ASSESSMENT OF BONE MARROW IRON STORES IN VARIOUSHEMATOLOGICAL DISORDERS AND ITS CORRELATION WITH SERUM FERRITIN

By

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In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

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"The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires."

- William Arthur Ward.

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LIST OF ABBREVIATIONS USED

IDA	Iron deficiency anemia
NFHS	National Family Health Survey
sTfR	Serum transferrin receptor
ITP	Idiopathic thrombocytopenic purpura
SF	Serum ferritin
ACD	Anemia of chronic disease
CRP	C - reactive protein
ESR	Erythrocyte sedimentation rate
MDS	Myelodysplastic syndrome
CMP	Common myeloid progenitor
GMP	Granulocyte macrophage progenitor
COPD	Chronic obstructive pulmonary disease
MEP	Megakaryocyte erythroid progenitor
CLP	Common lymphoid progenitor
BFU-E	Erythroid burst forming units
CFU-E	Erythroid colony forming unit
HSC	Hematopoietic Stem cell
CSF	Colony stimulating factor
BM	Bone marrow
TPO	Thrombopoietin
CFUMK	Megakaryocyte colony forming unit
DC	Dendritic cells

cDC	Conventional dentritic cells
pDC	Plasmacytoid dentritic cells
MSC	Mesenchymal stem cells
Tf	Transferrin
PAS	Periodic acid schiff
MPO	Myeloperoxidase
$Fe2^{+}$	Ferrous ions
$Fe3^{+}$	Ferric ions
TIBC	Total iron binding capacity
CMIA	Chemiluminescent microparticle immuno assay

ABSTRACT

BACKGROUND

Hematological disorders usually present with anemia. Bone marrow estimation of iron stores remains the gold standard, also serum ferritin estimation reflects the iron stores in the body and decreased levels indicate nutritional anemias. (Iron Deficiency, Megaloblastic anaemia). Ferritin also serves as an positive acute phase reactant wherein the levels increase in inflammatory conditions, liver diseases, malignancies etc. leading to false positivity in setting of anemia

OBJECTIVE

Assessment of Bone marrow Iron stores in various hematological disorders and its correlation with Serum Ferritin.

MATERIALS

Bone marrow aspirate was performed at the PSIS, sternum whichever was suitable for patients havimg hematological disorders. The smears were fixed in methanol and stained with Prussian for iron stores with a positive control of old hemorrhage/ hemosiderin laden macrophages. Peripheral blood was collected at the same setting for complete blood count and serum ferritin level estimation.

The iron stores were graded by Gale's grading method. Serum ferritin was estimated by the Chemiluminescent Microparticle Immunoassay method.

RESULTS

110 cases of bone marrow iron stores were studied. RBC disorders were predominant accounting to 55.5% of total 110 cases. Diminished iron stores were seen in RBC disorders 61.7%. WBC disorders had increased iron stores (20%) and serum ferritin in females. Serum ferritin was estimated in 70/100 cases. Co-relational co-efficient was 0.547 which inferred

that at diminished iron stores in body the serum ferritin levels was as high as >2000 ng/ml, whereas in normal to high levels of iron stores showed negligible ferritin levels. Serum ferritin levels ranged from 1.4-2001 ng/ml. RBC disorders showed high incidence of normal, high and low serum ferritin levels in males i.e. (50.0%), (55.6%), and (72.7%) respectively. Females showed normal and decreased ferritin values in RBC disorders increased in WBC disorders. Nutritional anemias had diminished iron stores (78.6%) with decreased ferritin levels as same was the case with non – nutritional anemias (100%). But normal ferritin levels also had diminished and increased iron stores.

CONCLUSION

Serum ferritin reflects the iron stores in the body in consistence with bone marrow iron stores. But the fact that its levels are increased during inflammatory conditions, liver diseases and malignancies serve us to be aware of such conditions and avoid the false positivity of the results.

KEY WORDS: Anemia, iron stores, serum ferritin, acute phase reactant.

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INTRODUCTION

Anemia is common worldwide and particularly in developing countries. Hematological disorders usually present with anemia. However bone marrow examination confirms the diagnosis by giving explanation for cytopenias and leukemias.[1] Combination of surrogate markers, namely Serum Ferritin (SF), serum Total Iron Binding Capacity (TIBC), and percentage saturation of transferrin are routinely employed to assess the iron status in clinically suspected cases of IDA (Iron Deficiency anemia).[2]

The National Family Health Survey (NFHS-3) conducted in 2005-06 estimates an alarmingly high prevalence of anemia in India, with 70% pregnant women and 24% adult men presenting with anemia.[2]

Assessment of the bone marrow biopsy iron stores is often necessary to resolve the diagnosis. Microscopic examination of bone marrow aspirate is the gold standard for assessing marrow iron store.[2]

Anemic patients have reduced hemoglobin and eventually low oxygen carrying capacity and therefore suffer from multiple co-morbidities, hence their serum ferritin and transferrin saturation are altered in many conditions other than iron deficiency.[2]

Therefore, these two parameters cannot be made the absolute criteria for diagnosing iron deficiency anemia. Diseases associated with defective reticuloendothelial system release of iron may be difficult to distinguish from the iron deficient state since serum iron parameters (serum iron, percentage saturation of transferrin < 15%) may overlap in such conditions.[2]

Serum ferritin reflects the body iron reserve and is generally the determinant of the anemic nature of the individual.[2] However, serum ferritin is an acute phase reactant whereby concentration increases during infections, inflammation, liver disease, malignancy, haemodialysis and thereby rendering the interpretation of normal or high serum ferritin values difficult in such situations.[2] Information about iron stores in bone marrow among various hematological disorders particularly in non-IDA patients are scarcely available. Also utility of serum ferritin in non-IDA patients is less understood in published literatures.[2]

Therefore, the study was carried out with a purpose of finding the behaviour of iron stores, its spectrum of activity in different hematological disorders and its correlation with serum ferritin.

OBJECTIVE

To assess the bone marrow iron stores in various hematological disorders and its correlation with serum ferritin.

REVIEW OF LITERATURE

The spectrum of hematological disorders is very wide. Bone marrow examination is a useful tool in reaching the final diagnosis in most of the cases.[1] The increasing prevalence of multiple co-morbidities among anemic patients with chronic diseases have made the use of serum ferritin more challenging in diagnosing iron deficiency.[2]Serum ferritin reflects the total body iron store and a low level is indicative of depleted iron stores and a hypoferremic state.[2]

The microscopic examination of stainable iron in the bone marrow aspirate smear is generally considered the reference standard for determining the body iron stores[3,4]. In normal healthy individual iron is stored in liver, muscle and bone marrow. Bone marrow iron is contained mainly in macrophages that release it to the developing erythron for the synthesis of hemoglobin. The commonest indication of bone marrow aspirate is pancytopenia (50%), followed by bicytopenia (36%).[3]

Conventionally, iron in bone marrow has been primarily assessed in marrow fragments which represent iron stores in the form of hemosiderin.[4] The body iron reserve is traditionally assessed by the biochemical markers of iron metabolism, namely serum ferritin, serum iron, total iron binding capacity.[4]

In view of all these different kind of investigations, serum ferritin and serum iron is performed and correlated with the results of stainable bone marrow iron.[5]

Bableshwar *et al*[2] conducted a study with intensive method of grading bone marrow iron stores on 80 subjects with anemia and no history of transfusion in preceding four weeks to assess bone marrow iron in fragments, in macrophages around fragments and in erythroblasts and to correlate the marrow iron stores with

serum ferritin. Out of 80 adults patients recruited in study, 60% (40/80) were male and 40% (32/80) were female. The minimum age was 16 years and maximum was 86 years, (average 41.6years). The intensive grading system revealed normal iron stores in 37.5% cases, depleted iron stores in 16.25% patients while 23.75% and 22.5% patients had functional iron deficiency and combined deficiency, respectively. Mean log ferritin concentration was significantly lower in patients with depleted iron stores in comparison to those with normal iron stores, functional iron deficiency or combined deficiency. They concluded that intensive marrow iron examination provides a clinically useful iron status classification which is of particular importance in cases of anemia of chronic diseases (ACD) characterized by functional iron deficiency as opposed to iron store depletion seen in iron deficiency anemia.

In a study conducted by Hughes *et al*[5], on the number of particles that should be examined to enable a confident examination of presence and absence of iron stores, and the quantity of iron in a bone marrow aspirate to be made. They observed that a minimum of seven particles must be examined to establish the absence of stainable iron and minimum of nine particles must be reviewed to see the maximum iron stores in 100% of samples to make judgment of whether iron stores are reduced, normal, or increased. Hence they concluded that the sensitivity of examination of bone marrow aspirates for iron stores can be optimised by increasing the number of particles reviewed to seven or more. This may require the staining of additional slides.

In a study conducted by Tripathi *et al*[6] Perl's Prussian blue stained bone marrow smears of 40 adult patients with moderate to severe anemia was performed and correlated with serum ferritin and serum iron. Gale's grading method revealed decreased iron stores in 30%, normal iron stores in 55% cases, and 15% cases had

increased iron stores. The co-relational coefficient between bone marrow iron grade and serum ferritin came out to be 0.59 with an F- test value of 55.10.The correlation coefficient between bone marrow grade and serum iron is 0.76 and the F-test value - 117. Hence they concluded that, although bone marrow iron grading is still gold standard but there exists a positive correlation between bone marrow iron with serum ferritin and serum iron thus iron status can be assessed by serum ferritin and serum iron, bone marrow being an invasive procedure can be avoided, also it will be helpful where bone marrow examination facility is not available.

In a study conducted by Donald T *et al*[7] the correlation between serum ferritin and bone marrow stainable iron was measured in 48 hospitalized patients. 41 of the plotted points had a correlation coefficient of 0.6853. Variations were most apparent in the group having no detectable marrow iron stores, where serum ferritin values ranged from 5-960 zg fL. This latter finding is most curious, because one should expect unusually low serum ferritin in such a case. 21 of the 48 patients studied, 3 with the lowest ferritin values had clear-cut iron deficiency. In several instances, increased serum ferritin values were associated with neoplasia. 5 patients whose bone marrow stores were evaluated by marrow sections, all but one of these patients had agnogenic myeloid metaplasia. Their serum ferritin ranged from 12 to 960 tg/L, and the corresponding bone marrow assays were negative for iron. Thus they concluded that, the serum ferritin can provide information similar to that obtained by the invasive bone marrow aspirate stained for iron. Of the two techniques, serum ferritin seems more routinely applicable because of the convenience of sampling. Quantification of serum ferritin is considerably more objective and reproducible than is the assessment of bone marrow iron. Serum ferritin also seems to be a more sensitive and specific index of declining iron stores than other measures of iron

deficiency. In contrast to indices such as percentage transferrin saturation and quantification of erythrocyte protoporphyrin, serum ferritin concentration becomes abnormal long before mobilizable iron stores are exhausted and before onset of clinically apparent anemia. Thus this assay is clinically valuable because of its sensitivity and convenience for assessing the status of physiological iron stores.

In a study conducted by Coenen J *et al*[8] determined serum ferritin, C-reactive protein (CRP), fibrinogen, and the erythrocyte sedimentation rate (ESR)in 73 patients with anemia of chronic disease. Normograms of CRP, ESR, or fibrinogen vs ferritin concentrations were constructed and used to estimate the iron stores in bone marrow. Iron stores estimated from the normograms were compared with the results of staining cytological bone marrow smears for iron. They observed that normograms of CRP, fibrinogen, or ESR (i.e. acute-phase reactants not influenced by changes in iron metabolism) vs ferritin are not suitable to correct for the acute-phase component of changes in ferritin concentrations.

In a study conducted by Pujara *et al*[9] bone marrow aspiration was performed in 73 patients with different haematological disorders. Marrow films were stained with hematoxylin and eosin stain, observed and then submitted for Prussian blue stain. They observed that, in iron deficiency anemia 92.7% of cases received iron store grade in marrow in range of 0-1. In megaloblastic anemia, 70% of cases received in the range of 3-4, whereas all cases of dimorphic anemia received in the range of 2-3 when compared to the normal range of 1-2.They concluded that, Prussian blue stain is simple and helpful technique to see and measure body iron store semi quantitatively. Furthermore, it helps to decide whether iron therapy would be useful or not.

BONE MARROW ASPIRATION

Bone marrow is the site of hematopoiesis in postnatal life. It is the largest organ in the body and widely distributed in skeleton and located within the cavities of bone. It mainly consists of hematopoietic cells, vascular sinusoids, fibroblasts, fat cells, and macrophages. Highly vascularized loose connective tissue is organized around bone vasculature which is located between trabeculae of spongy bone.[10]

Bone marrow aspiration studies are carried out principally to permit cytological assessment of bone marrow cells. However, in some clinical circumstances other tests—for example, cytogenetic and immunophenotypic analysis are equally important. In many patients, a trephine biopsy will be carried out as part of the same procedure. Bone marrow aspiration should be preceded by evaluation of the medical history and clinical features, examination of a blood film and assessment of results of a full blood count, other laboratory tests, and radiological investigations. This is essential to ensure that all appropriate tests are performed on the material obtained and to permit an adequate evaluation. For example, it is necessary to know whether the patient is receiving, or has recently received, any medication that may influence the blood count or bone marrow cytology to stimulate hematopoiesis.[11]

Even remote history of travel to an area where leishmaniasis or histoplasmosis is prevalent or previous irradiation of a site where a biopsy has been performed is of vital importance. [11]

BONE MARROW ARCHITECTURE - NORMAL ANATOMY

The Bone marrow aspirate and biopsy enables the assessment of bone marrow architecture, the distribution of cellular elements i.e. bone and stromal cells. The outermost elements of the biopsy are composed of collagenous periosteal connective tissue, followed by a zone of cartilage or cortical bone (depending on the age of the patient). After this the bone breaks up into a meshwork of trabeculae, between which are the intertrabecular spaces.[12]

Hematopoietic cells are present within these intertrabecular spaces and are supported by fat cells, stromal cells, histiocytes extracellular matrix and blood vessels.[12]

The intertrabecular areas can be divided into three zones which contain different hemopoietic cell types. (**Fig.1**) [12]

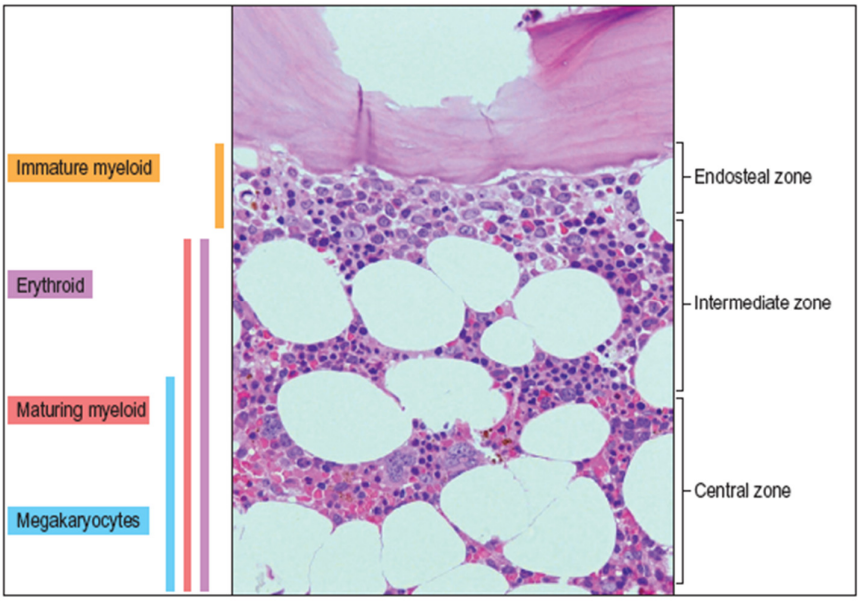


Fig.(1)[12]:Organization of the bone marrow: zones and distribution of various cell types. × 100.

1. Endosteal or paratrabecular zone: immediately adjacent to the trabecular bone and composed predominantly of myeloid precursor cells.

2. Intermediate zone: contains erythroid colonies and maturing myeloid cells

3. Central zone: in the center of the intertrabecular space contains mature adipose tissue and fat globules.

In addition to erythroid cells and maturing myeloid cells, this contains sinusoids and megakaryocytes. Small arteries and arterioles are often seen in the intermediate and central zones; these may be surrounded by cuffs of immature myeloid cells around them.[12]

Table.1: Cellularity varies for different ages [12]

Age	Cellularity
Newborn to 3 months	80–100%
Childhood	60–80%
20–40 years	60–70%
40–70 years	40–50%
>70 years	30–40%

The normal constituents of Bone marrow

The major categorization of the bone marrow constituents is done into:

a. Hematopoietic cells and; [12]

b. Stromal cells[12]

Hematopoietic cells are further classified into;

- **Erythropoiesis :**

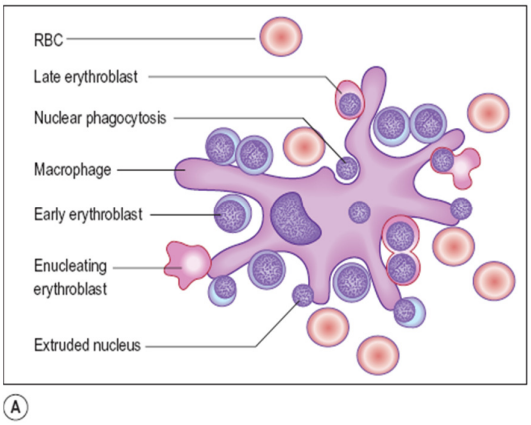


Fig:2(a)[12]. Figure shows erythroid island showing normal erythroid progenitors and mature RBC's.

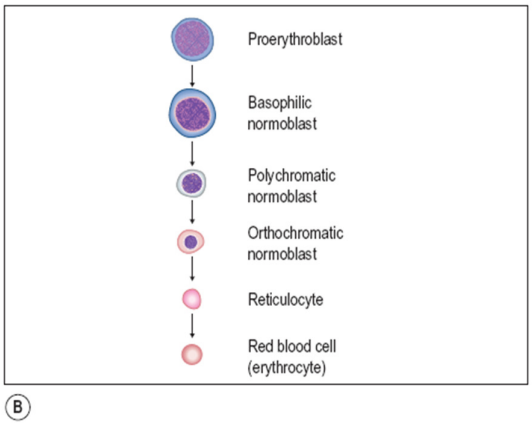


Fig:2(b)[12]. Figure shows normal erythroipoiesis

1. Common myeloid progenitor (CMP) gives rise to cells of all myeloid lineages (i.e. granulocytic, erythroid, megakaryocytic). The CMP subsequently generates granulocyte-macrophage progenitors (GMP) and megakaryocyte-erythroid progenitors (MEP).[12]
2. Common lymphoid progenitor (CLP), which gives rise to T- and B-lymphocytes and natural killer (NK) cells.[12]
3. The most immature lineage-specific erythroid progenitor cells are the erythroid burst-forming units (BFU-E) and the most mature are the erythroid colony-forming units (CFU-E).[12]
4. The CFU-E develop into proerythroblasts, the earliest morphologically recognizable BM red cell precursors. Proerythroblasts progress through several morphologically defined cytologic classes. These are, in order of

increasing maturity, the basophilic erythroblasts, early and late polychromatic erythroblasts and reticulocytes. (**Fig.2.a,b**) [12] (**Fig.4**) [12]

5. Within the BM, erythroblasts are present in erythroid islands (**Fig.3**)[12]composed of one or more central macrophages surrounded by one or two layers of erythroblasts.[12]

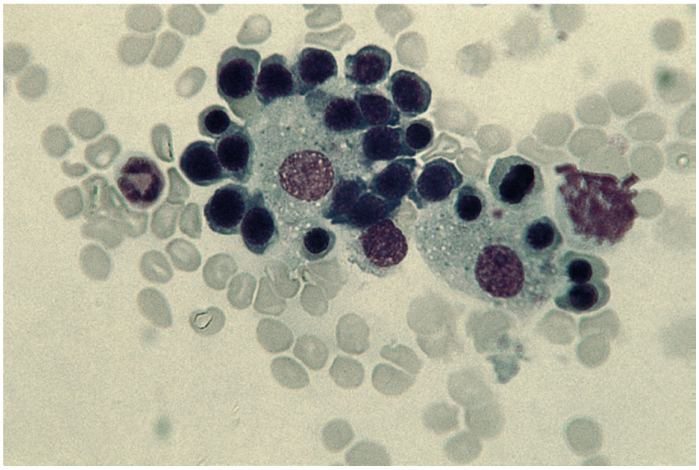


Fig.3[12]: Erythroid islands in normal bone marrow. (A) Erythroid islands consisting of early and late polychromatic erythroblasts surrounding macrophages.(MGG 100x).

