"STUDY OF DIMORPHIC ANEMIA IN ADULTS WITH REFERENCE TO BASIC ETIOLOGY"

By

DR. DEEPTHI.A

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IN

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Under the guidance of

DR. CSBR Prasad M.D
Professor of Pathology

DEPARTMENT OF PATHOLOGY

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DR. CSBR PRASAD M.D, Professor of Pathology,

Department Of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar.

Date: **DR. DEEPTHI.A**

Place : Kolar

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I have great pleasure in forwarding this to the university.

Date :
Place : Kolar

DR. CSBR PRASAD .MD
Professor
Department Of Pathology
Sri Devaraj Urs Medical College,
Tamaka, Kolar

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in

PATHOLOGY.

\

DR. B.N.Raghavendra Prasad .MD
Professor & HOD
Department Of Medicine
Sri Devaraj Urs Medical College,
Tamaka, Kolar.

Date :
Place : Kolar

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ENDORSEMENT BY THE HOD,

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under the guidance of **DR. CSBR PRASAD .**MD

Professor, Department Of Pathology

DR. M.L.HARENDRA KUMAR .MD,FICP
Professor & HOD
Department Of Pathology
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:

Place: Kolar

DR. M.B.SANIKOP .MS
Principal
Sri Devaraj Urs Medical College
Tamaka, Kolar

Date:

Place: Kolar

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This is to certify that the Ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved

DR. DEEPTHI.A

Post-Graduate student in the subject of

PATHOLOGY at **Sri Devaraj Urs Medical College, Kolar**

to take up the Dissertation work entitled

"STUDY OF DIMORPHIC ANEMIA IN ADULTS WITH REFERENCE TO BASIC ETIOLOGY"

to be submitted to the

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, TAMAKA, KOLAR , KARNATAKA

Member Secretary
Sri Devaraj Urs Medical College,
Kolar–563101

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DR. DEEPTHI A

DEDICATED

TO

MY BELOVED PARENTS

LIST OF ABBREVIATIONS

BM – Bone marrow

CBC – Complete Blood Count

CV – Co efficient of Variation

DA – Dimorphic Anemia

EDTA – Ethylene Diamine Tetra Acetic Acid

Hb – Hemoglobin

Hct – Hematocrit

IDA – Iron deficiency anemia

LHD % - Low hemoglobin density %

NFHS - National Family Health Survey

MA – Megaloblastic Anemia

MCH – Mean Corpuscular Hemoglobin

MCHC - Mean Corpuscular Hemoglobin Concentration

MCV – Mean Corpuscular Volume

MDS – Myelodysplastic Syndrome

MM – Multiple Myeloma

PCV – Packed Cell Volume

RBC – Red Blood cell

RDW (CV) – Red Cell Distribution Width (Coefficient Of Variation)

RDW (SD) – Red Cell Distribution Width (Standard Deviation)

UNICEF – The United Nations Children's Fund

WBC – White Blood Cell

WHO – World Health Organization

TRBC – Total Red Blood Cell Count

ABSTRACT

OBJECTIVES

1. To investigate the basic cause of dimorphic anemia in adult patients using hematological parameters.
2. To study serum vitamin B12, folate assays and bone marrow iron stores in dimorphic anemia

MATERIALS AND METHODS

The study was conducted at our institute on sixty two patients with dimorphic anemia. The hematological parameters, bone marrow, serum vitamin B12 and folate assays were studied in these patients to evaluate the basic etiology in dimorphic anemia.

RESULTS

Majority of our patients belonged to second decade of life. The mean hemoglobin concentration was 5.74±2.43gm/dl. Thirty four percent of our cases had pancytopenia. The mean MCV was 95.01±13.14 fl. Normal MCV was seen in 56.45% cases. The mean MCH was 29.31±8 pg. The mean RDW of 17 ± 2.61 %. Bone marrow examination showed hypercellular marrow in 67.74% cases, erythroid hyperplasia in 93.54 % cases. Megaloblastic maturation was seen in 32.36% cases and 64.52% patients showed both megaloblastic and micronormoblastic maturation, 50% of these cases showed dyserythropoiesis. The mean MCV and MCH were high in cases with megaloblastic maturation but, not statistically significant. Bone marrow iron stores showed deficient stores in 32.26% cases, normal stores in 56.45% cases and increased stores in 11.29% cases. The mean of MCH and MCHC in cases with adequate and deficient iron stores were statistically significant.

In our study, 43.55% cases had only folic acid deficiency, 19.35% cases had only vitamin B12 deficiency and 27.42% cases had both vitamin B12 and folic acid deficiency. Combined iron and vitamin deficiencies was seen in 25.8% cases. The mean MCV, MCH, MCHC and RDW were high in combined (vitamin B12 and folic acid) deficiency and was statistically significant.

CONCLUSION

The most common basic cause for dimorphic anemia was serum folate deficiency. Majority of cases with these deficiencies presented with pancytopenia. MCV alone is unreliable as a screening parameter for anemia associated with vitamin B12 or folate deficiencies. Concomitant deficiencies of vitamin B12 and/ or folate along with iron deficiency are not infrequent, one type of anemia may mask the other. Serum assays will be appropriate to establish the cause in cases with concomitant deficiencies, as it may result in discordant results with various other tests like hematological parameters.

Keywords : Dimorphic anemia, MCV, megaloblastic, vitamin B12, folate .

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INTRODUCTION

Anemia is the most common hematological disorder in all age groups. Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in women in reproductive age group (42%) and the lowest prevalence is in men (12.7%). The population group with the greatest number of individuals affected is non-pregnant women (468.4 million).[1]

One third of the world population suffers from anemia. India is one of the countries with very high prevalence rates. NFHS-3(National Family Health Survey) reveals the prevalence of anemia to be 70-80% in children, 70% in pregnant women and 24% in adult men.[2]

Dimorphic anemia has a complex pathogenesis with involvement of more than one deficiency state, usually due to deficiency of both iron and vitamin B12 or folic acid.[3]Its important to recognize masked megaloblastosis, where the anemia due to vitamin B12/folate deficiency is not accompanied by the classic findings of megaloblastic anemia in the peripheral blood.[4] This condition may be reflected by increased RDW in the presence of normal MCV, with dimorphic blood picture, showing two RBC populations, that is a combination microcytic hypochromic and macrocytic normochromic cells.[5] This should be further investigated to rule out iron deficiency and anemia of chronic disease. Myelodysplasia may be an underlying pathology in the older age group, as dimorphic anemia is characteristic of sideroblastic anemia.[6]

From the therapeutic point of view, it is important to differentiate the anemia in accordance with the type of disorder in the hematopoietic system.

Our study focuses on detecting the underlying basic pathology of dimorphic anemia, by estimating serum levels of vitamin B12, folic acid and bone marrow iron stores. This study signifies the importance of early diagnosis and treatment of specific vitamin deficiencies and thereby prevent the further life threatening consequences.

AIMS AND OBJECTIVE

1. To investigate the basic cause of dimorphic anemia in adult patients using hematological parameters.

2. To study serum vitamin B12, folate assays and bone marrow iron stores in dimorphic anemia.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORICAL ASPECTS

Anemia was well known as a disease entity to ancient Greek and Indian physicians. The word "Anemia" is derived from Greek word "Anemia" (an - not, naime - blood) meaning "without blood", which is decrease in the normal number of RBCs or less than normal quantity of hemoglobin in blood.[7,8]

William Hewson (1739-1774) "the father of hematology", published an opinion that because red cells were present in such abundance they had to be important.[9]

Sir William Harvey described blood as "the fountain of life and the primary seat of the soul. The marrow of our bones is the seed bed of our blood", hence, careful assessment of blood elements is the first step of approach to hematological disorder.[9]

In 1843, Gabriel Andral coined the term anemia and defined it as diminution of the globular elements (red blood cells) of the blood, noting that anemia can vary from mild to severe depletion of these elements.[10]

DEFINITION

Anemia is functionally defined as an insufficient RBC mass to adequately deliver oxygen to peripheral tissues. Anemia refers to reduction in oxygen carrying capacity and is defined as a clinicopathological condition, which is present when the hemoglobin level of the body is below the normal range for the age and sex of the individual.[11] World Health Organization (WHO) defines the lower limit of normal for hemoglobin concentration at sea level to be 12.0 g/dl in women and 13.0 g/dl in men.[12]

Anemia can develop if: (1) Erythrocyte loss or destruction exceeds the maximal capacity of bone marrow erythrocyte production or (2) The bone marrow erythrocyte

production is impaired. The classification of anemia based on these principles aids the physician in diagnosis.[13]

In 1930, Wintrobe conducted a study on more than 1,000 persons and tabulated his findings according to morphology into four groups: Macrocytic, Normocytic, Simple microcytic and Microcytic hypochromic. He gave a morphological classification of anemia based on their size and hemoglobin content using RBC indices, also called Wintrobe's indices.[13,14]

HEMATOLOGICAL PARAMETERS:

The routine laboratory tests used to screen for the presence of anemia are explained below.

Hemoglobin (Hb) – Hemoglobin is the main component of the red blood cell, is a conjugated protein that serves as a vehicle for the transportation of oxygen and carbon dioxide. The standard method of estimation of hemoglobin level is by using cyanmethemoglobin. This technique is used both in manual determinations and automated hematology analyzers.[15,16]

Hematocrit / Packed Cell Volume (Hct/PCV) - Hematocrit is the proportion of the volume of a blood sample that is occupied by red cells. The height of the column of red cells after centrifugation compared with the total blood sample volume yields the Hematocrit.

Total RBC count (TRBC) – RBC counts are usually performed at areas with automated instruments. In areas with limited resources, screening can be limited to either Hct or Hb.

Once anemia is established by the above parameters, it can be typed morphologically using RBC indices described below.

Mean Corpuscular volume (MCV) - MCV indicates the average volume of individual erythrocytes expressed in femtoliters (fl).[13]

MCV = Hematocrit / RBC count.

MCV is measured directly in few automated instruments or calculated from the RBC count and Hct.[17]The MCV is used to classify cells as normocytic, microcytic or macrocytic. An abnormality in the MCV gives a clue to the disease process of the hematopoietic system.[13]

Mean Corpuscular Hemoglobin (MCH) - MCH is a measure of the average hemoglobin content per red cell expressed in picograms (pg).

MCH = Hb / RBC count

It is a reflection of hemoglobin mass. It is calculated manually or by automated methods using hemoglobin and RBC counts.

Mean Corpuscular Hemoglobin Concentration (MCHC) - MCHC is the average concentration of hemoglobin in a deciliter of erythrocytes expressed in g/dl.

MCHC = Hb / (MCV x RBC count)

It represents the ratio of hemoglobin mass to the volume of red cells. MCHC indicates whether the general cell population is normochromic or hypochromic.[13]

Red Cell Distribution Width - Price-Jones, a British pathologist devised a curve for measuring variation in the diameter of erythrocytes by measuring the diameter of erythrocytes with an ocular micrometer and plotting the results as a distribution curve with the x-axis as the size and the y-axis as the cell count. The shape of the curve

indicates the presence of anemia. The normal curve is a typical Gaussian distribution. However, this method, called the Price – Jones curve could never become established for routine work, as measuring each erythrocyte diameter is very tedious.[18]

RDW is a quantitative measure of the variation in cell volume, an equivalent of the microscopic assessment of the degree of anisocytosis. RDW is derived from pulse height analysis and can be expressed either as the standard deviation (SD) in fl or as coefficient of variation (CV) in (%) of the measurements of the red cell volume.[17]RDW-SD is a direct measure across the RBC histogram and is theoretically a better and more accurate measure of RBC anisocytosis across the entire spectrum of MCV values. RDW-CV is calculated with the following formula:

RDW (CV) % = (Standard deviation of red cell width ÷ MCV) × 100 %

RDW CV shows better correlation according to published reports as an indicator of anisocytosis, if the MCV is in the normal range when anisocytosis may be difficult to detect. RDW CV has been found to have some value in distinguishing between IDA and thalassemia, and between megaloblastic anemia and other causes of macrocytosis.[17]The possible cause of anemia can be enumerated accurately, when RDW is used in conjunction with the MCV.[18]

Bessman et al in the early 1980s, proposed an improved classification of anemia based on MCV and RDW in which in addition to microcytic, normocytic and macrocytic anemia, new terms of homogeneous microcytic (normal RDW, low MCV) and heterogeneous microcytic (increased RDW, low MCV) were suggested.[19] RDW has been proposed to be more useful in early detection of nutritional deficiency anemia than other red cell parameters, such as iron, folate or vitamin B12 deficiency; even in non-

anemic individuals. RDW is also useful in identifying red cell agglutination and dimorphic cell populations.[20-22]

Low Hemoglobin Density % - (LHD%)

LHD% is a biomarker of hypochromasia. It is obtained in some Beckman coulter by calculation derived mathematically by sigmoid transformation of MCHC value. MCHC includes both the availability of iron over preceding 90 to 120 days and of the proper introduction of iron into intracellular hemoglobin. LHD% was calculated using the formula:[23]

$$\text{LHD\%} = 100^* \sqrt{1 - \left(\frac{1}{(1 + e^{1.8(30 - \text{MCHC})})} \right)}.$$

Square root is applied to further enhance numerical resolution in the region corresponding to the lower end of % hypo and to improve the differentiation between the normal and the abnormal among the blood samples having relatively low values of LHD%. LHD% is similar to other biomarkers of hypochromasia obtained from other automated blood counters – Sysmex and Abott. Studies have shown the significant correlation of serum ferritin levels with that of LHD%.[23]

Studies were conducted on the use of LHD% in chronic kidney disease patients, to know its importance in differentiating IDA from the group with anemia of chronic disease. Biochemical markers like serum ferritin are reliable parameters to diagnose IDA in an uncomplicated setting. Ferritin is an acute phase response protein, its levels may be high even in iron deficient stores, when associated with inflammation.[24]

LHD% is relatively inexpensive laboratory parameter, it can be performed simultaneously as a part of routine blood counts. It is an indirect marker of iron restricted

erythropoiesis and iron availability in the presence of inflammation and acute phase response.[23]In our study, LHD% is included to correlate it with bone marrow iron stores, which is the "Gold standard" for assessing body iron stores.

Table 1: Haematology Reference Values in Normal Adults[25]

Test	**MEN**	**WOMEN**
Hemoglobin	14-17 g/dl	12.3 -15.3 g/dl
Hematocrit	41.5 – 50.4%	36 – 45%
Red cell count	4.5 – 5.9 x 10^6/µl	4.5 – 5.1 x 10^6/µl
White cell count	4.0 – 11.0 x 10^3/ µl	4.0 – 11.0 x 10^3/ µl
MCV	80 – 96 fl	80 – 96 fl
MCH	27.5 – 33.2 pg	27.5 – 33.2 pg
MCHC	33.4 – 35.5 g/dl	33.4 – 35.5 g/dl
Platelet count	150 -450 x 10^3/ µl	150 -450 x 10^3/ µl
Reticulocyte count	0.5 – 2.5 %	0.5 – 2.5 %

The MCV, MCH, and MCHC reflects average values and may not adequately describe blood samples when mixed populations of red cells are present. In a dimorphic red cell population the indices may be normochromic and normocytic. In such cases, use of RDW and red cell histograms with blood smear examination will detect dimorphic populations.[26]

Peripheral Blood Smears

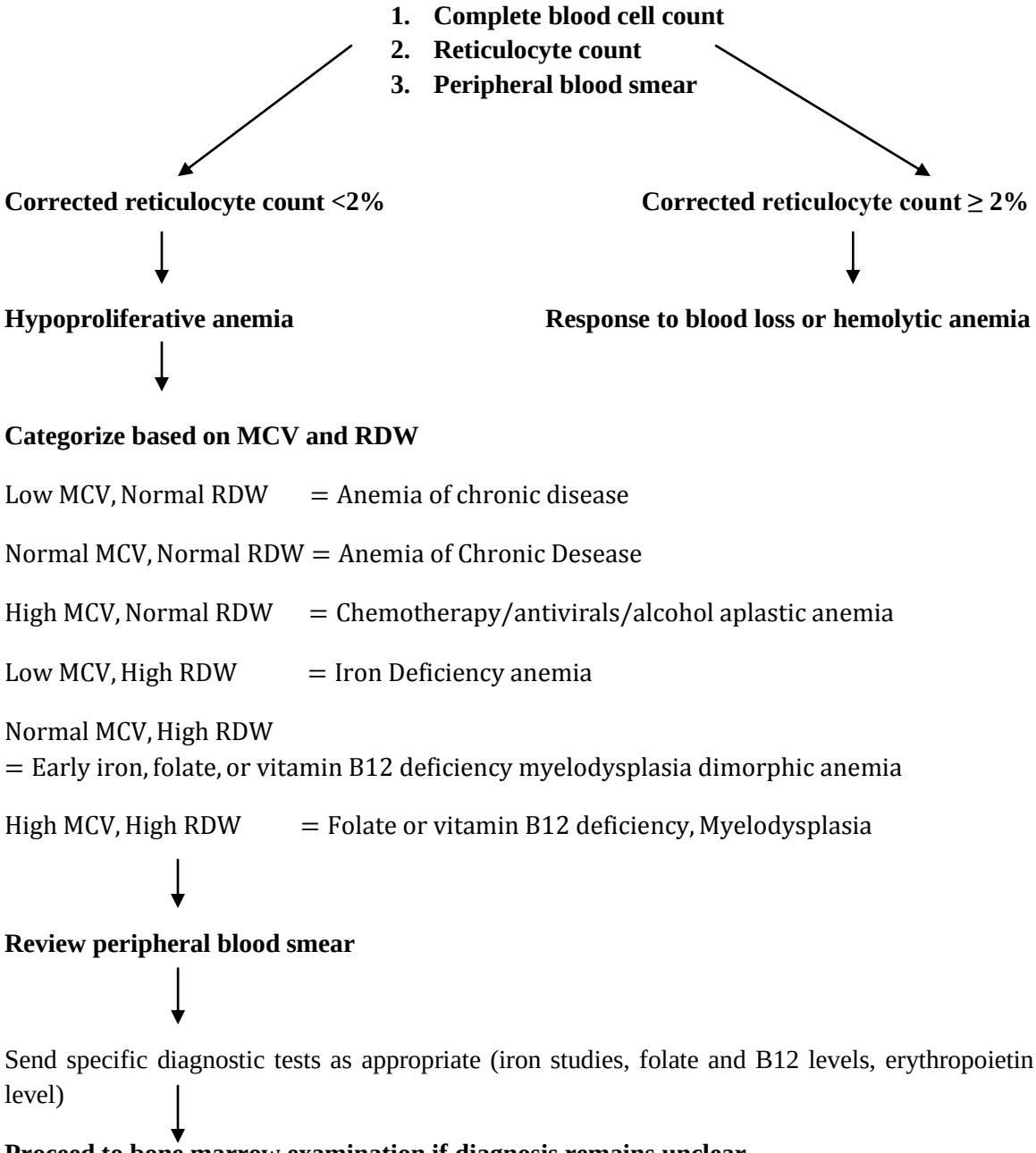
Paul Ehrlich (1854-1915), first person to study blood cell morphology by developing aniline dyes to stain blood films.[9]Blood films are prepared from samples of Ethylene diamine tetra acetic acid(EDTA) anticoagulated blood or non-anticoagulated blood (fingerprick procedure).

The peripheral blood smear examination remains a critical aspect of the diagnostic evaluation for anemia. It confirms the electronically determined classification of RBC size and it may reveal abnormal cell populations too small to affect the erythrocyte indices. For example, as iron deficiency develops, some microcytic cells are produced while MCV is still normal. Furthermore as B12 deficiency progresses, some characteristic oval macrocytes and hypersegmented neutrophils may appear long before the changes in MCV or Hb, especially when features of megaloblastic anemia is masked by presence of iron deficiency anemia.[5]

Dimorphic blood picture is characterized by two distinct populations of red cells, hypochromic microcytic and normochromic macrocytic cells. A Dimorphic blood film can be seen in several conditions.[5] Patients with hemolysis also show many variations in RBC size and shape therefore, reticulocyte count is helpful to differentiate hemolytic anemia from hypoproliferative anemia (nutritional deficiencies).

The classification of anemia is improved by MCV, RDW and reticulocyte count.

Chart 1: Approach to the differential diagnosis of anemia in the adult.[27]



Causes of Dimorphic anemia -

Anemia due to iron and vitamin B12 or folate deficiency.

In acquired sideroblastic anemia, as a feature of myelodysplasia.

Iron deficiency anemia responding to iron therapy.

Following blood transfusion, in patient with hypochromic anemia and sideroblastic anemia.[5]

The dimorphic anemia is particularly characteristic of sideroblastic anemias.[6]

IRON DEFICIENCY ANEMIA

Iron deficiency is the state in which the content of iron in the body is less than normal. It occurs in varying degrees of severity that merge imperceptibly into one another.[28]

William Harvey (1578–1637) postulated the circulation of the blood in 1628. In late 1920s and early 1930s, a distinct hypochromic anemia was described, which corresponds to iron deficiency anemia as we know it today. Hypochromic microcytic anemia was only fully recognized as iron deficiency potentially of dietary origin in the 1930s by Mackay's studies, which finally brought iron deficiency and IDA together. Davies (1931) and Witts (1931), defined the role of iron in hypochromic anemia in adults.[9]

Wintrobe & Beebe (1933) stated that 'there is only presumptive evidence that idiopathic hypochromic anemia develops because an individual is unable to meet the demands for hemoglobin or replace the normal loss of blood on account of defective utilization of blood building materials in the diet'.[9]

The clinical manifestations of iron-deficiency anemia appear to have been recognized in earliest times. A disease characterized by pallor, dyspnea and edema was described in about 1500 B.C. in the Papyrus Ebers, a manual of therapeutics believed to be the oldest complete manuscript. This ancient disease may have been due to chronic blood loss from hookworm infestation.[28]

In 1961, Maxwell Wintrobe, used the term “simple chronic anemia" for the normocytic anemia associated with the majority of infections and chronic systemic diseases. He described anemia associated with inflammation as a common subtype. Wintrobe proposed "profound alterations in iron and porphyrin metabolism" as the likely cause and referred to his own experiments that showed a decrease in erythrocyte survival in only 27 percent, which "could easily be met by increased erythropoiesis if the marrow functional capacity were not impaired”.[29]

MEGALOBLASTIC ANEMIA

Megaloblastic anemia has been recognized as a clinical entity for over a century. The first clinical description of pernicious anemia, which is one of the known causes of Megaloblastic anemia, has been attributed to Thomas Addison in 1849.[30]

In 1876, Sorenson described the presence of macrocyte in the blood in pernicious anemia. In 1880, Paul Erlich published a paper on “Findings in anemia”, named MEGALOBLASTS for the degenerated blood cells present in the group of anemia termed “progressive pernicious anemia”. In 1923, Naegali described hypersegmented neutrophils in peripheral blood film. In 1932, Tempka, Braun and Cooke described giant metamyelocyte and macropolycyte in the bone marrow.[31]

Megaloblastic anemia results from abnormal maturation of hematopoietic cells due to defective DNA synthesis. The megaloblastic anemia is due to the deficiency of cobalamin and/or folic acid. These vitamins are essential for DNA biosynthesis, deficiency of either vitamins results in asynchrony in the maturation of the nucleus and cytoplasm of rapidly regenerating cells. This asynchrony in hematopoietic system results in apoptosis, ineffective erythropoiesis, intramedullary haemolysis, anemia/pancytopenia and typical morphological abnormalities in the blood and marrow cells.[4,32,33]This ineffective erythropoiesis is accompanied by intramedullary hemolysis causing an elevated Lactate Dehydrogenase and indirect bilirubin in the serum.[4]

Megaloblastic anemia leads to substantial morbidity if unrecognized or misdiagnosed. Its etiology is multifactorial and may result from dietary deficiency, impaired absorption and transport or impaired utilization of these vitamins in DNA synthesis.

Megaloblastic anemia must be an important differential diagnosis in patients presenting with dimorphic anemia with leucopenia and thrombocytopenia. A Study done on 175 patients of pancytopenia found that 62% of them had Megaloblastic anemia. A Study on nutritional megaloblastic anemia reported from India, showed 78% were B12 deficient and 43.8% had pancytopenia.[34]

In developing countries like India, most cases of Megaloblastic anemia are due to nutritional deficiency of these micronutrients. It is hypothesized that bulk of the B12 is derived from bacterial contamination of food and water. Intrinsic factor available in small intestine absorbs B12 which is produced endogenously and contributes to individual's daily B12 requirement. They postulated that this may be a "complication of migration to

a more sanitized environment" resulting in diminished bacterial contamination of small intestine, leading to reduced endogenously available B12.[35]

Clinical features

Patient may be asymptomatic or present with pallor and generalized weakness. In severe anemia, patients may have dyspnea, tachycardia & cardio-pulmonary distress.

