Hemoglobin, Red blood cells, white blood cells and platelet count was 5.03±1.40 gm/dl, 3.42±9.5 lac, 11.74±3.8 thousand /mm3 and 1.55±2.7 lac/mm3 respectively. The mean MCV, MCHC, RDW, hematocrit and reticulocyte count was 87.0±16.8.4 fl, 29.03±4.5%, 20.45±5.5%, 19.8±6.9% and 1.29±0.5% respectively.

Majority 58(48.33%) of the study subjects had hemoglobin less than 5 gm/dl, 39(32.5%) study subjects had hemoglobin between 5.1 to 6gm/dl and 23(19.17%) had hemoglobin between 6.1 to 7gm/dl. The mean Hemoglobin present study was 5.03 ± 1.40 gm/dl.

Table 2: Distribution of study subjects according to Peripheral smear findings

Microscopic findings on Peripheral smear findings	Diagnosis	Total (%)
Microcytic hypochromic with Anisopoikilocytosis, Pencil cell	Iron deficiency anaemia	48 (40%)
Macro-ovalocytes, Hypersegmentedneutrophils, Poikilocytes, howell jolly body, cabot ring	Megaloblastic anaemia	43 (35.83%)
Microctic hypochromic and macro-ovalocytes	Dimorphic anaemia	23 (19.16%)
Normocytic Normochromic	Normocytic normochromic anaemia	3 (2.5%)
Normocytic with myelocytes and promyelocytes, Blast cells	Acute myeloid leukemia	3 (2.5%)
Total		**120**

Majority 48 (40%) of peripheral smear were suggestive of microcytic hypochromic, followed by macrocytic 43 (35.83%), microcytic and macrocytic 23 (19.16%), normocytic normochromic 3 (2.5%) and acute myeloid leukemia 3 (2.5%).

Out of 120 study subjects, bone marrow cellularity was hypercellular in majority 115 (95.83%) of study subjects and in 5(4.17%) patients it was hypocellular.

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Table 3: Distribution of study subjects according to Bone marrow aspiration findings

Bone marrow microscopic picture	Iron store	Diagnosis	Total (%)
Hypercellular micronormoblastic, Erythroid hyperplasia,	Reduced	Iron deficiency anemia	44 (36.67%)
Hypercellular macronormoblastic, Erythroid hyperplasia,	Adequate	Megaloblastic anaemia	43 (35.83%)
Micronormoblastic and megaloblastic,	Reduced	Dimorphic anaemia	23 (19.17%)
Hypercellular with blast cell >20%, Auer rod. MPO +	adequate	Acute myeloid leukemia	5 (4.16%)
Hypocellular with erythroid hypoplasia, Fatty marrow, decrease in erythroid, myeloid and megakaryocytic precursors	Adequate	Aplastic anaemia	4 (3.33%)
No erythroid precursors, normal myelopoisis and megakaryopoeisis	Adequate	Pure red cell aplasia	1 (0.83%)
Total			120 (100%)

Most study subjects 44(36.67%) had iron deficiency anaemia, followed by megaloblastic anaemia 43 (35.83%), dimorphic anaemia 23(19.17%), acute myeloid leukemia 5 (4.16 %) aplastic anaemia 4 (3.33%) and pure red cell aplasia1 (0.83 %) cases on bone marrow aspiration.

Figure. 1: Bone marrow aspirate of iron deficiency anaemia

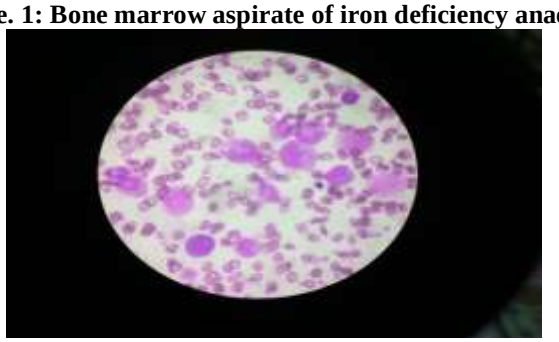

Figure. 2:Bone marrow aspirate of megaloblastic anaemia

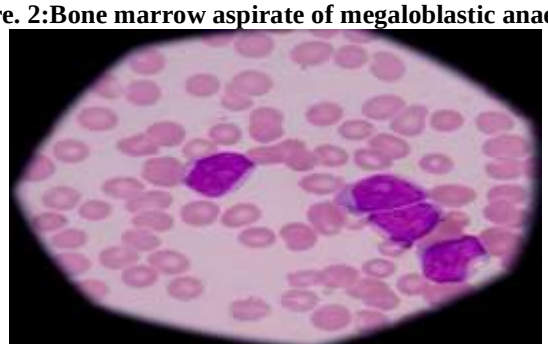

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Figure. 3:Bone marrow aspirate of aplastic anaemia