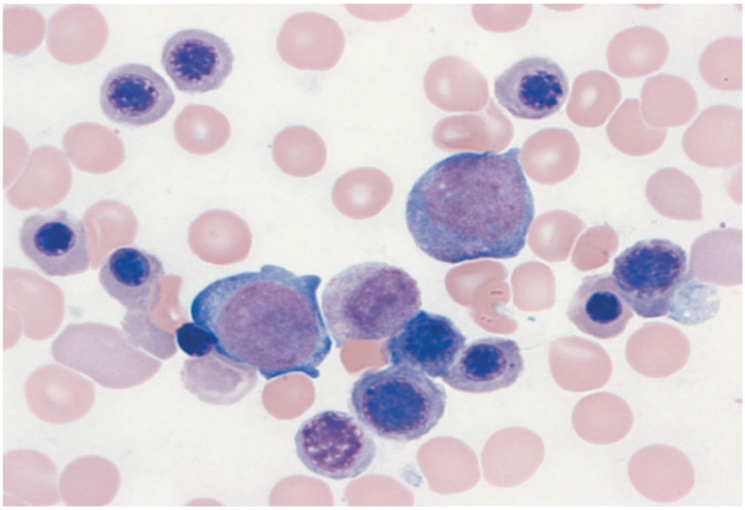


Fig.4[12]: Erythroblasts in a normal bone marrow aspirate showing all stages of erythropoiesis from pronormoblast to late polychromatic normoblasts. (May–Grunwald–Giemsa stain. 400x)

Granulopoiesis :

1. Granulopoesis is the production of granulocytic cells (neutrophils, eosinophils and basophils, and cells of the monocyte–macrophage series) within the BM.[12](**Fig.5**)[12] (**Fig.6**)[12]
2. It commences with the differentiation of the HSC to the common myeloid progenitor (CMP). The CMP further develops into the bipotent granulocyte-macrophage progenitor (GMP).[12]
3. The GMP differentiates into cells that are irreversibly committed to mature into granulocytic cells (CFU-G) or macrophages (CFU-M).[12]

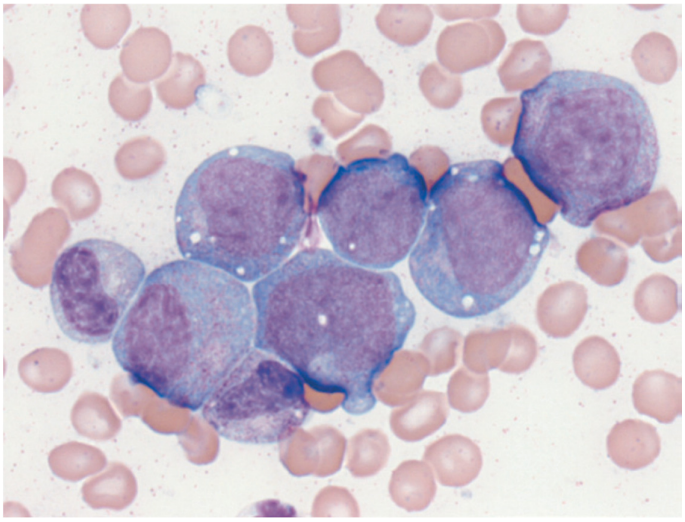


Fig.5[12]: Neutrophil granulocyte progenitor cells from a smear of normal bone marrow. Myeloblasts and promyelocyte. (May–Grunwald–Giemsa stain. 1000x)

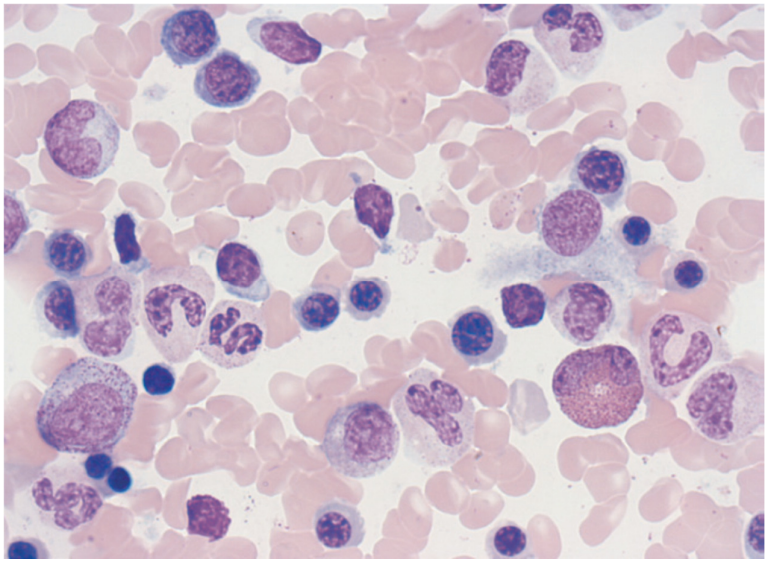


Fig.6[12]: Maturing granulocytic cells including neutrophil metamyelocytes, band forms and neutrophil myelocyte. (May–Grünwald–Giemsa stain. 1000x)

Monocytopoiesis (the mononuclear phagocyte system) :

1. Monocytopoiesis is the process by which peripheral blood monocytes and tissue macrophages are produced. The monocyte–macrophage lineage is derived from the GMP, the common progenitor for granulocytes and monocytes. Under the influence of GM-CSF, M CSF and IL-3, and up-regulation of a basic leucine zipper (bZIP) transcription factor, MafB, the GMP undergoes monocyte maturation pathway.[12]
2. The morphologically recognizable precursors of the monocyte series are, in order of increasing maturity, monoblasts, promonocytes and BM monocytes; only the first two of these undergo division. The blood monocytes leave the circulation after 20–40 hours and transform into tissuemacrophages.[12]
3. These are present in the BM, as well as other tissues, where they have a role in erythropoiesis and phagocytosis of cell debris.[12]

4. Normally, there is a constant loss of tissue macrophages (e.g. by shedding of alveolar macrophages), balanced by the formation of new macrophages from blood monocytes and to a small extent from the division of some existing macrophages. The system of cells concerned with macrophage production is called the ***mononuclear phagocyte system.*** [12]

Lymphopoiesis:

1. The common lymphoid progenitor (CLP), precursor of mature lymphocyte, arises from the differentiation of the HSC under the influence of IL-7 and FLT3.(**Fig.7**)[12]
2. These generate B-cell, T-cell and NK cell progenitors and give rise to all types of lymphocytes. Lymphopoiesis that occurs in normal BM is independent of antigenic stimulation and serves to supply the body with mature B-lymphocytes or with T-lymphoid progenitors that mature into T-cells in the thymus.[12]
3. The newly-formed mature B and T cells enter the circulation and then migrate to peripheral lymphoid tissues (spleen, lymph nodes, Peyer's patches, Waldeyer's ring).[12]

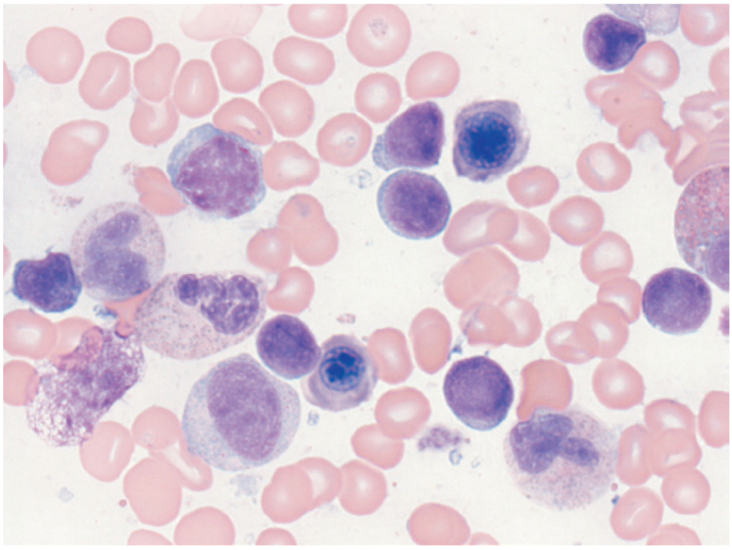


Fig.7[12]:Normal lymphoblast in normal pediatric marrow. Lymphoblasts are larger than lymphocytes with finer, less condensed chromatin. (May–Grunwald–Giemsa stain. 1000x)

Plasma cells :

Plasma cells comprise less than 1% of cells in normal BM. They are 14–20 μm in diameter and have deeply basophilic cytoplasm with a pale perinuclear zone corresponding to the site of the Golgi apparatus with cytoplasmic vacuolations and an eccentrically placed nucleus and moderate amount of condensed chromatin.[12](**Fig.8**)[12]

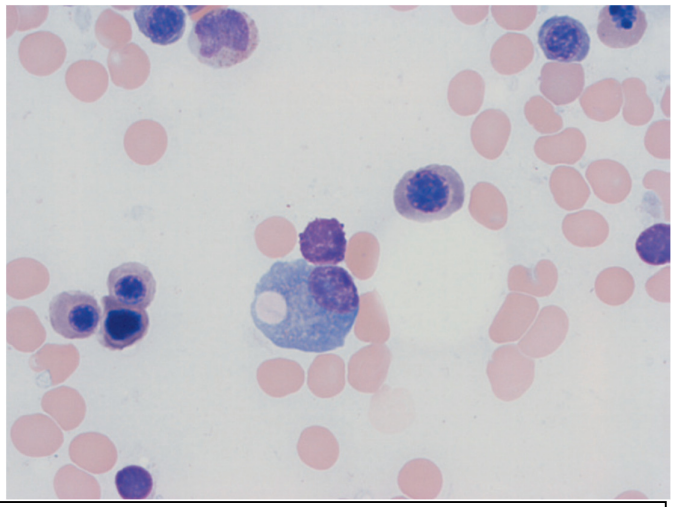


Fig.8[12]: Plasma cell showing a Russell body. (May–Grunwald–Giemsa stain. 400x)

Megakaryopoiesis:

1. Megakaryopoiesis is the process of development of megakaryocytes and platelets within the marrow. Humans generate 1011 platelets per day, and production can be increased 20 fold when in demand.[12]
2. Megakaryocytes are derived following a cascade of differentiation from the megakaryocyte erythroid progenitor (MEP). The bipotent MEP under the influence of thrombopoietin (TPO) which is the primary regulator of platelet production, IL-6 andIL-11 generates megakaryocyte colony-forming units (CFU-MK).[12]
3. CFU-MK are a diploid cell population, in which DNA synthesis and nuclear division (karyokinesis) is followed by cell division (cytokinesis). [12]
4. CFU-MK undergo further maturation to megakaryoblasts, the earliest morphologically recognizable member of the megakaryocyte series.[12]

Four types of megakaryocytic cells can be identified in Romanowsky-stained BM smears in increasing order of maturity:[12]

1. Megakaryoblasts (group I megakaryocytes)
2. Promegakaryocytes (group II megakaryocytes)
3. Granular megakaryocytes (group III megakaryocytes)which produce platelets.**(Fig.9)** [12]
4. 'Bare' nuclei.

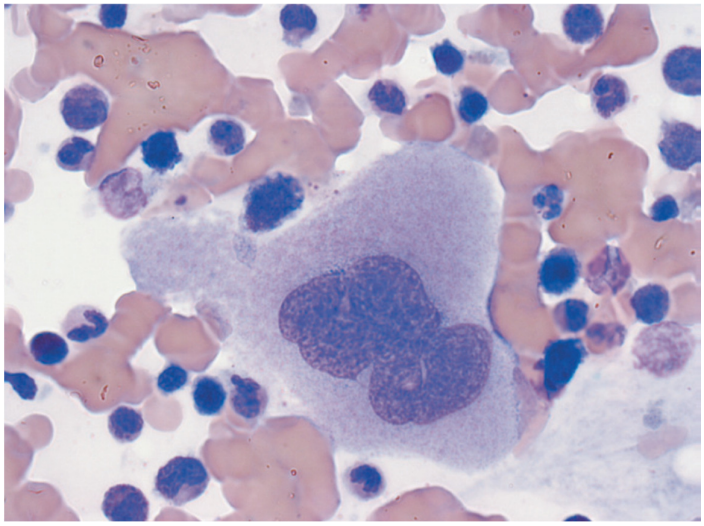


Fig.9[12]: Granulated megakaryocyte. May–Grunwald–(Giemsa stain: 400x).

Bone Marrow stromal cells

The stromal cells regulate hemopoiesis through direct contact and mediators i.e. adhesive ligands, synthesis of extracellular matrix and production of signaling molecules and cytokines.[12]

BM stromal cells include osteoblasts, endothelial cells, macrophages, non-phagocytic reticular cells (including myofibroblasts and sinusoidal adventitial cells) and mesenchymal stem cells. [12]

1. **Bone marrow macrophages**

Macrophages are derived from monocytes, as described above. Their role is to phagocytose cell debris and pathogens and to stimulate lymphocytes to respond to pathogens. They are located within erythroblastic islands (where they are involved in regulating erythropoiesis), plasma cell islands, lymphoid nodules, and adjacent to marrow sinusoids (forming part of the incomplete adventitial layer of the sinusoidal wall).[12]

2. **Dendritic cells** (DC)

DC are derived from hemopoietic progenitor cells and process antigen which they then present to other immune cells. There are two main subpopulations: conventional DC (cDC) and interferon-producing plasmacytoid (pDC).[12]

3. **Mast cells**

Mast cells differentiate from multipotent hemopoietic cells in the BM and have a close developmental relationship with basophils. After initial differentiation in the BM the most mature mast cell progenitors enter the blood, circulate and migrate into tissues where they proliferate and mature into mast cells.[12]

They have an abundance of electron-dense secretory granules.(**Fig.10**) These contain large amounts of mast cell mediators which include histamine, serotonin, cytokines (especially tumor necrosis factor), proteoglycans, lysosomal enzymes, heparin and chondroitin sulphates and mast-cell-specific proteases.[12]

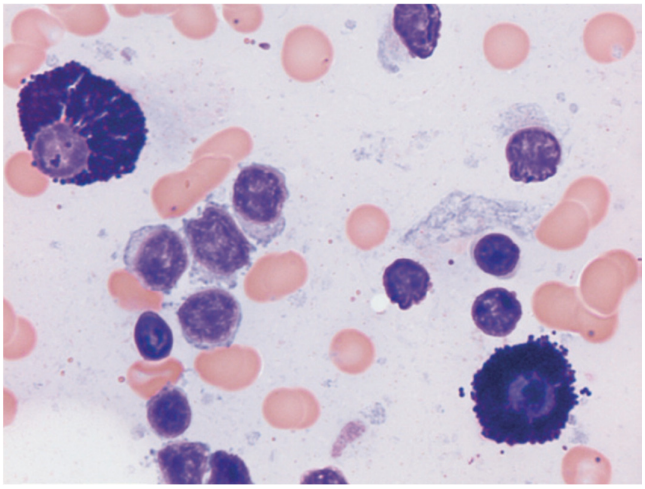


Fig.10[12]:Normal mast cells.The cytoplasm is packed with coarse granules . (May–Grunwald–Giemsa stain. 1000x).

4. **Mesenchymal stem cells**

Mesenchymal stem cells (MSC) comprise a population of non-hemopoietic stromal cells and are found in the BM. These are rare cells in the BM (<0.01%) and possess multilineage potential with the capacity to differentiate in vitro into chondrocytes, adipocytes and osteocytes, and osteoblasts.[12]

5. **Osteoblast**s

They may be seen in BM smears as single cells or small groups. They are ovoid or elongated, have a single small eccentric nucleus with small quantities of condensed chromatin and one to three nucleoli and have abundant lightly basophilic cytoplasm with indistinct margins.[12] Furthermore, the nucleus of an osteoblast does not show the heavily-stained coarse clumps of condensed chromatin that are characteristic of plasma cells.[12]

6. **Osteoclasts**

Osteoclasts are derived from undifferentiated cells of the monocyte-macrophage lineage or osteoclast differentiation factor. Osteoclasts are giant multinucleate cells with abundant pale-staining cytoplasm containing many fine azurophilic granules.(**Fig.11**) [12] The individual nuclei within a single cell are small, round or oval, uniform in size, and have a single prominent nucleolus.[12]

Osteoclasts must be distinguished from megakaryocytes, the other polyploid giant cells in the marrow. Unlike multinucleated osteoclasts, normal megakaryocytes have a single large lobulated nucleus.[12]

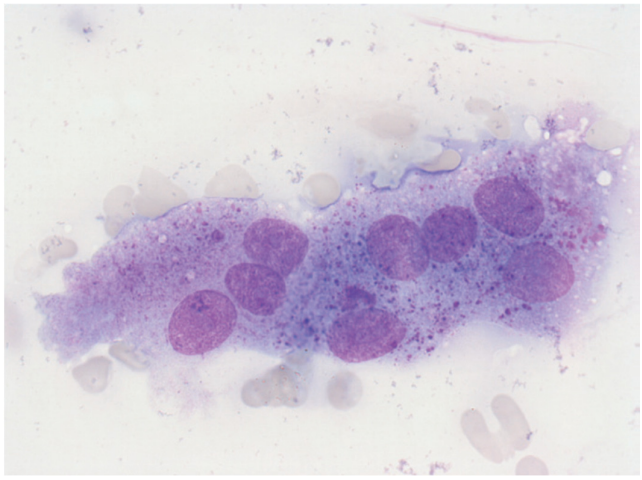


Fig.11[12]: A multinucleate osteoclast from a smear of normal bone marrow. (May–Grunwald–Giemsa stain. 1000x)

7. **Adipocytes**

The number of adipocytes is inversely related to the marrow cellularity.(**Fig.12**)[12]

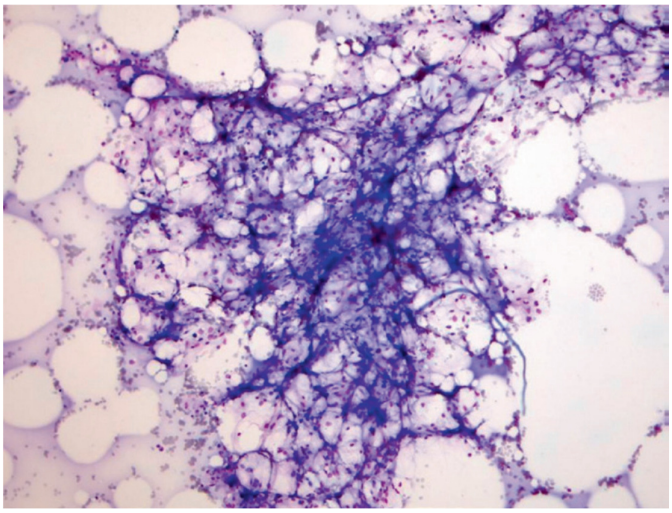


Fig.12[12]: Bone marrow smear showing hypocellularfragment composed predominantly adipocytes and few hemopoietic cells (aplastic anemia). May–Grunwald–Giemsa stain. × 100

Bone marrow sinusoids

Marrow sinusoids are thin-walled and composed of an inner complete layer of flattened endothelial cells, little basement membrane material, and an outer incomplete layer of adventitial cells (macrophages). They have numerous small pinocytotic vesicles along their luminal and abluminal surfaces. [12]

Marrow endothelial cells affect hemopoiesis by secreting stem cell factor, IL-6, IL-1α, IL-11, GM-CSF and G-CSF. They are also involved in controlling the entry and exit of hemopoietic stem cells and progenitor cells from the marrow and the exit of mature blood cells. [12]

The adventitial cells have long cytoplasmic processes some of these lie on the external surface of the sinusoid and others are found between surrounding hemopoietic cells.[12]

Bone marrow aspiration

Bone marrow differentiates into myeloid, erythroid and lymphoid cell lineages under the influence of cytokines or growth factors.[12]

Function: Hematopoiesis (**Fig.13**) [12] and supply mature hematopoietic cells into peripheral blood and respond to demands.[12]

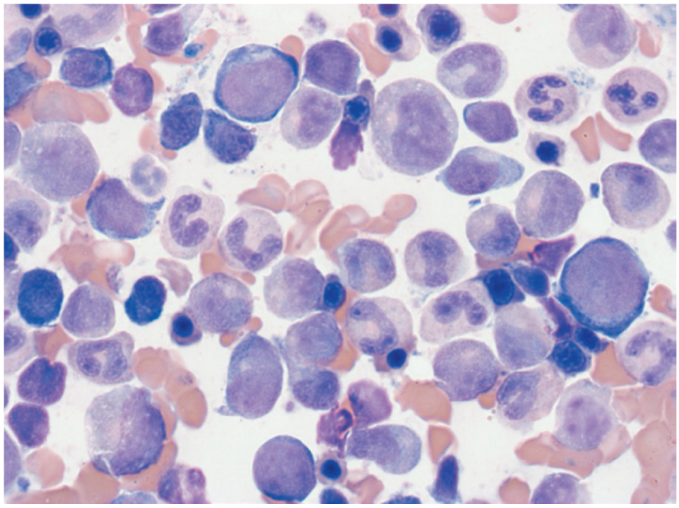


Fig.13[12:] Normal adult hematopoiesis in a bone marrow aspirate showing range of normal erythroid and granulocytic progenitors at different stages of differentiation. (May–Grunwald–Giemsa stain 400x)

Indications for bone marrow aspiration[11]

1. Unexplained microcytosis
2. Unexplained macrocytosis
3. Unexplained anaemia
4. Dyserythropoietic anemia is suspected
5. Unexplained thrombocytopenia
6. Investigation of pancytopenia (including suspected aplastic anaemia)
7. Investigation of a leucoerythroblastic blood film and suspected bone marrow infiltration
8. Investigation of suspected acute leukemia
9. Assessment of remission status after treatment of acute leukemia
10. Investigation of suspected MDS or Myelodysplastic / myeloproliferative disorder
11. Investigation of suspected chronic myeloidleukaemia
12. Follow up of chronic myeloid leukaemia

13. Investigation of suspected myeloproliferative disorder (polycythemia rubra vera, essential thrombocythemia, idiopathic myelofibrosis, or systemic mastocytosis)
14. Investigation of chronic lymphocytic leukemia
15. Investigation of suspected non-Hodgkin's lymphoma
16. Diagnosis and follow up of hairy cell leukemia
17. Staging of low grade non-Hodgkin's lymphoma
18. Investigation of multiple myeloma
19. Staging of high grade non-Hodgkin's lymphoma
20. Investigations of suspected storage disease
21. Investigation of fever of unknown origin
22. Suspected chromosomal disorders in neonates when rapid confirmation is required
23. Confirmation of normal bone marrow if bone marrow is being aspirated for allogenic transplantation.

Contraindications[13]

Contraindications to bone marrow aspiration/biopsy include ;

Absolute contraindication

1. Hemophilia and other major disorders of coagulation.

Relative contraindication

1. Skin infection

2. Osteomyelitis

3. Previous radiation therapy in the area of sampling and an uncooperative patient.

Planning the bone marrow aspirate[11]

It is necessary to plan a bone marrow aspiration to ensure not only that the procedure is safe and causes minimal discomfort to the patient but also that all appropriate specimens are taken into the correct anticoagulant and despatched to the correct laboratory. The appropriate supplementary tests may be better determined after the bone marrow aspirate has been examined.

Therefore, it is often useful to take a 10 ml sample into preservative free heparin and stain and examine a single film rapidly before deciding whether supplementary investigations are indicated which are most likely to be informative.

Choice of Needle[11]

Disposable needles are preferable. All of the standard needles are satisfactory for aspiration of the iliac crest. It is acceptable to remove the guard, if necessary, to provide a greater length of needle in obese patients. For sternal aspiration the needle selected should have a guard that screws along the needle—for example, a **Klima needle** because the guard cannot slip during the procedure.

A needle with a guard secured by a side screw for example a **Salah needle** is less satisfactory because the guard can slip, allowing the needle to penetrate further than was intended. Occasionally, in a very obese patient, it is necessary to use a trephine biopsy needle, which is generally longer than an aspiration needle, to obtain an aspirate from the iliac crest.

Patient Consent[11]

When bone marrow aspiration is to be performed, it is essential that an explanation is given in advance and that a consent form is signed.

Procedure for aspiration and preparation of slides[11]

Sites:[11]

- Sternum
- Iliac crest
- Anterior iliac spine
- Posterior iliac spine
- Spinous processes of lumbar vertebrae
- Medial aspect of tibia

Aspirate can be dealt in four ways: [11]

- Smears-direct
- Smears after centrifugation
- Particle smears
- Histological sections.

Aspirations films are suited for:[11]

- Wedge spread films
- Films of crushed marrow fragments
- Study of cell markers
- Cytogenetic study
- Ultrastructural examination
- Culture of microorganisms
- Culture of hemopoetic precursors
- Histological sections of fragments

Complications of both aspiration and biopsy[11]

- Cardiac and great vessel laceration
- Hemorrhage- if hemostatic defect is suspected, apply firm pressure
- Hemorrhage- also in abnormal vascularity of the bone as in Paget's disease
- Pneumothorax
- Sternomanubrial separation
- Breakage of the needle in patients with osteosclerosis.