Patients may have icterus, caused by accumulation of unconjugated bilirubin due to ineffective erythropoiesis.[4,32] Bleeding manifestations occur when thrombocytopenia is present.[36] Fever is seen when there is associated neutropenia. The lining epithelium of the gastrointestinal tract becomes atrophic in megaloblastosis. A vicious cycle of megaloblastosis leading to atrophy of mucosa, and subsequent malabsorption of the two vitamins, worsens megaloblastic anemia.[34]

Cobalamin deficiency may result in neurological deficits.[4,37]

Diagnosis

The anemia is macrocytic with increased MCV and is characterized by macro-ovalocytes. Vitamin B12 and folate deficiency complicated with iron deficiency can eliminate macrocytosis, resulting in dimorphic population of red cells, further confusing the diagnostic process. Basophilic stippling, Howell jolly bodies, nucleated red cells may be seen.

Leucopenia and thrombocytopenia is usually encountered. Hypersegmented neutrophils are the first to be seen, even before the changes in RBC indices and may be the only finding in cases with concurrent iron deficiency.

The bone marrow is hypercellular. The myeloid, erythroid ratio is normal or reduced, with erythroid hyperplasia. Megaloblasts and mild dyserythropoiesis are seen.

Early erythroid cells are over-represented in comparison with mature cells. Macrophages are increased. Granulopoiesis is also hyperplastic, showing giant metamyelocytes. Megakaryocyte may be normal or decreased and are hyperlobated.

Serum cobalamin and folate assay confirms the diagnosis and establishes the nature of deficiency. Up to 5% of patients with megaloblastic anemia may have vitamin B12 levels within reference range. Serum folate levels are usually low, may be high in patients with vitamin B12 deficiency, and normal with a recent diet rich in folates.[38]

The fact that vitamin B12 or folate deficiency may exist without any obvious hematological abnormalities has been known for several years.

MASKED MEGALOBLASTOSIS

The term "masked megaloblastosis" is reserved for conditions in which true cobalamin or folate deficiency with anemia is not accompanied by classic findings of megaloblastic anemia in the peripheral blood and bone marrow. This occurs in the presence of coexisting condition that neutralizes the tendency to generate megaloblastic cells (usually involving reduction in RBC hemoglobinization, as in iron deficiency).[4] Incomplete expression of megaloblastic maturation may be due to the combination of a reduced stimulus to erythropoiesis with impaired iron delivery.[39] A wide RDW in the presence of normal MCH or MCV may reflect either megaloblastic anemia or dimorphic anemia (macro-ovalocytes with microcytic hypochromic RBC).[4] Normal MCV can be seen in megaloblastic anemia, particularly in cases with coexistent iron deficiency.[40] Presence of giant metamyelocytes and hypersegmented neutrophils are pathognomonic, as the myeloid series are unaffected by deficient hemoglobinization.[39]

Thus, the recognition of masked megaloblastosis should initiate investigations to rule out iron deficiency, anemia of chronic disease or hemoglobinopathies. Maximal therapeutic benefit is observed by appropriate replacement with cobalamin or folate. Conversely, if treated with iron alone, megaloblastosis will be unmasked.[39]

MYELODYSPLASTIC SYNDROME (MDS)

MDS is a relatively uncommon cause of anemia, but is a more common cause in the elderly than in younger patients. This should be a diagnostic consideration when cytopenias range from an isolated anemia for 10 years progress to a rapidly evolving acute leukemia fatal within weeks.

MDS refers to a clonal disorder of the hematopoietic stem cell interrelated with other clonal bone marrow disorders such as the acute leukemias and the myeloproliferative syndromes. The dyspoiesis resulted from clonal expansion of a multipotent stem cell leads to impaired differentiation, resulting in cytopenia. The underlying clonal expansion of MDS was first suggested by Dacie, who noted a dimorphic population of red cells consistent with a clonal disorder. [41]

MDS transforms to acute myeloid leukemia (AML) in approximately 30% of cases. Recognition and enumeration of blast cells is of critical importance both in the diagnosis of AML and MDS.

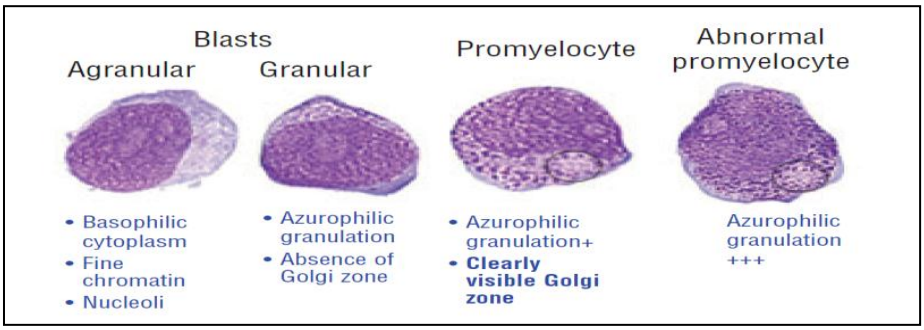


Figure 1: Blasts, promyelocytes and abnormal promyelocytes.[42]

Mahmoud et al reported that of 124 patients 75 years of age or older with macrocytic anemia, a definitive cause was ascertained by non-invasive techniques in 60% of the cases. Of the remaining 49 patients, all underwent a bone marrow procedure, but diagnosis of MDS could be made only in 6, and remaining 43 patients were suggested that they might have "MDS in evolution".[43]

The pathogenesis of MDS appears complex, may be due to years of environmental exposures resulting in genetic events. Low risk MDS groups appear to be predominantly pro-apoptotic. In high risk groups, there will be a shift in the equilibrium from predominant apoptosis towards unobstructed proliferation and leukemic transformation following genomic instability, subsequent karyotypic evolution and additional molecular mutations. Altered methylation patterns, causing genomic instability result in disease progression, increased cytogenetic abnormalities, leukemic transformation with increased blasts in one third of patients, and thereby, poor prognosis.[44]

Morphological features of MDS[41,44]

Peripheral blood: Dimorphic red cell population with anisopoikilocytosis and nucleated red blood cells, hyposegmention and hypogranulation of neutrophils.

Bone Marrow:

Dyserythropoiesis: Increased erythroblasts with megaloblastoid changes showing multinuclearity and nuclear fragments. Cytoplasmic abnormalities and ring sideroblasts are seen.

Dysgranulopoiesis: Nuclear abnormalities like hypolobulation, nuclear sticks and ring-shaped nuclei with hypogranulation

Dysmegakaryopoiesis: Micromegakaryocytes, large mononuclear forms, multiple small nuclei and reduced in number.

Dysplasia is the pathologic hallmark of the disease.[45]

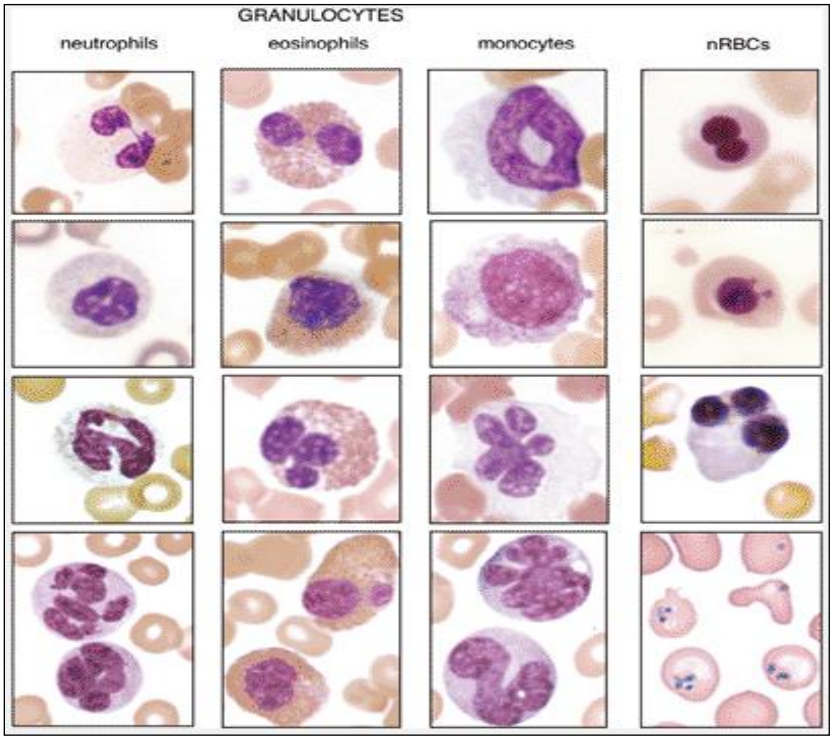


Figure 2: Dysplastic nuclear features in circulating cells. The right lower figure shows numerous Pappenheimer bodies.[46]

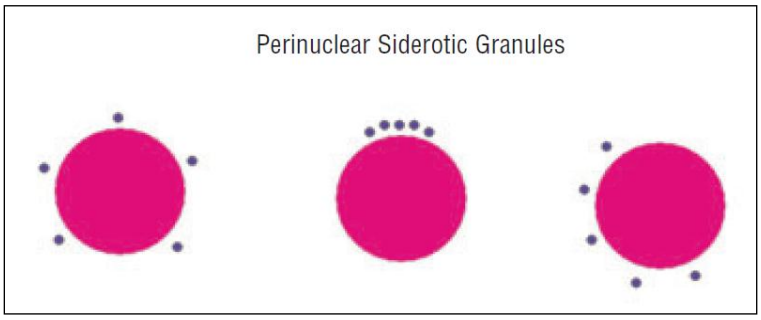


Figure 3: Siderotic granules in erythroblasts[42]

Ring sideroblasts: The prognosis of patients with pure sideroblastic anemia differs from that of patients with non-sideroblastic anemia.

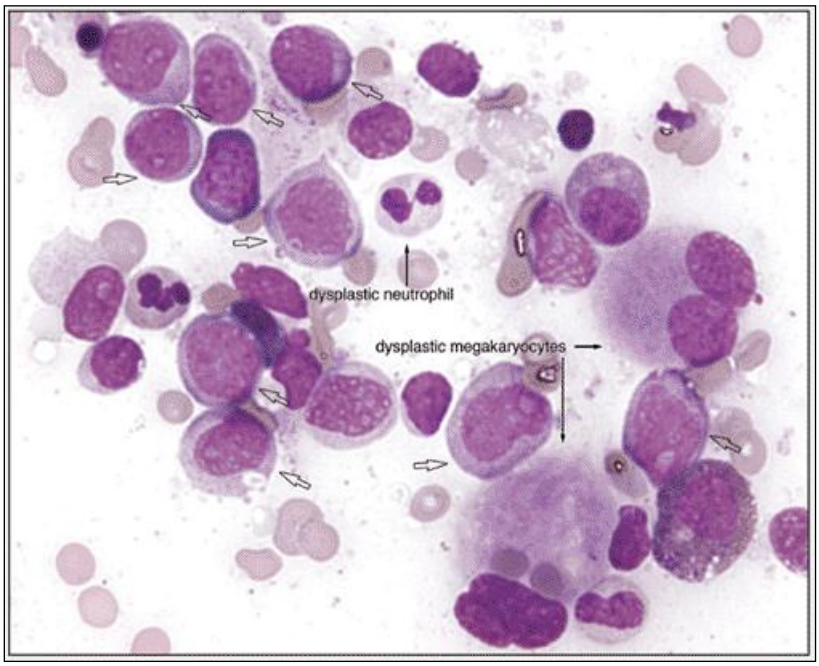


Figure 4: Aspirate smear of Refractory anemia with excess blasts, type 2 (RAEB-2) showing dysplastic small megakaryocytes (micromegakaryocytes), a dysplastic hypogranular/hypolobulated neutrophil, and increased numbers of blasts (open arrows).[46]

Cytogenetic abnormalities are only found in 40-60 % of cases of MDS, and even less frequent in low-grade lesions and 95% of therapy related MDS.[47]

Minimal diagnostic criteria for patients with myelodysplastic syndromes, as recommended by the International Working Conference (2007).[48]

A. Prerequisite criteria (both 1 and 2 required)

1. Constant cytopenia in one or more of the following cell lineages:

- Erythroid (hemoglobin <11 g/dL)
- Neutrophilic (absolute neutrophil count <1.5 X 10^9/L) or
- Megakaryocytic (platelets <100 X 10^9/L)

2. Exclusion of all other hematopoietic or non-hematopoietic disorders as the primary

reason for cytopenia /dysplasia

B. MDS-related decisive criteria (at least one required)

- Dysplasia in ≥ 10% of all cells in at least one of the following lineages in the BM smear: Erythroid, neutrophilic, or megakaryocytic, or >15% ringed sideroblasts (iron stain)
- 5%–19% Blast cells in the BM or PB
- Typical chromosomal abnormality (by conventional karyotyping or fluorescence in situ hybridization [FISH])

C. Co-criteria (for patients fulfilling "A" but not any of the "B" criteria above and those who otherwise show typical clinical features [e.g., transfusion-dependent macrocytic anemia]) (atleast one required)

- Abnormal phenotype of BM cells clearly indicative of a monoclonal population of erythroid and/or myeloid cells, determined by flow cytometry.
- Clear molecular signs of a monoclonal cell population on X-inactivation assay, gene chip profiling, or point mutation analysis (e.g., RAS mutations)
- Markedly and persistently reduced colony formation ± cluster formation of BM and/or circulating progenitor cells by colony-forming unit assay.

In patients suspected to have MDS clinically, with unexplained cytopenias and absent morphological findings, it is best to monitor these patients with non-invasive procedures, genetic and immunophenotypic analysis. But, diagnosis of MDS is avoided till the morphological criteria is satisfied.

There are various classification systems for MDS, like FAB, IPSS and WHO. WHO classification is most commonly followed system for classification of MDS

Table 2 : WHO classification of MDS[46]

Disease	Peripheral Blood	Bone Marrow
Refractory Anemia (RA)	Unicytopenia or bicytopenia Rare blasts	Erythroid dysplasia only < 5% blasts < 15% ring sideroblasts
Refractory Anemia with Ring Sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia ≥ 15% ringed sideroblasts < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia No or rare blasts No Auer rods $< 1 \times 10^9$/L monocytes	Dysplasia >10% of cells of ≥ 2 myeloid cell lines < 5% blasts in marrow > 15% ringed sideroblasts
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenias < 5% blasts	Unilineage or multilineage dysplasia 5% to 9% blasts No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenias 5% to 19% blasts	Unilineage or multilineage dysplasia 10% to 19% blasts ±Auer rods
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenia only No blasts	Unilineage dysplasia in granulocytes or megakaryocytes , < 5% blasts, cytogenetic abnormality +
MDS associated with isolated del(5q)	Anemia < 5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobated nuclei < 5% blasts No Auer rods, Isolated del(5q)

The WHO 2008 classification recommends using a hemoglobin level of <10 g/dl and an absolute neutrophil count level of $<1.8 \times 10^9$ /L.

The diagnosis of MDS is a "diagnosis of exclusion". After excluding:

Vitamin B12 and/or folate deficiency

Proven exposure to heavy metals or recent cytotoxic therapy

Ongoing inflammation including HIV and Cancer

Chronic liver disease /alcohol use.[41,49]

In certain cases, MDS diagnosis can be made without clear evidence of morphologic dysplasia, such as chronic myelomonocytic leukemia (CMML), if monocytes are persistently elevated or if a recurrent cytogenetic abnormality is detected in patients with sustained unexplained cytopenia (''presumptive MDS'' by WHO 2008 criteria).[50]

Poor technical quality of the specimen is a common obstacle in the accurate diagnosis of MDS.

Table 3: International prognostic scoring system for MDS.[50]

	Score Value				
Prognostic Indicator	0	0.5	1	1.5	2
Bone Marrow Blasts (%)	<5	5–10	—	11–20	21–30
Karyotype[a]	Good	Intermediate	Poor	—	—
Cytopenias[b]	0–1	2–3	—	—	—

	IPSS			
	Low	Intermediate 1	Intermediate 2	High
Score	0	0.5–1	1.5–2	>2.5
Percent MDS	33	38	22	7
Median Survival (Months)[c]	5.7	3.5	1.1	0.4
Progression to AML (25%)[c]	9.4	3.3	1.1	0.2

[a] Karyotype: Good→ Normal (46XX or XY); -Y; del(5q); del(20) [*alone*] Intermediate→*All* that is not Poor or Good (*Other*) Poor→ Complex (>3); Chromosome 7 anomalies.
[b] Cytopenia: ANC <1800; Hemoglobin <10; Platelets <100.
[c] Without therapy.

ANATOMY OF BONE MARROW

Adult marrow weighs about 1300 to 1500 gms. It produces about 6 billion cells per kilogram of body weight per day. The marrow takes only few hours to few days to undergo complete transformation. This rapid transformation involving the whole organ is evidenced by the fact that a small biopsy or aspirate sample is representative of the whole marrow.[51]

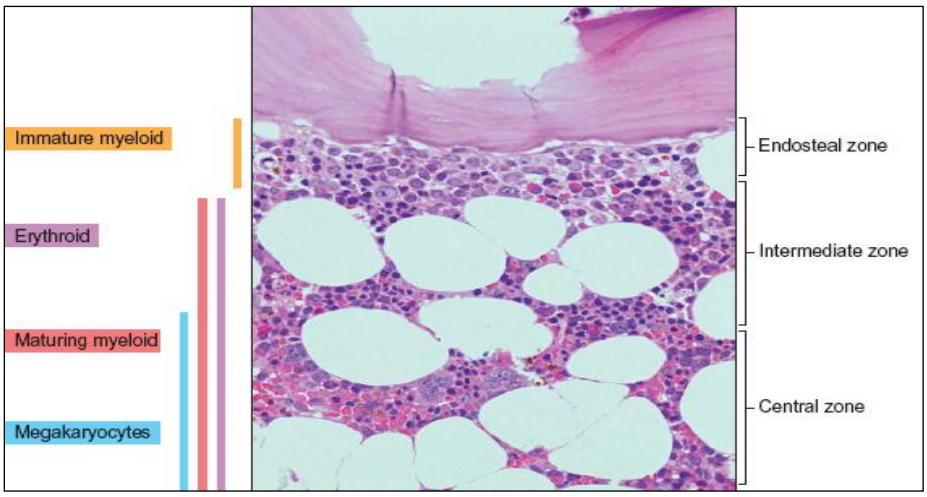


Figure 5: Histology of bone marrow[51]

ORIGIN AND DIFFERENTIATION OF HAEMOPOIETIC CELLS.

Hematopoiesis takes place in the inter sinus spaces in a controlled way by a complex array of stimulatory and inhibitory cytokines. The medullary stroma – endothelium and reticular cells are the sources of these cytokines.[51]

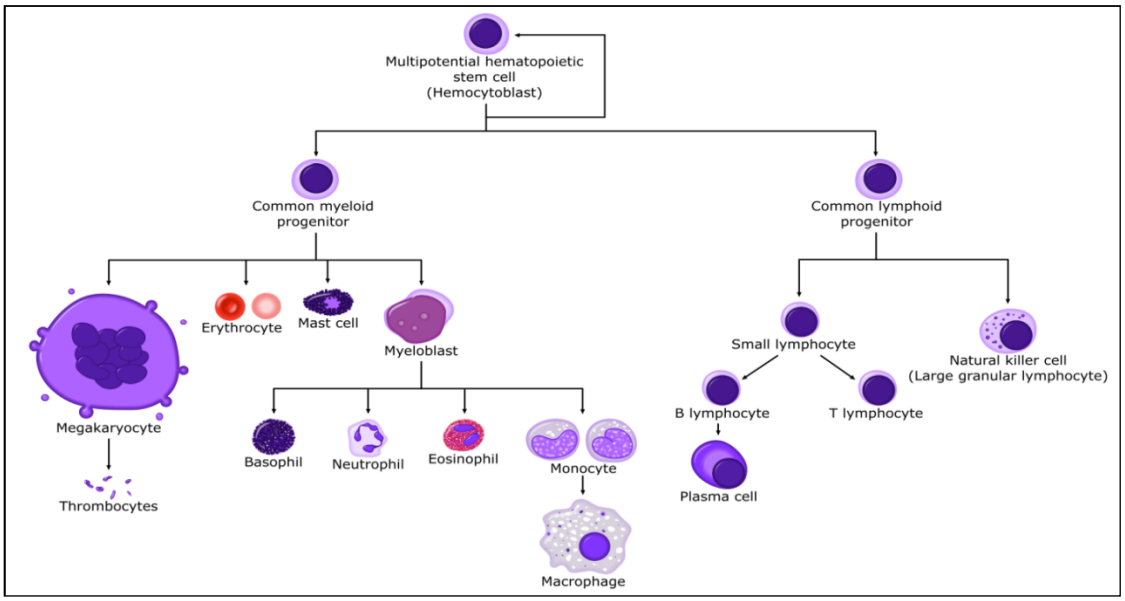


Figure 6: Origin and differentiation of haematopoietic cells[51]

CELLULARITY OF THE MARROW

The marrow cellularity is expressed as the ratio of the volume of haemopoietic cells to the total volume of the marrow space (cells plus fat and other stromal elements). Cellularity varies with the age of the subject and the site.

The most reliable assessment of cellularity is based on biopsy specimen. An estimate of percentage of marrow space occupied by haemopoietic elements plus stroma is the parameter to assess cellularity. Erythroid cellularity and adequacy of megakaryocyte are evident at low power. The granulocytic series is identifiable at all developmental stages.

The myeloid/erythroid ratio is the ratio of total granulocytes to total normoblasts. In adults, this ratio varies from 2:1 to 4:1

Table 4: Normal ranges for differential counts[52]

	95% range
Myeloblasts	0-3
Promyelocytes	3-12
Myelocytes(neutrophil)	2-13
Metamyelocytes	2-6
Neutrophils	22-46
Myelocytes(eosinophil)	0-3
Eosinophils	0.3-4
Basophils	0-0.5
Lymphocytes	5-20
Monocytes	0-3
Plasma cells	0-3.5
Erythroblasts	5-35
Mega karyocytes	0-2
Macrophages	0-2

METHODOLOGY

PART II-RESEARCH METHODOLOGY

RESEARCH METHODOLOGY:

Source of Data:

Patients with dimorphic anemia admitted at R.L.Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

Clinical data was obtained in each case.

Ethical clearance was obtained for the study from the institutional ethical board.

Method of Collection of Data:

A. Study Setting:

This study was carried out at Department of Pathology, R.L.Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

B. Study Design:

Cross sectional study

C. Study Duration:

The study was conducted from December 2011 to August 2013

D. Study Population and Sample Size:

Of 130 cases with dimorphic anemia admitted at R.L.Jalappa Hospital & Research Centre during the study period, 62 patients fulfilling the inclusion and exclusion criteria were studied.

Inclusion criteria:

Patients above the age of 20 years with

Hemoglobin <12 gm/dl (for females)

Hemoglobin <13 gm/dl (for males)

MCV- normal or high

RDW >15

Peripheral smear showing dimorphic blood picture or RBC histogram showing two peaks.

Patients consented to undergo bone marrow aspiration.

Exclusion criteria:

Patients with increased reticulocyte count

Pregnant women.

Patients who had received blood transfusions.

Sample size: 62

E. study procedure:

Collection of blood samples:

2ml of venous blood sample was drawn by venipuncture (antecubital vein) under aseptic precautions and was collected in EDTA vacutainers.

3 ml of fasting venous blood sample was collected in plain tube, without anticoagulant.

Analysis of the blood samples:

STEP 1: The EDTA anticoagulated samples were analyzed in an automated cell counter (Beckman Coulter AC T diff 2). The following parameters were obtained:

Total WBC count

RBC Count

Hemoglobin

MCV

MCH

MCHC

RDW

Platelet count.

The WHO cut off value for hemoglobin to diagnose anemia among women was 12 gm/dl and men was 13 gm/dl.[12]

LHD% (Low Hemoglobin density) was calculated by mathematical transformation of the MCHC value, using the formula used in Beckman coulter instruments.

$$\text{LHD\%} = 100^{*}\sqrt{1-\left(\frac{1}{(1+e^{1.8(30-\text{MCHC})})}\right)}.$$

LHD% is a variable for assessment of hypochromia.[54] It is an indirect marker of iron restricted erythropoiesis and iron availability in the clinical settings influenced by inflammation and acute phase response.LHD% can be used in diagnosis and monitoring the response to therapy in a reasonable manner.[23,53,54]

Optimal cut off point is 10.4 %. LHD% > 10.4% was considered as increased, and implies iron deficiency.[53]

STEP 2: Peripheral smear examination:

Peripheral smears were prepared from samples of patients fulfilling inclusion criteria. Blood smears are prepared by the wedge method. A drop of blood is placed in the middle of the slide approximately 1 to 2 cm from one end. A spreader slide is placed at a 30- to 45-degree angle and moved backward to make contact with the blood drop. Then the spreader slide is moved rapidly forward, to obtain a film of 3 to 4 cm long. These air-dried blood smears were stained with Leishman's stain, modified Romanowsky stain.

Procedure: The smear is covered with 10-15 drops of Leishman's stain. After two minutes, double the volume of buffer solution is added. After 10 minutes, the smear is washed by flooding the slide with tap water and the slide is dried by allowing to stand vertically in a rack.