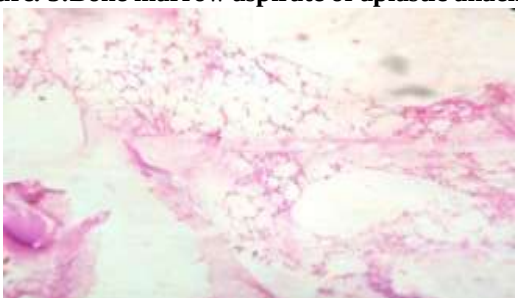
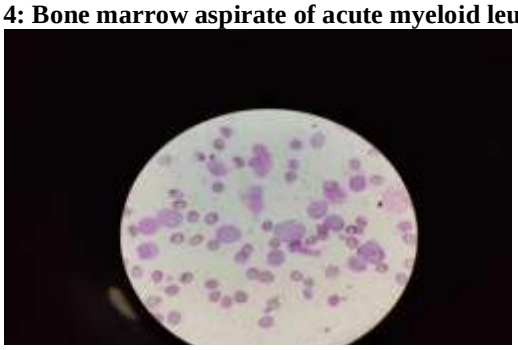


Figure. 4: Bone marrow aspirate of acute myeloid leukemia



VI. DISCUSSION

The encouraging results of present study revealed that bone marrow aspiration is a valuable procedure to investigate the causes of severe anaemia and also to establish accurate diagnosis and initiation of appropriate treatment.

The prevalence of anaemia in India is very high across all groups. Among the adult population in rural India, anaemia is prevalent among 50% of the women and 44.3% of the men. It is commonly encountered in clinical practice among all age groups. The prevalence of severe anaemia is on the rise causing morbidity and complication and burden to the patients. Although the etiology is myriad, with a comprehensive history, clinical examination and routine laboratory tests, a diagnosis is often established in majority. However, in few subsets of patients, due to lack of laboratory facilities and specific hematological procedures (bone marrow examination), the further investigations for establishing the causes of severe anaemiais often hindered.

In present study cellularity on bone marrow aspiration in majority of study subjects was hypercellular,i.e. in 115(95.83%) of total 120 study subjects and hypocellular in 5(4.17%) patients.Hypercellular bone marrow was found in 44(36.67%) cases of iron deficiency anemia, 43(35.83%) cases of megaloblastic anemia, 23(19.17%) cases of dimorphic anemia and 5(4.16%) cases of acute myeloid leukemia.Reddy et al[6]studied 50 study subjects presenting with anaemia. They observed that bone marrow was hypercellular in 94% cases. Out of these 28(56%) study subjects had macrocytic anaemia 18(36%) had microcytic blood picture with iron deficiency anaemia and 2 (4%) had leukemia. Pudasini et al[7] studied 57 study subjects of anaemia. Maximum no. of bone marrow aspirate was hypercellular i.e. 61.4%. Among them Erythroid hyperplasia 12(21%), Megaloblastic anemia 7 (12.3%), Acute leukemia 7 (12.3%), Infective pathology 7 (12.3%) and Multiple myeloma 2 3.5% were diagnosis on bone marrow aspirate.

Out of 120 study subjects bone marrow aspirate of 5 (4.16%) study subjects was hypocellular.Hypocellular bone marrow was observed in 4(3.3%) study subjects of aplastic anemia and 1 (0.83%) patient of pure red cell aplasia.Reddy et al[6] studied 50 study subjects presenting with anaemia. Out of 50 study subjects bone marrow aspirate was hypocellular in 4% cases i.e. aplastic anemia.These observations well matched with present study.Pudasini et al[7] studied 57 study subjects of anaemia hypoplastic anemia was seen in 3 cases (5.3%). In all cases of hypoplastic anemia the marrow was hypocellular and all 3 lineages of cell were suppressed. Bone marrow aspiration findings were correlated with peripheral smear which also showed pancytopenia.

In present study, iron deficiency anaemia44 (36.67%) was the most common diagnosis followed by megaloblastic anaemia 43 (35.83%), anaemia dimorphic anaemia23 (19.17%), acute myeloid leukemia 5 (4.16 %) aplastic 4 (3.33%) and pure red cell aplasia 1(0.83 %) based