Procedure

The patient should be questioned with regard to allergy to local anaesthetic or any antiseptics or sedatives that might be used. Sterile gloves should be worn and aseptic precautions must be taken. On safety grounds, it is preferable to use disposable needles. This not only eliminates the risk of transmission of infection to the current or future patients but also avoids the necessity for cleaning non-disposable needles, a procedure that may be hazardous for staff. [11]

When aspirating from the sternum, it is of vital importance to aspirate only from the first part of the body of the sternum (or from the manubrium) and to judge the depth carefully.

The needle that is used for infiltrating with local anaesthetic can be used to measure the depth of the periosteum, the guard is set accordingly so that the needle will penetrate only 5–6 mm beyond the depth of the periosteum. Particular care must be taken in patients with suspected multiple myeloma and in elderly patients who may have osteoporosis - inadvertant penetration through the sternum is more likely in these patients if the needle guard is not set correctly or if a needle with an insecure guard is used. [11]

When aspirating from the posterior superior iliac spine, the guard can safely be set so that the needle can penetrate up to 1 cm beyond the surface of the bone.[11]

The skin, subcutaneous tissues, and periosteum should be infiltrated. It is important to wait until the local anaesthetic has had time to take effect and to test for adequacy of anaesthesia before proceeding. The patient must be warned of the possibility of suction pain before it occurs and be reassurance that it will be brief. If suction is distressing, slowing the rate of aspiration is indicated.[11]

Technical considerations

When aspirating from the sternum, the needle should be inserted slightly to one side of the midline because bone marrow is less cellular in the midline. The needle should be inserted at right angles to the surface of the bone. In carrying out a biopsy from the posterior superior iliac spine, it should be inserted into the bone in the direction of the anterior superior iliac spine.[11]

Needle should be inserted through the bone rotating in clock wise direction. Needle is firmly fixed in cortex of bone before trocar is removed. Then aspirate is obtained by too and fro motion. The minimum amount of bone marrow needed for the tests indicated should be aspirated because the greater the volume of marrow aspirated the more dilution by peripheral blood occurs.[11]

Aspirate about 0.25 ml first, this being used for spreading films, and then to use a second syringe to obtain a further sample. Ideally, films should be spread immediately without any anticoagulant being added. However, if there has been any difficulty in aspiration and it is thought that the specimen might clot rapidly, part of the aspirate should be put into anticoagulant. Slides are prepared by placing the drop of blood and smearing it towards the frosted end.[11]

Any residual bone marrow aspirate can be left in the syringe until it clots and can then be put out into formal saline and submitted for histological examination. Alternatively, the bone marrow can be expelled immediately into formalin, which disperses the particles; they can then be collected as a cytoblock or into a filter.[11]

Processing and staining of bone marrow films

The films should be thoroughly fixed in methanol. Bone marrow films should be stained with a Romanowsky stain, such as May-Grunwald-Giemsa or Wright-Giemsa.

A film with at least one fragment, preferably more, should be used for a Perls' stain (for assessment of iron stores) on all initial bone marrow biopsies.[11]

All iron stains should be performed together with a control slide, containing fragments, from a patient known to have iron in bone marrow fragments.[11]

Assessing and reporting bone marrow films[11, 14]

Preparation	: Satisfactory /Adequate or Inadequate
Cellularity	: Normocellular/ Hypocellular / Hypercellular
Erythropoiesis	: Normoblastic /Megaloblastic/ Micronormoblastic.
Myelopoiesis	: Morphology/ Number/ Maturation.
M:E ratio	: Normal / Reversed.
Lymphocytes	: Morphology/ Number/
Megakaryocytes	: Normal/ Hypolobulated/ Hyperlobulated.
Plasma cells	: Present/ Absent/ Number.
Abnormal cells	: If any.
Iron Stores	: Grade of iron stores according to Gales method.
Serum ferritin	: in ng/l
Impression	:

Bone marrow iron stores

Functions of iron

Iron is a vital element in all aerobic organisms, and it plays critical functions in the human body, largely attributable to its oxygen-carrying capacity. The amount of iron present in a healthy adult is 2-4 grams.[15]

Iron is a binding site for oxygen in heme containing proteins, such as hemoglobin in red blood cells and myoglobin in muscle. Heme consists of a ferrous (Fe2+) iron complex within protoporphyrin IX.[15]

Porphyrin is a cyclic macromolecule that binds divalent and trivalent metals like iron to form complexes. **Thus, hemoglobin and myoglobin perform the important function of oxygen transport in human body.** [15]

Iron is a redox (reduction-oxidation reaction) active transition metal that exists mainly in two oxidative states—ferrous ($Fe^{2+)}$ and ferric (Fe^{3+}). **Iron actively participates in electron transfer chains by reducing ferric iron to ferrous iron and supplying proton (H+) ions to the cytochromes.** [15]

Heme, therefore, is an essential component of cytochrome proteins, which comprise the electron transport chain. In addition, iron is also an integral part of iron-sulfur (Fe-S) proteins, which are present in mitochondrial enzymes, thus playing an important role in mitochondrial single electron transport. These Fe-S proteins, also known as Fe-S clusters, appear in different forms based on equal number of iron and sulfur ions, with the most common Fe-S clusters being 2Fe-2S and 4Fe-4S. [15]

Regulation of iron availability

Iron availability is tightly regulated by an array of proteins, including transferrin (Tf) and ferritin, which are responsible for iron transport and storage, respectively. [15]

These proteins bind iron in the ferric (Fe3+) form, which is non-reactive. This helps inhibit the reaction of free iron with oxygen, which can lead to free radical formation and cell injury. [15]

Iron availability in the body is regulated through intestinal absorption, post translational regulatory mechanisms, recycling of iron in macrophages and cellular storage of iron, also it is regulated by transport of iron through the small intestine,which depends on the rate of erythropoiesis and the reduction in body iron stores. [15]

Erythropoeisis takes place in bone marrow. The ferrous iron absorbed from the diet and transported by the plasma protein transferrin, as well as iron recycled by reticuloendothelial macrophages, is taken up by the bone marrow erythroblasts. Excess iron is stored in the form of ferritin primarily in hepatocytes. [15]

Assessment of Iron stores in bone marrow

Perl in (1867) discovered Prussian blue method for iron store estimation in bone marrow.[9] **Principle**: Given by Perl was, Ferric iron deposits in tissue (present mostly as ferric iron within the storage protein ferritin) react with the soluble ferrocyanide in the stain, to form insoluble Prussian blue dye (a complex hydrated ferric ferrocyanide substance) in situ.[9] They are then visualizable microscopically as blue or purple deposits, within cells.

Perls' acid ferrocyanide stain identifies one small blue–black granule in up to 30% of polychromatic erythroblasts.[9,16] These iron-containing siderotic granules are

randomly distributed in the cytoplasm, and erythroblasts containing such granules are termed sideroblasts.[9 ,16-17]

The term ring sideroblasts is used when there are five or more perinuclearsiderotic granules.

Erythroid cells at all stages of maturation frequently contain coarse acid phosphatase-positive perinuclear granules. Normal erythroblasts are periodic acid-Schiff (PAS), Sudan black and myeloperoxidase (MPO)-negative.[9 ,16-17]

Grading for iron storage[9,16-17] (Figs.:14-19)[17]

0 **:** No stainable iron.

1 **:** Small iron particles just visible in reticulum cell in oil immersion objective.

2 **:** Small, sparse iron particle in low power field.

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 marrow details.

Interpretation of iron stores: [9, 17]

Minimum seven particles are required to see for the depletion of iron stores:

They are interpreted as:

- 0 :Absent iron stores
- 1, 2: Normal
- 3: Slightly increased
- 4: Moderately increased
- 5, 6: Markedly increased

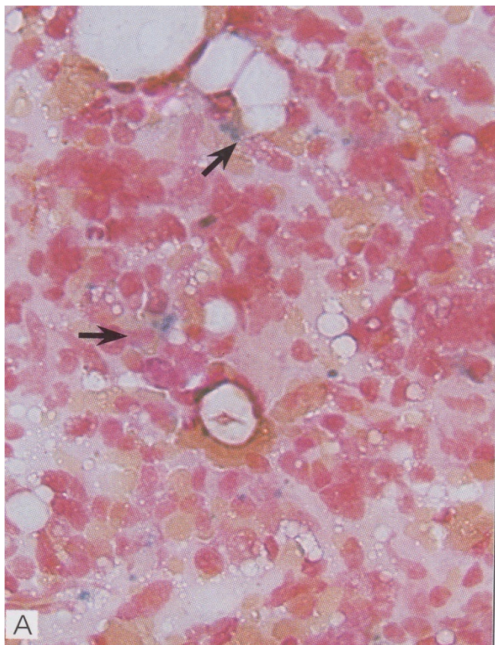


Fig.14[17]: Grade 1 iron stores[17]

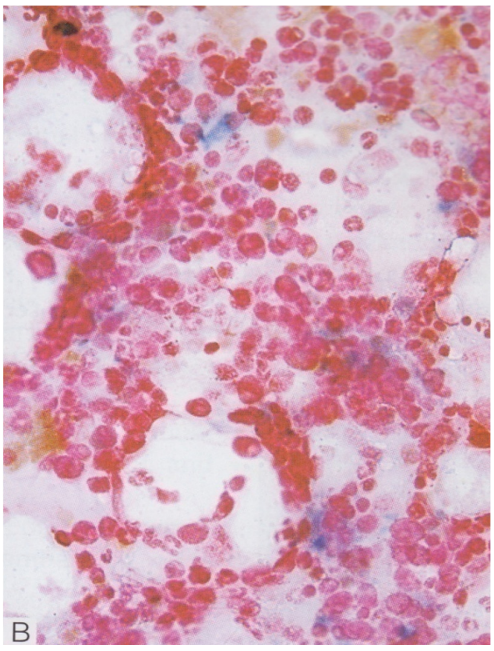


Fig.15[17]: Grade 2 iron stores[17]

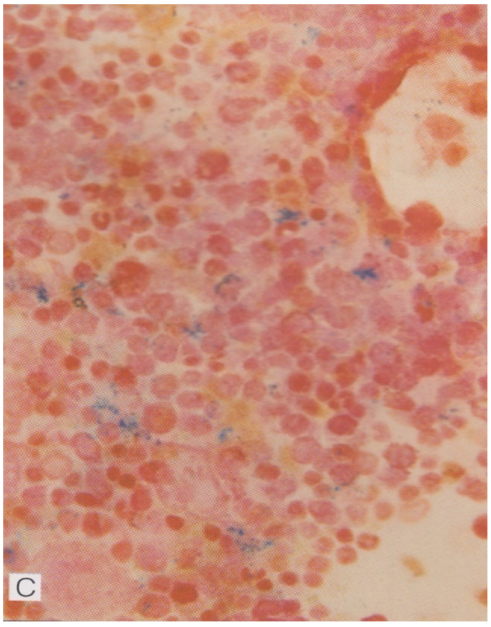


Fig.16[17]: Grade 3 iron stores[17]

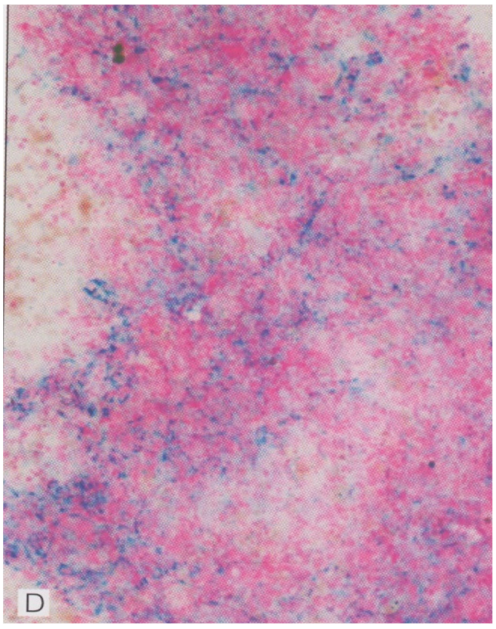


Fig.17[17]: Grade 4 iron stores[17]

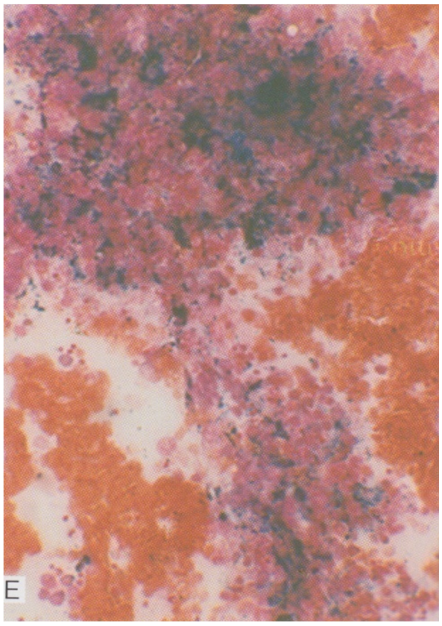


Fig.18[17]: Grade 5 iron stores[17]

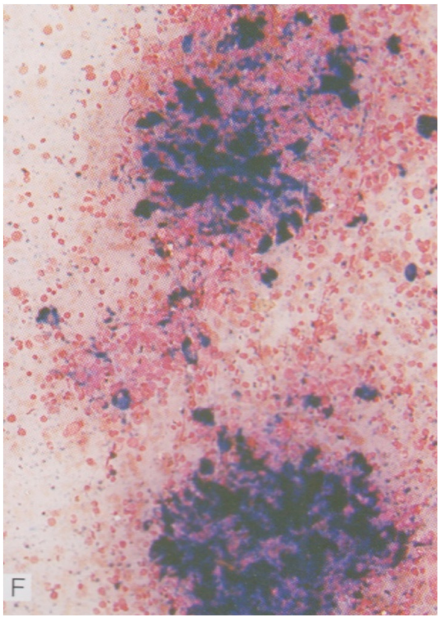


Fig.19[17]: Grade 6 iron stores[17]

Proteins in iron metabolism

Ferritin

Ferritin is the intracellular storage form of iron, found chiefly in the cytoplasm of the reticulo-endothelial system. Ferritin is an iron storage protein characterized by 24 subunits. These subunits are comprised of heavy (H) and light (L) chains, which form a hollow apoferritin shell and hold nearly 4500 atoms of iron.[18,19]Apoferritin binds iron in its ferrous (Fe^{2+}) form and then oxidizes it to ferric (Fe^{3+}) iron. In this form iron is non-reactive and thus, non-toxic to the cells.[15,18] The H subunit of ferritin has ferroxidase activity, which is an essential characteristic of this iron storage protein. The heavy subunit is found mainly in heart tissue, and the light subunit is found in liver cells. [15,18]

Once iron is absorbed in the duodenum, it is transported across the enterocyte and is either transported to cells in the transferrin-bound form, or the surplus iron is stored in the form of ferritin. [15,18]

When iron is required for erythropoiesis, it is released from ferritin. Thus, ferritin levels can reflect an early stage of iron deficiency in a normal healthy adult and are considered a sensitive biomarker of iron deficiency. However, chronic inflammatory conditions can alter serum ferritin levels, resulting in a false estimation of iron stores. This can be explained by alterations in ferritin due to the inflammatory response.[18]

Ferritin is a positive acute-phase protein, which means that its levels rise in response to inflammation. Stimulation of interleukins such as IL-1 and IL-6 gives rise to increased transcription of ferritin. Thus, chronic inflammatory conditions cause increased serum ferritin levels.[15, 18-20]

Inflammatory cytokines can also cause down regulation of *Fpn,* which results in sequestration of iron in the macrophages.[13] Therefore, in inflammatory conditions or chronic disease, serum ferritin can be elevated and might not serve as an appropriate measure of iron status.[13]The concentration of the serum ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. Values peak among men between 30-39 years of age and then tend to remain constant until about 70 years of age.[15]Among women serum ferritin values remain relatively low until menopause and then rise. Serum ferritin concentrations have been documented to give an accurate indication of the amount of storage iron not only in healthy individuals but also in cases of iron deficiency or iron over load.[15]

Values less than 20ug/l indicate negative iron balance or decreased stores. In patients with acute or chronic diseases it can be up to 100ug/l with an absence of stainable bone marrow iron.[21,22]

Transferrin saturation is the ratio of serum iron concentration and TIBC expressed as percentage. The most reliable diagnostic parameter to identify absolute iron deficiency in patients with chronic inflammation is the ratio of serum transferrin receptor concentration to the log of serum ferritin concentration. [15]

Serum transferrin receptor protein is released by the erythroid precursors in the blood and their levels are increased in iron deficiency anemia which needs highly sophisticated technique.[15]

One of the most common diagnostic problems encountered by clinicians is the distinction between absolute and functional iron deficiency. [15]In absolute or true iron deficiency there is absence or decreased endogenous iron stores where as in functional iron deficiency there is lack of utilization of endogenous iron[23,24.] It is important to differentiate between the two from the treatment point of view. [2,15]

Estimation of serum ferritin

Several procedures are available for the immunological quantification of serum ferritin. The most widely used method for the estimation of serum ferritin is Chemiluminescent Microparticle Immunoassay (CMIA).[7]

Biological Principle of the procedure

This uses a two step immunoassay technique to determine the presence of ferritin in human serum and plasma using CMIA technology with flexible assay protocols referred to as Chemiflex.[7,19]

First step: Sample and anti-ferritin coated paramagnetic microparticles are combined. Ferritin in the serum binds to the anti-ferritin coated microparticles. After washing, anti- ferritin acridinium labelled conjugate is added to second step. [19]

Second step: Pre trigger and trigger solutions are then added to the reaction mixture. The resulting chemiluminescent reaction is measured in relative light units (RLU's). A direct relation exists between amount of ferritin in the body and the RLU's.[19]

Specimen collection

Human serum collected in serum separator tubes or plasma collected in tripotassium EDTA and lithium heparin can be used.[19]For optimal results, serum and plasma should be free from fibrin, RBC's or other particulate matter. The results are interpretated as follows:

Normal values:[19]

Table.2: Table showing the normal ranges of serum ferritin it's mean and 95% CI in males and females.

Normal range	Mean ng/ml	Central 95% interval ng/ml
Males	75.62	21.81 – 274.66
Females	39.42	4.63 – 204.00

Reference Range: **M: 36 to 262 ng/ml**[24]

F: 24 to 155 ng/ml[24]

Elevated levels may reflect iron overload but will be increased in liver disease, inflammation or malignant disease.

In the presence of inflammation, a level of **> 100 ug/l** generally excludes iron deficiency.[6]

Therefore the current study provides the useful information that, ferritin is the principle iron storage compound in the body and serum ferritin concentration results from the leakage of tissue ferritin.

Serum ferritin reliably reflects the storage iron in the absence of inflammation and a reduced level serves as a sensitive early indicator of depletion of body iron store.[2,7,15] However, the fact that ferritin is also an acute phase response protein undermines its predictive ability in the setting of anemia co-existing with infection and inflammation. [2,7,15] Recently, serum transferrin receptor and zinc protoporphyrin have been used as more accurate indicators of iron status.[14] However, their use in developing nations is restricted by the limited availability of assay facility and higher cost.[14]

MATERIALS AND METHODS

Source of data

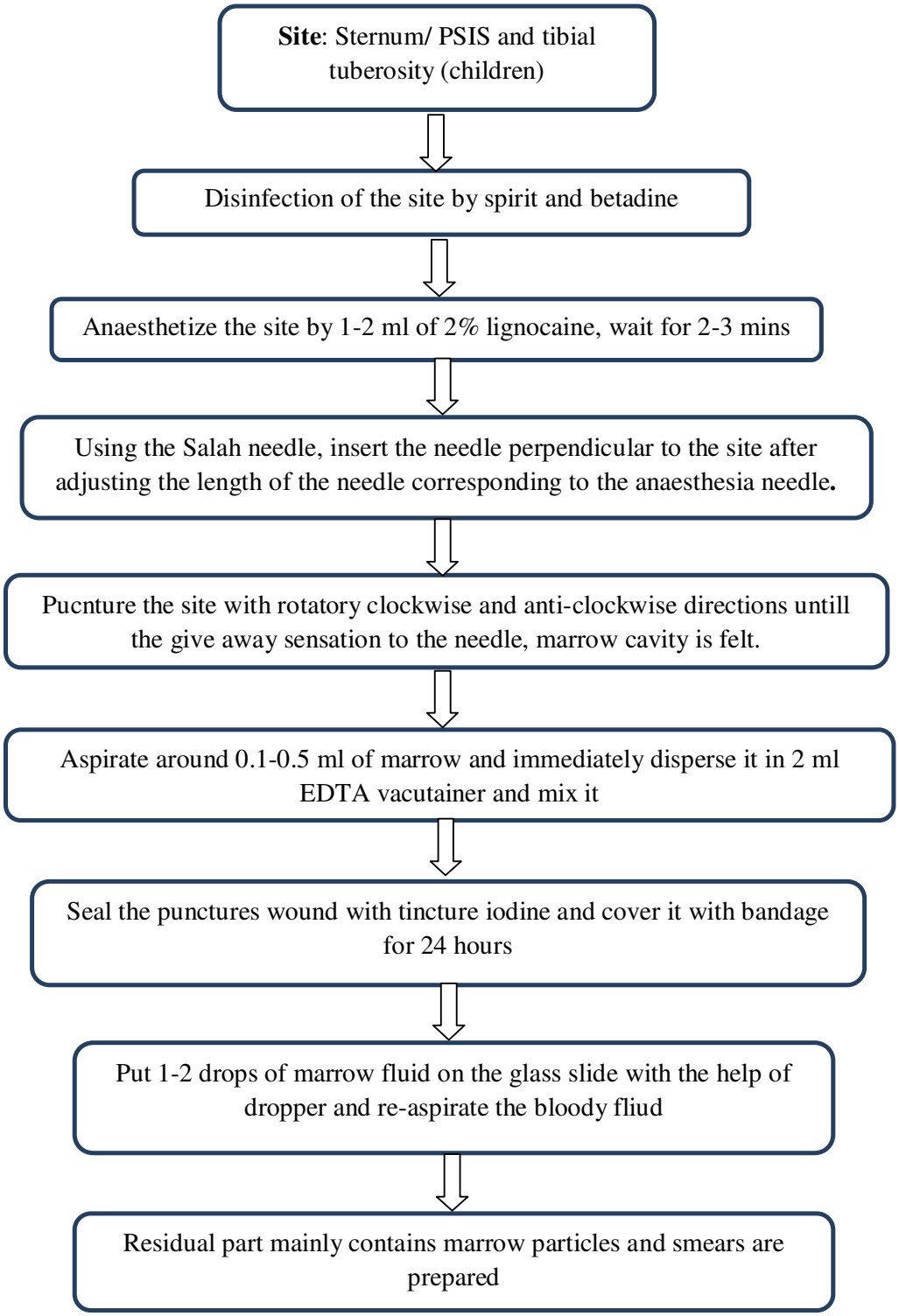
Patients presenting with hematological disorder in whom diagnostic bone marrow evaluation was indicated, and referred to the Department of pathology in BLDEU'S Shri B.M.Patil Medical College, Hospital and Research centre, Vijayapura was included from the study period of 1st November 2013 to 30th September 2015.