The Leishman's stain formulation contains methylene blue, sodium carbonate, eosin and acetone free methanol. Buffer used is a mixture of disodium hydrogen phosphate and potassium dihydrogen phosphate. Methyl azures are basic dyes that impart a violet-blue coloration on binding to the acidic components of the cell, such as nucleic acids and proteins. The eosin reacts with the basic cellular elements imparting a reddish hue to cytoplasmic components and hemoglobin. A properly stained slide has a pink tint. The red cells will have salmon pink coloration and leukocytes have purplish-blue nuclei.

Smears were studied under oil immersion lens of light microscope to look for RBC morphology for any variations in size, shape, hemoglobin distribution and presence of cellular inclusions.

WBC number and morphology like nuclear lobes, cytoplasmic granules were evaluated.

Platelet number and morphology were evaluated.

Smears were reported after analyzing the hematological parameters and RBC morphology. Samples reported as dimorphic anemia were considered for further tests.

STEP 3: Reticulocyte stain was done on samples reported as dimorphic anemia.

Two drops of 1% brilliant cresyl blue stain and two drops of well mixed blood specimen was transferred into a small test tube with the help of Pasteur pipette. Test tube is left undisturbed for 15 minutes in the incubator at 37ºC. After 15 mins, one small drop of the

mixture was transferred to a clean, grease-free glass slide. A thin smear was prepared with the help of spreader slide. Smear was air dried.

The smear was examined under low power objective to select a thin portion of the smear where red cells are evenly distributed. Reticulocytes were identified by the fine, deep violet filaments arranged in a network and fine dot like structure.10 consecutive fields or reticulocytes per 1000 red cells were counted and the percentage was calculated.

Normal value: 0.5 -2.5%

Patients with increased reticulocyte count were excluded from the study.

STEP 4: Bone marrow aspiration was performed.

Bone marrow aspiration was performed using Jamshidi needle after obtaining informed consent from patient or patient's relative for the procedure.

Site: Posterior iliac spine, with patient in the lateral position.

Procedure: Aspiration site was prepared and cleaned with antiseptic solution. Skin and the area down to the periosteum were infiltrated with 2ml of 2% lignocaine.

The bone marrow aspiration needle with the stylet was introduced into the site by gentle screwing motion, after adjusting the guard to appropriate length. The outer plate of bone was pierced with a gentle boring motion. As the marrow cavity was entered, sensation of 'giving in' was experienced. Then, the stylet was removed and marrow material was aspirated using 10 ml disposable syringe, using a suction of 0.5 ml.

The aspirate was transferred to a petri dish containing 3.8% Sodium citrate anticoagulant. The needle was withdrawn and puncture site was sealed with tincture benzoin swab. Patient was advised to lie in supine position for 2 hours.

The aspirate material from the anticoagulant solution is transferred to a set of slides and was spread on the glass slides to obtain cell trails. These smears were air dried completely and stained with Giemsa stain.

Smears were examined for-

Cellularity: Cellularity was determined under low power, based on percentage of haematopoietic cells in marrow particles, this is affected by the age of patient.

Normocellular / Hypocellular / Hypercellular

200 cell differential was performed under High power, using categories erythroid, myeloid, lymphoid and plasma cells.

M: E ratio was obtained from 200 cell differential count. It is the ratio of neutrophil and neutrophil precursor cells to erythroid precursors, it varies from 2:1 to 4:1

Erythropoiesis: Erythroid hyperplasia was noted. All stages of maturation were identified. Maturation pattern – Normoblastic/ micronormoblastic/ megaloblastic maturation, any maturation abnormalities and dyserythropoiesis was noted.

Myelopoiesis: All stages of maturation were identified. Any maturation defect- like increased blast percentage and morphological abnormalities like giant metamyelocytes and dysplastic changes were recorded.

Megakaryopoiesis: Number of megakaryocytes/ low power field, immature forms and dysplastic changes were noted.

Others – Plasma cells, lymphocytes, mast cells – differential and dysplastic changes and Parasites were noted.

STEP 5: Perls' staining was done on bone marrow aspirates.

Smears were fixed in methanol and were treated with freshly prepared solution of equal parts of

2% Aqueous potassium ferrocyanide

2% Hydrochloric acid (Hcl) - for 30 minutes.

Washed with distilled water for 5 minutes

Counter stain with 1% neutral red for 30 seconds.

Washed with water.

Smears were dried and mounted.

Assessment of iron stores in bone marrow was performed using Perls' Prussian blue reaction, which is considered as "Gold standard" test. But, mere presence of stainable iron doesn't define the quantity resolubilized and incorporated iron into the developing erythron. For this reason, along with routine Gale's method of grading system, intensive grading system was used to differentiate iron deficiency anemia from functional iron deficiency.

Smears were first assessed according to Gale's Histological Grading Method. Smears with at least seven fragments were assessed.

0 – No iron granules seen.

1 – Small granules in reticulum cells only, under oil immersion.

2 – Few small granules visible, under low power.

3 – Numerous small granules in all marrow particles.

4 – Large granules in small clumps

5 – Dense large clumps of granules

6 – Very large deposits obscuring the marrow cells.

Smears were interpretated as deficient, normal and increased iron stores based on the above mentioned grade as follows -

Grade 0 – Iron deficiency

Grade 1 – Diminished iron stores.

Grade 2 & 3 – Normal iron stores.

Grade 4 to 6 – Increased iron stores.

Perls' stained smears were also looked for ring sideroblasts, in sideroblastic anemia.

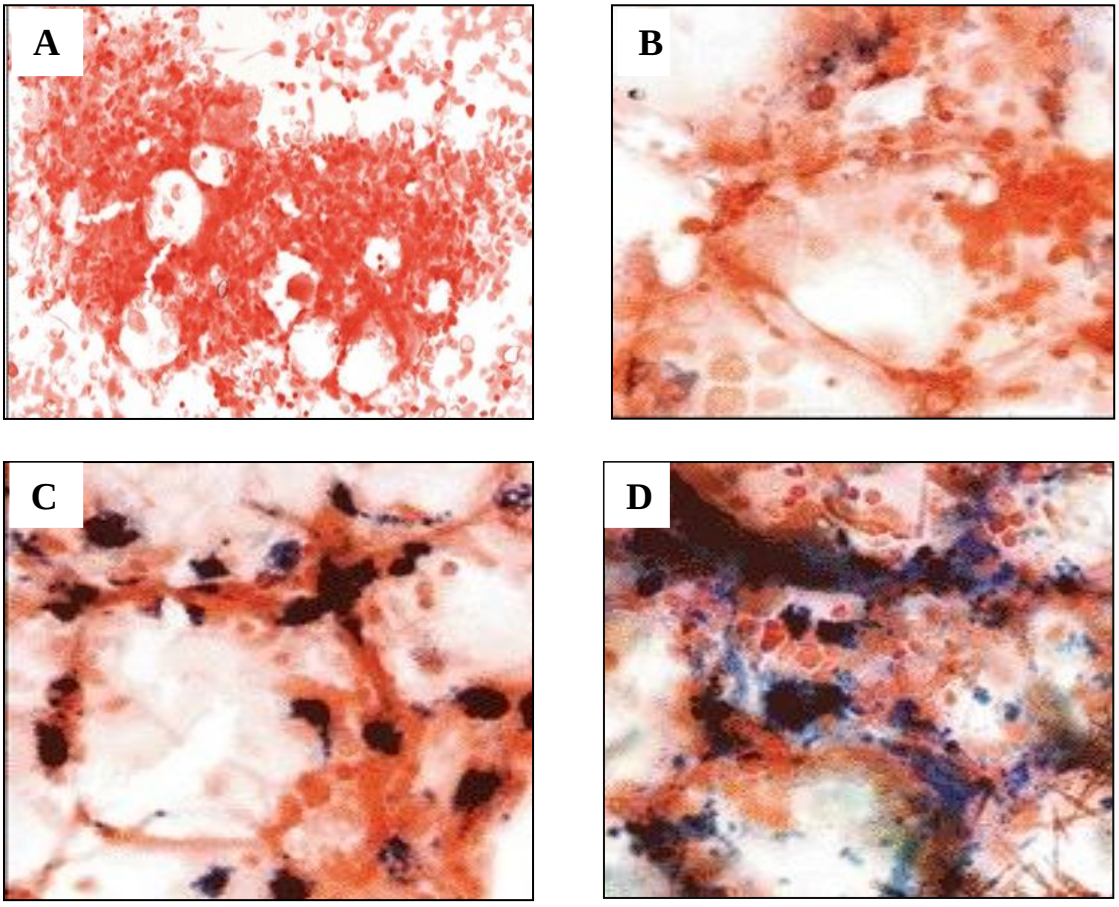


Figure 7 : Bone marrow aspirate smears stained with Prussian blue for iron stores. Higher magnification views. A. Absent iron stores with no visible blue staining, B. Normal, C. Moderately increased and D. Severely increased.[55]

Perls' stained smears were also assessed using **Intensive histological grading method.**[56] Fragments, macrophages and erythroblasts were looked for iron particles. Iron particles in the fragments are considered positive only when grade ≥ 2, according to Gale's grading system.

Erythroblasts were considered positive, when more than 30% erythroblasts are showing iron particles. Positive macrophage iron, when iron is present in reticular cells.

Results were interpretated as normal iron stores, functional iron deficiency, iron stores deficiency and both functional and iron stores deficiency, as described below

Table 5: Iron status category by Intensive grading method.[56]

Fragment	Macrophage	Erythroblast	Iron status category
Present	Present	Present	Normal
Present	-	Present	
Present	Present	-	Functional iron deficiency
Present	-	-	
-	Present	Present	Iron stores deficiency
-	-	Present	
-	Present	-	Functional and iron stores deficiency
-	-	-	

STEP 6: Serum Vitamin B12 and folic acid assays

3 ml of fasting venous blood of selected patients was collected in plain tube. The sample was centrifuged and the serum was separated.

Serum Vitamin B12 and folate assays were performed by fully automated chemiluminescent immunoassay.

Serum folate, in this study, serum folate level was as per Axysm folate test kit package insert, where reference range was > 5.38 ng/ml

Serum vitamin B12, in this study, serum B12 level will be interpreted as per Axysm B12 test kit package insert where reference range was 211 – 911 pg/ml.

Kit validation reference is Chen IW, Sperling MI, Heminger IA. Vitamin B12. In: Pesce AJ, Kaplan LA, editors. Methods in clinical chemistry. St. Louis:CV Mosby, 1987. p569-73.

STEP 7: CYTOGENETICS

Conventional Cytogenetics was performed on suspected cases of MDS.

1 – 1.5 ml of venous blood sample was in a heparin vacutainer (green cap) and mixed gently. Vigorous shaking of the vacutainer is avoided, to prevent blood lysis.

Peripheral blood lymphocyte culture

Day 1

8 – 10 drops of whole blood was added to a sterile centrifuge tube containing 8 ml of sterile RPMI – 1640 chromosome medium [Gibco, BRL] supplemented with 20% fetal bovine serum, antibiotics.

300µl of Phytohemagglutinin (PHA) – M form was added, gently agitated and closed with the stopper tightly.

The cultures were incubated for 70 hrs at 37º C. The culture vials were gently agitated at an interval of 24 hrs to avoid the lymphocytes from clumping and settling down at the bottom.

Day 4

Harvesting of the cultures

After 68 hrs of incubation, 50 µl of colcemid (10µg/ml) was added to the cultures. This was done to arrest the cell division at metaphase stage. Again incubated for additional 60 minutes. Centrifuged for 3 – 5 mins at 1500 rpm. 0.5 ml of supernatant was discarded. The cells were subjected to hypotonic treatment by adding 5 ml of 0.075 M KCl with constant agitation on a vortex mixer.

The culture was incubated for 8 minutes at 37º C and centrifuged at 1500 rpm for 3-5 minutes.

The supernatant was discarded and freshly prepared fixative (Methanol: Acetic acid in the ratio 3:1) was added drop by drop with constant agitation on a vortex mixer.

The contents were centrifuged and the supernatant was discarded. The procedure was repeated twice by adding fresh fixative.

The culture setup was transferred to Falcon tube and centrifuged at 2000 rpm for 5 minutes.

Preparation of slides:

The supernatant was almost removed completely without disturbing the cell pellet. The cells were re-suspended gently in 0.5-2 ml of freshly prepared fixative. The final concentration of cell suspension was adjusted visually after determining the cell concentration by preparing a test slide.

Slides pre – cleaned with ethyl alcohol were used. A layer of fixative was run on the dry slides with a Pasteur pipette and a drop of the cell suspension was placed on the wet slide from a height of 4-6 inches. Slides were air dried

The slides were incubated at 65º C overnight before doing G – banding (GTG – banding).

GTG – banding using trypsin and Giemsa stain

Preparation

Coplin jar 1: Trypsin working solution:

50 ml of normal saline + 5 mg of trypsin powder (Sigma Aldrich co). Mixed thoroughly and allowed for 5-10 minutes at room temperature before commencing the banding procedure.

Coplin jar 2: 50 ml of normal saline.

Coplin jar 3: 42 ml of distilled water + 2.5 ml each of Na_2HPO_4 and NaH_2PO_4 buffer solutions + 3 ml of water - soluble Giemsa stain solution. Mixed well and the top layer was removed using a filter paper.

Coplin jar 4: 50 ml of distilled water.

Slides incubated at 65º C were dipped in trypsin working solution (Coplin jar 1) for about 20-35 seconds. Rinsed in normal saline (Coplin jar 2). Stained in buffered Giemsa (Coplin jar 3) for 10 minutes. Again rinsed in distilled water (Coplin jar 4). Slides are air dried. The banded slides were mounted with coverslips using DPX mounting solution and observed after they were dried.

Individual chromosomes were recognized by their size and their banding pattern following staining (Giemsa staining [G-banding])

The results of cytogenetic analysis are displayed visually according to standard conventions.

STATISTICAL ANALYSIS

Percentage of each cause was calculated.

The mean and standard deviation were used for statistical analysis. ANOVA test was used to test the variation of mean of hematological parameters in various groups and its significance.

Fisher's exact test was used to determine association between independent and dependent categorical variable. p value of < 0.05 was considered statistically significant.

RESULTS

RESULTS

A total of 62 cases of dimorphic anemia were studied during the study period, conducted at R.L.Jalappa Hospital attached to Sri Devaraj Urs Medical College.

Age Distribution:

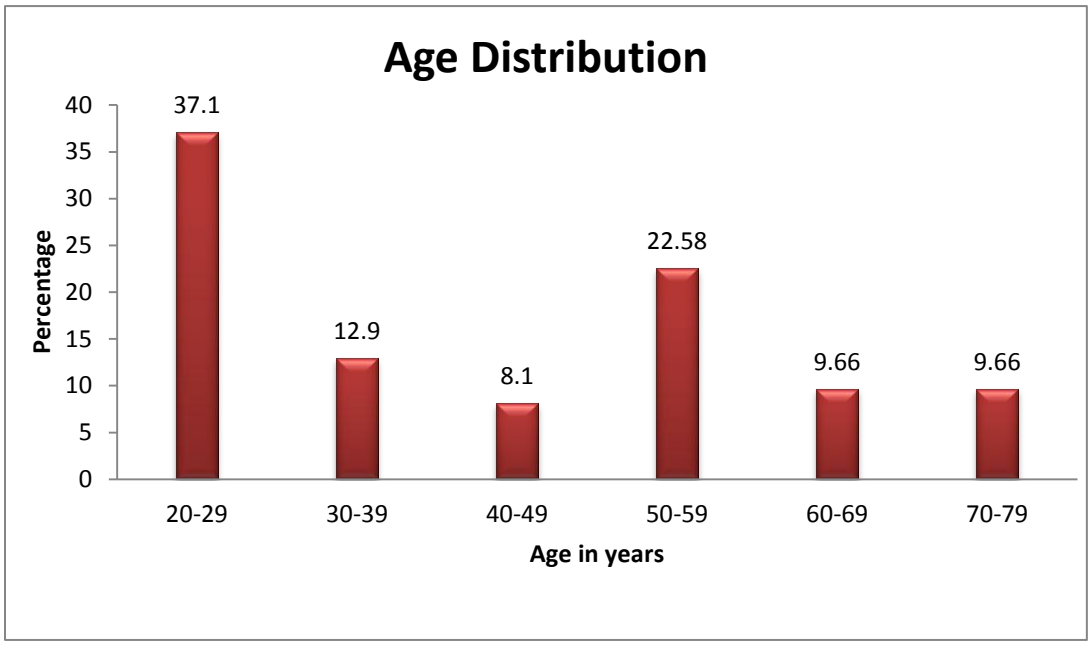
The age group of the cases in our study ranged from 20 to 75 years with mean age of 40.95±17.61 years.

Table 6: Age distribution of the cases

Age (years)	No. of cases	Percentage (%)
20-29	23	37.1
30-39	8	12.9
40-49	5	8.1
50-59	14	22.58
60-69	6	9.66
70-79	6	9.66
Total	**62**	**100**

Majority of patients belonged to 20 – 29 years constituting 23 (37.1%) cases, followed by 50 – 59 years constituting 14 (22.58%) cases and 8 (12.9%) cases belonged to 30 – 39 years.

Chart 2: Age Distribution



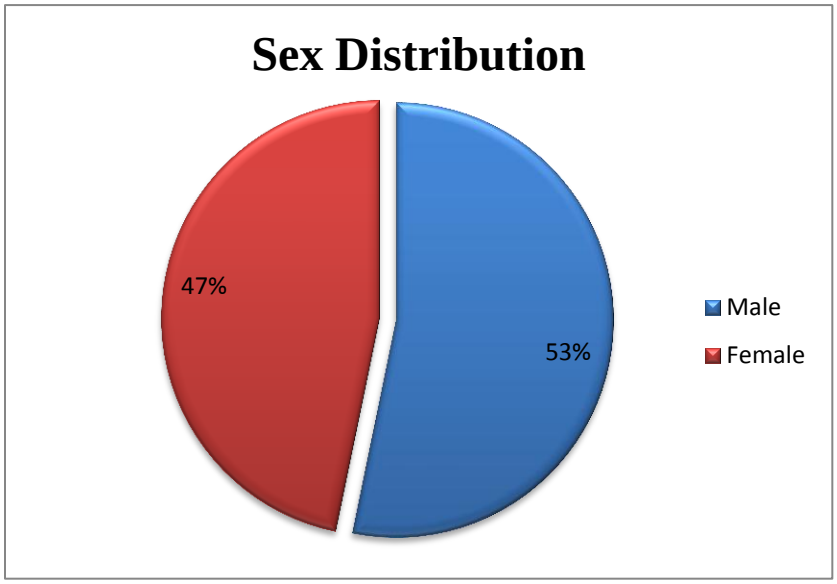
Sex Distribution:

33(53.2%) were male and 29(46.8%) were female. Male: Female ratio was 1.1

Table 7: Gender Distribution

Gender	**No. of cases**	**Percentage (%)**
Male	33	53.23
Female	29	46.77
Total	62	100

Chart 3: Gender Distribution



Age and Gender Distribution

Table 8: Age and Gender Distribution

Age (years)	Female	Male	Total
20-29	13	10	23
30-39	2	6	8
40-49	2	3	5
50-59	7	7	14
60-69	4	2	6
70-79	1	5	6
Total	29	33	62

The age distribution in females ranged from 20 to 70 years and in males ranged from 20 to 75 years. The mean age in female was 39.51±16.91 and mean age in male was

42.21±18.36. Majority of cases were seen distributed in second decade in males and females.

Clinical Manifestations:

Table 9: Frequencies of observed clinical manifestations

Symptoms and Signs	Number	Percentage (%)
Fatiguability	37	59.68
Respiratory distress	15	24.19
Fever	31	50
Bleeding	6	9.68
Pallor	62	100
Icterus	7	11.29
Edema	8	12.9
Hepatomegaly	8	12.9
Splenomegaly	10	16.13
Others	18	29

The commonest presenting complaint was fatiguability in 37 (59.68%) cases, followed by fever in 31(50 %) cases. 15 (24.19%) cases presented with respiratory distress. 6 (9.68%) patients had bleeding manifestations in the form of epistaxis, bleeding per rectum and blood tinged vomitus. Other symptoms like headache, chest pain, pain abdomen, joint pain and diarrhea were seen in 18 (29%) cases.

Chart 4: Frequency of presenting complaints

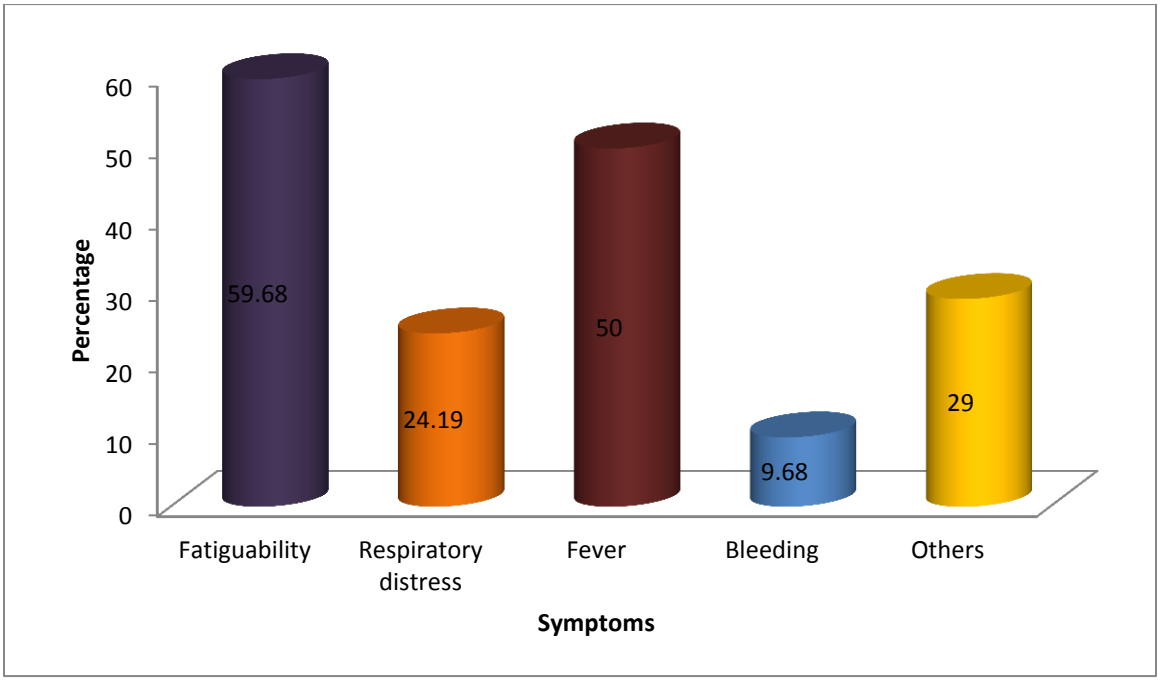
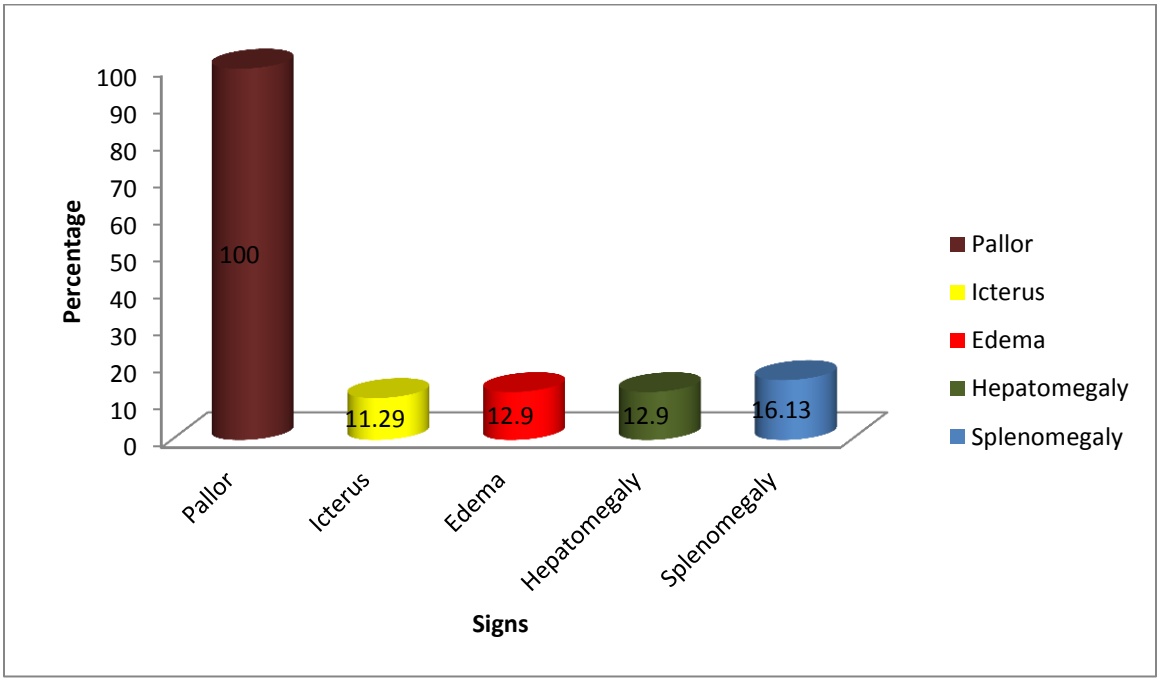


Chart 5: Frequency of signs observed



Pallor was present in all patients. Icterus was seen in 11.29% (7/62). Splenomegaly was seen in 16.13% (10/62) and hepatomegaly in 12.9% (8/62) of patients.