on bone marrow aspiration. In present study, the most common cause of severe anaemia observed was nutritional anaemia. Among these cases with nutritional anaemia, iron deficiency was the most common cause, which was similar to the findings of Dasharatham et al[8] in Indian health scenario.Dashratham et al[8] studied bone marrow aspirate of 50 study subjects of severe anemia and observed that iron deficiency anaemia (54%) most common, followed by dimorphic (30%), megaloblastic (4%) and normocytic normochromic (16%).Iron deficiency is the most common micro-nutrient deficiency associated with anaemia, while folate deficiency and Vitamin B12 deficiency are ranked as the second and third most prevalent micro-nutrient deficiencies associated with anaemia.However, studies have also documented contrast findings. Studies conducted by Reddy et al[6] studied 50 study subjects of anaemia. Megaloblastic reaction was seen in 52 % cases, microcytic in 20% cases, normoblastic in 18% cases each and normoblastic in 4% cases. Nutritional anaemia was the most important and common cause of severe anaemia. Merla et al,[9] studied 331 patients. Bone-marrow aspiration revealed 88 (26.59%) cases of micronormoblastic and macronormoblasticmaturation(dimorphic), 56 (16.92%) cases micronormoblastic maturation (iron deficiency), 38 (11.48%) cases of megaloblastic maturation, 23 (6.95%)cases of Leukemia, Plasma cell myeloma in 7(2.11%), lymphoma in 6 (1.81%), hypoplastic marrow in 5 (1.51%) cases haemophagocyticLymphohistiocytosis in 4 (1.21%),3 (0.91%) cases of myelodysplastic syndrome, 2 (0.60%)casesof idiopathic thrombocytopenic purpura. Sharma et al[10] have documented megaloblastic anaemia as the most common cause of anaemia, which has raised the concerns about Vitamin B-12 and folate deficiency in the population. Megaloblastic anaemia was the predominant cause of anaemia and was seen in 23 cases (28.6%) followed by acute leukemia seen in 20 cases (24.3%), out of which 8 cases (40%) were of acute lymphoid leukemia (ALL) and 12 cases (60%) were of acute myeloid leukemia (AML) (M2 and M3). There were 13 cases (15.2%) of erythroid hyperplasia with micronormoblast in 6(7.13%) cases. 5(6%) cases showed megaloblastoid type of picture.

This changing trend difference may be due to better iron supplementation or changing socioeconomic status. Whereas acute leukemia has been reported by Egesie et al[11] to be commonest followed by combined megaloblastic and iron deficiency anaemia (nutritional deficiency anaemia). Egesie et al[11] studied 185 study subjects of anaemia. The commonest cause of anaemia was acute leukemia is (n=45: 24.3%); followed by combined megaloblastic and iron deficiency anaemia (nutritional deficiency anaemia) (n=34: 18.4%); and bone marrow failure (Aplastic anaemia) (n=20: 10.8%). Bone marrow aspiration alone failed to identify causes of anaemia in a few study subjects (n=6: 3.2%). High prevalence ofdimorphic anaemia was also found present study i.e. 23(19.17%) out of 120 patients.Pooja Garg et al[12] studied 1015 cases of anaemia.Dimorphic anaemiawas found in 17.5% (178/) of all cases of anaemia. Other studies conducted in India showed theincidence of dimorphic anaemia is found to be 12.5%.The high prevalence of dimorphic anemiacan be explained by the occurrence of nutritional deficiencies. Nutritional deficiencies of iron andfolate commonly occur together, more commonly. Iron deficiency occurring concurrently withmegaloblastic anaemia has been reported in many areas. In some cases, the megaloblastosismay be intermediate in degree but may become more marked after administration of iron, and some cases of severe dimorphic anaemia do not respond initially to iron therapy, possibly due to concomitant severe folate and occasionally vitamin B12 deficiency.

In present study, in bone marrow aspiration of 5(4.17%) cases were suggestive of acute myeloid leukemia. Egesie et al[11] identified the causes of anaemia by bone marrow aspiration in 50 study subjects and documented acute leukemia (24.3%), followed by (18.4%) combined megaloblastic with iron deficiency anaemia (nutritional deficiency anaemia) and (10.8%) aplastic anaemia as the most common causes.They also observed that in majority (96.8%) of the cases sent for bone marrow aspiration, had the cause of their anaemia identified. However, bone marrow aspiration alone failed to identify the cause of anaemia in only a few (3.2%) cases.Reddy et al[6] studied bone marrow aspirate of50 study subjects of anaemia and found acute myeloid leukemia in 2% cases. Overall, present study showed that bone-marrow aspiration is a valuable diagnostic tool for the confirming diagnosis and explaining the causes for severe anaemia. This can help in early diagnosis of underlying condition and effective management.

Studies have shown that aspiration of bone marrow is a valuable diagnostic tool and helps to confirm diagnosis of various hematological and non-hematological disorders. Bone-marrow aspiration provides direct observation and assessment of red blood cell (RBC) precursor's presence of abnormal maturation of red blood cells

and amount and distribution of cellular pattern can be determined.

VII. CONCLUSION

In present study, based on bone marrow aspiration, iron deficiency anaemia (36.67%) was the commonest diagnosis followed by megaloblastic anaemia (35.83%), dimorphic anaemia (19.17%). Bone marrow aspiration is a safe invasive technique. Bone marrow aspiration should not be a first line investigation. It is often indicated after initial evaluation of the peripheral blood and other ancillary tests.

Conflict of Interest: None to declare

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