Methods of collection of data.

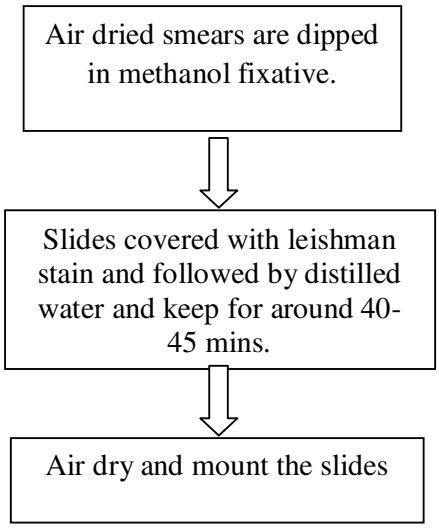
Bone marrow aspirate was obtained from the PSIS (Posterior Superior Iliac Crest)/iliac crest/sternum/ whichever was suitable observing strict aseptic precautions. The smears were stained with Perl's Prussian blue stain for iron assessment along with the routine Leishman stain. One positive control slide, (slide of old haemorrhage with hemosiderin laden macrophages) was taken. Peripheral venous blood was collected at the same setting for complete blood count (CBC) and serum ferritin level estimation.

The Bone Marrow iron stores were graded by Gale's grading method (grade 0-grade 6). The serum ferritin was evaluated by the CMIA method.

FLOWCHART DEPICTING THE OPERATIVE PROCEDURES FOR THE ABOVE MENTIONED

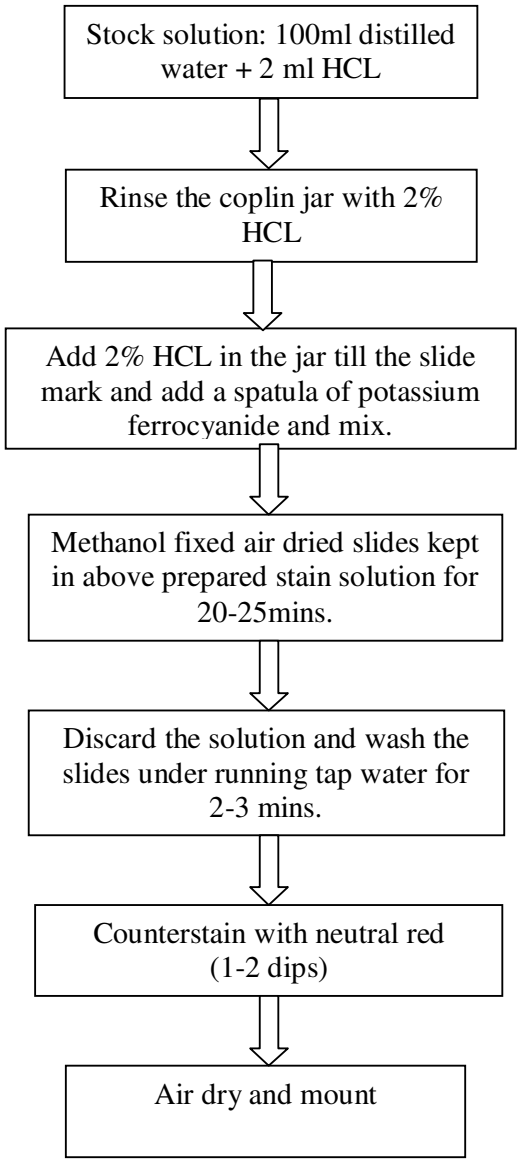


LEISHMAN STAIN [14]



PERL'S STAIN [16-17]

(Modified)



Sample Size:

In a study titled, "Intensive method of assessment and classification of the bone marrow iron status" by Bableshwar *et al*[2] The Gales method of grading Iron stores in marrow fragments revealed the normal iron stores in 61.25%.

Considering the proportion of normal iron stores 61.25% at 95% confidence and at 15% allowable error sample size is 110 using the formula,

$$n = \frac{(Z\alpha)^2 \, p \times q}{L^2}$$

Where, p = prevalence rate,

q = 100 – p,

L = allowable error,

Zα = 1.96 for α value.

Hence, 110 cases of various hematological disorders were studied to assess the bone marrow iron stores and its correlation with serum ferritin.

Statistical analysis:

Data was expressed with diagrams and percentages. Results were analysed using ANOVA test and Z test and p value.

Inclusion criteria: All patients of various hematological disorders in whom bone marrow aspiration was indicated were included in the study.

Exclusion criteria:

1. Aspirates of inadequate material or dry tap were excluded.
2. Patients receiving treatment of iron supplementation.

3. Patients who had undergone recent blood transfusion (preceding four weeks) were excluded.

RESULTS

General considerations:

A total of 110 cases of bone marrow examination were examined along with serum ferritin. Out of total 110 cases serum ferritin was estimated in 70 number of cases.

Bone marrow iron stores were grouped into three groups[14]

1. **Diminished:** iron grades **0,1**
2. **Normal** : iron stores **2,3**
3. **Increased**: iron grades **4,5,6**

Serum ferritin levels were grouped into[24]

1. **Normal :** Males: 36 - 262ng/ml

   Females: 24 -155 ng/ml

2. **Increased:** Males: >265ng/ml

   Females: >155ng/ml

3. **Decreased:** Males: <30 ng/ml

   Females: <20ng/ml

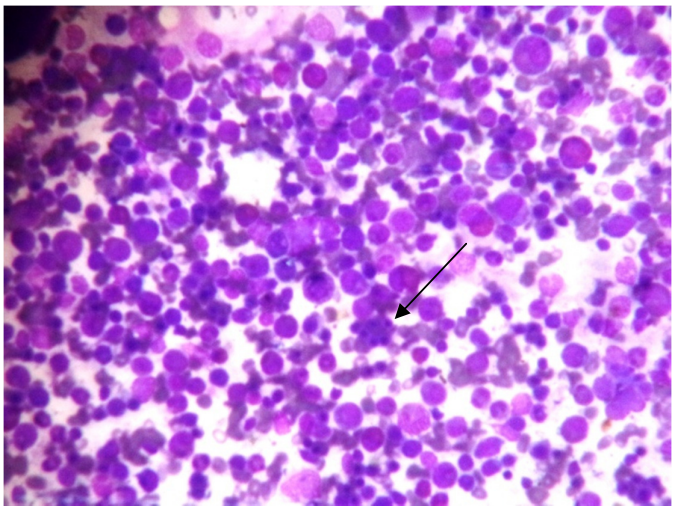


Fig.20: Bone marrow aspirate showing high cellularity with predominant increase in micronormoblasts with an erythroid island (arrow) in a case of micronormoblastic erythroid hyperplasia Leishman stain (400x)

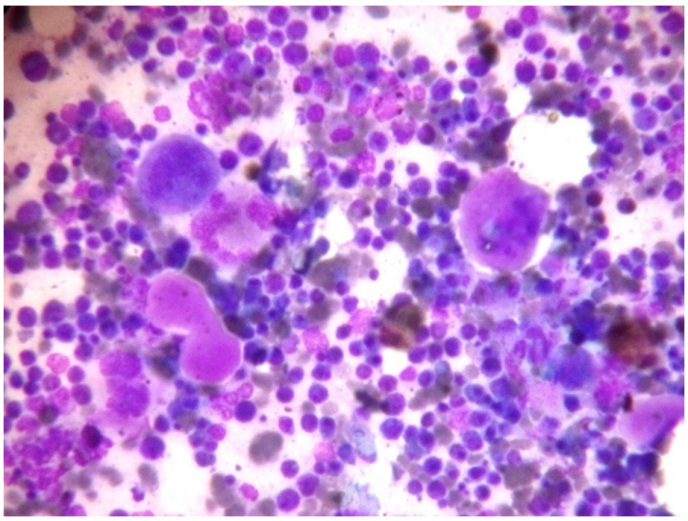


Fig.21: Bone marrow aspirate in a patient showing increase in megakaryocytes some of which have hypolobulation in a case of ITP. (megakaryocytic hyperplasia) Leishman stain (400x)

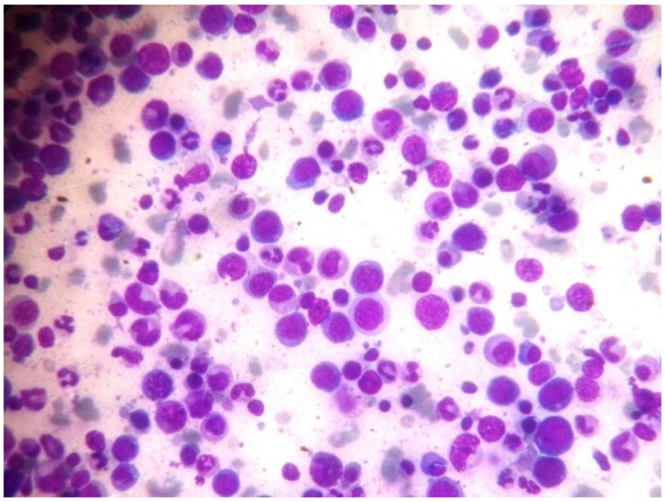


Fig.22: Bone marrow aspirate in a patient showing megaloblasts having sieve like nucleus having megaloblastic erythroid hyperplasia. Leishman stain (400x)

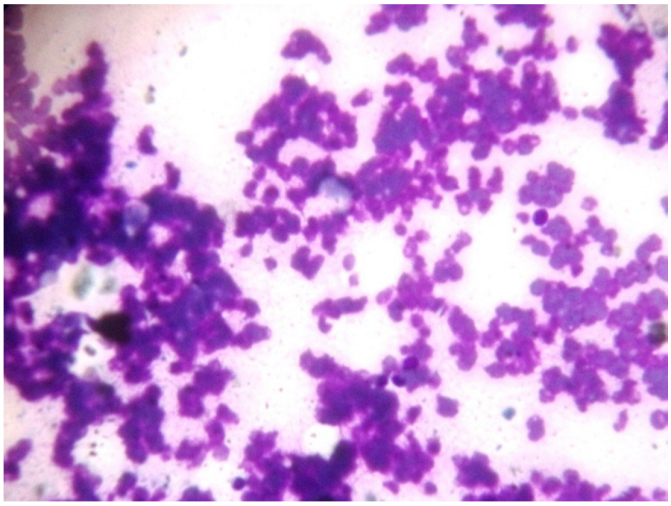


Fig.23: Bone marrowaspirate showing a hypocellular smear. Leishman stain (400x)

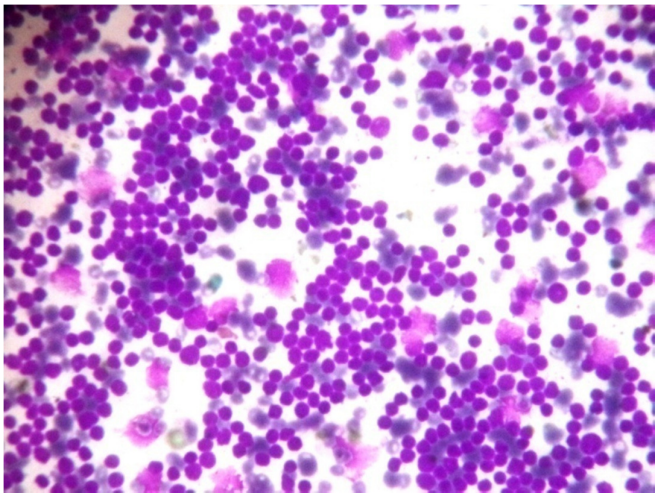


Fig.24: Bone marrow aspirate showing hypercellular smear almost most of the marrow is occupied by lymphoblasts in a case of ALL –L2 Leishman stain (400x)

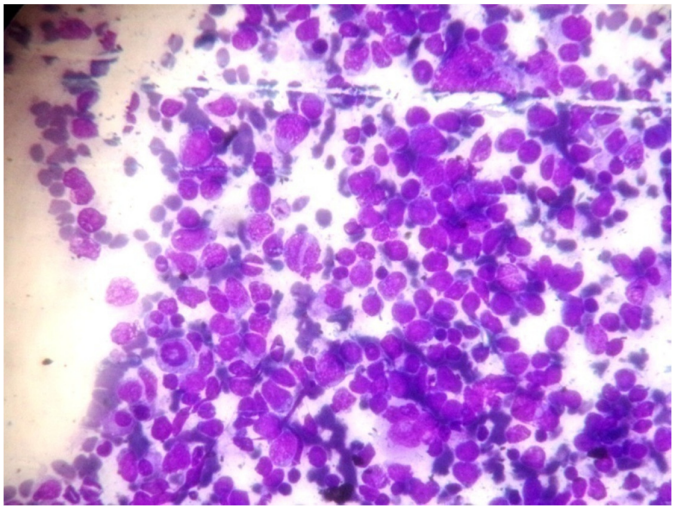


Fig.25: Bone marrow aspirate showing Myelodysplastic cells having nuclear bridging and nuclear notching in a patient with MDS Leishman stain (400x)

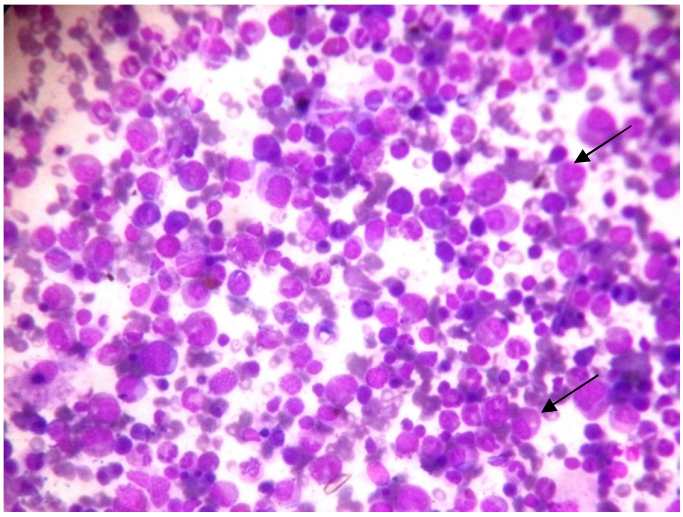


Fig.26: Bone marrow aspirate showing plasmacytosis with two plasma cells showing Rusell body (arrow) in a patient with Multiple myeloma. Leishman stain (400x)

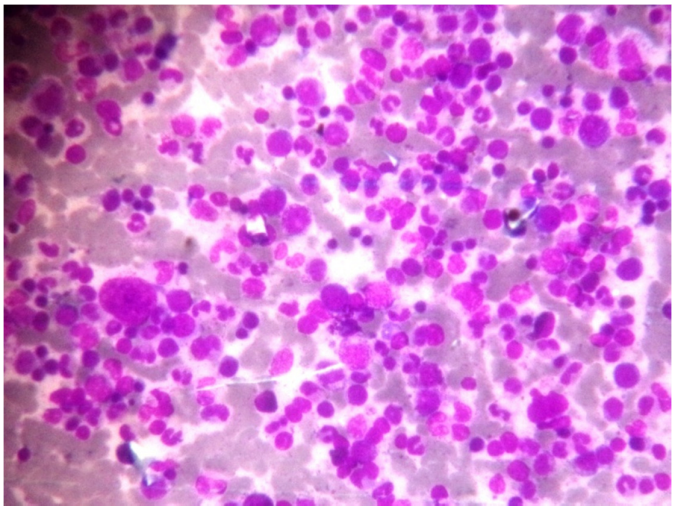


Fig.27: Bone marrow aspirate showing high cellularity with increase in all cell lineages with hypolobulation of the megakaryocyte in a case of polycythemia vera. Leishman stain (400x)

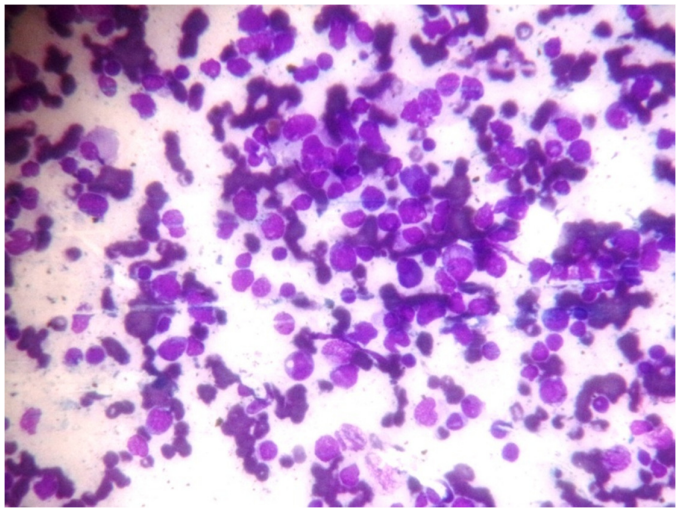


Fig.28: Bone marrow aspirate showing high cellularity with increase in myeloblasts in a case of AML. Leishman stain (400x)

The bone marrow iron stores stained were as follows ;

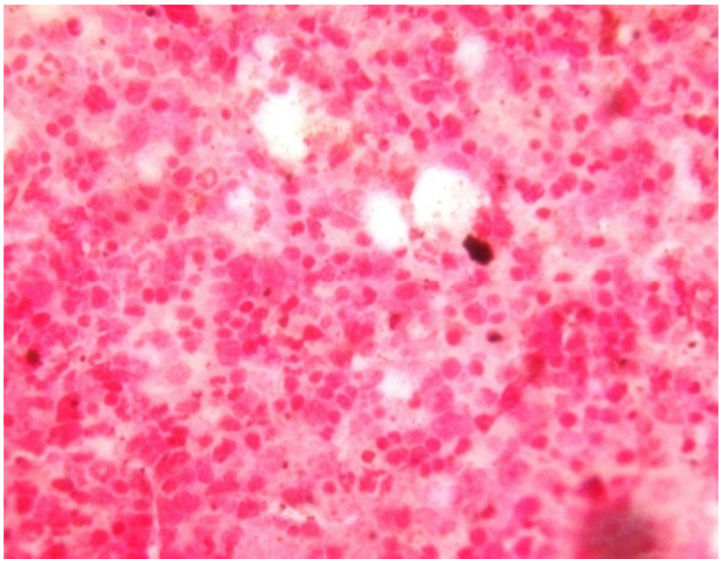


Fig. 29: Grade 0 iron store in diluted /hypocellular marrow. Perl's stain.400X

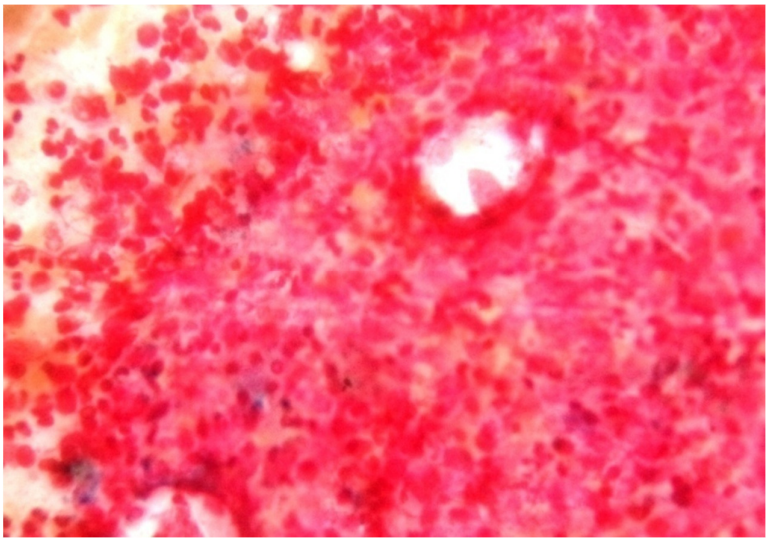


Fig. 30: Grade 1 iron store in a case of EH with micronormoblastic pattern. Perl's stain : 400X

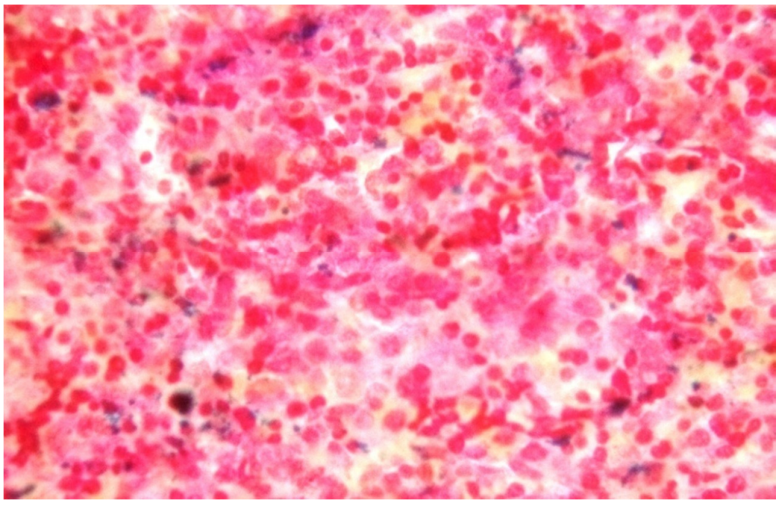


Fig. 31: Grade 2 iron stores in a case of EH with megaloblastic pattern. Perl's stain: 400X

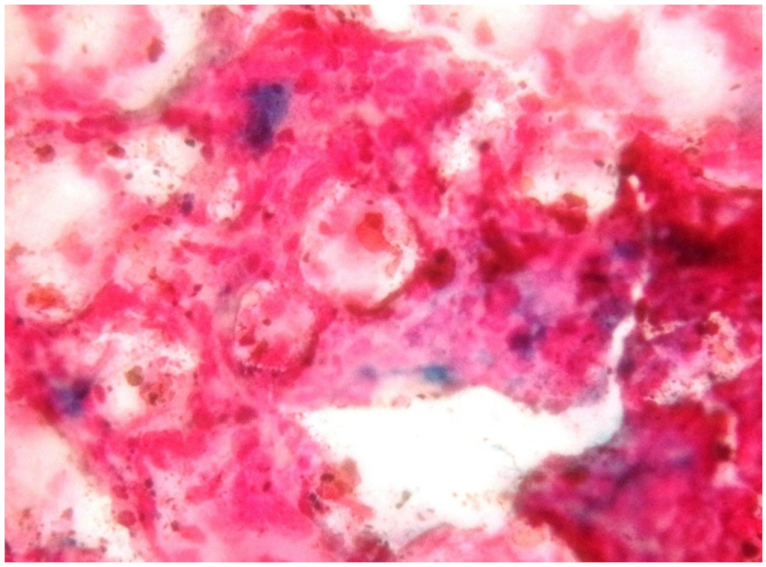


Fig.32: Grade 3 iron stores in a case of normocellular morrow with excess blasts. Perl's stain: 400x