Hematological parameters

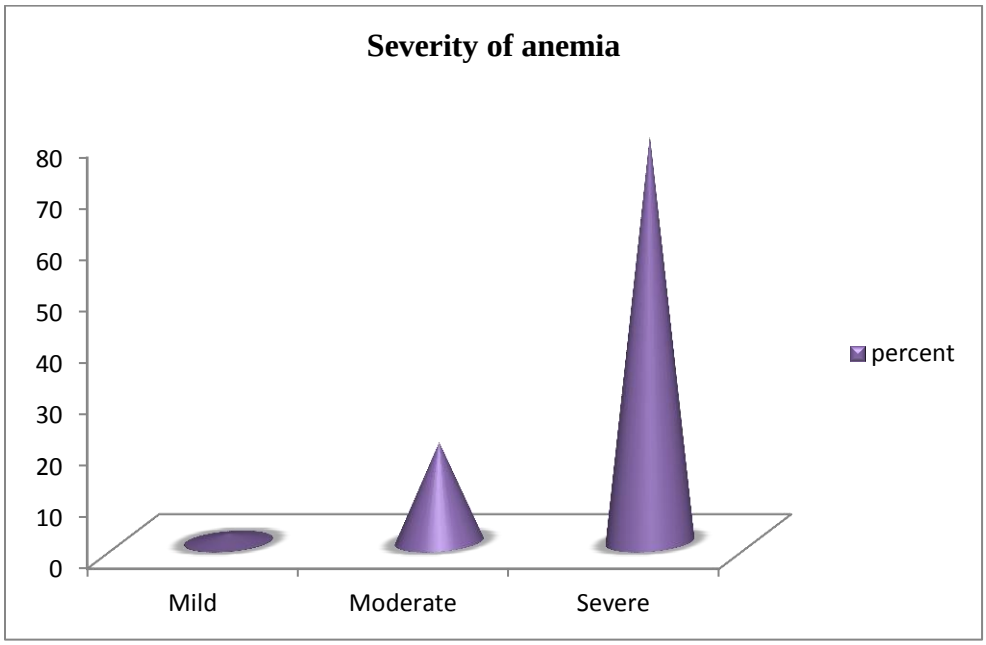
Hemoglobin

The mean hemoglobin concentration was 5.74±2.43gm/dl. The hemoglobin concentration ranged from 1.49 gm/dl to 10.5 gm/dl

Table 10: Severity of anemia[57]

Severity of anemia-Hb (gm/dl)	Number of patients	Percentage (%)
Mild (> 10)	1	1.61
Moderate (8 – 10)	12	19.35
Severe (< 8)	49	79.03
Total	62	100

Chart 6: Severity of anemia



Severe anemia was seen in 49 (79.03%) cases, moderate anemia in 12 (19.35%) cases and mild anemia in only one (1.61%) case.

Cytopenia

Out of 62 cases studied, 22 (35.48%) cases had only dimorphic anemia, 19(30.65%) cases had bicytopenia, either dimorphic anemia with leucopenia or thrombocytopenia and 21(33.87%) cases had pancytopenia (dimorphic anemia with leucopenia and thrombocytopenia)

Table 11: Distribution of cases based on cytopenia

Cytopenia	Number of patients	Percentage (%)
Only dimorphic anemia	22	35.48
Bicytopenia	19	30.65
Pancytopenia	21	33.87
Total	62	100

Chart 7: Distribution of cytopenia

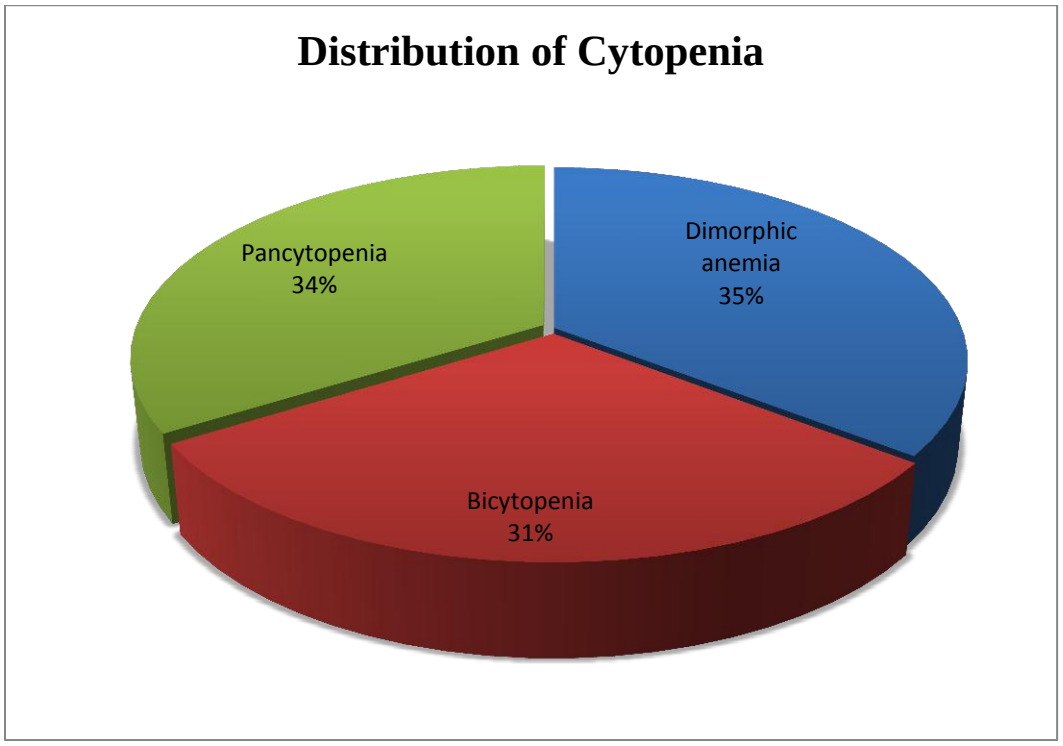
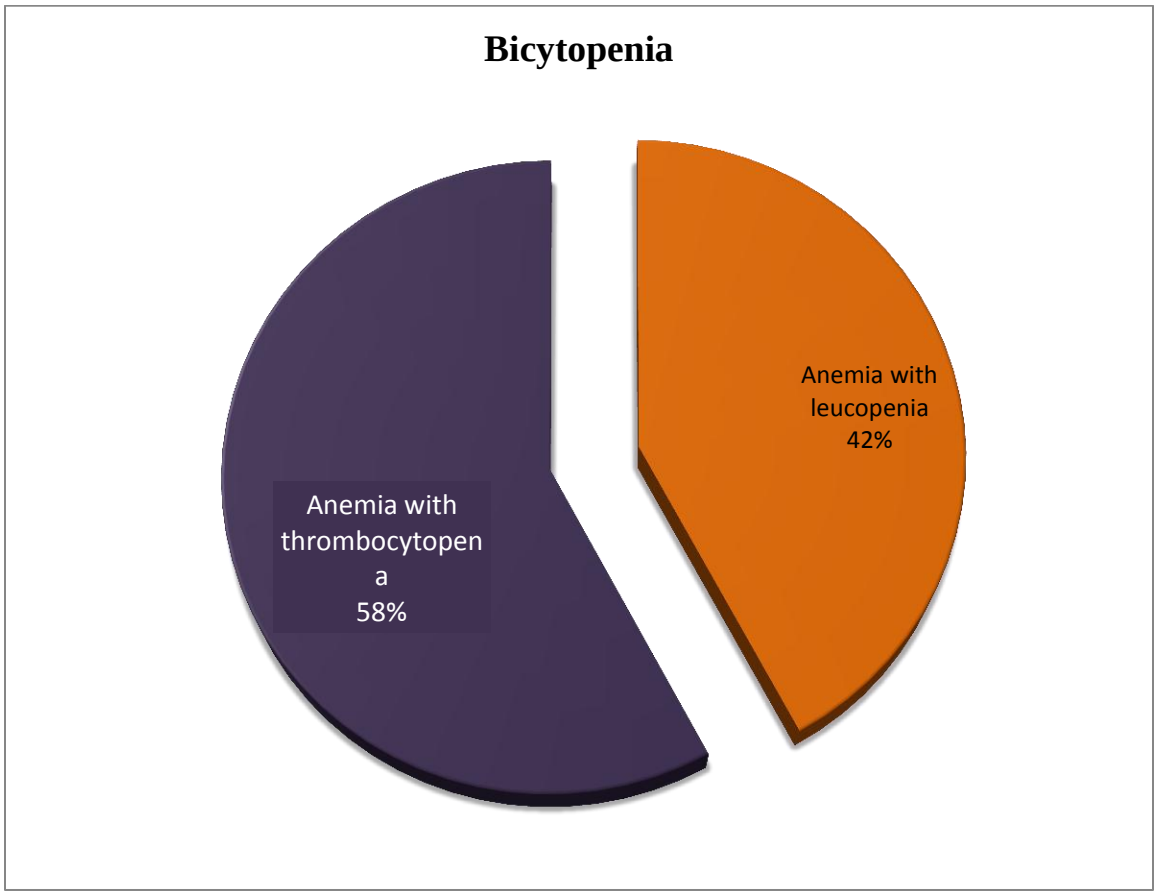


Table 12: Distribution of cases of Bicytopenia with leucopenia or thrombocytopenia

Bicytopenia	**Number (19)**	**Percentage (%)**
Anemia with leucopenia	8	42.11
Anemia with thrombocytopenia	11	57.89
Total	19	100

Chart 8: Distribution of cases with Bicytopenia



In this study, Out of 19 cases with bicytopenia, 11(57.89%) cases had thrombocytopenia and 8 (42.11%) cases had leucopenia. Thrombocytopenia and anemia was the most common form of bicytopenia, constituting 57.89%.

RBC indices

Mean corpuscular volume ranged from 80 to 120 fl. The mean MCV was 95.01±13.14 fl. MCV was <97 fl in 35 (56.45%) patients and high MCV was seen in 27 (43.55%) patients. Among the patients with high MCV, 63% were male and 37% were female. Among the patients with normal MCV, male were only 45.7% while female were 54.29%.

Mean corpuscular hemoglobin ranged from 16.2 to 44.8 pg with mean MCH of 29.31±8 pg. Mean corpuscular hemoglobin concentration ranged from 25.4 to 40.5 g/dl with mean MCHC of 32.22 ± 2.85 gm/dl. The RDW ranged from 15.1 to 25.9 %, with mean RDW of 17 ± 2.61 %

Table 13: Age Distribution with Raised RDW and MCV

Age Groups (Years)	Raised RDW with Normal MCV	Raised RDW with Increased MCV	Total
20-39	20	11	31
40-59	11	8	19
60-79	4	8	12
Total	35	27	62

In our study, 62% of cases less than 60 years of age showed normal MCV and 66.67 % of cases more than 60 years of age showed increased MCV.

Chart 9: Distribution of cases with Raised RDW and MCV

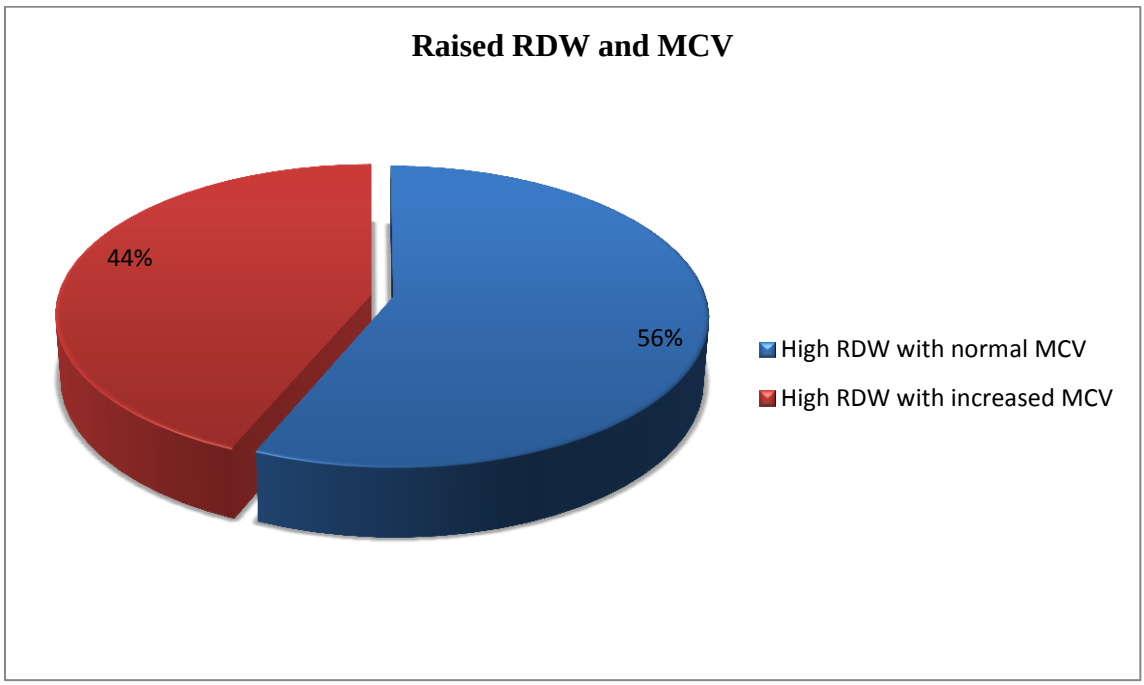
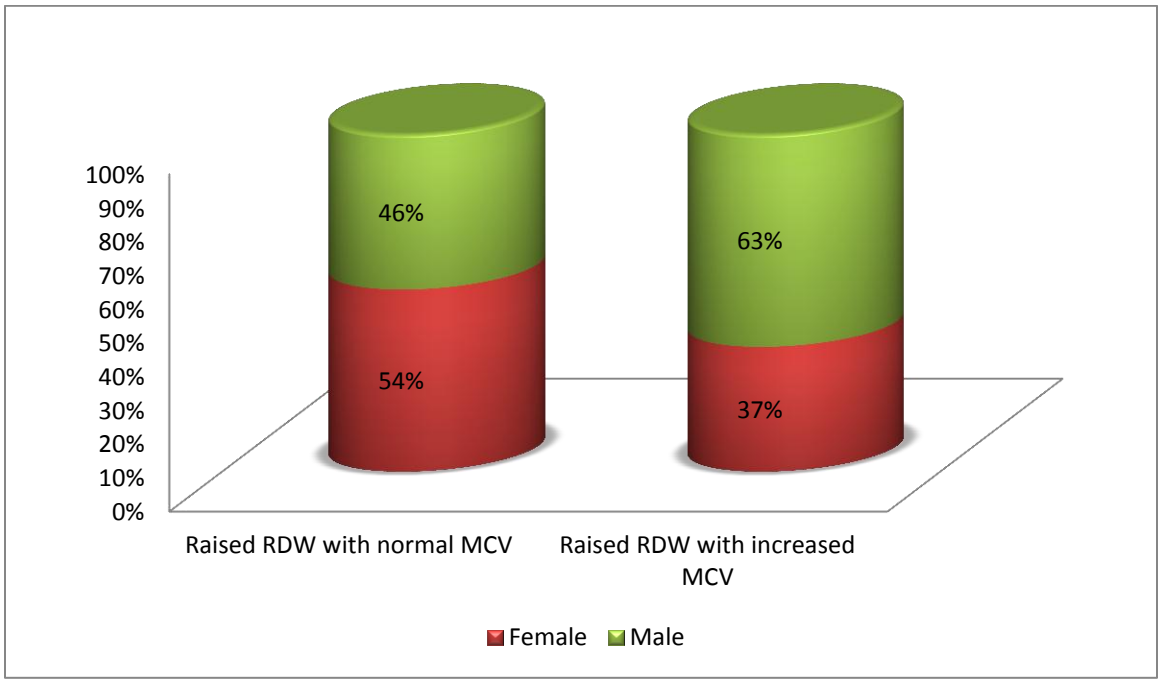


Table 14: Gender distribution with raised RDW and MCV

Raised RDW and MCV	Male	Female	Total
Raised RDW with Normal MCV	16	19	35
Raised RDW with Increased MCV	17	10	27
Total	33	29	62

In our study, among males 17 cases (63%) showed increased MCV and 16 cases (45.71%) showed normal MCV. Among females, 10 cases (37%) showed increased MCV and 19 cases (54.29%) showed normal MCV. So, predominantly males showed increased MCV compared to female.

Chart 10: Gender based distribution of raised RDW and MCV



Bone marrow examination

Bone marrow examination showed normocellular marrow in 20 (32.36%) cases and hypercellular marrow in 42 (67.74%) cases. None of our cases showed hypocellular marrow.

Table 15: Distribution based on Bone marrow cellularity

BM Cellularity	**Number**	**Percentage (%)**
Normocellular	20	32.36
Hypercellular	42	67.74
Total	62	100

Chart 11: Distribution of cases based on Bone marrow cellularity

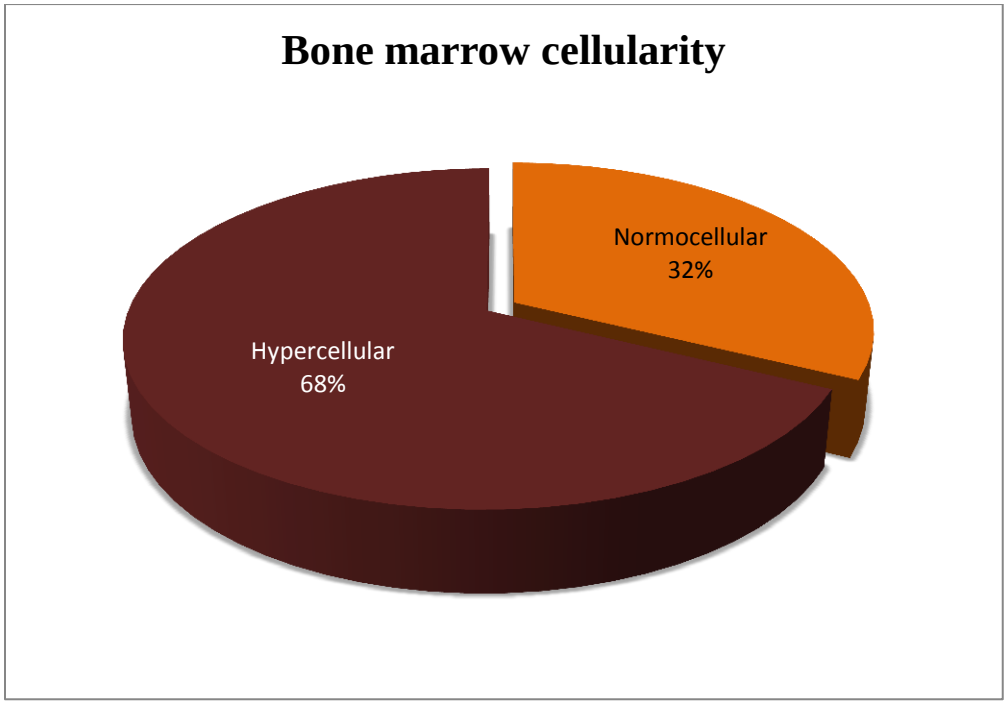


Table 16: Correlation of Hematological parameters with Bone marrow cellularity

Hematological parameters	Normocellular	Hypercellular
Hb	6.68±2.45	5.29±2.31
TLC	4.98±2.47	6.81±10
Platelet count	2.02±1.19	1.32±0.93
MCV	90.5±10.47	97.64±13.17
MCH	24.44±7.25	31.63±7.42
MCHC	30.94±2.47	32.83±2.83
RDW	16.58±1.63	17.20±2.96

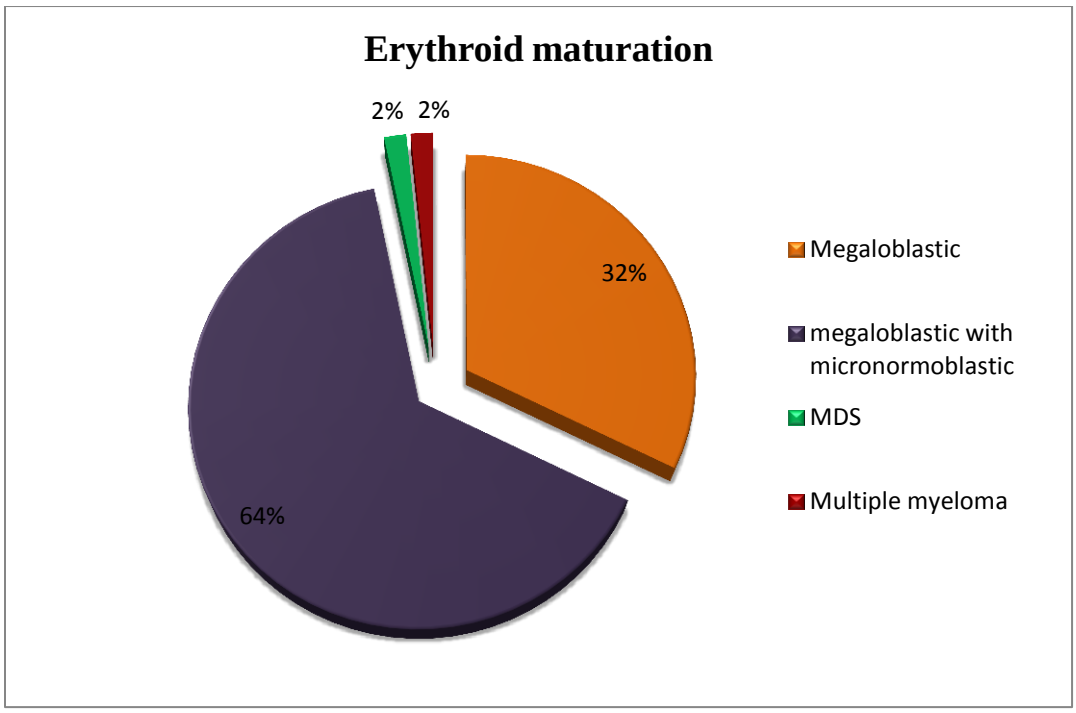
The mean MCV and MCH were high in cases with hypercellular marrow and showed no statistical significance.

In our study, Erythroid hyperplasia was seen in 58 (93.54 %) cases, which is indicative of bone marrow response to anemia.

Table 17: Distribution of cases based on Erythroid maturation

Erythroid maturation	Number	Percentage (%)
Megaloblastic maturation	20	32.26
Megaloblastic with micronormoblatic maturation	40	64.52
Megaloblastic - MDS	1	1.61
Normoblastic maturation - Multiple myeloma	1	1.61

Chart 12: Distribution of cases based on Erythroid maturation



Megaloblastic maturation was seen in 20 (32.36%) and 40 (64.52%) patients showed both megaloblastic and micronormoblastic maturation. 31 (50%) patients showed dyserythropoiesis, all of these cases also showed giant metamyelocytes and 5 (8%)

patients showed only giant metamyelocytes without dyserythropoiesis. Among patients with dyserythropoiesis, 13 cases showed only megaloblastic maturation.

A 40 year old male patient presented with fever and moderate anemia with Hb of 7.3 gm/dl, along with leucopenia and thrombocytopenia. Bone marrow showed features of myelodysplastic syndrome, with erythroid hyperplasia showing dyserythropoiesis, dysmyelopoiesis with 9% of blasts. The iron stores were increased.

A 65 year old female presented with pathological fracture and anemia with Hb of 7 gm/dl. Peripheral blood smear examination showed only features of dimorphic anemia. BM showed normoblastic erythroid maturation with features of multiple myeloma, having 40% plasmablasts.