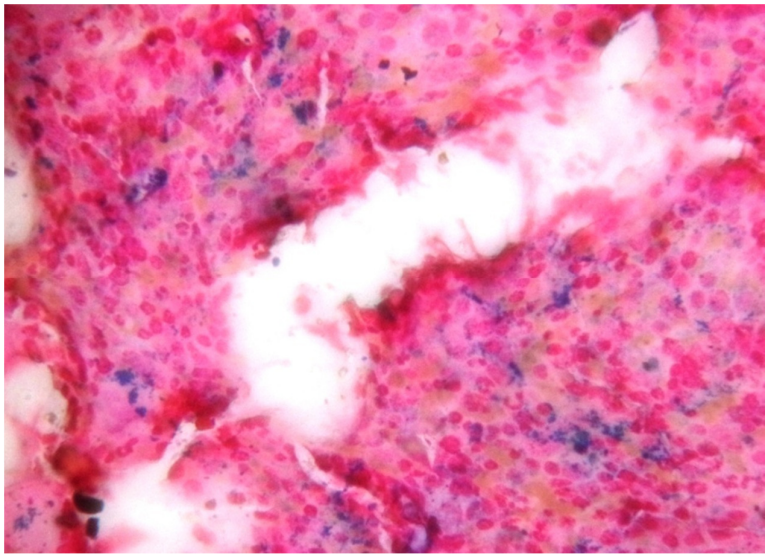


Fig. 33: Grade 4 iron stores in a case of **AML-M4**. Perl's stain : 400X

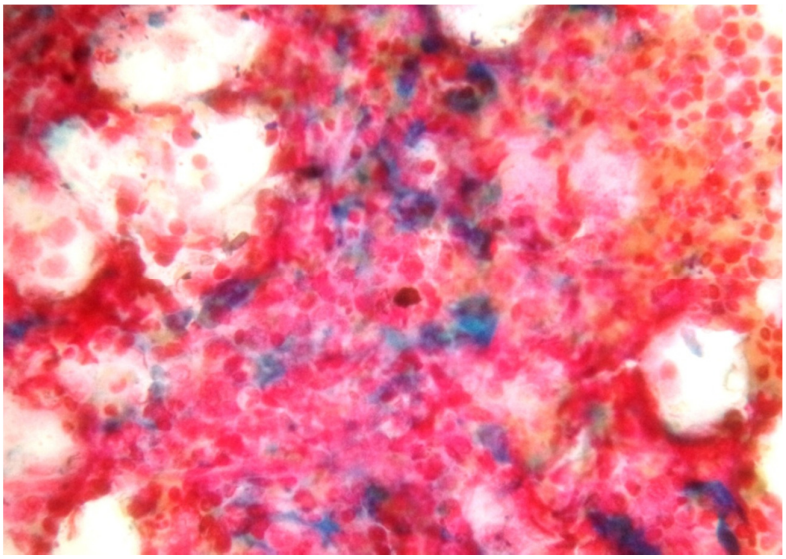


Fig.34: Grade 5 iron stores in a case of **Megakaryocytic hyperplasia with EH.** Perl's stain: 400X

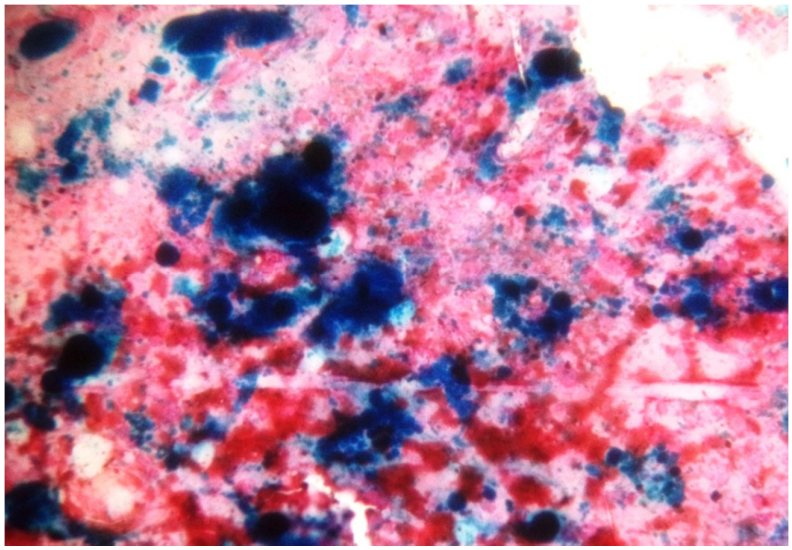
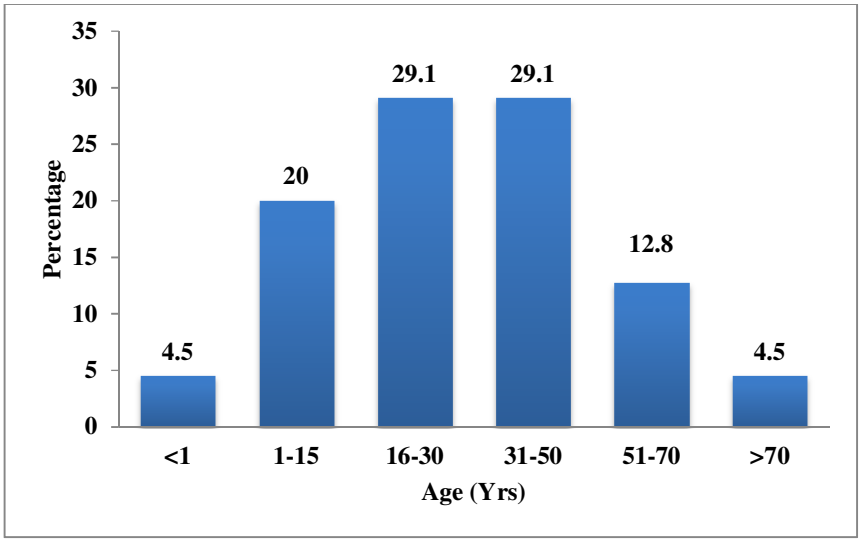


Fig.35: Grade 6 iron stores in a case of Mild hypercellular marrow with plasmacytosis in Multiple Myeloma. Perl's stain : 400X

Table.3: Percentage distribution of Age

Age (Yrs)	N	Percent (%)
<1	5	4.5
1-15	22	20
16-30	32	29.1
31-50	32	29.1
51-70	14	12.8
>70	5	4.5
Total	110	100

Fig.36: Bar diagram showing age-wise distribution

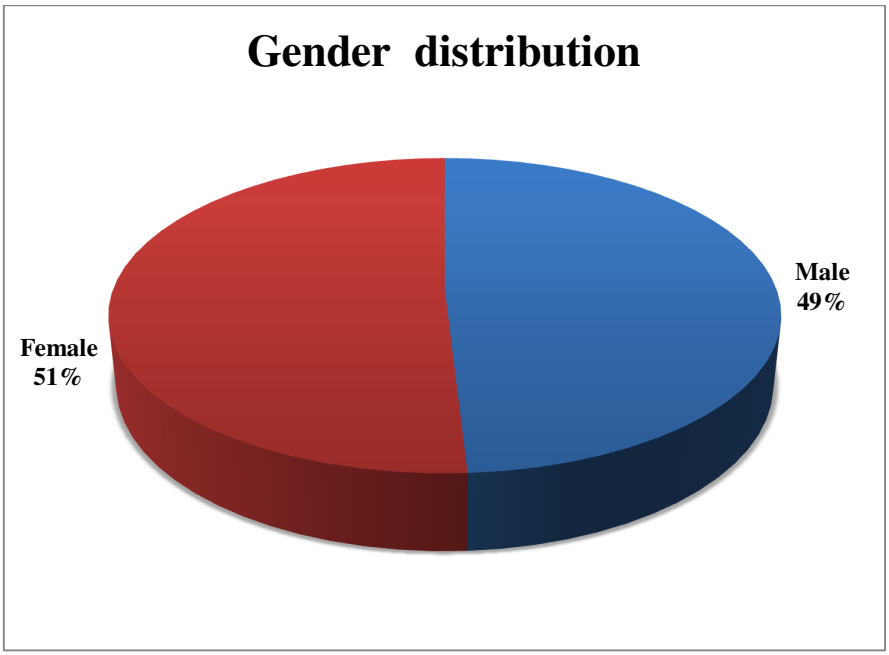


In the present study, out of 110 cases the maximum number of cases were under 16-50 years of age group accounting to 29.1 % (64 cases).

Table.4: Percentage distribution of gender

Sex	N	Percent (%)
Male	54	49.1
Female	56	50.9
Total	110	100

Fig.37: Pie-chart depicting the percentage distribution of gender

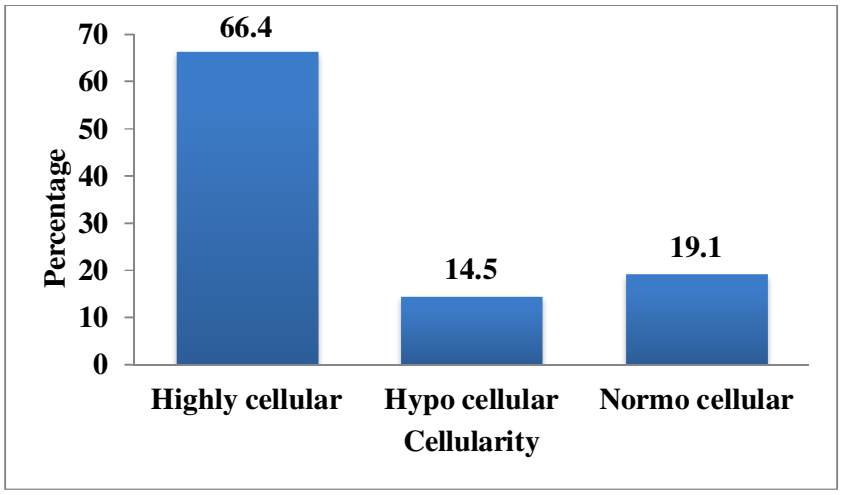


In the current study, the female population was 51% (56 cases) and male population was 49% (54 cases).

Table.5: Percentage distribution of the cellularity of the bone marrow smears

Cellularity	N	Percent (%)
Highly cellular	73	66.4
Hypocellular	16	14.5
Normocellular	21	19.1
Total	110	100

Fig.38: Bar diagram showing percentage distribution of cellularity

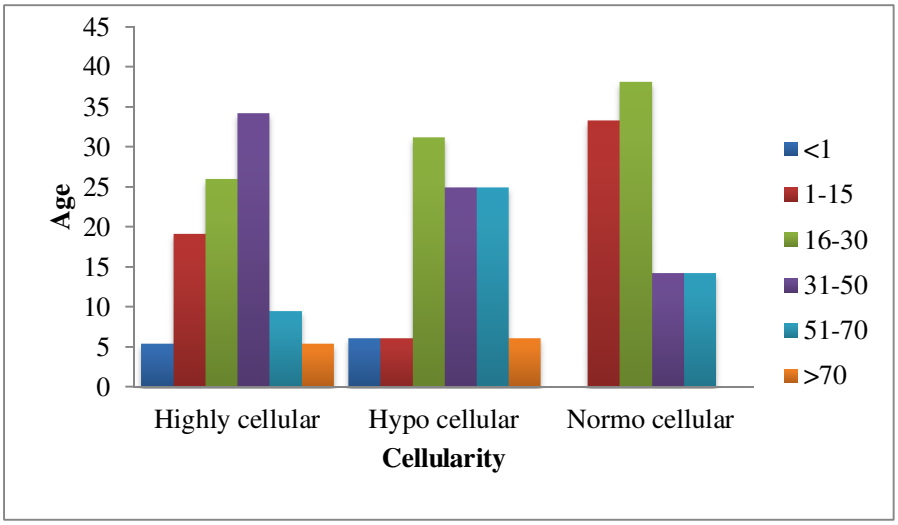


The cellularity of the bone marrow aspirates consisted high cellularity in 66.4%, 19.1% were normocellular and 14.5% were hypocellular. Predominant population was highly cellular smears.

Table.6: Table showing the relation between age and cellularity

Age (Yrs)	Highly cellular		Hypo cellular		Normo cellular		Total		p value
	N	%	N	%	N	%	N	%	
<1	4	5.5	1	6.2	0	0.0	5	4.5	0.342
1-15	14	19.2	1	6.2	7	33.3	22	20.0	
16-30	19	26.0	5	31.2	8	38.1	32	29.1	
31-50	25	34.2	4	25.0	3	14.3	32	29.1	
51-70	7	9.6	4	25.0	3	14.3	14	12.7	
>70	4	5.5	1	6.2	0	0.0	5	4.5	
Total	73	100.0	16	100.0	21	100.0	110	100.0	

Fig.39: Bar diagram depicting the relationship between the age and cellularity



In hypercellular smears the majority of the cases were from 31-50 years of age group i.e.34 % (25 cases), and lowest 4 cases each i.e.(5.5%) were from <1 and >70 years of age.

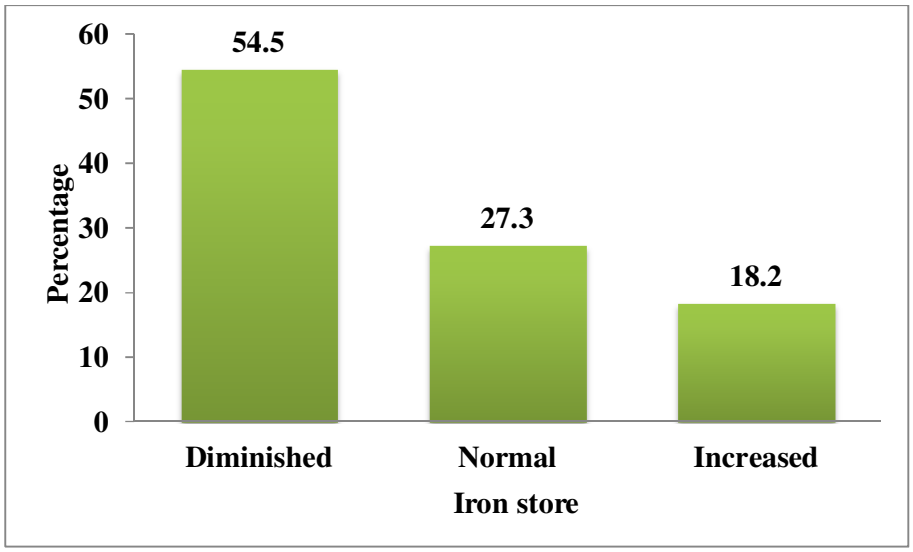
Hypocellular smears (**Fig.23**) showed highest incidence in 16-30 years of age group i.e. 31.2% (5 cases), and lowest in <1, 1-15, >70 i.e.6.2% (1 case each).

Normocellular cellularity showed highest incidence in 16-30 age group 38.1% (8 cases) and lowest in >70, and <1 age group 4.5% (5 cases).P value was 0.342

Table.7: Percentage distribution of Iron store

Iron store	N	Percent (%)
Diminished	60	54.5
Normal	30	27.3
Increased	20	18.2
Total	110	100

Fig.40: Bar diagram depicting percentage distribution of iron stores

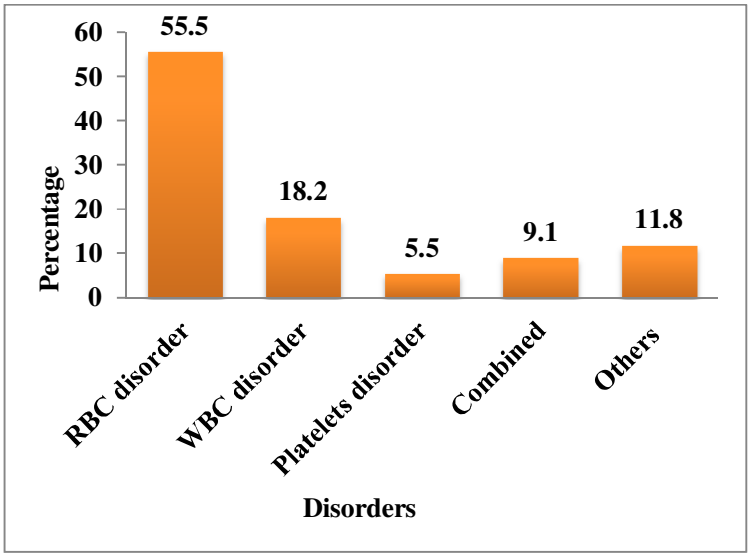


This table depicts the incidence of iron stores. Diminished iron stores among the patients were highest accounting to total of 60/110 cases i.e.(54.5%), followed by normal 30 cases(27.3%) and finally increased stores 20 cases (18.2%).

Table.8: Percentage distribution of various disorders

Disorders	N	Percent (%)
RBC disorder	61	55.5
WBC disorder	20	18.2
Platelet disorder	6	5.5
Combined	10	9.1
Others	13	11.8
Total	110	100

Fig .41: Bar diagram showing percentage distribution of disorders

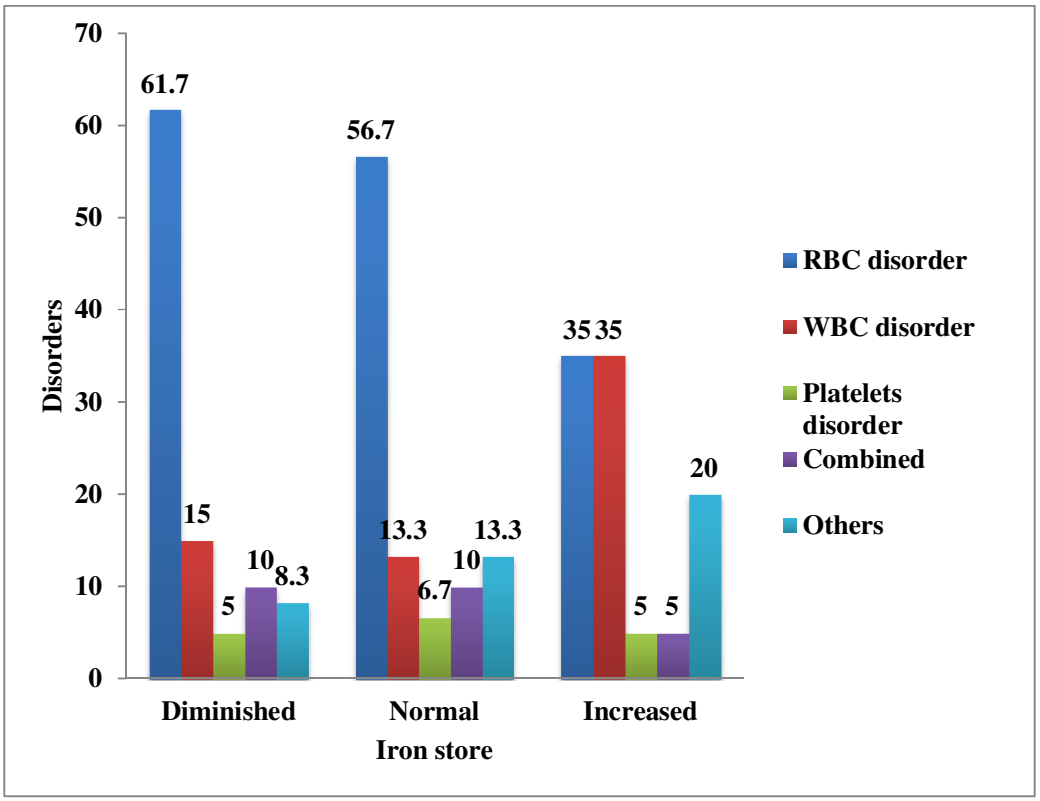


Among all the various hematological disorders studied RBC disorders were predominant i.e.55.5% (61 cases), followed by WBC i.e. 18.2% (20cases), platelet disorders were 5.5% (6 cases), combined disorders were 9.1% (10 cases) and others comprising of 11.8% (13 cases).

Table.9: Table showing the iron stores in various hematological disorders

Disorders	Diminished		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
RBC disorder	37	61.7	17	56.7	7	35	61	55.5	0.421
WBC disorder	9	15	4	13.3	7	35	20	18.2	
Platelet disorder	2	5	3	6.7	1	5	6	5.5	
Combined	6	10	3	10	1	5	10	9.1	
Others	5	8.3	4	13.3	4	20	13	11.8	
Total	60	100	30	100	20	100	110	100	

Fig.42: Bar diagram showing relationship between iron stores and various hematological disorders



RBC disorder with diminished iron stores showed peak incidence i.e.61.7% (37 cases), followed by normal iron stores 56.7% (17 cases), 35% (7 cases) had increased iron stores with RBC disorders.

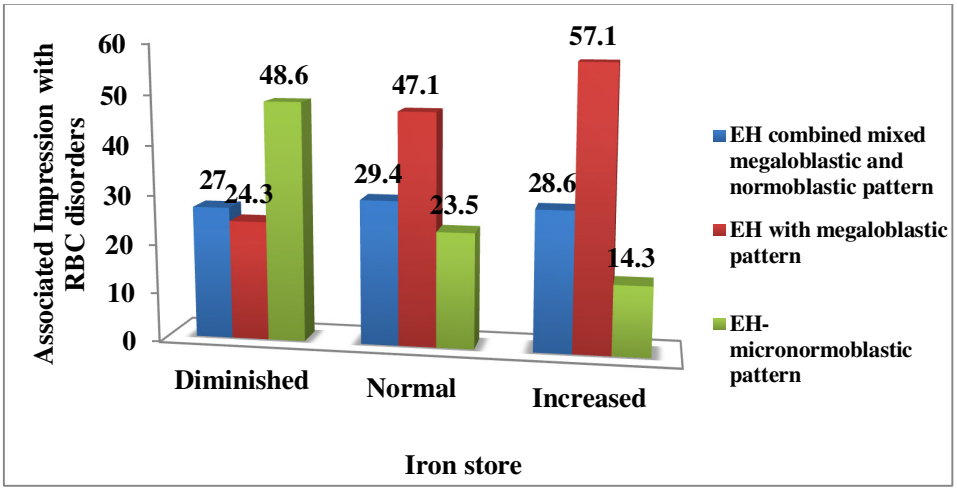
WBC disorders showed highest peak in diminished iron stores i.e. 9/20 cases, followed by increased iron stores i.e.7/20 (35%), cases and normal iron stores with WBC disorders were 4/20 (20%) cases.

Platelet disorders showed predominantly normal iron stores number being 2/6(66%) cases. Combined disorders showed predominantly diminished iron stores i.e.6/10 cases (60%). Other groups also showed predominantly diminished iron stores i.e. 5/13 cases (38.4%). p value was 0.421 which showed that the iron stores were distributed among all hematological disorders and not only confined to RBC disorders.

Table.10: Table is depicting the relationship between the various RBC disorders and the iron stores

RBC disorders	Diminished		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
EH combined mixed megaloblastic and normoblastic pattern	10	27.0	5	29.4	2	28.6	17	27.9	**0.201**
EH with megaloblastic pattern	9	24.3	8	47.1	4	57.1	21	34.4	
EH-micronormoblastic pattern	18	48.6	4	23.5	1	14.3	23	37.7	
Total	37	100.0	17	100.0	7	100.0	61	100.0	

Fig.43: Bar diagram showing association between RBC disorders and iron stores

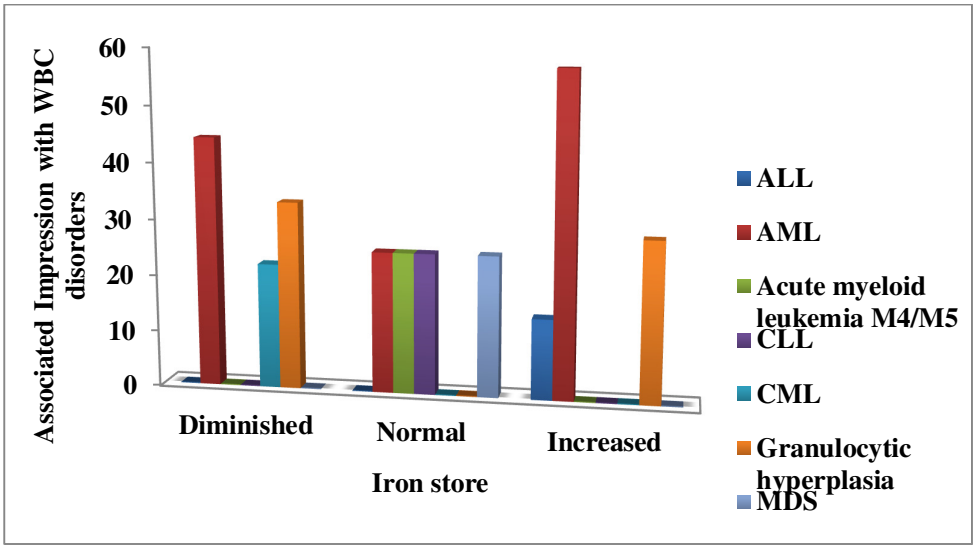


Erythroid hyperplasia with micronormoblastic pattern (**Fig.20**) had significantly diminished iron stores i.e.48.6% (14 cases), followed by mixed megaloblastic and micronormoblastic pattern i.e.27.0 % (10cases), normal and increased iron stores were found in erythroid hyperplasia with megaloblastic pattern (**Fig.22**) having 47% (8 cases and 4 cases) respectively. P value for this group was 0.201

Table.11: Table showing relationship between WBC disorders and iron stores

WBC disorders	Diminished		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
ALL	0	0.0	0	0.0	1	14.3	1	5.0	0.111
Myeloid hyperplasia with atypical cells.(AML)	4	44.4	1	25.0	4	57.1	9	45.0	
Acute myeloid leukemia M4/M5	0	0.0	1	25.0	0	0.0	1	5.0	
CLL	0	0.0	1	25.0	0	0.0	1	5.0	
CML	2	22.2	0	0.0	0	0.0	2	10.0	
Granulocytic hyperplasia	3	33.3	0	0.0	2	28.6	5	25.0	
MDS	0	0.0	1	25.0	0	0.0	1	5.0	
Total	9	100.0	4	100.0	7	100.0	20	100.0	

Fig.44: Bar diagram depicting the iron stores in various WBC disorders

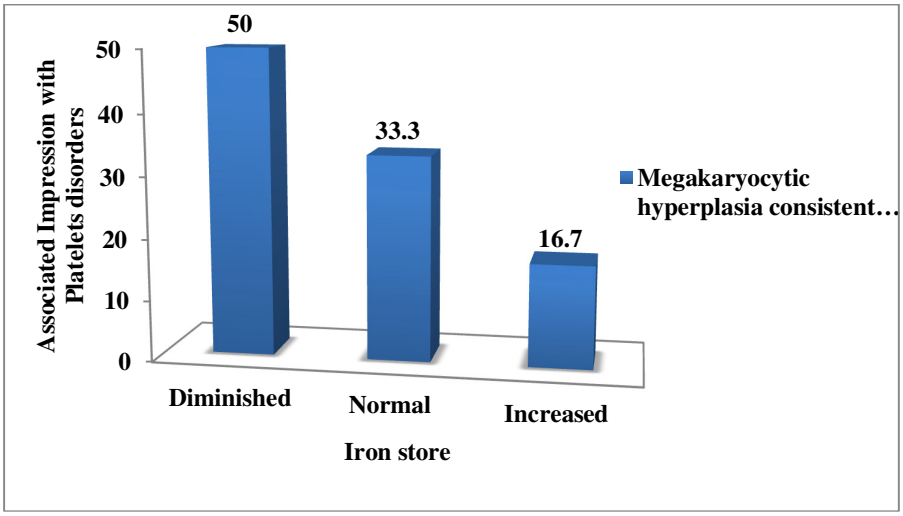


Increased iron stores were seen predominantly in myeloid hyperplasia with atypical cells i.e.57.1% (4 cases), AML-M4 5 cases (**Fig.28**), MDS (**Fig.25**) and ALL (**Fig.24**) showed predominantly normal iron stores 25% (1 case), CML and Granulocytic hyperplasia showed diminished iron stores i.e.22.2% (2 cases) and 33.3% (3 cases) respectively. ALL showed minimal increased iron stores with 14.3% (1 case).p value being 0.111

Table.12: Association between megakaryocytic hyperplasia and iron stores

Platelets disorder	Diminished		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
Megakaryocytic hyperplasia consistent with ITP	3	50.0	2	33.3	1	16.7	6	100.0	NA

Fig.45: Bar diagram showing the association between megakaryocytic hyperplasia and iron stores

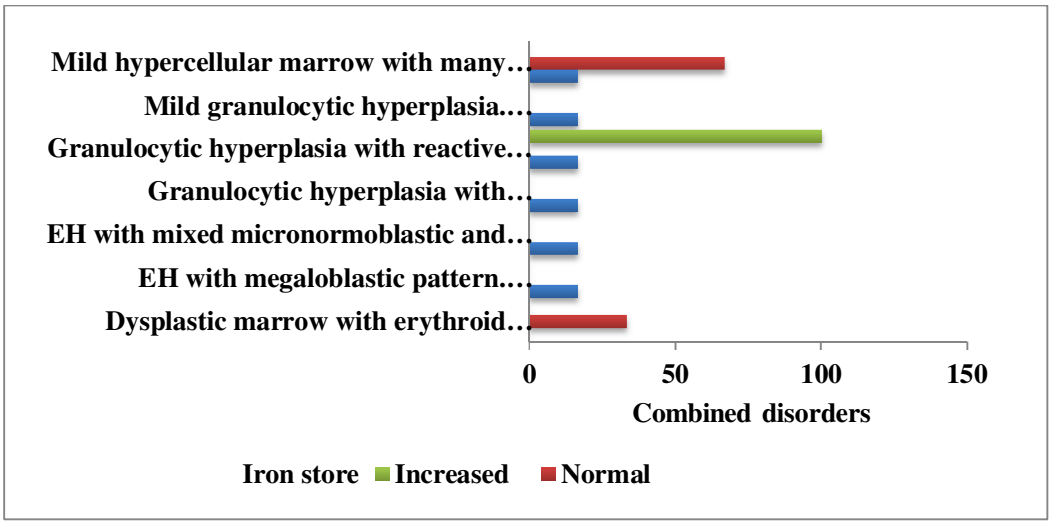


Three cases of megakaryocytic hyperplasia (**Fig.21**) i.e. consistent with ITP had peak with diminished iron stores 50.0% (3 cases) followed by normal i.e.33.3% (2 cases) and increased iron stores i.e.16.7% (1 case).

Table.13: Association of various combined hematological disorders and iron stores.

Combined disorder	Diminished		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
Dysplastic marrow with erythroid hyperplasia	0	0.0	1	33.3	0	0.0	1	10.0	0.543
EH with megaloblastic pattern and Myelodyspalsia	1	16.7	0	0.0	0	0.0	1	10.0	
EH with mixed micronormoblastic and megaloblastic pattern and myeloid shift to left	1	16.7	0	0.0	0	0.0	1	10.0	
Granulocytic hyperplasia with micronormoblastic changes	1	16.7	0	0.0	0	0.0	1	10.0	
Granulocytic hyperplasia with reactive plasmacytosis	1	16.7	0	0.0	1	100.0	2	20.0	
Mild granulocytic hyperplasia (infection induced turnover)	1	16.7	0	0.0	0	0.0	1	10.0	
Mild hypercellular marrow with many atypical cells	1	16.7	2	66.7	0	0.0	3	30.0	
Total	6	100.0	3	100.0	1	100.0	10	100.0	

Fig.46: Showing combined hematological disorders and its correlation with iron stores



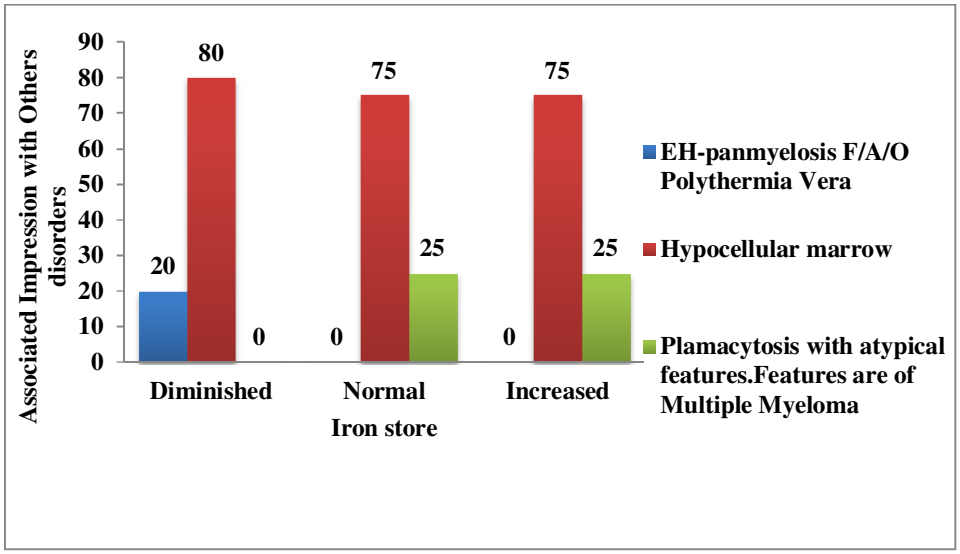
Diminished iron stores were seen in EH with megaloblastic pattern and Myelodyspalsia, EH with mixed micronormoblastic and megaloblastic pattern and myeloid shift to left, Granulocytic hyperplasia with micronormoblastic changes and reactive plasmacytosis, mild granulocytic hyperplasia ?infection induced turnover and mild hypercellular marrow with many atypical cells showing 16.7 % of the population 16.7% i.e.(1 case each).

Normal iron stores were seen in dysplastic marrow with EH and hypercellular marrow with atypical cells having 33.3 % (1 case), 66.1% (2 cases).Increased iron stores were seen in 10.1% (1 case) reactive plasmacytosis. p value (0.543) was statistically insignificant as highest percentage was seen in hypercellular marrow with atypical cells with normal iron stores.

Table.14: Table showing the association between various other hematological disorders and iron stores.

Others	Diminished		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
EH-panmyelosis F/A/O Polycythemia Vera	1	20.0	0	0.0	0	0.0	1	7.7	0.311
Hypocellular marrow	4	80.0	3	75.0	3	75.0	10	76.9	
Plasmacytosis with atypical / Multiple Myeloma	0	0.0	1	25.0	1	25.0	2	15.4	
Total	5	100.0	4	100.0	4	100.0	13	100	

Fig.47: Bar diagram depicting the association of other hematological disorders and iron stores



Iron stores were diminished predominantly in hypocellular marrow 80% (4 cases) followed by polycythemia vera 20% (1 case) (**Fig.27**). Normal iron stores were seen in hypocellular marrow only 75% (3 cases), and in multiple myeloma 25%, 1 case (**Fig.26**).Increased iron stores were seen predominantly in hypocellular marrow 75% (3 cases), p value being statistically insignificant (0.311)

Table.15: Descriptive statistics of Serum ferritin

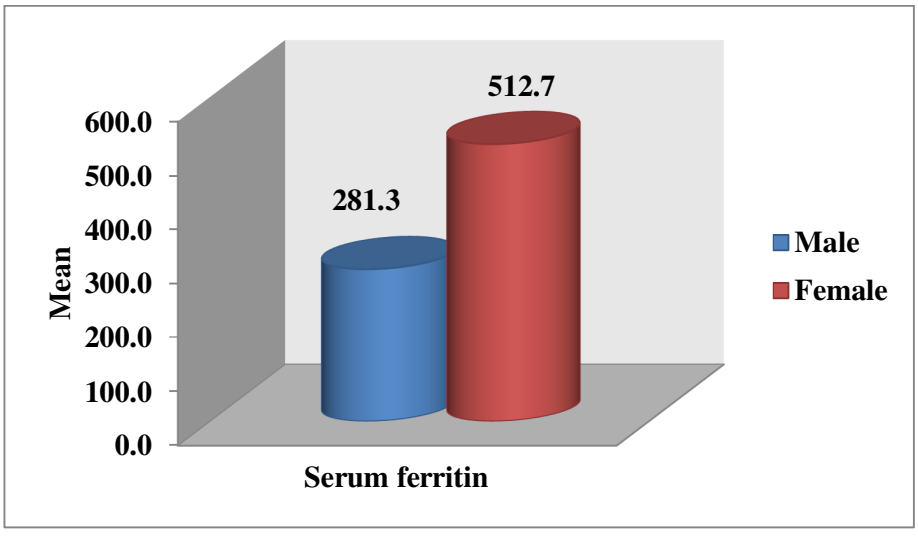
	N	Minimum	Maximum	Mean	SD
Serum ferritin	70	1.4	2001	400.3	619.7

Serum ferritin values could be estimated in 70 out of 110 cases which were ranging from 1.4ng/ml to 2001ng/ml and the mean being 400.3

Table.16: Percentage distribution of Serum ferritin among males and females

	Sex	N	Mean	SD	p value
Serum ferritin	**Male**	34	281.3	403.4	0.119
	Female	36	512.7	759.6	

Fig.48: Bar diagram showing the mean and the SD with the total incidence of the serum ferritin values among the males and females

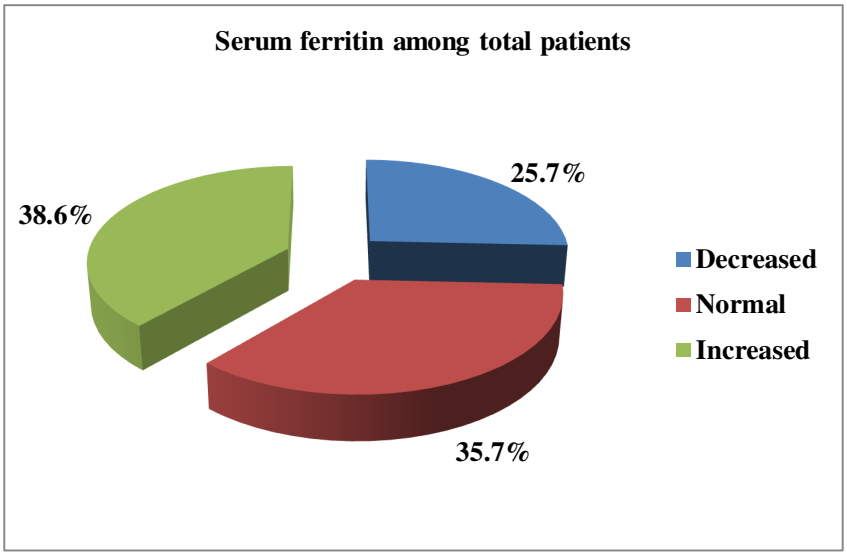


A total of 34 males and 36 females serum ferritin was estimated mean being 281.3 and 512.7 respectively. p value was 0.119

Table.17: Distribution of Serum ferritin between male and females

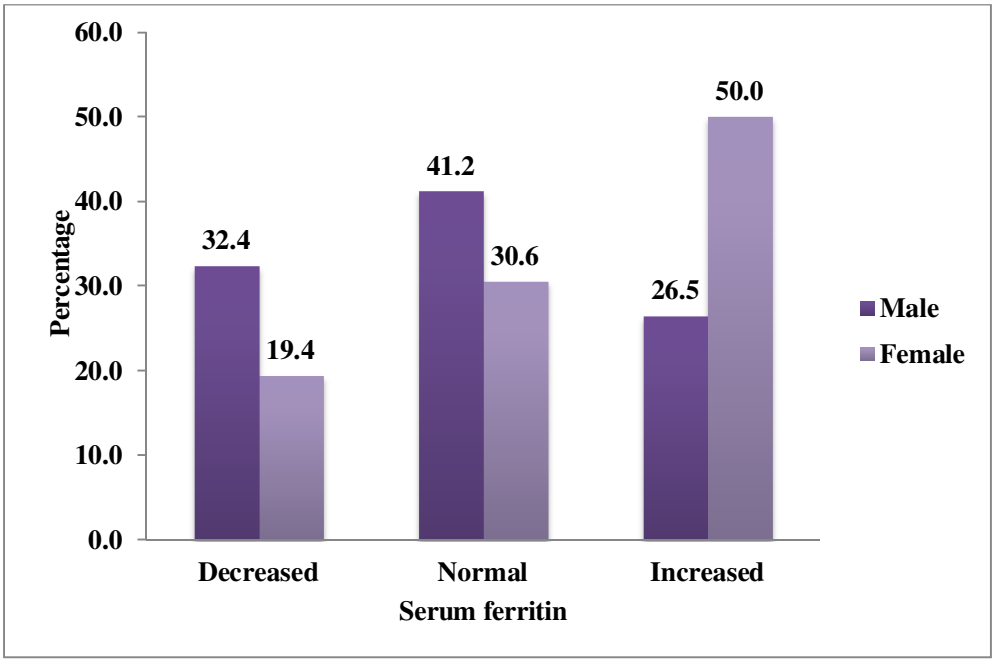
Serum ferritin	Male		Female		Total		p value
	N	Percent (%)	N	Percent (%)	N	Percent (%)	
Decreased	11	32.4	7	19.4	18	25.7	0.123
Normal	14	41.2	11	30.6	25	35.7	
Increased	9	26.5	18	50.0	27	38.6	
Total	34	100.0	36	100.0	70	100.0	

Fig.49: Pie chart showing the total distribution of serum ferritin



The table shows the total number of patients with decreased serum ferritin was 18 cases (25.7%), normal 25 cases (35.7%) and increased were the highest i.e.27 cases (38.6%).

Fig.50: Serum ferritin gender wise

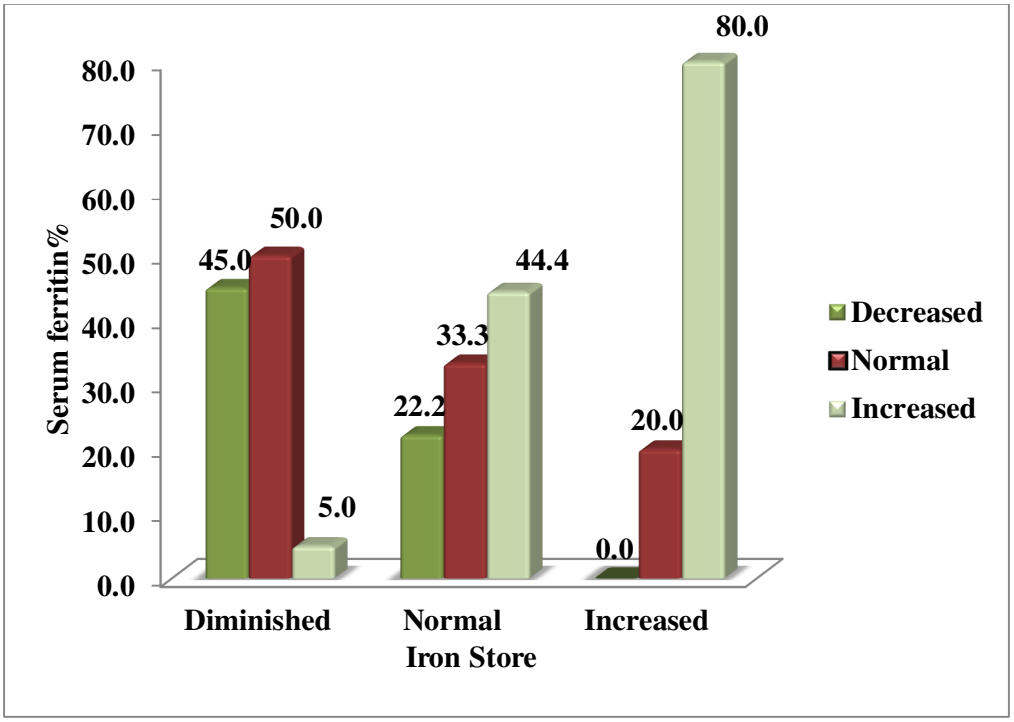


The percentage distribution among males and females were as follows; males had predominantly normal ferritin levels in 41cases (41.2%), decreased in 11 cases (32.4%), 9 cases of increased serum ferritin levels (26.5%). p value was 0.123

Table.18: Association of Serum ferritin and Iron store among males

Serum ferritin	Iron Store								p value
	Diminished		Normal		Increased		Total		
	N	%	N	%	N	%	N	%	
Decreased	9	45.0	2	22.2	0	0.0	11	32.4	0.007
Normal	10	50.0	3	33.3	1	20.0	14	41.2	
Increased	1	5.0	4	44.4	4	80.0	9	26.5	
Total	20	100.0	9	100.0	5	100.0	34	100.0	

Fig.51: Bar diagram showing the relationship of serum ferritin and iron stores among males



In males, decreased serum ferritin levels were predominantly observed in diminished iron stores in 9 cases (45.0%), 2 cases were of normal iron stores (22.2%), and no cases with increased stores.