Table 18: Correlation of Hematological parameters with erythroid maturation

Parameters	**Megaloblastic**	**Megaloblastic with micronormoblastic**	**MDS**	**Normoblastic - Multiple myeloma**
Hb	5.78±2.79	5.65±2.3	7.3	7
TLC	8.53±13.67	5.08±3.68	1.8	9.9
Platelet count	1.25±0.87	1.37±0.84	0.87	1.96
MCV	98.35±12.20	94.1±13	81	99
MCH	31.53±7.75	28.44±8.05	31.9	16.9
MCHC	32.49±2.4	31.87±2.82	40.5	32.3
RDW	16.83±2.3	17.17±2.8	15.3	15.5

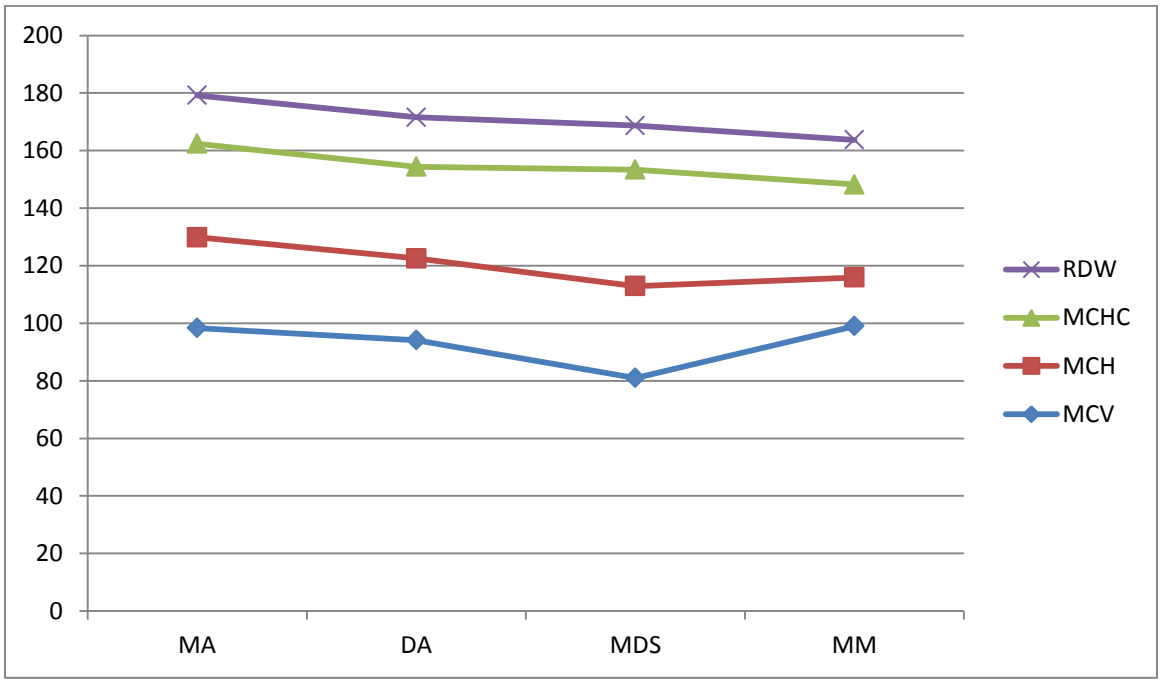
In cases with megaloblastic maturation, hemoglobin ranged from 1.59 to 10.5gm/dl and mean Hb was 5.78 gm/dl. In cases with megaloblastic and micronormoblastic maturation, Hb ranged from 1.49 to 9.6 gm/dl and mean Hb was 5.65 gm/dl.

The mean MCV was 98.35 fl in cases with megaloblastic maturation and 94.1 fl in cases with megaloblastic and micronormoblastic maturation.

The mean MCH was 32.49 pg in cases with megaloblastic maturation and 28.44 pg in cases with both megaloblastic and micronormoblastic maturation.

RDW ranged from 15.1% to 23 % in cases with megaloblastic maturation and 15.1% to 25.9% in cases with megaloblastic and micronormoblastic maturation.

Chart 13: Mean of RBC indices among different groups based on Erythroid maturation.



The mean MCV and MCH were high in cases with megaloblastic maturation, as compared with patients with both micronormoblastic and megaloblastic maturation. But, the difference was not statistically significant, with p value = 0.2 by independent t- test.

Table 19: Distribution of cases in respect to based on Cytopenia and Erythroid maturation

Cytopenia	Megaloblastic (n = 20)	Megaloblastic with micronormoblastic (n = 40)
Dimorphic anemia alone	9 (45%)	12 (30%)
Bicytopenia	3 (15%)	16(40%)
Pancytopenia	8 (40%)	12 (30%)
Total	20	40

In our study, 40% of patients with megaloblastic maturation presented with pancytopenia. 30 % of patients with both megaloblastic and micronormoblastic maturation presented with pancytopenia.

In patients with megaloblastic maturation, 3(15%) cases showed normocellularity and 17 (85%) cases showed hypercellularity. In patients with dimorphic maturation, 16 (40%) cases showed normocellularity and 24 (60%) cases showed hypercellular bone marrow.

Bone marrow iron stores estimation

The iron status were assessed on Perls' stained bone marrow aspirate smears of these 62 cases, both by Gale's method and intensive method

Bone marrow iron grade ranged from grade 0 to grade 5.

According to Gale's grading system, iron deficient stores was seen in 20 (32.26%) cases, normal stores in 35 (56.45%) cases and increased stores in 7 (11.29%) cases.

Table 20: Bone marrow iron status category results.

Method	Iron status category	No.	Percentage (%)
Gale's method	Normal	35	56.45
	Increased	7	11.29
	Iron deficiency	20	32.26
Intensive grading method	Normal	20	32.26
	Functional iron deficiency	22	35.48
	Iron store deficiency	5	8.06
	Functional & iron store deficiency	15	24.19

The intensive grading system demonstrated normal marrow iron stores in 20 (32.26%) cases, functional iron deficiency in 22 (35.48%) cases, only iron store deficiency in 5 (8%) cases, both functional and iron store deficiency in 15 (24.19%) cases.

According to intensive grading method, most of the cases predominantly showed functional iron deficiency.

Table 21: Correlation of Hematological parameters with iron stores.

Parameters	Adequate iron stores	Iron deficient state
Hb	5.86±2.4	5.46±2.31
MCV	95.94±14.24	92.8±9.6
MCH	30.3±8.52	25.79±6.85
MCHC	32.71±2.85	30.93±2.23
RDW	17.01±2.85	16.71±1.56

The mean of MCH and MCHC showed statistically significant difference between the cases with adequate iron stores and deficient iron stores with p – value < 0.05, obtained by one - way ANOVA test.

Iron status based on LHD%

LHD% is a biomarker of hypochromasia. LHD values were utilized as a predictor of iron status. This value is derived using MCHC values. 10.4 was considered as optimal cut off. LHD% of > 10.4 is used as predictor of iron deficiency.[23]

Table 22: LHD % in different iron status categories (Gale's method)

Method - Iron status category	**LHD %**	
Gale's method	**≤ 10.4**	**>10.4**
Normal	20	15
Increased	3	4
Iron deficiency	1	19

On comparison of LHD% with Gale's method of evaluation of iron stores, 20 patients with normal LHD% had normal iron stores and 15 patients with increased LHD% had normal iron stores. 3 patients with normal LHD% showed increased iron stores and 4 patients with increased LDH % (iron deficiency) showed increased iron stores and 1 patient with normal LHD% had iron deficient stores and 14 patients with increased LHD% had iron deficient stores.

LHD% is strongly significant on comparison of categories obtained from Gale's method of assessing iron stores, with p-value < 0.001, obtained using Fisher exact test.

LHD% and intensive grading method for iron stores

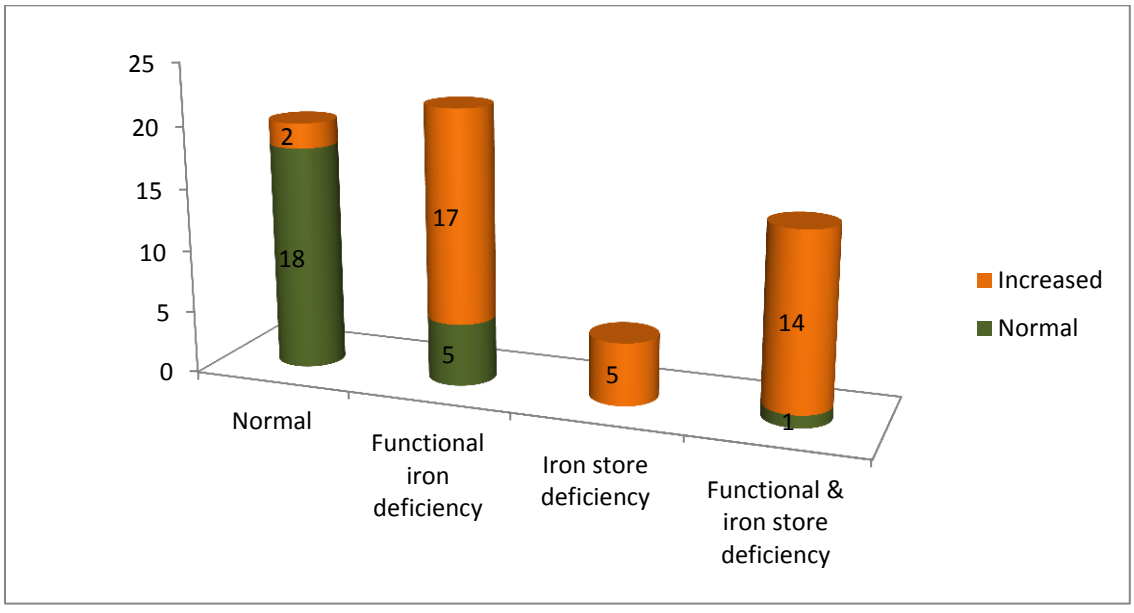
LHD% was statistically significant with the groups obtained from intensive grading system.

Only applying Fisher exact test, LHD% was strongly significant between normal iron stores and functional iron deficiency anemia and also both functional and iron store deficiency, with p-value < 0.001

Table 23: LHD % in different iron status categories (Intensive grading system)

Method - Iron status category	**LHD %**		**Median of LHD %**
Intensive grading method	**≤ 10.4**	**>10.4**	
Normal	18	2	2.24
Functional iron deficiency	5	17	33.49
Iron store deficiency	-----	5	32.16
Functional & iron store deficiency	1	14	19.42

Chart 14: LHD% and iron status category obtained from intensive grading system



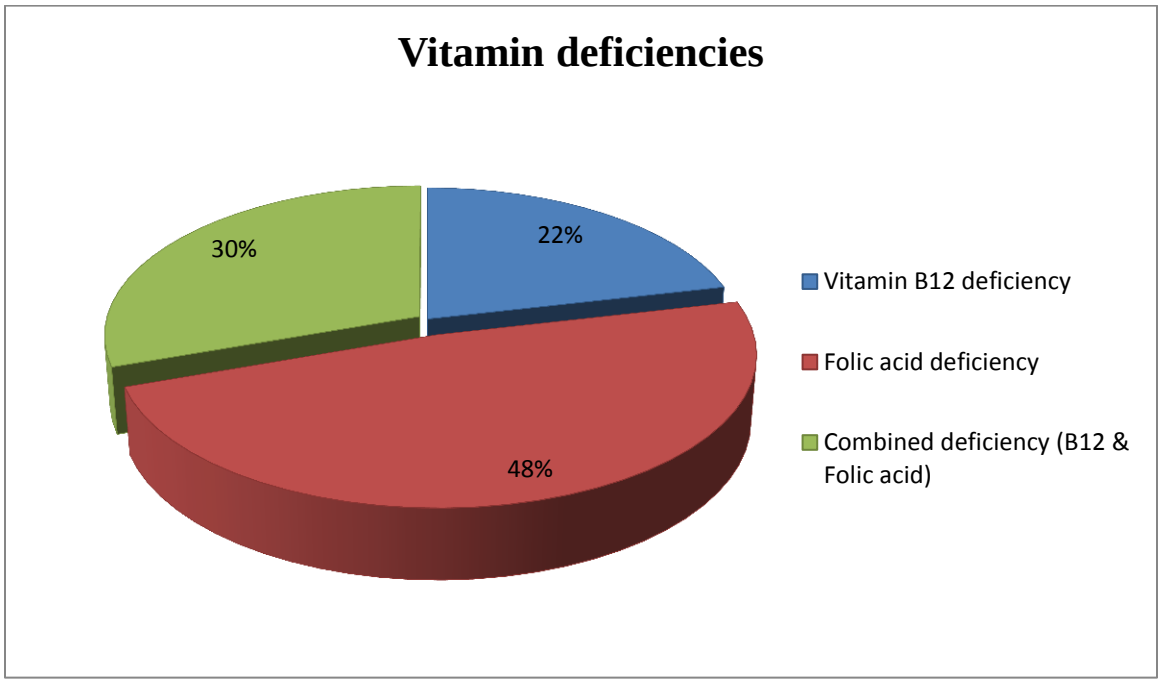
Etiology based on Serum vitamin B12 and folic acid levels

The mean of serum folate levels obtained was 5.86 ng/ml. The mean of serum vitamin B12 levels obtained was 417.9 pg/ml

Table 24: Distribution of cases in respect to etiology – vitamin B12 and folic acid deficiency

Causes	Number	Percentage (%)
Vitamin B12 deficiency	12	19.35
Serum Folic acid deficiency	27	43.55
Both vitamin B12 and folic acid deficiency	17	27.42
Normal	6	9.67

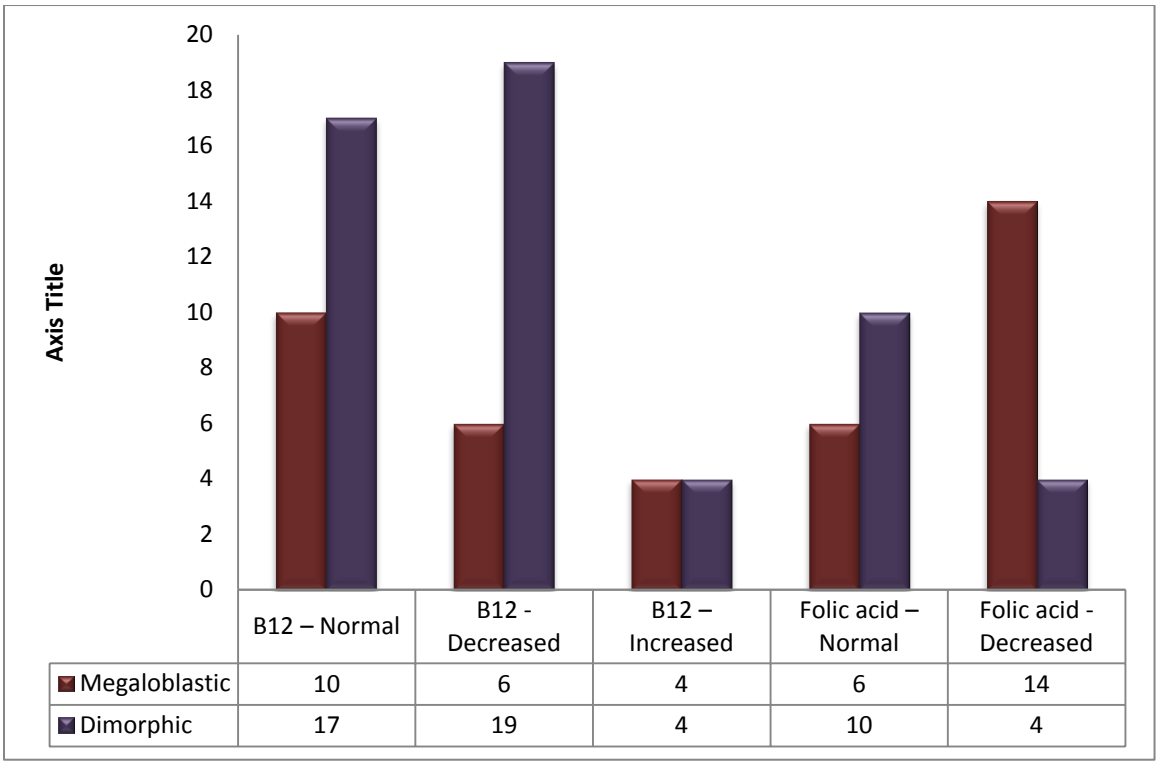
Chart 15: Distribution of cases in respect to vitamin B12 and folic acid deficiencies



In our study, 27 (43.55%) cases showed only folic acid deficiency. 12 (19.35%) cases showed only vitamin B12 deficiency. 17 (27.42%) cases showed both vitamin B12 and

folic acid deficiency. 6 (9.67%) cases showed normal levels of serum vitamin B12 and folic acid. 38.6% of folate deficient patients had vitamin B12 deficiency as well, while 58.6% of vitamin B12 deficient patients were having co-occurrence of folate deficiency.

Chart 16: Distribution of cases with respect to Vitamin B12 & folate levels among two groups - megaloblastic maturation and micronormoblastic with megaloblastic maturation.



	B12 – Normal	B12 - Decreased	B12 – Increased	Folic acid – Normal	Folic acid - Decreased
Megaloblastic	10	6	4	6	14
Dimorphic	17	19	4	10	4

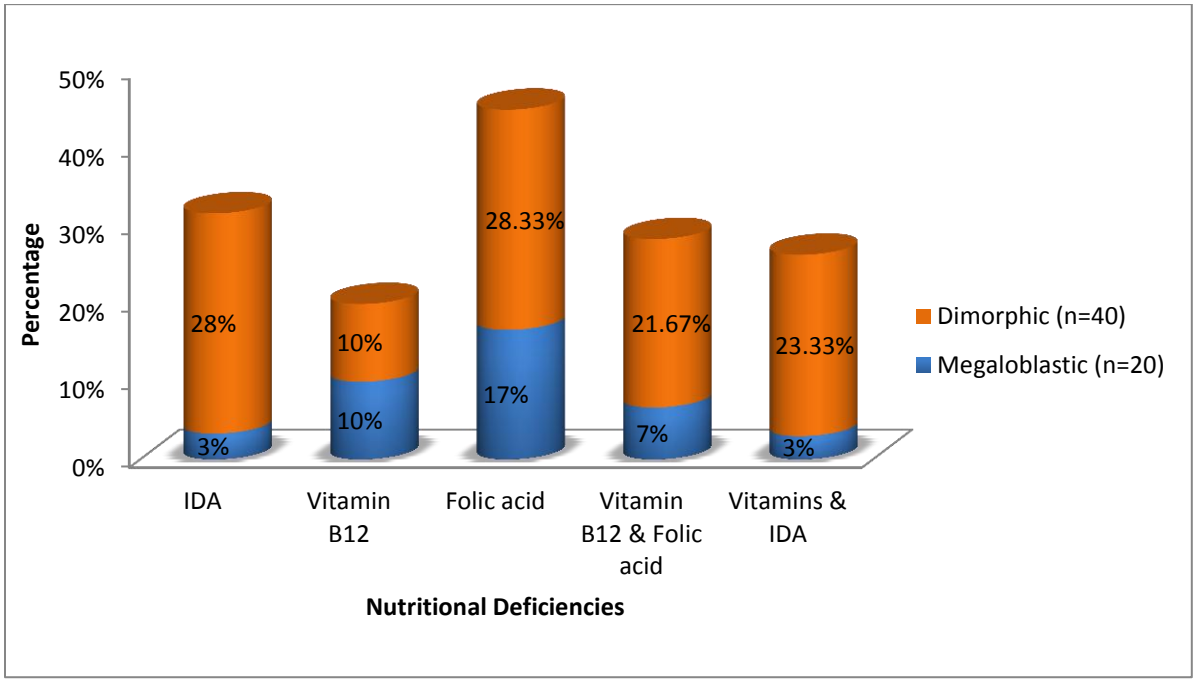
Among patients with megaloblastic maturation, 10 (50%) patients showed only serum folic acid deficiency, 6 (30%) patients showed only vitamin B12 deficiency and 4 (20%) patients showed both vitamin B12 and folic acid deficiency.

Among patients with both megaloblastic and dimorphic maturation, 17 (42.5%) patients showed only folic acid deficiency, 6 (15%) patients showed only vitamin B12 deficiency and 13 (32.5%) patients showed both vitamin B12 and folic acid deficiency.

Table 25: Distribution of cases with Iron deficiency anemia, vitamin B12 and folic acid deficiency in megaloblastic anemia and dimorphic anemia (micronormoblastic and megaloblastic)

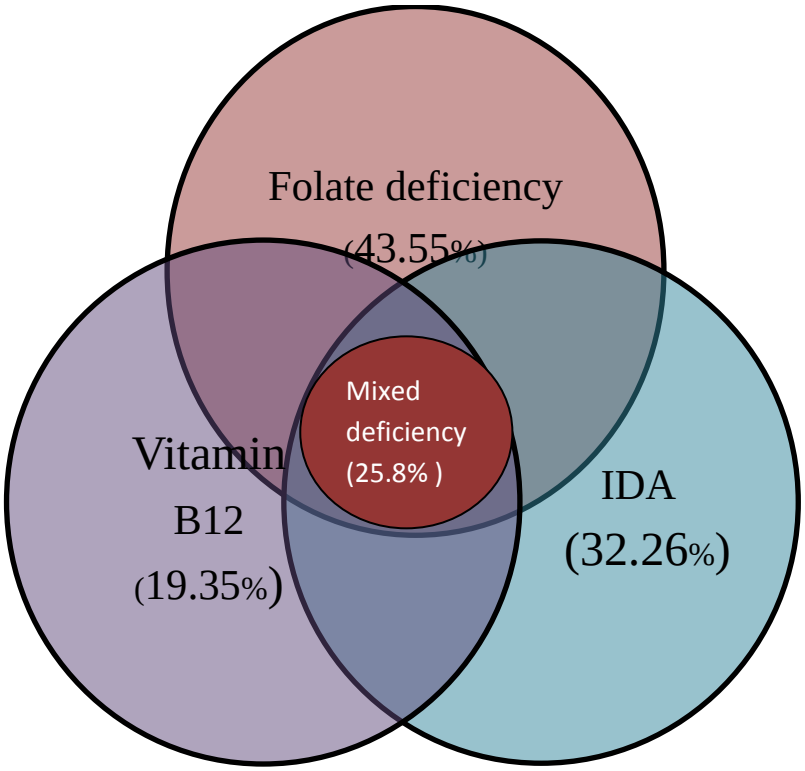
Deficiencies	Megaloblastic (n=20)	Dimorphic (n=40)
IDA	2	17
Vitamin B12	6	6
Folic acid	10	17
Vitamin B12 & Folic acid	4	13
Vitamins & IDA	2	14

Chart 17: Distribution of cases with Iron deficiency anemia, vitamin B12 and folic acid deficiency in megaloblastic anemia and dimorphic (micronormoblastic and megaloblastic)



All cases of megaloblastic anemia showing deficient iron stores (IDA) are associated with vitamin deficiency (either vitamin B12 or folic acid). Among patients with both micronormoblastic and megaloblastic maturation, 17 (42.5%) cases were associated with deficient iron stores (IDA), of which only 14(35%) cases showed mixed deficiency of both iron and vitamins.

Chart 18: Distribution of cases based on iron, vitamin B12 and folate deficiencies.

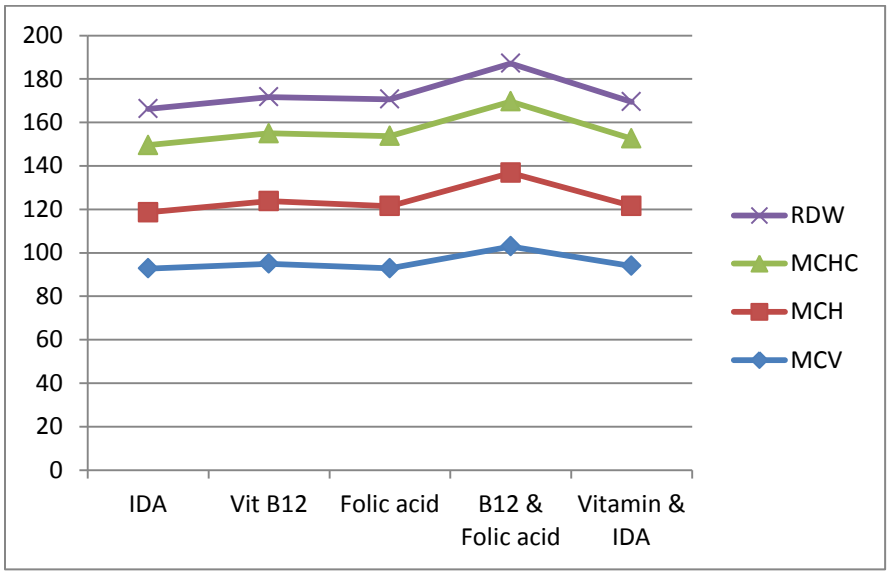


In our study, 25.8% cases were associated with both iron and vitamin B12 and / folate deficiency (mixed deficiency)

Table 26: Mean of Hb and RBC indices in patients with nutritional deficiencies

Parameters	IDA	Vitamin B12 deficiency	Folate deficiency	Combined (B12 &folate deficiency)	Mixed (Iron & vitamin deficiency)
Hb	5.46±2.31	6.36±2.6	5.6±2.4	4.76±2.27	4.94±2.27
MCV	92.8±9.6	95±12.58	92.85±11.21	102.94±13.41	93.93±9.84
MCH	25.79±6.85	28.77±8.18	28.67±7.29	33.83±7.22	27.63±6.38
MCHC	30.93±2.23	31.18±2.5	32.22±2.52	32.88±3.12	30.99±2.43
RDW	16.71±1.56	16.75±1.99	16.96±2.72	17.48±3.10	16.97±1.64

Chart 19: Mean MCV, MCH, MCHC, RDW and various nutritional deficiencies



MCV, MCH, MCHC and RDW were high in combined (vitamin B12 and folic acid) deficiency compared to all other groups. MCV and MCH were statistically significant between IDA cases and combined (vitamin B12 and folic acid) deficiency with p value of < 0.05 and < 0.01 respectively, obtained by ANOVA test.

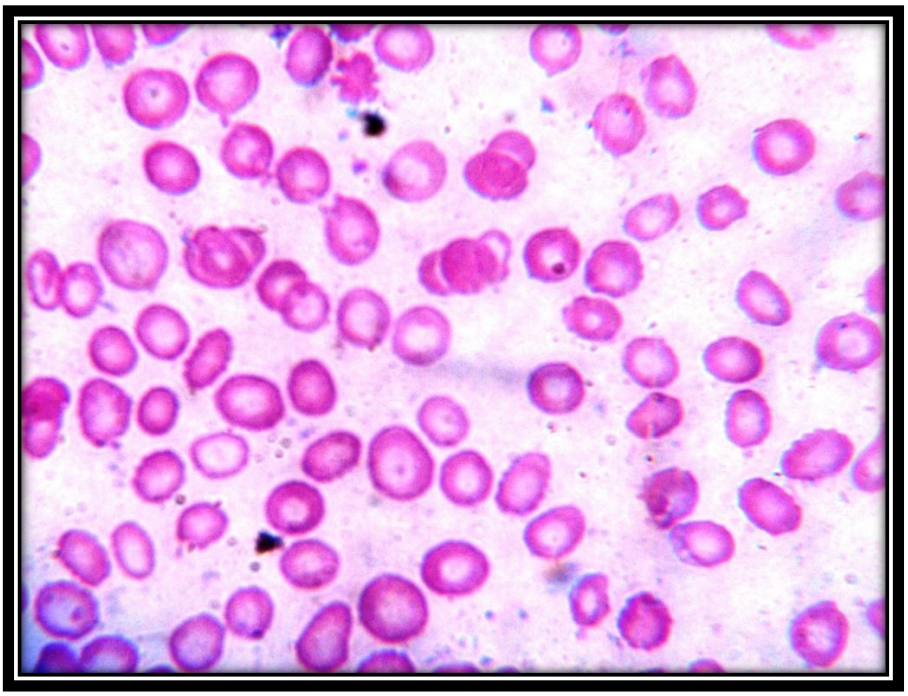


Figure 8: Photomicrograph of peripheral blood smear showing dimorphic blood picture.(Leishman stain 1000X)

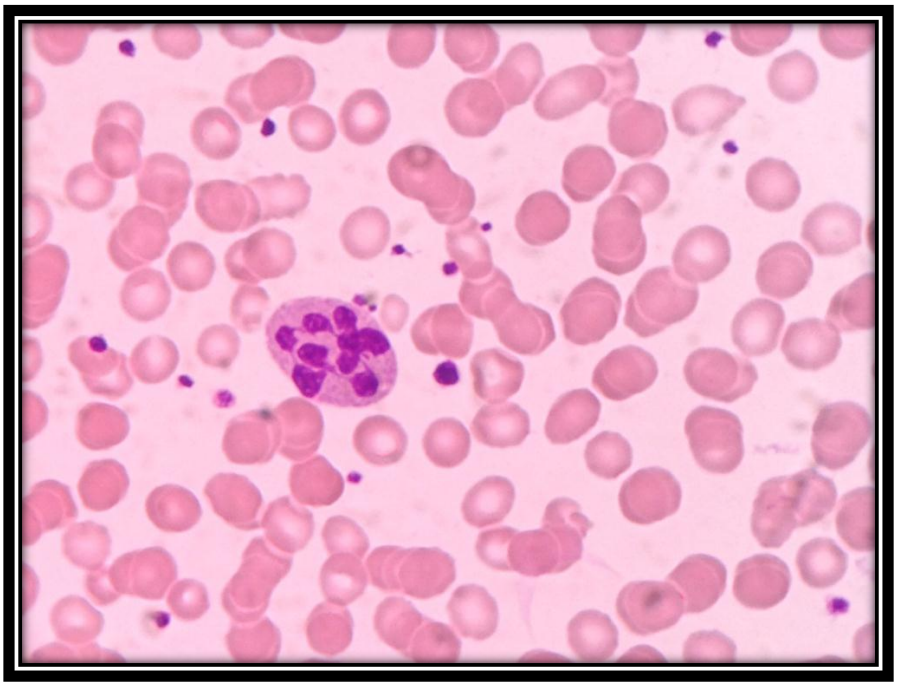


Figure 9: Photomicrograph of peripheral blood smear showing hypersegmented neutrophils.(Leishman stain 1000X)

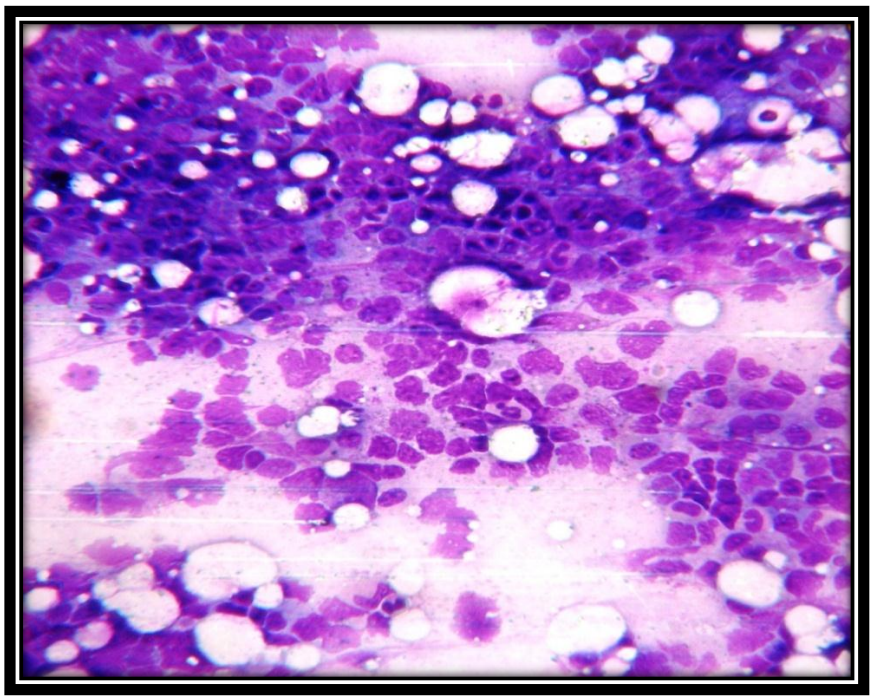


Figure 10: Photomicrograph of Bone marrow aspirate smear showing hypercellular marrow.(Giemsa stain 400X)

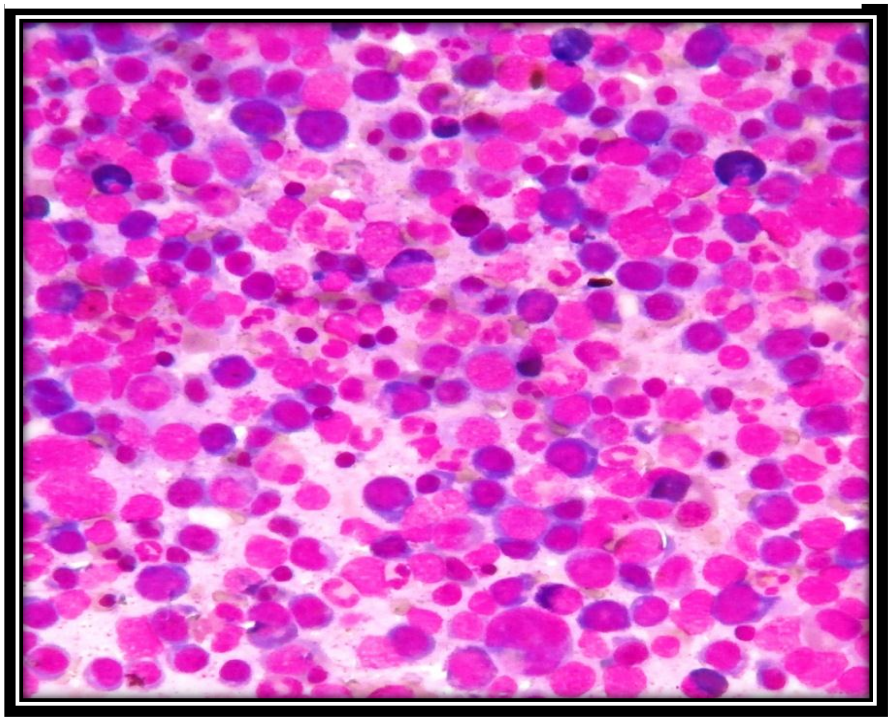


Figure 11: Photomicrograph of Bone marrow aspirate smear showing erythroid hyperplasia.(Giemsa stain 400X)

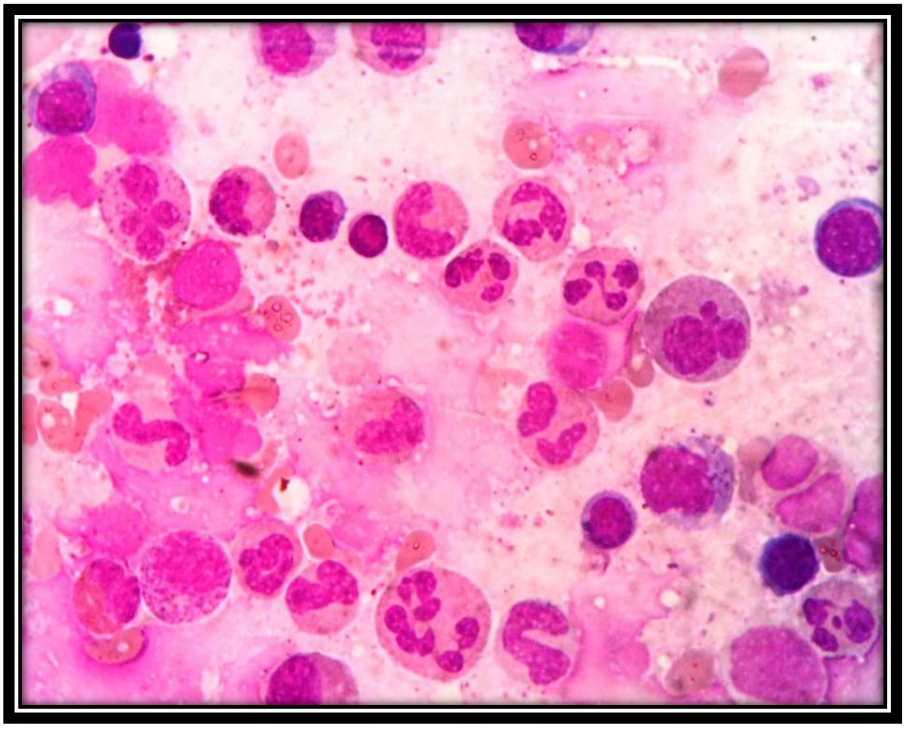


Figure 12: Photomicrograph of Bone marrow aspirate smear of megaloblastic anemia showing dyserythropoiesis and hypersegmented neutrophil (Giemsa stain 1000X)

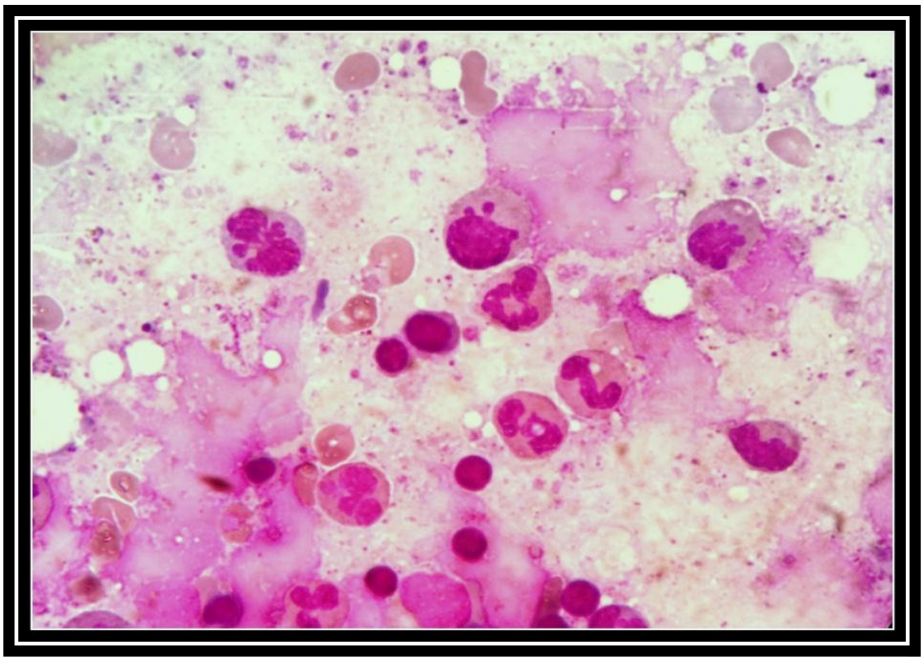


Figure 13: Photomicrograph of Bone marrow aspirate smear of megaloblastic anemia showing dyserythropoiesis (Giemsa stain 1000X)

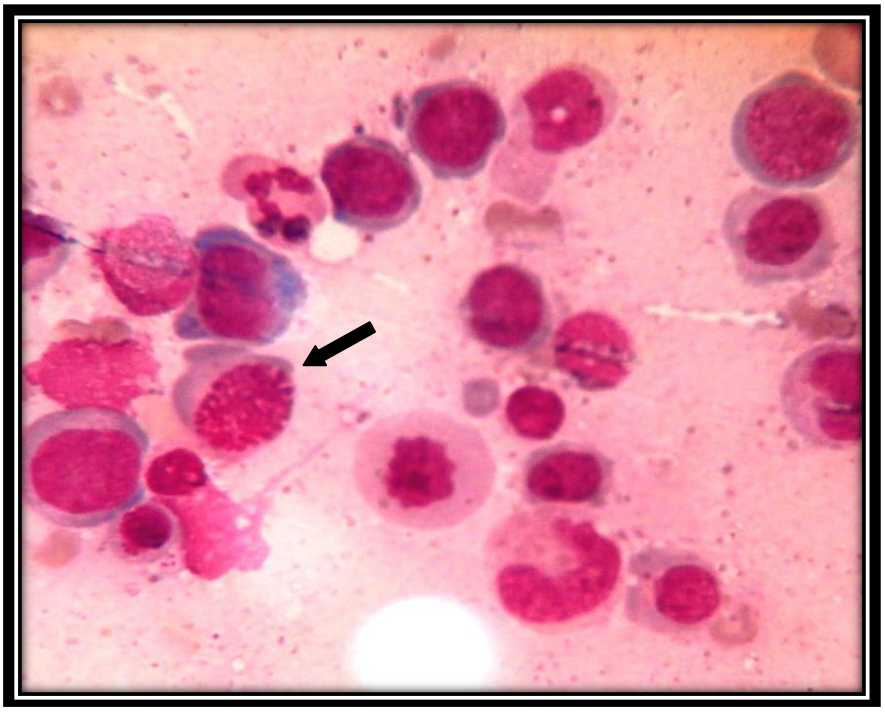


Figure 14: Photomicrograph of Bone marrow aspirate smear of megaloblastic anemia showing giant metamyelocyte and megaloblast (arrow) (Giemsa stain 1000X)

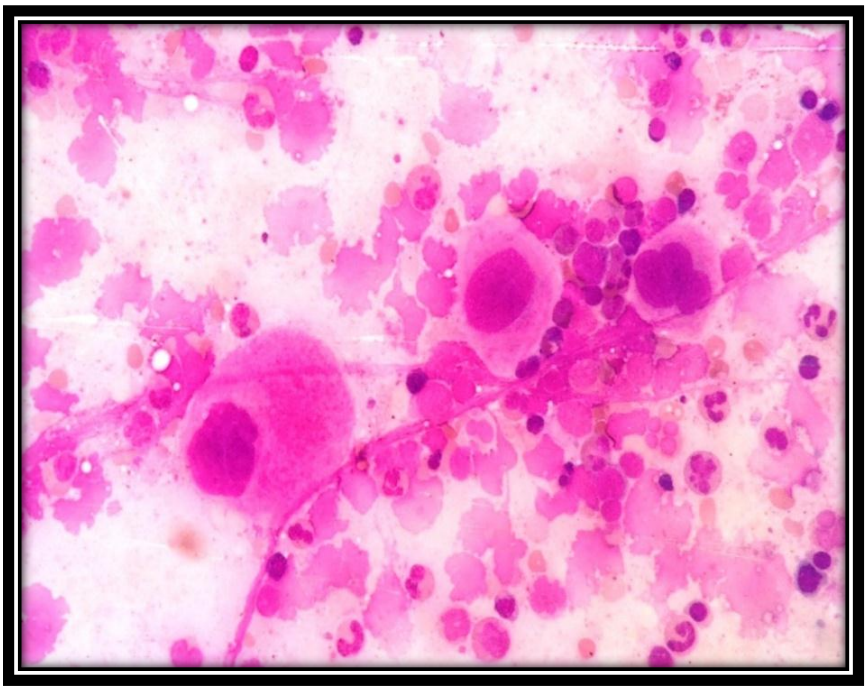


Figure 15: Photomicrograph of Bone marrow aspirate smear of megaloblastic anemia showing increased number of megakaryocytes, immature forms (Giemsa stain 1000X)

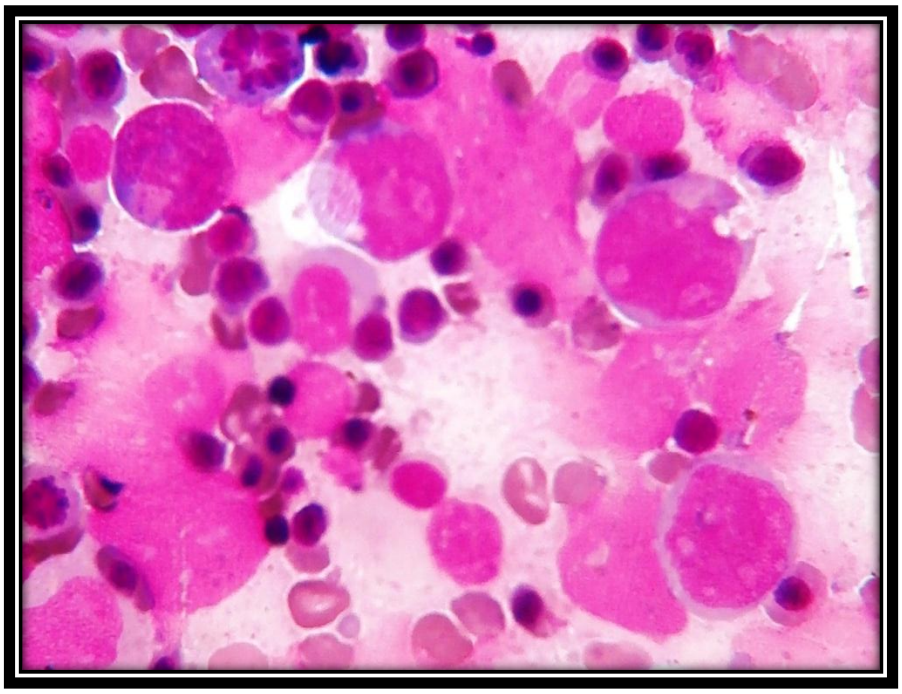


Figure 16: Photomicrograph of Bone marrow aspirate smear of MDS showing myeloblasts (Giemsa stain 1000X)

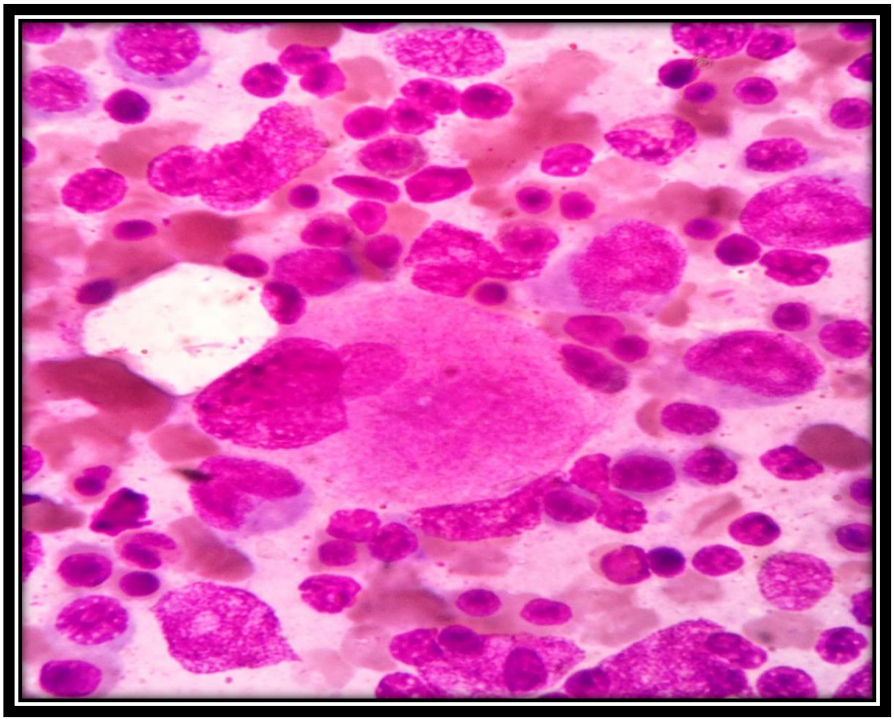


Figure 17: Photomicrograph of Bone marrow aspirate smear of MDS showing dysplastic megakaryocyte (Giemsa stain 1000X)

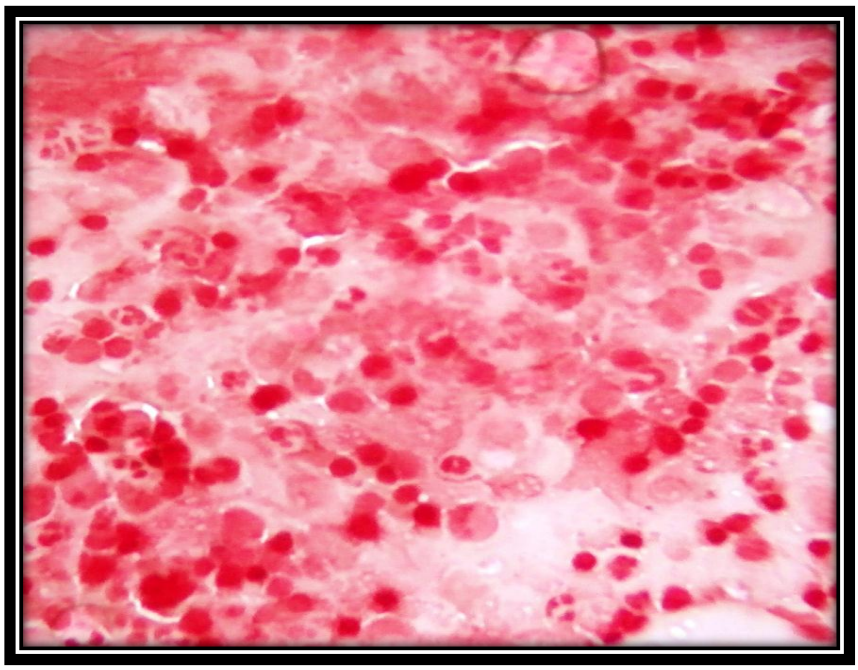


Figure 18: Photomicrograph of Perls' stained Bone marrow aspirate smear with absent iron stores (1000X)

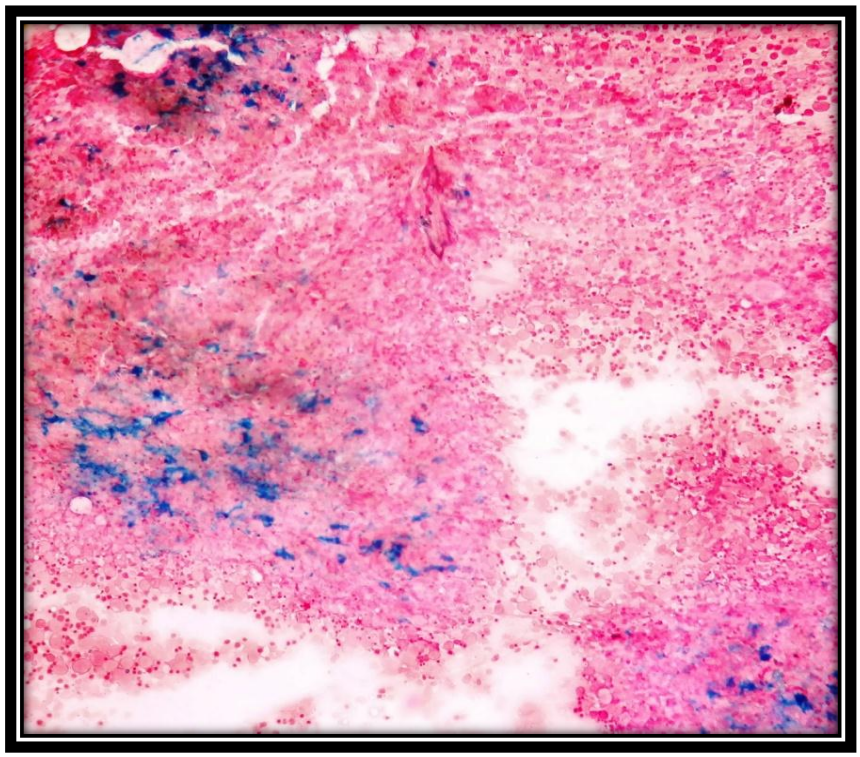


Figure 19: Photomicrograph of Perls' stained Bone marrow aspirate smear with increased iron stores of case of MDS (400X)

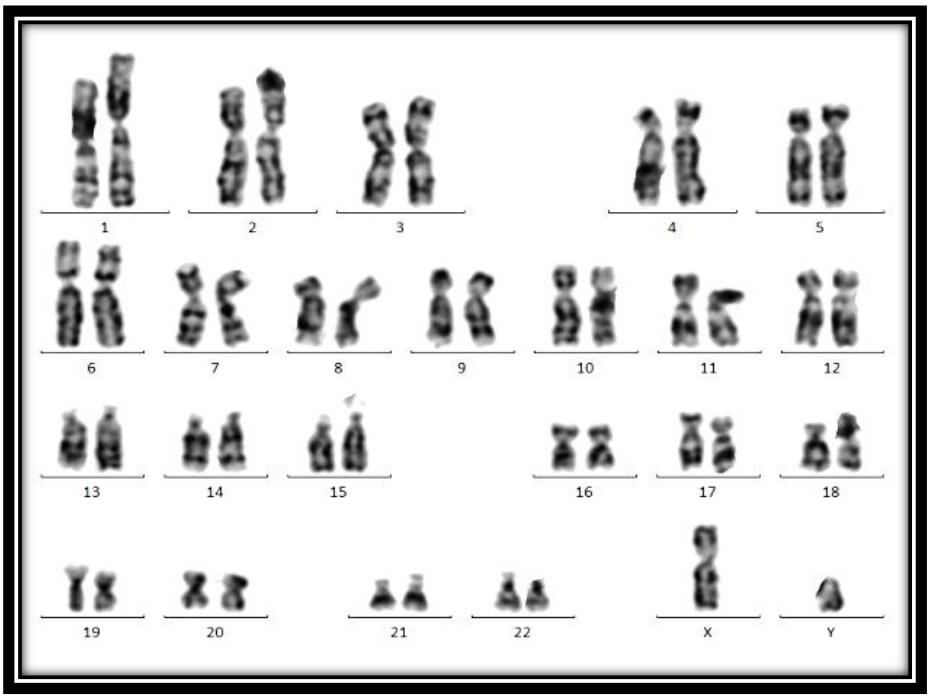


Figure 20: Karyogram of a case of MDS, showing normal karyotype (44,XY)

DISCUSSION

Discussion

Anemia is the most common treatable problem in developing countries.[58] In country like India, most common cause of anemia is nutritional deficiencies. Good proportion of cases show combined deficiency of iron, vitamin B12 and folic acid, where multiple factors affect the diagnostic parameters, resulting in discordant results of tests like bone marrow morphology, iron stores and iron studies.[59]Megaloblastic anemia is the second commonest nutritional anemia next to iron deficiency anemia in our country. People with low socio- economic group are the ones who are mainly affected.