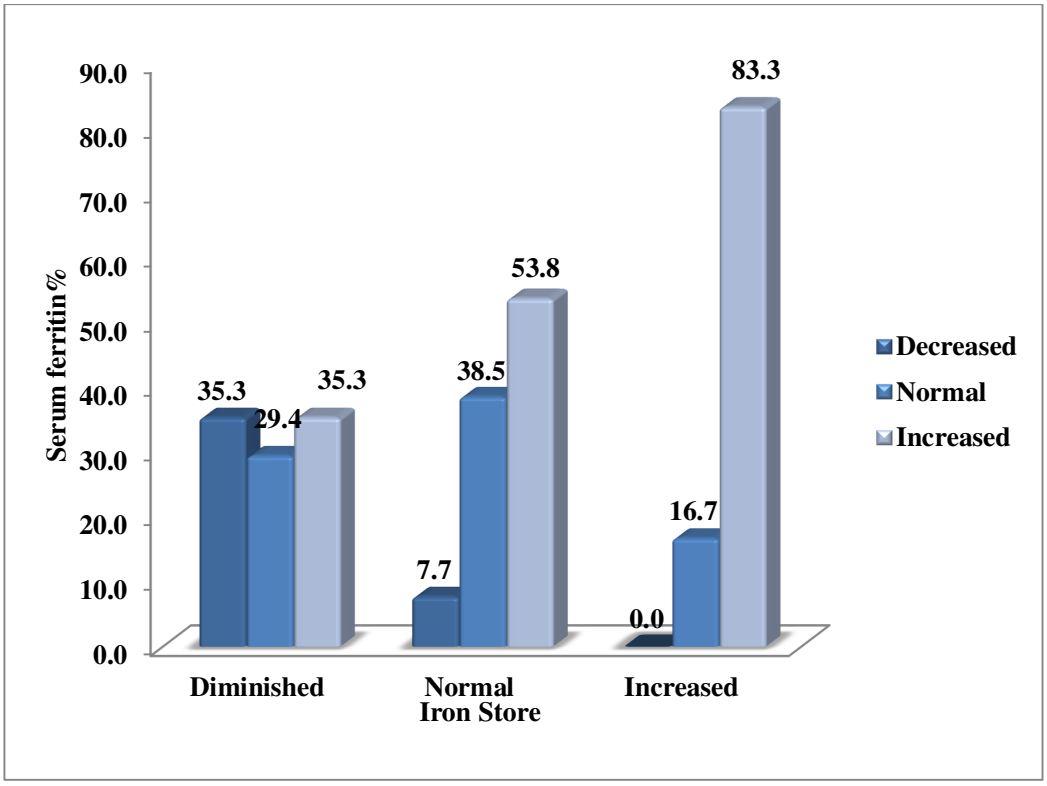
Normal serum ferritin levels were seen highest again in diminished iron stores in 10 cases (50.0%), 3 cases of normal iron stores (33.3%), and 1 case of increased iron stores.

Increased serum ferritin levels were seen mainly in 4 cases of increased iron stores (80%), 4 cases of normal stores (44.4%) and 1 case (5.0%) of decreased iron stores. p value was 0.007

Table.19: Relationship of Serum ferritin and Iron store among females

Serum ferritin	Iron Store								p value
	Diminished		Normal		Increased		Total		
	N	%	N	%	N	%	N	%	
Decreased	6	35.3	1	7.7	0	0.0	7	19.4	0.134
Normal	5	29.4	5	38.5	1	16.7	11	30.6	
Increased	6	35.3	7	53.8	5	83.3	18	50.0	
Total	17	100.0	13	100.0	6	100.0	36	100.0	

Fig. 52: Bar diagram showing the relationship of serum ferritin and iron stores among females



In females, decreased serum ferritin values were found predominantly found in diminished iron stores accounting for 6 cases (35.3%), followed by 1 case of normal iron stores (7.0%).

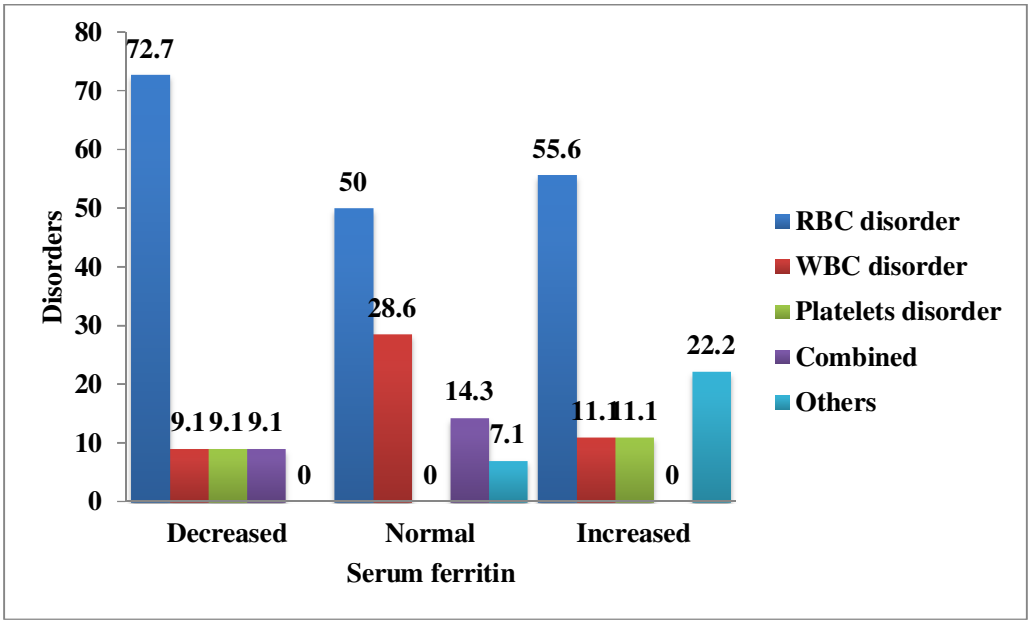
Normal serum ferritin levels were seen highest in 5 cases of diminished iron stores (29.7%), 5 cases (38.5%) of normal iron stores, and 1 case of increased iron stores (16.5%).

Increased serum ferritin levels showed predominantly normal iron stores i.e. 7 cases out of 13 i.e. (53.8%) and 5 cases out of 6 showed normal stores (83.3%), and 6 cases out of 17 showed diminished iron stores (35.3%).

Table.20: Relationship of Disorders and Serum ferritin among males

Disorders	Decreased		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
RBC disorder	8	72.7	7	50	5	55.6	20	58.8	0.466
WBC disorder	1	9.1	4	28.6	1	11.1	6	17.6	
Platelets disorder	1	9.1	0	0	1	11.1	2	5.9	
Combined	1	9.1	2	14.3	0	0	3	8.8	
Others	0	0	1	7.1	2	22.2	3	8.8	
Total	11	100	14	100	9	100	34	100	

Fig.53: Bar diagram depicting the percentage incidence of serum ferritin levels and various disorders among males.



Decreased serum ferritin values were predominantly observed in RBC disorders which accounted for 8 cases out of 11 (72.7%), and one case each of WBC (9.1%), platelet (9.1%), and combined disorders (9.1%).

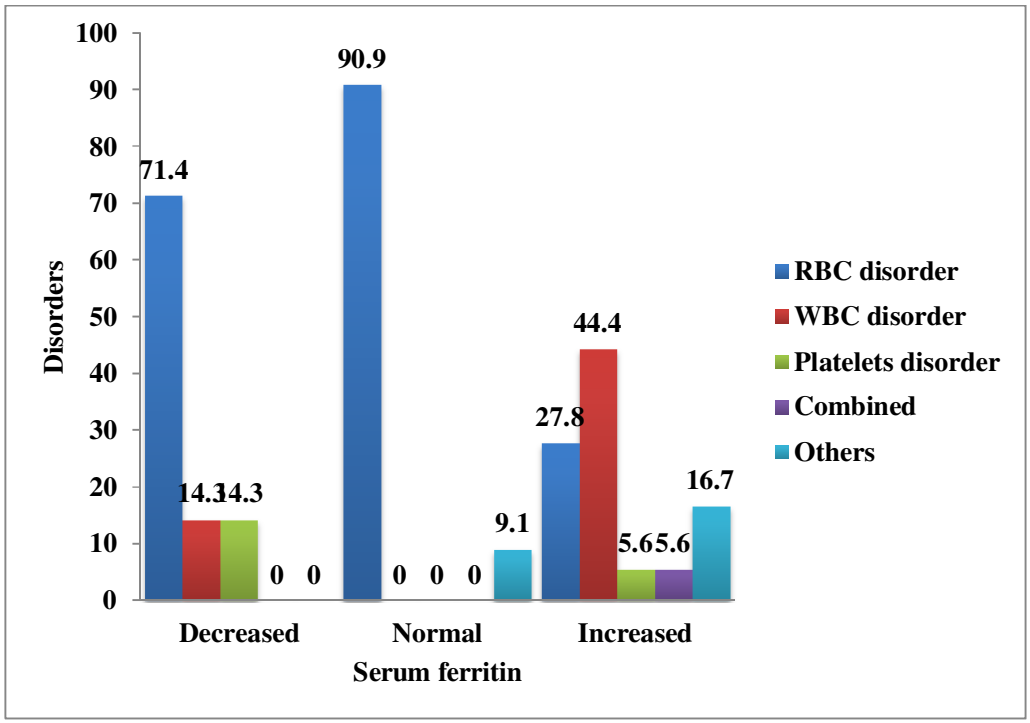
Normal serum ferritin values were seen in 7/14 (50%) of RBC disorders, WBC disorders showed 4 cases (28.6%) of normal levels, followed by 2 cases of combined disorders (12.3%) and 1 case of other disorders (7.1%).

Increased serum ferritin levels were seen in 5/9 cases i.e. (55.6%) of RBC disorders, followed by 2 cases of other disorders 22.2%, 1 case each WBC and platelet disorders (11.1%). p value was 0.466

Table.21: Association of Disorders and Serum ferritin among females

Disorders	Decreased		Normal		Increased		Total		p value
	N	%	N	%	N	%	N	%	
RBC disorder	5	71.4	10	90.9	5	27.8	20	55.6	0.061
WBC disorder	1	14.3	0	0	8	44.4	9	25	
Platelets disorder	1	14.3	0	0	1	5.6	2	5.6	
Combined	0	0	0	0	1	5.6	1	2.8	
Others	0	0	1	9.1	3	16.7	4	11.1	
Total	7	100	11	100	18	100	36	100	

Fig.54: Bar diagram depicting the percentage incidence of serum ferritin levels and various disorders among females.



Decreased serum ferritin levels were predominantly seen in 5 cases out of 7 (71.4%) of RBC disorders and 1 case each of WBC and platelet disorders (14.3%).

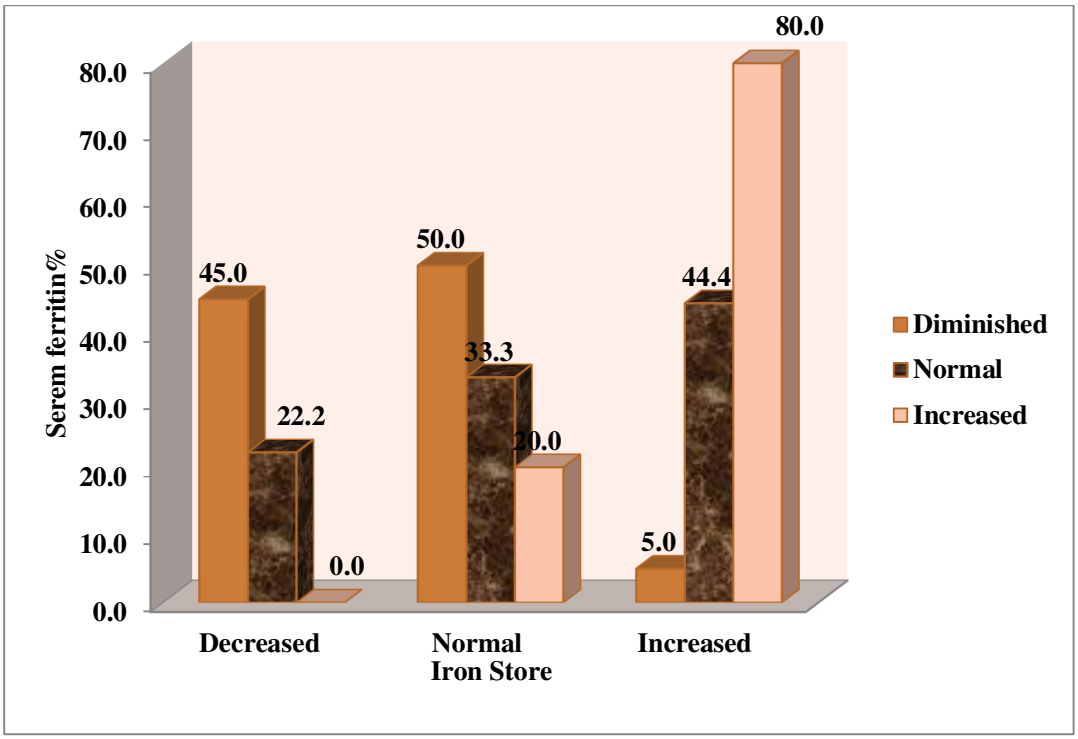
Normal ferritin levels were predominantly seen in RBC disorders 10/ 11 cases (90.9%), and 1 case of other disorders (9.1%).

Increased ferritin levels were seen mainly in 8 cases of WBC disorders (44.4%), followed by 5 cases (27.8%) of RBC disorders, 1 case each of platelet and combined disorders (5.6%), and 3 cases (16.7%) of other disorders. p value was almost statistically significant.

Table.22: Association of Serum ferritin and Iron Stores among patients having non-nutritional Anaemia

Iron Store	Serum ferritin								p value
	Decreased		Normal		Increased		Total		
	N	%	N	%	N	%	N	%	
Diminished	3	100.0	2	50.0	2	16.7	7	36.8	0.060
Normal	0	0.0	0	0.0	5	41.7	5	26.3	
Increased	0	0.0	2	50.0	5	41.7	7	36.8	
Total	3	100.0	4	100.0	12	100.0	19	100.0	

Fig.55: Bar diagram showing the relation between the serum ferritin and iron stores in non- nutritional anemias.



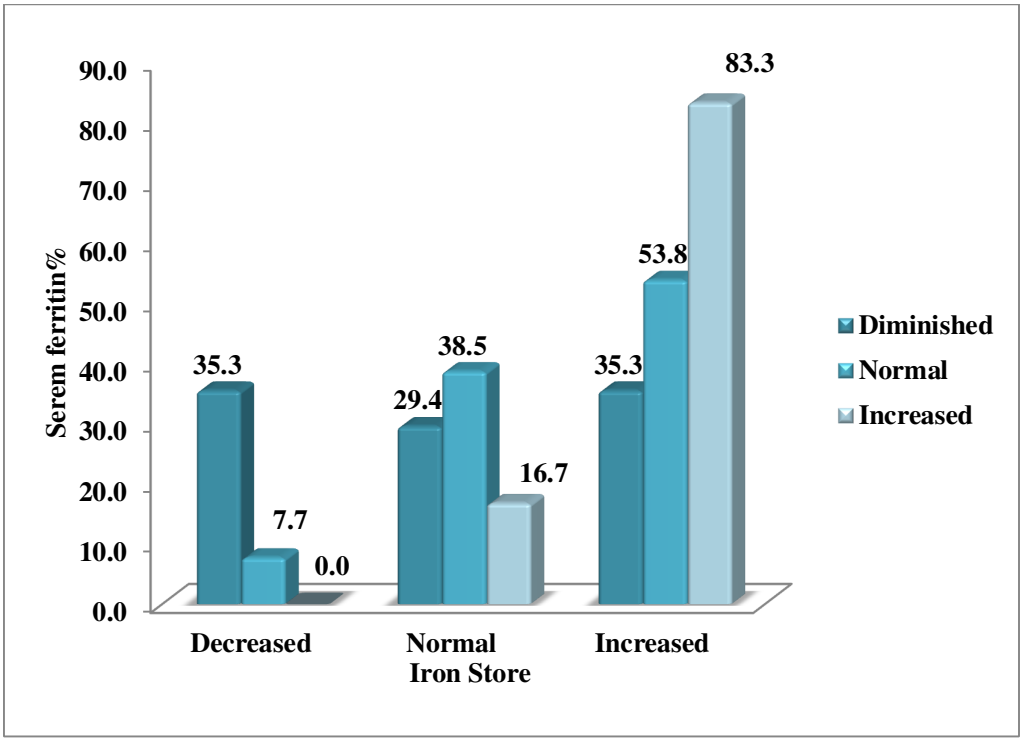
All cases of decreased serum ferritin levels showed diminished iron stores i.e. 3 cases (100%). 2 cases each of normal serum ferritin levels were associated with diminished and increased iron stores (50%).

Increased ferritin levels were associated with 2 cases of diminished iron stores (16.7%), 5 cases of normal and increased (41.7%) iron stores. P value was statistically significant 0.060.

Table.23: Association of Serum ferritin and Iron Stores among patients having nutritional Anaemia

Iron Store	Serum ferritin								p value
	Decreased		Normal		Increased		Total		
	N	%	N	%	N	%	N	%	
Diminished	11	78.6	11	61.1	4	36.4	26	60.5	0.023
Normal	3	21.4	7	38.9	4	36.4	14	32.6	
Increased	0	0.0	0	0.0	3	27.3	3	7.0	
Total	14	100.0	18	100.0	11	100.0	43	100.0	

Fig.56: Bar diagram showing relation between the iron stores and serum ferritin in nutritional anemia

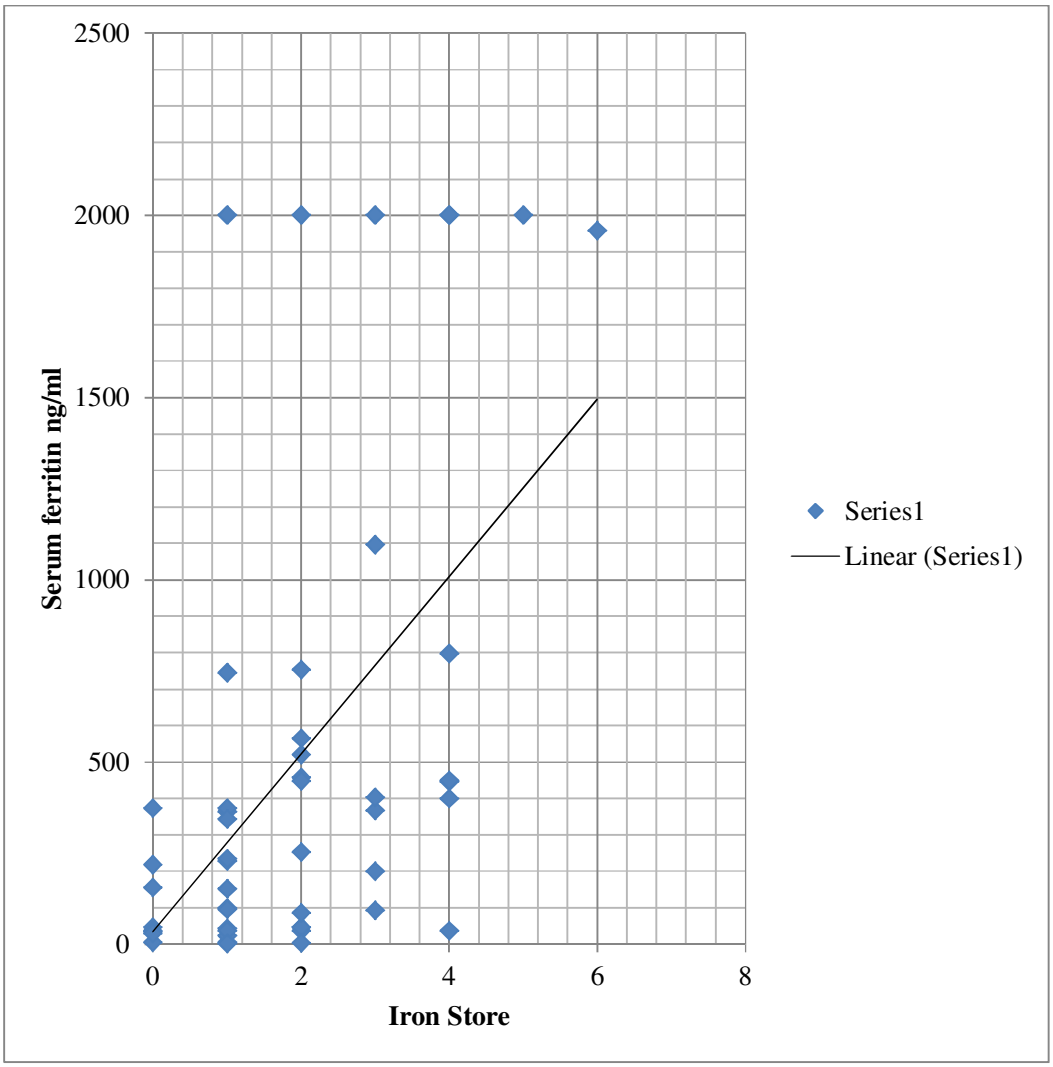


Decreased serum ferritin levels were predominantly seen in patients with diminished iron stores 11/14 cases (78.6%), normal iron stores with decreased ferritin levels were 3 cases (21.4%), and no case of increased iron stores with decreased ferritin.

Normal ferritin levels were mainly found in diminished iron stores 11/18 cases (61.1%), normal iron stores with normal ferritin levels were 7 cases (38.9%), with no case having increased levels.

Increased ferritin levels were associated highly with both decreased and normal stores 4 /11 cases (36.4%), with 3 cases (27.3%) having increased stores. P value was statistically significant 0.023

Table.24: Correlation of Serum ferritin and Iron store



This scattergram shows a weak correlation between serum ferritin and bone marrow iron stores as the iron stores were decreased i.e. Grade 0-1, serum ferritin was low, normal serum ferritin levels showed low to normal iron stores, and increased iron stores showed raised serum ferritin levels i.e. around 2000ng/ml

But some cases with granulocytic hyperplasia with infection induced turnover showed high levels of serum ferritin even with decreased iron stores.

And also in turn where there were normal iron stores, few cases showed low serum ferritin levels. Coefficient value being 0.547

DISCUSSION

Blood disorders have a varied diversity among different age groups. They usually range from anemias to advanced hematological malignancies.[25] However; the pattern of these disorders is quite different in developing countries than from developed countries. Bone marrow examination is quite a valuable test which has become very important these days for the diagnosis of hematological disorders.[25-27]Bone marrow aspiration provides reliable information regarding bone marrow cellularity, its architecture and the stage of maturation of different blood cells. [25-27]

The gold standard to estimate iron stores is to stain a bone marrow aspirate for iron.[2,14] The World Health Organization recommends that a serum ferritin concentration of <12 μg/l indicates depleted iron stores in children aged <5 years of age, while a concentration of <15 μg/l indicates depleted iron stores in those >5 years of age.[28-30]

Since 1970's the fact that the measurement of serum ferritin concentration can reflect the total body iron store and an acute phase response has been known. Understanding how to interpret the concentration of serum ferritin in the presence of infection is difficult, and various approaches have been suggested.[31]

Increased iron stores were observed in majority in RBC and WBC disorders. Diminished iron stores were present with normal and increased ferritin levels. In present study a total of 110 cases were studied for bone marrow iron stores and 70/110 cases had got their serum ferritin estimated. Out of total 110 cases, 54 were males and 56 were females. Predominant distribution was hypercellular marrow with RBC disorders being the predominant disorder. Diminished iron stores were mainly seen in RBC disorders.