Age Incidence

Majority of cases in this study were in younger age group of 20 – 39 years of age. The age distribution in this study was similar to the study by Baker et al, the incidence in general was in 20 – 40 years age group. The highest incidence in both male and female was in the age group of 20-29 years.

Sex Distribution

In the present study, incidence was almost equal in both male and female with male: female ratio of 1.1. There was no clear male preponderance seen. Studies on pancytopenia in India by Khan S et al and Kumar et al, showed male preponderance in contrast to our study.

Clinical manifestations

In this study, the commonest presenting complaint was fatiguability in 59.68% (37/62) followed by fever in 50 % (31/62). Frequencies of these symptoms were similar to the other studies. Tahlan et al also showed the incidence of fever in megaloblastic anemia varied from 28% to 60%. It may be attributed to leucopenia or premature

destruction of hematopoietic precursors and release of intracellular substances functioning as pyrogens.[59,60] Pallor was universally present in all patients. Icterus was seen in 11.29% (7/62).

Hematological parameters

The present study showed, only dimorphic anemia in 22 (35.5%) cases and anemia with leucopenia or thrombocytopenia in 19 (30.65%), pancytopenia in 21(33.87%) cases. Study conducted by Khan S et al[59] showed similar results in respect to pancytopenia in 38.8% cases, but anemia with leucopenia in 15.53% and anemia with thrombocytopenia in 13.5% patients

Table 27: Comparison with other studies in respect to cytopenias

	Present study	**Khan et al[59]**
Only anemia	35.5%	32.1%
Anemia with leucopenia	12.91%	15.53%
Anemia with thrombocytopenia	17.72%	13.5%
Pancytopenia	33.87%	38.87%

RBC indices

In our study, MCV was < 97 fl in 35 (56.45%) patients and high MCV was seen in 27 (43.55%) patients, our finding is consistent with a study where high MCV was seen in 41.7% patients.[59] Bhatia et al showed normal MCV in few cases with vitamin B12 deficiency and also in cases with low ferritin levels. Normal MCV may be because of concomitant iron deficiency, excessive fragmentation of red cells and anemia of chronic disease.[59] RDW is useful in differentiating megaloblastic anemia (high MCV with high RDW) and other macrocytic anemia (high MCV and normal RDW).[17]

Bone marrow examination

Our study showed normocellular marrow in 32% cases and hypercellular marrow in 68% cases, which is similar to the study by Metikurke SH et al where 57.72% cases had hypercellular marrow and 36.02% cases had normocellular marrow.

Megaloblastic maturation was seen in 32.36% of our patients and megaloblastic with micronormoblastic maturation in 64.52% of our patients. Megaloblastic bone marrow is hypercellular, with accumulation of primitive cells. Megaloblastic anemia may be associated with normoblastic or micronormoblastic maturation.[61]

One of our patients with pancytopenia, showed features of MDS. On conventional cytogenetics, no abnormality was identified. The underlying clonal expansion of MDS was first suggested by Dacie, who noted a dimorphic population of red cells consistent with a clonal disorder.[41] Mahmoud et al reported that, of 124 patients 75 years of age or older with macrocytic anemia, a definitive cause was ascertained by non-invasive techniques in 60% of the cases. Of the remaining 49 patients, all underwent a bone marrow procedure, but diagnosis of MDS could be made only in 6, and remaining 43 patients were suggested that they might have "MDS in evolution".[43]

Another patient presented with pathological fracture, with only features of dimorphic anemia on complete haemogram and peripheral blood smear. BM showed normoblastic maturation with features of multiple myeloma.

Bone marrow iron stores

In uncomplicated megaloblastic anemia, iron stores are normal or increased, both in reticuloendothelial cells and erythroblasts. Ineffective erythropoiesis with increased intramedullary cell death and also peripheral red cell destruction because of their

defective membrane architecture, makes the assessment of true iron stores difficult resulting in discordant results.

Assessment of iron stores in bone marrow is considered as "Gold standard" test.[54] In this study, bone marrow iron stores were assessed by Gale's method of grading system and intensive grading system. Only the presence of stainable iron does not define the quantity resolubilized and incorporated iron into the developing erythron. Thus, it is important to differentiate iron deficiency anemia from functional iron deficiency.[54] Functional iron deficiency is a state in which there is insufficient incorporation of iron into erythroid precursors in the face of apparently adequate body iron stores.

Megaloblastic anemia with adequate iron stores and low iron stores was present in 65% and 10% respectively, similar to other studies as shown in the table.[59,62] In 65% cases with adequate stores, 46.16% showed functional iron deficiency.

Table 28: Comparison with other studies in respect to percentage of cases of megaloblastic anemia showing normal iron stores and low iron stores.

	Adequate iron stores	**Low iron stores**
Khan et al[59]	70.87%	29.12%
Tahlan et al[62]	69.1%	17%
Present study	65%	10%

In this study, bone marrow iron stores were assessed by both Gale's method and intensive method. The Gale's grading revealed normal stores in 56.45% cases, iron deficient stores in 32.26% cases and increased stores in 11.29% cases. The intensive grading system demonstrated normal iron stores only in 32.26% cases, functional iron deficiency in 35.48% cases, only iron store deficiency in 8.06% cases, both functional

and iron store deficiency in 24.19%cases. This indicates that most of the cases with normal, decreased or increased iron stores on Gale's method, predominantly showed functional iron deficiency on intensive method. This may be attributed to ineffective erythropoiesis seen in megaloblastic anemia, which is responsible for poor incorporation of iron into red cells resulting in functional iron deficiency.[63]

LHD% (Low hemoglobin density)

Studies on LHD% have proved this as a reliable parameter for study of iron status. LHD% is an hypochromic biomarker. It is an indirect marker of iron restricted erythropoiesis and iron availability in the clinical settings influenced by inflammation and acute phase response. Damodhar et al studied, LHD% with the serum iron markers and concluded that it can be used in the absence of iron profile, as an useful predictor of iron deficiency. In our study, LHD% was compared with the bone marrow iron stores. LHD% was correlated with bone marrow iron stores and was statistically significant, in normal iron stores and iron deficient states. LHD% can be used in diagnosis and monitoring the response to therapy in a reasonable manner.[23,53,54]

Serum Vitamin B12 & Folate assays

Folic acid and vitamin B12 deficiency impairs DNA and folate synthesis resulting in impaired and ineffective erythropoiesis. A study on adolescents in Venezuela, observed high prevalence folic acid deficiency with iron deficiency of 80 to 90%.[63]

Vitamin B12 deficiency or folate deficiency is associated with elevated homocysteine levels, major risk factor of occlusive vascular diseases. Homocysteine stimulates vascular smooth muscle proliferation which is a hallmark of atherosclerosis. One of our patient with deficiency presented with chest pain and was diagnosed to have anteroseptal

myocardial infarction, another patient presented with cerebrovascular accident with upper limb weakness. This presentation may be due to long standing elevated levels of homocysteine, which may be because of deficiency.[64]

The present study shows isolated folate and vitamin B12 deficiencies in 43.5% and 19.3% of patients respectively. Combined folate and vitamin B12 deficiencies are seen in 27.42% cases. The present study showed folate deficiency as the major cause of megaloblastic anemia. Severe folate deficiency may result in low vitamin B12 levels, which is corrected on monotherapy with folic acid alone. Upto 5% of patients with megaloblastic anemia may have results within reference range.[38]

Table 29: Comparison of percentage of cases with vitamin B12, folate and combined deficiencies in our study with other studies conducted on various other groups

Studies	Study group	Folate defn	B12 defn	Combined
Premkumar et al[58]	Adults-pancytopenia	7.14%	81%	3.51%
Suarez T et al[63]	Adolescents	91.02%	19.23%	---
Clarke et al[64]	Older people	7%	6%	---
Bhende et al[65]	Nutri. MA	54.7%	7%	7.3%
Singh B et al[66]	population	---	43%	---
Bhardwaj et al[67]	Adolescent girls	25%	----	---
Present study	Adults-DA	43.55%	19.35%	27.42%

Racial and ethnic factors may influence the normal levels and metabolism of these vitamins. Variation of nutritional deficiencies across the geographical regions is associated with nutritional profile and associated infectious and inflammatory diseases.[67] The laboratory diagnosis of folate deficiency has been more difficult than that of vitamin B12. Serum folate levels are markedly affected by a short period of dietary deprivation

and recent alcohol ingestion.[68] Dietary deprivation may be reason for more patients with folate deficiency in our study, as 62.9% patients in our study were severely anemic with severe illness. 50% of patients presented with fever. Studies have shown that folate store depletion may be consequence of rise in temperature.[36]

Majority of our patients were male, alcoholism along with nutritional deficiency may be the reason for more of folate deficient patients. Megaloblastic anemia in adults due to vitamin B12 deficiency is associated with neurologic abnormalities, none of our patients presented with neurological symptoms.

Its postulated that, reduced endogenously available B12 may be a "complication of migration to a more sanitized environment".[34] In the present study, population studied was from rural areas, and as a result vitamin B12 deficiency may not be as prevalent as shown by other studies. Few cases with high vitamin B12 levels may be attributed to the leucocytosis in these patients.

Iron deficiency masks the expression of vitamin B12 and folate deficiency. If the vitamin deficiency predominates, full morphologic expression of megaloblastic hematopoiesis is seen, but response to vitamin will be incomplete are absent. If iron deficiency predominates, morphologic expression of vitamin deficient state is limited to only myeloid series. Thus, its very important to know the basic etiology and treat efficiently.

In the present study 25.8% of cases showed both iron and vitamin deficiency. In a study by Suarez T et al[63] on adolescents showed simultaneous iron and folate deficiency in 30.76% of anemic cases. As iron status was assessed on bone marrow iron stores, it may not be reliable due to discordant results obtained in cases of masked megaloblastosis

due to associated iron deficiency anemia. In therapeutic point of view, accurate identification of iron, vitamin B12 and folic acid deficiencies are important.

Folate supplementation in the presence of occult vitamin B12 deficiency corrects the hematological signs but neurological symptoms remain unaltered or may get precipitated.[66] When iron therapy is given to the patient with both megaloblastic and iron deficiency anemia, the bone marrow findings of megaloblastic anemia is unmasked and symptoms persist. Other than hematological and neurological manifestations, vitamin B12 and folate deficiencies are less commonly associated with occlusive cardiovascular diseases, osteoporosis and pathological fractures.[64]

Megaloblastic anemia develops over a period of time and most of them are well compensated. Urgent blood transfusion or any form of therapy is not indicated before collection of serum samples for vitamin assays. Assays alone determine which vitamin is deficient. Megaloblastic anemia results in life threatening complications if unrecognized and not treated adequately.

SUMMARY

The present study was conducted from December 2011 to August 2013. A total of sixty two cases of dimorphic anemia were studied in respect to clinical and hematological profile. Following are the observations made -

The peak incidence of dimorphic anemia was seen in second decade, followed by fifth decade. In both male and female the peak incidence was seen in second decade. There was no clear male preponderance in our study.

Majority of patients presented with severe anemia constituting 79.03% cases with mean hemoglobin of 5.74 gm/dl.

Of the 62 cases of dimorphic anemia, 35.5% cases presented with only anemia, 12.9% cases showed associated leucopenia and 17.7% cases showed associated thrombocytopenia and 33.87% cases had pancytopenia.

MCV was normal in 56.45% cases with megaloblastic anemia, could be because of concomitant iron deficiency. MCV alone is unreliable as a screening parameter for anemia associated with vitamin B12 or folate deficiencies. Reliability of MCV can be improved when RDW, RBC histogram and peripheral blood smear examination are considered.

On BM examination of these cases showed, erythroid hyperplasia in 93.54% cases, hypercellular marrow in 67.74% cases and dyserythropoiesis in 50% cases. Megaloblastic maturation was seen in 32.36% cases and megaloblastic with micronormoblastic maturation was seen in 64.52% cases. One case showed features of MDS and another case showed features of multiple myeloma.

Bone marrow iron stores revealed adequate iron stores in 56.45% cases and iron deficient stores in 32.26%. Assessment of iron stores was improved by intense BM examination, which further classified a category – functional iron deficiency, which is of particular importance in areas with high rates of inflammatory condition and also in megaloblastic anemia, associated with functional iron deficiency due to ineffective erythropoiesis. 59.67% cases showed functional iron deficiency.

The mean of MCH and MCHC were statistically significant between the cases with adequate iron stores and deficient iron stores. LHD% known as predictor of iron deficient state, showed statistically significant correlation with the BM iron stores.

Our study showed predominantly folate deficiency, seen in 43.55% cases showed and vitamin B12 deficiency in 19.35%. Both vitamin B12 and folic acid deficiency in 27.42% cases. Iron deficient state with vitamin deficiency was seen in 25.8% cases.

The mean MCH was statistically significant between the cases with iron deficient state and vitamin B12 & folate deficiency. Thus, MCH may be used as specific marker of iron deficiency in borderline cases with normal MCV.

Vitamin B12 and folic acid deficiency should be considered as differential diagnosis for wide spectrum of cases, particularly for anemia of presumed iron deficiency type or cytopenias.

The definite evidence of diagnosis of megaloblastic anemia by bone marrow examination has been undoubtedly proven as a major diagnostic tool, and is a gold standard, when costly investigations like vitamin B12, Folic acid assay are not easily available in our country. But, to establish the nature of deficiency vitamin B12 and folate assays are essential.

In cases of combined nutritional deficiencies of iron, vitamin B12 and folic acid, peripheral blood examination, bone marrow examination and serum ferritin levels may not be of much use for definitive diagnosis, as morphological features of vitamin B12 and/or folic acid deficiencies are masked by iron deficiency. In such situations vitamin B12 and folate assays play a major role. Though, these tests are expensive, its evaluation and early diagnosis of deficiencies of vitamin B12 and folic acid may result in providing cost effective health services, by reducing the hospital stay, unnecessary iron therapy and repeated hospital admissions and blood transfusion.

CONCLUSION

In the present study, the most common basic cause for dimorphic anemia was serum folate deficiency. Majority of cases with these deficiencies presented with pancytopenia.

Good number of cases showed normal MCV even in the presence of deficiency of vitamin B12 and folic acid. Thus, MCV alone is unreliable as a screening parameter for anemia associated with vitamin B12 or folate deficiencies, especially in cases associated with iron deficiency.

Iron assessment can be greatly improved by a more intense marrow examination. LHD% can be used as predictor of iron status, which is cost effective and obtained from the instrument with routine haemogram report.

Concomitant deficiencies of vitamin B12 and/ or folate along with iron deficiency are not infrequent, one type of anemia may mask the other. In the present study, concomitant deficiency was seen in 25.8% of our cases. It is important to establish the correct diagnosis and avoid inappropriate therapy, which may hide the real deficiency resulting in disastrous results.

Serum assays will be appropriate to establish the cause in cases with concomitant deficiencies, as it may result in discordant results with various other tests like hematological parameters, BM morphology and BM iron stores including serum ferritin levels. Thus, hematologic parameters alone are likely to miss deficient cases.

BIBLIOGRAPHY

1. De Benoist B, McLean E, Egli I, Cogswell M. WHO Global Database on Anemia: Worldwide prevalence of anemia 1993–2005; p7.
2. IIPS National Family Health Survey 1998-99 (NFHS-2): Available from: http://www.nfhsindiaorg/india2.html.
3. Galgali SR, Puttaswamaiah RN. Periodontal findings in a patient with Dimorphic anemia. Int J Clin Dental Sci 2011;2(1):33-37
4. Antony AC.Megaloblastic anemias. In: Hoffman R, Benz EJ, Silberstein LE, Heslop H, Weitz J, Anastasi J editors. Hematology Basic principles and practice. 6th Edition. Churchill Livingstone;2012.p519-56.
5. Bain B J. Blood cell morphology in health and disease.In: Lewis S M, Bates I, editors. Dacie and Lewis Practical Haematology, 10thEdition.Churchill Livingstone:Elsevier Ltd;2011.p79-114.
6. Perkins SL. Examination of the blood and bone marrow. In: Greer JP, Foester J, Rodgers GM, editors.Wintrobe's Clinical Hematology. 12th Edition. Philadelphia: Lippincott Williams & Wilkins;2009:p1-20
7. Merriam-Webster Dictionary - Anemia Retrieved on May 25, 2009
8. E Medicine – Anemia, Chronic : Article by Fredrick M Abrahamian, DO, FACEP. Emedicine.com. 2009-12-07.
9. Historical Review. Br J haematol 122. 2003 Blackwell publishing Ltd.554-62
10. Andral G. Essaid'HematologiePathologique. Fortin, Masson and Cie, Paris, 1843.

11. Bunn HF. Anemia. In: Wilson, Isselbacher B, Petersdorf, Martin, Fauci, Roof, editors. Harrison's Principles of Internal Medicine. Vol.1. 12th ed. New York: McGraw Hill; 1991.p344.
12. WHO, UNICEF.UNU. Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva, World Health Organization, 2001.Available at http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/index.html
13. Mckenzie SB, Otto CN. Introduction to Anemia.In: Mckenzie SB, Williams JL, editors. Clincal laboratory hematology, 2nd Edition. New Jersey: Pearson Education; p146-71.
14. Wintrobe. Classification and treatment on the basis of differences in average volume and hemoglobin content of red corpuscles. Arch Intern Med 1934; 54: 256-79.
15. NCCLS. Reference and standard procedure for quantitative determination of hemoglobin in blood. Villanova, PA: NCCLS, 2000.
16. Recommendations for reference method for hemoglobinometry in human blood (ICSH standard 1986) and specifications for international haemoglobincyanide reference preparation (3rd edition). International Committee for Standardization in Haematology; Expert Panel on Haemoglobinometry. Clin Lab Haematol 1987; 9(1): 73-79.

17. Bain BJ, Lewis SM, Bates I. Basic haematological techniques.In: Bain B J, Bates I.editors. Dacie and Lewis Practical Haematology, 10th Edition.Churchill Livingstone: Elsevier Ltd;2011.p11-24
18. England JM, Down MC. Red cell volume distribution curves and the measurement of anisocytosis. Lancet 1974;1:701-3
19. Simel DL. Is the RDW-MCV classification of anaemia useful ? Clin Lab Haematol 1987;9:349-59.
20. Lawrence A, Young M, Cooper A. Red cell histograms in the diagnosis of diseases. In: Simpson E, ed. Hematology Beyond the Microscope. New York: Technicon Instruments;1984:155–64.
21. Ryan DH. Examination of blood cells.In: Lichtman MA, Kipps TJ, Seligsohn, editors.In: Williams Hematology. 8th Edition. New York : The McGraw-Hill Companies;2010.
22. Bessman DJ, Gilmer PR, Gardner FH. Improved classification of Anemias by MCV and RDW. Am J Clin Pathol 1983;80(3):322-6
23. Urrechaga E, Borque L, Escanero JF. Erythrocyte and reticulocyte indices on the LH 750 as potential markers of functional iron deficiency. Anemia 2010;2010:1-7
24. Urrechaga E, Borque L, Escanero JF. Assessing Iron Status in CKD Patients: New Laboratory Parameters, Chronic Kidney Disease, Göőz M (Ed.), ISBN: 978-953-51-0171-0, In Tech, DOI: 10.5772/25645.(2012). Available from: http://www.intechopen.com/books/chronic-kidney-disease/assessing-iron-status-in-ckd-patients-new-laboratory-parameters

25. Lewis SM. Reference ranges and normal values.In: Bain B J, Bates I, editors. Dacie and Lewis Practical Haematology, 10th Edition.Churchill Livingstone: Elsevier Ltd;2011.p11-24.
26. Constantino BT. The red cell histogram and the dimorphic red cell population. Lab Med 2011;42:300-8.
27. Systematic approach to the evaluation of anemia. Available at: http://www.rspady.com/Pages/Links.asp?Action=Content&LinkID=687&ParentLinkID=158.
28. Beutler E. Disorders of Iron Metabolism. In: Marshall A, Beutler E, Seligsohn U, Kaushansky K, Kipps TJ**.** editors. William's hematology.7th ed. New York: Mc Graw Hill-Medical Publishing Division; 2006.p518-33.
29. Ganz T. Anemia of Chronic Disease. In:Marshall A, Beutler E, Seligsohn U, Kaushansky K, Kipps TJ**,** editors. William's hematology.7th ed. New York:Mc Graw Hill-Medical Publishing Division; 2006.p561-70.
30. Addison T. Anemia – disease of the suprarenal capsules. London Med Gazette 1849;43:517-8.
31. Chanarin. The megaloblastic anemias: Black well scientific publications. Oxford and Edinburgh:1969.
32. Ryan DH, Cohen HJ. Bone marrow aspiration and morphology. In :Hoffman R, Benz EJ, Shathil SJ, Furie B, Cohoen HJ,Silberstein LE et al,editors.Haematology basic principles and practice, 3rd Edition. Philadelphia: Churchill Livingstone 2002; p2248-460.

33. Carmel R. Megaloblastic anemias:Disorders of impaired DNA synthesis. In: Greer JP, Foester J, Rodgers GM, editors.Wintrobe's Clinical Hematology. 12th Edition. Philadelphia: Lippincott Williams & Wilkins;2009.p1367-97.
34. Khandari U, Sharma A. Megaloblastic anemia: prevalence and causative factors. Natl Med J India 2007;20(4):172-5.
35. Chandra J. Megaloblastic Anemia: back in focus. Indian J Pediatr 2010;77(7):795-9.
36. Iqbal SP, Kakepoto GN, Iqbal SP. Vitamin B12 deficiency-a major cause of megaloblastic anemia in patients attending a tertiary care hospital. J Ayub Med Coll Abbottabad 2009;21(3):92-4.
37. Frenkel EP, Yardley DA. Clinical and laboratory features and sequelae of deficiency of folic acid (folate) and vitamin B12 (cobalamin) in pregnancy and gynecology. Hematol oncol clin North Am 2000;14(5):1079-98.
38. Hamilton M, Blackmore S. Investigation of megaloblastic anemia-cobalamin, folate and metabolic status. In: Lewis S M, Bain B J, Bates I,editors.Dacie and Lewis Practical Haematology,10th Edition.Churchill Livingstone:Elsevier Ltd; 2011.p161-86
39. Spivak JL. Masked megaloblastic anemia. Arch Intern Med 1982;142:2111-5
40. Weyden MVD, Rother M, Firkin B. Megaloblastic maturation masked by Iron deficiency:a biochemical basis. Br J Hematol 1972;22:299-306.
41. Kouides PA, Bennett JM. Morphology and classification of myelodysplastic syndromes. Hematol Oncol Clin North Am 1992;6(3):485-99.

42. Mufti GJ, Bennett JM, Goasguen J, Bain BJ, Baumann I, Brunning R, et al. Diagnosis and classification of myelodysplatic syndrome: International working group on morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. Haematologica 2008;93(11):1712-7

43. Rosenfeld C, List A. A hypothesis for the pathogenesis of myelodysplastic syndromes: implications for new therapies. Leukemia.2000;14(1):2-8.

44. TefferiA, Vardiman JW. Myelodysplastic syndromes. N Engl J Med 2009;361:1872-85

45. Asif N, Hassan K, Qazi H, Zafar T. Myelodysplastic syndrome: Megaloblastic anemia to Aplastic anemia to Refractory anemia with excess Blast in transformation. Int J Pathol 2008;2:1-4.

46. Chun K. Myelodysplastic Syndromes.In: Tkachuk DC, Hirschmann JV, editors.Wintrobe's Atlas of clinical Hematology.1st Edition. Lippincott Williams & Wilkins;2007.p95-104

47. Vardiman JW. Hematopathological Concepts and Controversies in the diagnosis and Classification of Myelodysplastic Syndromes. Hematology Am Soc Hematol Educ Program 2006:199-204.

48. Wang SA. Diagnosis of Myelodysplastic Syndromes in Cytopenic Patients. Hematol Oncol Clin North Am 2011;25(5):1085–110.

49. Bartl R, Frisch B, Baumgart R. Morphologic classification of the myelodysplastic syndromes (MDS): combined utilization of bone marrow aspirates and trephine biopsies. Leuk Res 1992;16(1):15-18

50. Komroji RS, Zhang L, Bennett JM. Myelodysplastic syndromes classification and risk stratification. Hematol Oncol Clin North Am 2010;24(2):443-57.

51. Bain B J. Normal Bone marrow. In: Clark D M, Wilkins B S.Bone marrow pathology, 4th Edition.Wiley-Blackwell Ltd;2010.p1-41

52. Bates I. Bone marrow biopsy. In: Lewis S M, Bain B J, Bates I.editors. Dacie and Lewis Practical Haematology, 10th Edition.Churchill Livingstone:Elsevier Ltd; 2011: p130

53. Damodar S, Raghunath ST, Murthy S, Jayanthi KJ, Latha BRS. Low Hemoglobin Density as a Measure of Iron Status. Indian J Hematol Blood Transfus 2013; 29(2):75-76

54. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I. Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol 2013;161(5):639-648.

55. Barth D, Hirschmann JV. Anemia. In: Douglas TC, Hirschmann JV, editors. Wintrobe's Atlas of clinical hematology.1st edition. Lippincott Williams & Wilkins;2007.p14.

56. Bableshwar RS, Roy M, Bali A, Patil PV, Inumella S. Intensive method of assessment and classification of the bone marrow status: A study of 80 patients. Indian J Pathol Microbiol 2013;56(1):16-19.

57. WHO. Haemoglobin concentrations for the diagnosis of anemia and assessment of severity. Vitamin and Mineral Nutrition Information System.Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1) (http://www.who.int/vmnis/indicators/haemoglobin.pdf, accessed on Jan 2012).

58. Premkumar M, Gupta N, Singh T, Velpandian T. Cobalamin and folic acid status in relation to the etiopathogenesis of pancytopenia in adults at a tertiary care centre in North India. Anemia 2012;2012:1-12.

59. Khan S, Raziq F, Qureshi H. Association of megaloblastic anemia with peripheral cytopenias. J Postgrad Med Inst 2009;23(1):46-50.

60. Manuel K, Padhi S, Varghese RGB. Pyrexia in a patient with megaloblastic anemia: A case report and literature review. Indian J Med Sci 2013;38(2):198-202.

61. Metikurke SH, Rashmi K, Bhavika R. Correlation of Bone marrow aspirate, biopsies and touch imprint findings in pancytopenia. J Hematol 2013 May [cited 2013 June] 2(1):8-13. Available from: http://www.jh.elmerpress.com/index.php/jh/article/view/76/53

62. Tahlan A, Bansal C, Palta A, Chauhan S. Spectrum and analysis of bone marrow findings in anemic cases. Indian J Med Sci 2008;62(8):336-9.

63. Suarez T, Torrealba M, Villegas N, Osorio C, Garcia-Casal MN. Iron, folic acid and vitamin B12 deficiencies related to anemia in adolescents from a region with a high incidence of congenital malformations in Venezula. Arch Latinoam Nutr 2005;55(2):118-23.

64. Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P et al. Screening for vitamin B12 and folate deficiency in older persons. Am J Clin Nutr 2003;77(5):1241-7.

65. Bhende YM. Some experiences with nutritional megaloblastic anemia. J Postgrad Med 1965;11(4):145-55.

66. Singh B, Gupta VK, Venkatesan M. Burden of vitamin B12 deficiency in urban population in Delhi, India: A hospital based study. Int J of Pharma & Bio Sci 2011;2(1):521-7.

67. Bhardwaj A, Kumar D, Raina SK, Bansal P, Bhushan S, Chander V. Rapid assessment for coexistence of vitamin B12 and iron deficiency anemia among adolescent males and females in Northern Himalayan state of India. Anemia 2013;2013:1-5.

ANNEXURE – I

INFORMED CONSENT FORM

I undersigned _____ have been explained in my own language about the procedure of bone marrow aspiration and the risks and complications of the procedure. After understanding the purpose of this procedure and its complications. I agree to undergo the procedure.

Subject name and signature/ Thumb impression DATE:

Parents / Guardians name / Thumb impression DATE:

Signature of the person taking consent DATE:

ANNEXURE – II

PROFORMA

Name : **Case No.:**

Age / Sex : Bone marrow report no.:

Address : Hospital No.:

Presenting complaints:

Fatigue / Dyspnoea / Palpitations / Giddiness

Fever / Infection

Bleeding diathesis

Others -

Past history:

Blood transfusions in past

Diabetes / Hypertension / Renal disease

Treatment history : Drugs /Exposure to radiation / Chemotherapy

Any surgeries -

Family history :

Malignancy /Anemia /Bleeding disorders /Genetic illness

Personal history:

Diet : Vegetarian / Non-Vegetarian. Appetite :

Alcohol intake : Bowel / Bladder habits :

General physical examination :

Pulse: BP:

Respiratory Rate: Febrile / Afebrile

Pallor / Jaundice / Lymphadenopathy / Edema

Others:

Systemic examination :

CVS :

RS :

CNS :

PA:

Clinical Diagnosis :

INVESTIGATIONS

Complete Haemogram :

RBC		MCV	
Hb		MCH	
Hct		MCHC	
WBC		RDW	
Platelet		LHD%	

Peripheral Smear :

Reticulocyte count –

Bone marrow No.:

Cellularity : Hypocellular/ Normocellular/ Hypercellular

M: E ratio :

Erythropoiesis :

Leucopoiesis :

Megakaryopoiesis :

IMPRESSION :

Special stain : Pearls stain

Serum Vitamin B12 levels :

Serum folate levels :

Other tests :

SIGNATURE OF GUIDE **SIGNATURE OF CANDIDATE**

KEYS TO MASTER CHART

M – Male

F – Female

\+ Present

\- Absent

Symptoms –

1 - Fatigaubility

2 - Fever

3 - Respiratory Distress

4 - Bleeding manifestations

Signs –

P – Pallor

I – Icterus

H – Hepatomegaly

S – Splenomegaly

L – Lymphadenopathy

E – Edema

Complete blood counts

Hb – Hemoglobin

RBC – Red Blood Cell Count

WBC – White Blood Cell Count

PLT – Platelet Count

MCV – Mean Corpuscular Volume

MCH – Mean Corpuscular Hemoglobin

MCHC - Mean Corpuscular Hemoglobin Concentration

RDW – Red Cell Distribution Width

PBS – Peripheral Blood Smear Impression

DA – Dimorphic anemia

DA with NL - Dimorphic anemia with neutrophilic leucocytosis

DA with leuco - Dimorphic anemia with leucopenia

DA & thrombo - Dimorphic anemia with thrombocytopenia

Bone marrow findings

I - Cellularity

Normo - Normocellular

Hyper - Hypercellular

II – Erythroid Series

EH – Erythroid Hyperplasia

N - Normoblastic maturation

MI - Micronormoblastic maturation

ME - Megalobalstic maturation

DE – Dyserythropoiesis

III – Impression

Megaloblastic – Megaloblastic maturation

Dimorphic – Megaloblastic and micronormoblastic maturation

RAEB-2 – Refractory anemia with excess blasts - 2

MM – Multiple Myeloma

Iron Stores

IGM – Intensive grading method

F – Fragments

M – Macrophage

E – Erythroblast

LHD% - Low Hemoglobin Density

Case No.	Age	Sex	Hp no.	BM No.	Symptoms				Signs						Complete blood counts									PBS	Bone marrow findings								Iron stores					Vitamin assays	
																Counts			RBC indices						Cellularity	Erythroid Series					Impression		Pearl's stain					
																																IGS			Gal			
					1	2	3	4	P	I	H	S	L	E	Hb	RBC	WBC	PLT	MCV	MCH	MCHC	RDW			EH	N	MI	ME	DE			F	M	E	e's	LHD%	B12	Folate
1	29	F	744909	40/11	-	+	-	-	+	-	-	+	-	-	7	3.6	8.2	180000	89	18	29.8	18	DA	Normo	+		+	+	-	Dimorphic		-	+	-	1	76.8	814	2.5
2	20	M	751195	44/11	-	-	+	+	+	+	-	-	-	-	6	1.7	2	20,000	92	32	35.2	18	Pancytopenia	Hyper	+		+	+	+	Dimorphic		-	-	-	1	0.93	195	5.1
3	70	M	752154	45/11	-	-	+	-	+	-	-	-	-	-	7	2.2	17	1,91,000	99	26	31.8	21	DA with NL	Hyper	+		-	+	+	Megaloblastic		+	-	-	2	19.4	119	21
4	55	F	760939	46/11	+	-	+	-	+	-	-	-	-	+	3	1.3	3.5	25,000	90	29	32.2	16	Pancytopenia	Normo	+		+	+	+	Dimorphic		-	+	-	1	13.7	230	4.8
5	20	M	763355	47/11	-	+	-	-	+	-	-	-	-	-	9	2.2	3.8	10,000	112	41	36.3	15	Pancytopenia	Normo	+		+	+	+	Dimorphic		+	-	-	3	0.34	512	3.2
6	30	M	768798	1/12	-	-	+	-	+	+	+	-	-	-	2	1.2	3.5	237000	87	32	34	16	DA with leuco	Hyper	+		+	+	+	Dimorphic		+	+	-	2	2.73	416	4.2
7	26	F	782095	3/12	+	+	-	-	+	-	-	-	-	+	4	1	3.2	50,000	106	40	37.3	15	Pancytopenia	Hyper	+		-	+	-	Megaloblastic		+	+	-	2	0.14	113	1.8
8	40	M	783450	4/12	+	+	-	-	+	+	-	-	-	-	4	1	3.2	154000	103	41	39.3	26	DA with leuco	Hyper	+		+	+	-	Dimorphic		+	+	-	2	0.02	182	3.4
9	58	M	787060	5/12	+	-	-	-	+	-	-	-	-	-	9	3.7	4.4	300,000	86	25	32.5	19	DA	Normo	+		+	+	+	Dimorphic		-	-	-	1	11.5	673	1.9
10	40	F	793765	6/12	-	+	+	-	+	-	-	-	-	+	9	2.9	6.1	93,000	95	31	32.3	15	DA&thrombo	Hyper	+		-	+	-	Megaloblastic		+	+	-	2	12.5	872	2.9
11	55	M	795994	8/12	+	-	-	+	+	-	-	-	-	-	4	2.2	1.8	91,000	80	20	28.2	17	Pancytopenia	Normo	+		+	+	+	Dimorphic		+	-	-	2	98	180	20
12	23	F	797503	9/12	+	-	+	-	+	-	-	-	-	+	8	2.4	14	30,000	82	30	35.8	16	DA&thrombo	Hyper	+		+	+	+	Dimorphic		+	+	+	2	0.54	312	2.8
13	25	M	793846	10/12	+	-	-	-	+	-	-	-	-	-	9	3.8	5.3	150000	86	24	31.1	16	DA	Normo	-		-	+	-	Megaloblastic		+	+	-	3	34.8	150	5.6
14	50	F	807936	12/12	+	-	-	-	+	-	-	-	-	-	5	1.6	3.2	40000	95	31	32.4	15	pancytopenia	Hyper	+		+	+	+	Dimorphic		-	+	-	1	11.5	481	2.75
15	32	F	816351	13/12	+	-	-	-	+	-	-	-	-	-	3	1	3	39,000	93	31	33.5	23	pancytopenia	Hyper	+		-	+	+	Megaloblastic		+	+	+	3	4.28	1020	4.1
16	55	F	818376	14/12	+	+	-	-	+	-	-	-	-	-	5	3.2	6.2	400,000	80	16	28.5	17	DA	Normo	+		-	+	+	Megaloblastic		+	+	-	2	96.8	812	3.2
17	55	F	802679	15/12	+	+	-	-	+	-	-	-	-	-	6	2	2.6	3,00,000	91	30	33.3	15	DA with leuco	Normo	+		+	+	+	Dimorphic		+	-	+	2	5.12	930	2.4
18	21	M	820352	16/12	-	-	+	-	+	-	+	-	+	-	3	1.1	6.9	60,000	98	31	32.1	18	DA&thrombo	Hyper	+		+	+	-	Dimorphic		-	+	-	1	14.9	184	3.8
19	75	M	806346	17/12	+	+	-	-	+	-	-	-	-	-	8	4.1	13	3,22,000	90	21	31.2	16	DA with NL	Hyper	+		+	+	-	Dimorphic		-	-	+	1	32.2	1099	24
20	21	F	821348	19/12	+	-	+	-	+	-	-	-	-	-	3	0.9	1.9	45,000	98	34	34.9	22	pancytopenia	Hyper	+		-	+	+	Megaloblastic		+	+	+	4	1.22	45	0.4
21	20	M	820274	20/12	-	-	+	-	+	+	-	+	-	-	3	4.3	4.3	125,000	125	42	33.1	22	DA&thrombo	Hyper	+		+	+	-	Dimorphic		+	+	+	2	6.13	198	4.6
22	35	M	840640	23/12	-	+	-	-	+	-	-	-	-	-	6	2.1	5	1,50,000	97	35	36	15	DA	Hyper	+		-	+	+	Megaloblastic		+	+	+	3	0.45	597	2.3
23	70	M	839439	24/12	-	+	-	-	+	-	-	-	-	-	9	2.9	7.5	235,000	98	31	32.4	16	DA with NL	Normo	+		+	+	-	Dimorphic		-	+	-	1	11.5	813	2.1
24	23	M	838765	26/12	+	+	-	-	+	-	-	-	-	-	10	2.5	5.5	180,000	115	39	33.7	16	DA	Hyper	+		-	+	+	Megaloblastic		+	+	+	3	3.57	217	1.5
25	27	M	832444	27/12	+	-	-	-	+	+	-	-	-	-	5	3.6	3	150000	82	18	27.1	15	DA with leuco	Hyper	+		+	+	-	Dimorphic		+	-	-	2	99.7	193	1.2
26	35	F	846611	28/12	-	+	-	-	+	-	-	-	-	-	8	4.2	12	185000	82	18	27.6	15	DA with NL	Hyper	+		+	+	-	Dimorphic		+	-	-	2	99.3	314	1.6
27	62	M	848984	29/12	+	-	-	-	+	-	+	+	-	-	3	1.7	4.1	24,000	109	34	31.5	15	DA&thrombo	Normo	+		+	+	-	Dimorphic		-	+	-	1	25.1	345	2.6
28	70	F	848888	30/12	+	-	+	-	+	-	-	-	-	-	8	2.9	4.2	60000	110	38	32.8	16	DA&thrombo	Normo	+		+	+	-	Dimorphic		+	-	-	2	8.02	200	1.8
29	32	M	853170	31/12	-	+	-	-	+	-	-	-	-	-	3	0.7	1.3	90,000	120	41	30.9	16	pancytopenia	Hyper	+		-	+	+	Megaloblastic		+	+	-	4	40.6	126	5.8
30	65	F	851785	32/12	+	-	-	-	+	-	-	-	-	+	7	4.2	9.9	196000	99	17	32.3	16	DA	Normo	-	+	-	-	-	MM		-	-	+	1	12.5	734	5.7
31	60	F	858165	33/12	-	+	-	-	+	-	+	+	-	-	10	3.6	1.8	3,08,000	87	26	30.9	16	DA with leuco	Normo	+		+	+	-	Dimorphic		+	+	-	2	40.6	208	11
32	70	M	858979	35/12	-	+	+	-	+	-	-	-	-	-	5	1.4	1.6	90,000	121	38	32.3	15	pancytopenia	Hyper	+		+	+	-	Dimorphic		+	+	-	2	12.5	116	1.3
33	27	F	858512	36/12	+	+	-	-	+	-	-	-	-	-	10	3	3.5	66,000	108	33	30.4	15	pancytopenia	Hyper	+		-	+	-	Megaloblastic		+	+	+	4	57.2	164	3.4
34	55	F	862858	37/12	+	-	-	+	+	-	-	+	-	-	5	2.6	2.3	90,000	82	18	27.4	16	pancytopenia	Hyper	+		-	+	+	Megaloblastic		-	+	-	1	99	944	1.9
35	20	F	856498	38/12	+	+	-	-	+	-	-	+	-	-	4	1	11	1,20,000	103	39	31.4	15	DA&thrombo	Hyper	+		-	+	+	Megaloblastic		+	+	-	4	27.3	112	18

Case No.	Age	Sex	Hp no.	BM No.	Symptoms				Signs						Complete blood counts								PBS	Bone marrow findings							Iron stores					Vitamin assays	
															Hb	Counts			RBC indices					Cellularity	Erythroid Series					Impression	Pearl's stain				LHD%		
																									E H	N	M I	ME	DE		IGS F	IGS M	IGS E	Gal e's		B12	Folate
					1	2	3	4	P	I	H	S	L	E		RBC	WBC	PLT	MCV	MCH	MCHC	RDW															
36	25	F	866259	40/12	-	+	-	-	+	-	+	+	-	-	6	3	2.8	150,000	80	20	28.8	15	DA with leuco	Normo	-		+	+	-	Dimorphic	+	+	-	2	94.7	318	3.6
37	22	M	868717	41/12	+	-	-	-	+	-	-	-	-	-	3	2.3	7	2,95,000	85	19	25.4	17	DA	Normo	+		+	+	-	Dimorphic	-	-	-	0	99	146	6
38	20	F	868326	42/12	+	+	-	-	+	-	+	+	-	-	5	1.6	3.1	150,000	93	29	30.9	15	DA with leuco	Hyper	+		+	+	-	Dimorphic	-	-	-	0	40.7	158	4.2
39	40	M	870593	43/12	-	+	+	-	+	-	-	-	+	+	4	1.6	17	1,42,000	89	27	33.4	25	DA with NL	Hyper	+		+	+	+	Dimorphic	+	+	+	4	4.68	1183	0.9
40	20	F	870720	44/12	+	+	-	-	+	-	-	-	-	-	9	4.5	3.8	204,000	84	19	29.8	15	DA with leuco	Normo	-		+	+	-	Dimorphic	-	-	+	1	76.8	270	9.1
41	36	M	871239	46/12	+	+	-	-	+	-	-	-	-	-	6	4.6	8.1	271,000	88	28	31.8	15	DA	Hyper	+		+	+	+	Dimorphic	-	+	+	1	19.4	159	6.1
42	50	F	872604	2/13	-	+	-	-	+	-	-	-	-	-	7	1.7	4.2	1,00,000	112	39	35.1	15	DA&thrombo	Hyper	+		+	+	-	Dimorphic	+	+	+	2	1.02	121	3.1
43	56	M	843534	3/13	-	-	+	+	+	-	-	-	-	-	5	3.2	3.4	1,20,000	98	34	34.5	22	DA&thrombo	Hyper	+		+	+	+	Dimorphic	+	+	+	3	1.74	312	2.1
44	53	M	880148	5/13	+	+	-	-	+	-	-	-	-	+	7	2.2	3.2	90,000	99	26	31.8	21	Pancytopenia	Normo	+		+	+	-	Dimorphic	-	+	-	1	19.4	201	20
45	22	M	846052	6/13	+	+	-	-	+	+	+	-	-	-	7	2.5	2.3	58000	89	28	30.8	15	Pancytopenia	Hyper	+		+	+	-	Dimorphic	+	+	-	3	43.8	817	2.9
46	21	F	898282	8/13	+	+	-	+	+	-	-	-	-	-	6	1.4	2.6	55000	113	41	35.8	15	Pancytopenia	Hyper	+		+	+	+	Dimorphic	+	+	+	2	0.54	30.5	7.2
47	60	M	899954	9/13	+	-	-	-	+	-	-	-	-	-	7	3.4	8.4	3,55,000	80	22	31.2	16	DA	Hyper	+		-	+	+	Megaloblastic	+	+	-	3	32.2	1487	2.9
48	43	F	902480	12/13	+	-	-	-	+	-	-	-	-	-	7	4.2	9.9	196000	80	17	29.5	16	DA	Hyper	+		+	+	-	Dimorphic	-	+	+	1	68.1	271	24
49	65	F	904866	13/13	+	+	-	-	+	-	+	+	-	-	11	2.1	16	4,70,000	98	36	32.3	17	DA with NL	Hyper	-		-	+	-	Megaloblastic	+	+	-	4	12.5	204	12.1
50	20	F	904535	14/13	+	-	-	-	+	-	-	+	-	-	10	5.6	1.9	93000	80	17	31.1	20	Pancytopenia	Normo	+		+	+	-	Dimorphic	+	-	-	2	34.8	719	10.8
51	60	F	903844	16/13	+	-	-	-	+	-	-	-	-	-	8	4.6	4.9	240,000	82	20	32.8	17	DA	Normo	+		-	+	+	Megaloblastic	+	+	+	3	8.02	45	7.4
52	30	M	925095	18/13	-	+	-	-	+	-	-	-	-	-	3	2	3.1	150000	80	16	28.3	16	DA with leuco	Hyper	+		+	+	-	Dimorphic	+	+	-	2	97	1199	2.9
53	40	M	922269	20/13	-	+	-	-	+	-	-	-	-	-	7	1.2	1.8	87000	81	32	40.5	15	pancytopenia	Hyper	+		-	+	+	RAEB-2	+	+	+	5	0.01	861	24
54	52	M	928903	21/13	-	-	+	-	+	-	-	-	-	+	7	1.9	13	217000	102	35	34	16	DA with NL	Hyper	+		-	+	-	Megaloblastic	+	-	+	2	2.73	1000	4.8
55	52	F	930428	22/13	+	-	-	-	+	-	-	-	-	-	3	1	2.2	68,000	100	36	34.5	17	Pancytopenia	Hyper	+		-	+	+	Megaloblastic	+	+	+	3	1.74	367	4.3
56	30	M	932487	23/13	-	+	-	+	+	+	-	-	-	-	8	2.7	5	75,000	92	30	32.5	15	DA&thrombo	Hyper	+		+	+	+	Dimorphic	+	-	+	2	10.4	150	1.6
57	29	F	931636	24/13	+	+	-	-	+	-	-	-	-	-	2	1.2	8.5	447000	83	20	27.2	18	DA	Normo	+		+	+	+	Dimorphic	-	-	-	0	99.7	118	5.2
58	20	M	938448	25/13	-	+	-	-	+	-	-	-	-	-	3	0.9	0.7	46000	86	29	33.9	23	pancytopenia	Hyper	+		+	+	+	Dimorphic	+	+	+	3	0.49	214	1.6
59	57	M	938045	26/13	+	-	-	-	+	-	-	-	-	-	2	3.4	2.2	68000	103	36	34.5	17	Pancytopenia	Hyper	+		-	+	+	Megaloblastic	+	-	+	2	1.74	123	2.1
60	52	M	942269	27/13	+	-	+	-	+	-	-	-	-	-	2	6.5	4.4	74000	120	41	31.3	17	DA&thrombo	Hyper	+		-	+	-	Megaloblastic	-	-	-	0	29.6	415	3
61	70	M	895557	29/13	-	+	-	-	+	-	-	-	-	-	1	6.7	3.3	89000	128	45	32.4	16	Pancytopenia	Hyper	+		+	+	-	Dimorphic	+	-	+	2	11.5	192	4.1
62	23	F	950609	30/13	+	-	-	-	+	-	-	-	-	-	5	1.6	2	30000	96	31	32	19	Pancytopenia	Hyper	+		+	+	+	Dimorphic	-	+	-	1	16.3	209	2.9