Serum ferritin values was evaluated in 70/110 patients with value ranging from 1.4-2001ng/ml and mean being 400.3. Males having 281±403.4, and females having 512±759.6

Eleven (32.4%), of males had decreased ferritin levels, 14 (41.2%) had normal levels, and 9 (26.5%) cases showed increased ferritin levels. Females mainly had increased ferritin levels in 18 (50.0%). Males were found to have increased iron stores and increased serum ferritin in (80.0%), normal iron stores had increased ferritin levels in 4/9 (44.4%), diminished stores had normal ferritin stores in 10/20 (50%). In females the proportion of increased iron stores to raised ferritin levels were highest (83.3%). RBC disorders showed highest incidence in all normal, increased and decreased iron stores 7/11(50%), 5(55.6%), 8/11(72.7%) cases respectively. Among females also decreased, and normal ferritin values were associated with RBC disorders 5/7 (71.4%), 10/11(90.9%) respectively. But increased levels was observed in WBC disorders 8/18(44.4%).

Non- nutritional anemia showed a good statistically significant correlation between iron stores and ferritin levels. Decreased ferritin levels having diminished iron stores were seen in 3/3 (100%), increased iron stores showed normal and increased iron stores (41.7%).

Nutritional anemias had predominantly decreased iron stores with diminished ferritin values in 11/14 (78.6%), but increased ferritin levels had normal and diminished iron store levels.

Granulocytic hyperplasias (infection induced turnover) and myeloid hyperplasias had increased serum ferritin concentrations (>2000ng/ml) inspite of having diminished or absent iron stores in 3/5 cases (33.3%).

Hence, this study showed that serum ferritin reflects body iron stores, values < 12ng/ml showed absent iron stores and >500ng/ml showed increased iron stores. Although it acts as an indicator of body iron stores it also acts as an positive acute phase reactant, whereby values are raised inspite of diminished iron stores.

Table. 25 : Showing comparison of the gender- wise distribution amomg different studies

Study	Total participants	Male	Female
Thomason *et al*[32]	6	3	3
Coenen *et al*[8]	73	30	43
Kotru *et al*[22]	55	31	24
Anjum *et al*[25]	168	92	76
Pudasini *et al*[1]	57	27	30
Present study	110	54	56

Kotru *et al*[22] showed a total number of 55 cases, (31 males and 24 females) similarly, Pudasini *et a1*[1] showed 57 total cases in which 27 were males and 27 were females.

According to Thomason *et al*[32] in total of 6 cases, 3 cases each of males and females. Coenen *et al*[8] showed total of 73 cases 30 male and 43 females. Anjum *et al*[25] reported 168 cases in which 92 were males and 76 were females.

In present study, we reported a total of 110 cases out of which 54 were males and 56 were females which was consistent with Pudasini *et al*[1], Coenen *et al*[8] wherein the female population was slightly increased.

Table.26: Table depicting the age-wise distribution between various studies

Study	Thomason *et al*[32]	Jain *et al*[31]	Anjum *et al*[25]	Pudasini *et al*[1]	Present study
Age groups studied	44-80 years	18-58 years	1-100 years	9 months -75 years	5months - 78 years

Age groups studied were from 5 months to 78 years of age which was similar to Pudasini *et al*[1], Thomason *et al*[32] studied the participants from 44-80 years of age. Jain *et al*[31] studied the age groups from 18-58 years and Anjum *et al*[25]studied the age groups from 1-100 years.

Table.27: comparison of cellularity between different studies

Cellularity Study	Normocellular	Hypocellular	Hypercellular
Kotwal *et al*[33]	37 (67.3%)	8(14.5)	10(18.2%)
Present study	21 (19.1%)	16(14.5%)	73 (66.4%)

Kotwal *et al*[33] reported total of 37 (67.3%) normocellular subjects, 8 (14.5%) hypocellular and 10(18.2%) hypercellular subjects. In our study we found that, total of 73 (66.4%) subjects were having hypercellular smears, whereas 16 (41.5%) and 21 (19.1%) were hypocellular and normocellular smears respectively.

Table.28: Showing comparison of various disorders among different studies

Distribution of disorders	Anjum *et al*[25]	Pudasini *et al*[1]	Ali *et al*[18]	Pujara *et al*[9]	Present study
Micronormoblastic EH	20	16	69	42	23
Megaloblastic EH	31	7	12	10	21
Normoblastic EH	8	-	41	7	17
Acute leukemia	23	7	7	-	11
CML	4	-	8	1	2
CLL	2	-	5	-	1
Multiple myeloma	5	2	9	-	3
Thalassemia	-	-	-	1	1
Hypocellular marrow	-	-	81	3	10
Aplastic anemia	6	2	10	-	1
MDS	1	2	-	-	1
ITP	2	6	-	2	6
Polycythemia Vera	-	-	-	-	1
Total cases	102	42	242	66	98

Pudasini *et al*[1] series reported 16out of 48 cases were of microcytic anemia and EH, 7 cases each of megaloblastic hyperplasia and acute leukemias, 2 cases each of multiple myeloma, aplastic anemia, MDS and 6 cases of ITP.

Ali *et al*[18] reported 69 cases of micronormoblastic EH, 12 cases of megaloblastic anemia, 41 cases of normoblastic hyperplasia, 7,8,5 cases of Acute leukemia, CML, CLL respectively and 9,81, and 10 cases of Multiple myeloma, hypocellular marrow and aplastic anemia respectively.

Pujara *et al*[16]showed 42 cases of micronormoblastic EH, 10 cases of megaloblasticanemia, 7 cases of normoblastic hyperplasia, 1 case each of CML and thalassemia, 3 cases of hypocellular marrow and 2 cases of ITP.

Anjum *et al*[25] reported 20 cases of micronormoblastic EH, 31 cases of megaloblastic EH, 8 cases of normoblastic EH, 23 cases of acute leukemia, 4 cases of CML, and 2, 5, 6 cases of CLL, multiple myeloma and aplastic anemia respectively and 1, 2 cases of MDS and ITP respectively.

Present study had similar results with Anjum *et al*[25] with predominant population of the study being the EH (micronormoblastic and megaloblastic patterns).

Table.29: Comparison of iron stores in the patients in different studies

Iron stores / Study	Normal	Decreased	Increased
Nielsen *et al*[34]	10	19	5
Donald *et al*[7]	18	27	3
Coenen *et al*[8]	8	26	39
Jain *et al*[31]	60	24	16
Tripathi *et al*[6]	55	30	15
Present study	30	60	20

In studies conducted by Tripathi *et al*[6], Jain *et al*[31], normal iron stores were predominant, whereas decreased iron stores were predominant in Donald *et al*[7] Coenen *et al*[8] showed high frequency in increased stores. Nielsen *et al*[34] showed increased iron stores. Our study correlated and was similar to Coenen *et al*[8] with higher frequency of increased iron stores.

Table.30: Comparison between serum ferritin levels in various studies

Serum ferritin	Normal	Increased	Decreased
Rahma *et al*[3]			
Stainable iron	25	14	1
Unstainable iron	3	1	8
Thomason *et al*[32]	1	0	5
Ali *et al*[8]	69	63	113
Pujara *et al*[9]	58	0	12
Present study			
Males	14	9	11
Females	11	18	18

The studies discussed in Table: 30 predominant population was having normal iron stores i.e. in Rahman *et al*[3], Ronald *et al*[32], Pujara *et al*[9].Pujara*et al*[9] reported predominant population of decreased iron stores.

In the present study, normal ferritin values were observed in 14 males and 11 females, increased levels were seen in 9 male sand 18 females and decreased levels were seen in 11 male sand 18 females.

Table.31: Table showing the comparison between iron stores, patient incidence and serum ferritin levels between various studies

Study	Iron stores	Patient incidence	Serum ferritin
Phiri *et al* [4]	Normal	66.2%	2.7 mean log
	Deficiency	33.8%	1.8 mean log
Bableshwar *et al*[2]	Normal	37.25%	2.13mean log
	Iron store deficiency	16.25%	0.91mean log
	Combined	22.5%	2.64 mean log
Tripathi *et al*[6]	Normal	22 cases	48.4(27.2)mean(SD)
	Increased	6 cases	21.8(5.8) mean (SD)
	Decreased	12 cases	220(115.1)mean(SD)
Donald *et al*[7]	Normal	18 cases	84-428 mg/L
	Increased	9 cases	113-418 mg / L
	Decreased	19 cases	40-213 mg/L
Rahman *et al*[3]	Present	1 case	<100 ng/ml
		25 cases	100-500 ng/ml
		14 cases	>500 ng/ml
	Absent	8 cases	<100 ng/ml
		3 cases	100-500 ng/ml
		1case	>500 ng/ml
Present study	Normal	11/22 (50%)	Normal
	Increased	9/11 (81%)	Increased
	Diminished	16/37 (43.24%)	Decreased to normal

Table.31 highlights the incidence of iron stores, and their serum ferritin values. Phiri *et al* [4] showed that normal iron stores had high incidence 66.2% 2.7 log ferritin (which means the logarithmic conversion of the normal units of serum ferritin values i.e. ng/ml), decreased stores had 33.8% had 1.8 log ferritin. Bableshwar *et al*[2] showed normal in 37.25% with 2.13 log ferritin. Iron stores deficiency was observed in

16.25% with 0.91 log ferritin, combined deficiency showed 22.5% incidence with 2.64 log ferritin. Tripathi *et al*[6] reported highest population of normal iron stores with 48.2 ±27.2 (mean ± SD ferritin).

Donald *et al*[7] observed predominantly decreased number of stores in 19 cases with normal range of serum ferritin levels of 40-213 mg/l. Rahman *et al*[3] observed the iron stores as present and absent. 25 cases had iron stores present with ferritin levels being 100-500 ng/ml, 14 cases of present stores with >500 ng/ml stores. Absent stores were seen in 8 cases and their ferritin values were <100 ng/ml.

Present study had predominantly decreased iron stores 16/37 (43.2%) cases with decreased to normal stores followed by normal stores in 11/22 (50%) cases having predominantly normal stores. Our study had similar findings with Donald *et al*[7] and Rahman *et al*[3] but was in discordance with Phiri *et al* [4], Bableshwar *et al*[2], and Tripathi *et al*[6].

Table .32: Table showing comparison between iron stores and serum ferritin among various disorders among different studies

Study	Disorders	Iron stores	Serum ferritin
Ali *et al*[18]	Iron depletion/deficiency	Normal	<12 ng/ml
Kotwal *et al*[33]	HIV	Diminished	Raised
Mazza *et al*[35]	Portal cirrhosis	Normal	200-400 ng/ml
	Renal failure	Normal	>2000 ng/ml
Nielsen *et al*[34]	Rheumatoid arthritis	Normal	Raised
Donald *et al*[7]	Iron deficiency anemia (IDA)	Decreased	Low
	Anemia of chronic disease (ACD)	Raised with low Hb gm %	Raised
Kotru *et al*[22]	Iron deficient (ID)	Absent BMI	17 <ng/ml
	Iron replete (IR)	Absent BMI	91<ng/ml
Present study	RBC disorders	Diminished	<12 ng/ml
	Hematological malignancies	Normal	20-300 ng/ml
	Granulocytic/myeloid hyperplasia with infection induced turnover.	Diminished/normal	>2000 ng/ml

Table: 32 indicated that serum ferritin was profoundly low in depleted iron stores whereas it was high in patients clinically presenting with low haemoglobin levels and low iron stores with chronic inflammatory conditions.

Kotwal *et al*[33]reported diminished iron stores and raised serum ferritin levels in tuberculosis patients. Our study had similar results with Ali *et al*[18], Kotru *et al*[22], Donald *et al*[7]and had discordance results with Nielsen *et al*[34], Mazza *et al*[35] and Kotwal *et al*[33].

Ali *et al*[18]reported total of 248 cases in whom 69 patients reported no marrow iron stores, 20 among them were having serum ferritin of >20 mg/l (2 were receiving parental iron therapy, 2 had blood transfusion), no obvious pathology was traced in the other 16 patients. The other patients had various other pathological conditions viz. alcoholic liver disease, chronic liver hepatitis, acute renal failure due to glomerulonephritis.

Intragumtornchai *et al*[36] reported a total of 72 patients in whom the predominant patients having absent iron stores were also having serum ferritin <50 mg/l. Hence they indicated that serum ferritin was very useful test for the diagnosis of iron deficiency anaemia in patients with liver cirrhosis.

Coenen *et al*[8]reported that serum ferritin levels of <70µg/ml were iron deficient. Pujara *et al*[9]showed positive correlation between low serum ferritin levels and absent iron stores.

Nadeem *et al*[20] assessed the accuracy of serum ferritin which showed 75.90% in IDA and 79.71% in ACD and indicated that it was more specific for ACD where decreased iron stores indicated IDA and increased macrophage iron with decreased siderocytes and sideroblasts was diagnostic of ACD. Our study had similar results with Coenen *et al*[8] and Intragumtornchai *et al*[36] that stated diminished iron stores were seen in low serum ferritin levels. Discordance of the results were observed with Pujara *et al*[9].

In the present study it was observed that, serum ferritin levels were diminished in 18/70 (25.71%) and well correlated with decreased iron stores in 9/20 (45%). Also ferritin being an acute phase reactant serum ferritin levels were elevated in 27/70 cases (38.57%) having decreased iron stores in 1/20 (5%). Hence we would like to record that serum ferritin is a useful non-invasive investigating parameter in evaluation of anemia especially in nutritional type of anemias, wherein ferritin levels directly reflects the total body iron stores. Conversely, raised ferritin values indicate increased iron stores or more importantly may be raised as an acute phase reactant. Therefore, raised ferritin values should be correlated with clinical findings and by investigating other CBC parameters like Hb gm%., ESR, MCV or other acute phase reactants e.g. CRP.

Bone marrow examination remains the gold standard for demonstration of iron stores and correlation with marrow cellular morphology.

SUMMARY

- In a study of total 110 patients 54 were male and 56 were females. Predominant population was observed in 16-30 (58.1%) years of age group. The bone marrow smear were predominantly hypercellular accounting for 66.4%.
- The predominant disorders observed were RBC disorders (55.5%), followed by WBC (18.2%).
- Micronormoblastic hyperplasia was the highest (48.6%), with predominantly diminished iron stores as should be expected. Megaloblastic hyperplasia also showed good number of patients had diminished stores. Increased iron stores were also seen in megaloblastic pattern accounting for (57.1%).
- Amongst WBC disorders, acute leukemias (45.0 %) were highest showing both diminished and increased iron stores. Combine disorders comprised 10% of total disorders and had predominantly hypercellular marrow with many atypical cells (30%) which had normal iron stores.
- Hypocellular marrow showed predominantly diminished iron stores as expected while polycythemia vera also showed diminished stores where as multiple myeloma showed both increased and normal iron stores.
- Megakaryocytic hyperplasia showed predominantly showed diminished iron stores (50%). Combined hematological disorders showed highest incidence of granulocytic hyperplasia along with many atypical cells 30% which showed 66.7%of them having normal iron stores.
- Serum ferritin was carried out in 70 out of 110 cases. Serum ferritin levels were normal range i.e. 36-155 ng/ml in males and 24 – 155 ng/ml in females.
- Values lower than these, within the range and greater than these values were labeled as increased, normal and increased levels respectively.

- Serum ferritin levels ranged from 1.4-2001 ng/ml and mean was 400.3. Predominantly, serum ferritin values were normal (41.2%) in males and increased in females (50.0%).
- RBC disorders showed high incidence of normal to high and low serum ferritin levels in males i.e. (50.0%), (55.6%), and (72.7%) respectively. Whereas normal and decreased serum ferritin values were seen more commonly in RBC disorders in females and increased iron stores was found in WBC disorders predominantly.
- Nutritional anemias showed higher incidence with correlation of diminished iron stores (78.6%) with decreased ferritin levels as same was the case with non – nutritional anemias (100%). But normal ferritin levels also had diminished and increased iron stores.
- Decreased ferritin values were well correlated in (45%) of patients with diminished iron stores. Also ferritin values were elevated as acute phase reactants in (5%) with diminished iron stores.
- Hence, raised ferritin is a useful tool to evaluate patients with nutritional type of anemias where decreased values reflect reduced stores in the body, but conversely it also acts as an acute phase reactant whereby values are elevated in patients having diminished iron stores.
- Hence, in such settings ferritin values should be correlated clinically along with CBC parameters like Hb gm%., MCV, ESR and other acute phase reactants like CRP etc.

CONCLUSION

- A total of 110 cases were studied with their bone marrow aspiration and serum (70 cases) done.
- Bone marrow aspirates were stained with perl's stain and graded with grades 1-6 accordingly.
- To conclude, this study predominantly observed that the patients with micronormoblastic pattern i.e. IDA had diminished iron stores with normal and decreased serum ferritin.
- Also hematological malignancies had majority of normal to increased iron stores with normal ferritin values.
- Granulocytic hyperplasias, myeloid hyperplasia which were due to infection induced turnover showed decreased iron stores with increased >2000 ng/ml serum ferritin acting as an acute phase reactant.
- RBC disorders was found to be associated with decreased iron stores, WBC disorders showed elevated iron stores.
- Non –nutritional anemias showed diminished and increased iron stores with normal serum ferritin levels.
- Nutritional anemias also showed predomianatly diminished stores with decreased serum ferritin levels but also few indicated that diminished iron stores had increased ferritin levels, particularly in megaloblastic anemias.
- Hence, to conclude this study showed that serum ferritin is a reliable indicator of iron stores in the body along with its function of positive acute-phase reactant whereby levels increase in inflammatory conditions e.g. tuberculosis, rheumatoid arthritis, liver cell damage, and malignancies which are certainly not related to the body iron store.

- Non- nutritional anemia which also includes ACD are associated with chronic infectious, inflammatory, traumatic or neoplastic illness. In such clinical settings, the concentrations of hemoglobin levels as well as iron measured in serum are low. Ferritin concentrations in such conditions if measured should be interpreted cautiously. Nevertheless, if serum ferritin are corrected for the acute-phase response, it could still serve as an important estimate for the iron stores in bone marrow, even in patients with ACD.
- To diagnose or to exclude iron-deficiency anemia in patients with chronic disease, the relation between ferritin concentrations and other acute phase reactants like CRP ,along with other CBC parameters like Hb gm% concentrations and MCV, ESR and bone marrow iron status estimation can be of help and aid in the diagnosis.
- Ferritin is a very useful indicator of the iron status of the individual but also acts as a positive acute phase reactant hence interpretation of the body iron stores in such conditions should be made cautiously.
- But bone marrow iron stores evaluation still remains the gold standard for the assessment of iron status of the individual.

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ANNEXURES – I

Ethical clearance

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Assessment bone marrow iron stores in various hematological disorders and its correlation with serum ferritin"

Name of P.G. student Dr. Shruti Kulkarni
Department of Pathology

Name of Guide/Co-investigator Dr. Mahesh H. Karigoudar.
Professor of Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

Annexure – II

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT : ASSESSMENT OF BONE MARROW IRON STORES IN VARIOUS HEMATOLOGICAL DISORDERS IN CORRELATION WITH SERUM FERRITIN.

PRINCIPAL INVESTIGATOR : Dr. SHRUTI KULKARNI.
P.G. DEPARTMENT OF PATHOLOGY

P.G.GUIDE : Dr. MAHESH. H. KARIGOUDAR M.D, DNB
PROFESSOR,
DEPARTMENT OF PATHOLOGY

PURPOSE OF RESEARCH:

I have been informed that this study is done to assess the bone marrow iron stores in various hematological disorders and its correlation with serum ferritin.

PROCEDURE:

Iunderstand that I will undergo detailed clinical history, thorough clinical examination and after which bone marrow aspiration will be performed and subjected to bone marrow examination for the study of iron stores.

RISK AND DISCOMFORTS:

I understand that, I may experience pain and discomforts during the bone marrow examination. This is mainly the result of my condition and procedures of this study are not expected to exaggerate these feelings which are associated with usual course of treatment.

BENEFITS:

I understand that my participation in the study will have no direct benefit to me other than the potential benefit of the treatment.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is used for publications the identity of patient will not be revealed.

REQUST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations. I have read and fully understood this consent form. Therefore I agree to participate in the present study.

_____ _____

Investigator Date

_____ _____

Signature of Witness Date:

STUDY SUBJECT CONSENT STATEMENT:

I confirm that **Dr. SHRUTI KULKARNI** has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to signature) Date

Annexure - III

PROFORMA

NAME	:	OP/IP No.	:
AGE	:		
SEX	:	D.O.A	:
RELIGION	:	D.O.D	:
OCCUPATION	:		
RESIDENCE	:		

Presenting Complaints :

Past history :

Personal history :

Family history :

Treatment history :

General physical examination:

Pallor	present/absent	
	Icterus	present/absent
	Clubbing	present/absent
Lymphedenopathy	present/absent	
Edema	present/absent	
	Built	poor/average/well

VITALS: PR: RR:

BP: TEMPERATURE:

WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

Haematological investigations:

Biochemical Investigations:

Radiological Investigations:

Proforma for bone marrow aspiration :

Preparation	: Satisfactory /Adequate or Inadequate
Cellularity	: Normocellular/ Hypocellular / Hypercellular
Erythropoiesis	: Normoblastic /Megaloblastic/ Micronormoblastic.
Myelopoiesis	: Morphology/ Number/ Maturation.
M:E ratio	: Normal / Reversed.
Lymphocytes	: Morphology/ Number/ Morphology.
Megakaryocytes	: Normal/ Hypolobulated/ Hyperlobulated.
Plasma cells	: Present/ Absent/ Number.
Abnormal cells	: If any.
Iron Stores	: Grade of iron stores according to Gales method.
Serum ferritin	: in ng/l
Impression	:

KEY TO MASTER CHART

BM	-	Bone marrow
PS	-	Peripheral smear
NCHC	-	Normocytic Hypochromic smear
MCHC	-	Microcytic hypochromic
F/A/O	-	Features Are Of
EH	-	Erythroid Hyperplasia
hpf	-	high power field
ng/ml	-	nano grams/ml
AML	-	Acute Myeloid Leukemia
CML	-	Chronic myeloid Leukemia
ALL	-	Acute Lymphoblastic Leukemia
CLL	-	Chronic Lymphocytic Leukemia
ITP	-	Idiopathic Thrombocytopenic Purpura
MDS	-	Myelodysplastic Syndrome
Sat	-	Satisfactory
Hyp	-	Hypercellular
Hypo	-	Hypocellular
Dilu	-	Diluted
Norm	-	Normocellular
m	-	months
cell	-	Cellular