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Incidence of Bone Marrow Findings in Hematological Disorders Based on Age And Gender : An Observational Study

*** Dr Sharique Ahmad**	Associate Professor, Department of Pathology, Era's Lucknow Medical college, Lucknow-226003, India. * Corresponding author
Dr Shoaib Irfan	Junior Resident, Department of Pathology, Era's Lucknow Medical college, Lucknow-226003, India
Dr Pankhudi Gupta	Junior Resident, Department of Pathology, Era's Lucknow Medical college, Lucknow-226003, India
Prof Syed Riaz Mehdi	Professor, Department of Pathology, Era's Lucknow Medical college, Lucknow-226003, India
Dr Zeashan Haider Zaidi	Statistician, , Department of Community Medicine, Era's Lucknow Medical college, Lucknow-226003, India

ABSTRACT

Bone marrow examination is useful in the diagnosis of both haematological and non haematological disorders. Indications have included the diagnosis, staging, and therapeutic monitoring for lymphoproliferative disorders such as chronic lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma, hairy cell leukaemia, and multiple myeloma. Furthermore, evaluation of cytopenia, thrombocytosis, leukocytosis, anaemia, and iron status can also be done. This is also an important tool in the diagnosis of non-haematological disorders like storage diseases, granulomatous lesions, metastatic carcinoma, Pyrexia of unknown origin (PUO), and disseminated infection in immunocompromised hosts. The two most important techniques used for the diagnosis are bone marrow aspiration and bone marrow trephine biopsy which are complementary to each other. Aspiration of the marrow is primarily utilised for cytological assessment with analysis directed towards morphology and obtaining a differential cell count. Biopsy is essential for diagnosis in a dry tap or blood tap which occurs when the marrow is fibrotic or densely cellular. In the present study, consecutive three years of indoor patients with hematological disorders based upon age and gender were evaluated for the clinical diagnosis by doing bone marrow aspiration and bone marrow trephine biopsy in a Medical College Hospital.

KEYWORDS : Bone Marrow, Haematological, Aspiration, Biopsy, Age, Sex.

Introduction

Sir William Harvey described blood as "the fountain of life and the primary seat of the soul. The marrow of our bones is the seedbed of our blood"[1], careful assessment of the blood elements is often the first step in assessment of hematological function and diagnosis. Haematopoiesis begins in the yolk sac at 3rd week of intrauterine life and at birth in bone marrow. Bone marrow is made up of cellular elements of hematopoietic stem cells and stroma of bone marrow, which forms a unique microenvironment in which hematopoietic progenitors proliferate and differentiate. The adult haematopoietic system includes tissues and organs involved in the proliferation, maturation and destruction of haematopoietic cells. These organs and tissues include the bone marrow, thymus, spleen and lymph nodes. Bone marrow is the site of myeloid, erythroid, and megakaryocytic as well as lymphoid cell development.[2] Red marrow contains 40% fat cells, 40% water, and 20% hematopoietic cells, whereas yellow bone marrow is composed of 80% fat cells, 15% water, and 5% hematopoietic cells.[3] In an adult, the red marrow is mainly located in the appendicular skeleton in the metaphysis and near the vertebral endplate (the metaphyseal equivalent), due to well-developed vascularity.[4,5]Examination of the marrow is critically important in the study and management of wide variety of haematological disorders. 6

Objectives

To find the incidence of Bone marrow findings in hematological disorders based upon the age and gender of the patients referred to the pathology department for bone marrow study and evaluation..

Materials and Methods

Patients presenting with pancytopenia on initial work up requiring bone marrow aspirate and bone marrow biopsy at Era's Lucknow Medical College and Hospital, Lucknow from January 2012 to January 2015 are included in this study.

The bone marrow aspiration was performed at the posterior superior iliac spine using Klima needle. As bone marrow clots faster than peripheral blood, films are made from the aspirated material at bedside without delay. Remaining amount is transferred to an EDTA vacutainer. Usually 0.2 - 0.3 mL is sufficient for a morphological diagnosis. It has been found that more than 0.3 mL increases the peripheral blood dilution. After taking the marrow for smears, the trephine or the biopsy specimen was obtained by Jamshidi needle.

Before the biopsy specimen was transferred to 10% neutral buffered formalin, touch imprints were made by gently rolling the bony core across a clean glass slide. This along with the aspiration smears were stained with Leishmann stain. Special stains - Periodic acid Schiff stain, Myeloperoxidase, Sudan black and Perls' stains were used wherever indicated.

Bone marrow aspirate smear

The bone marrow aspirate smear is a preparation designed to spread the cellular material of the marrow so that Leishmann stain can reveal essential cellular details.

The bone marrow aspirate is evaluated for: 1), cellularity of the fragments; 2), erythropoiesis - cellularity, maturation pattern and any cytological abnormalities; 3), myelopoiesis - cellularity, maturation pattern and any abnormalities; 4), M:E ratio; 5), megakaryopoiesis - number, morphology, presence of immature forms; 6), lymphocytes; 7), plasma cells; 8), parasites/abnormal cells/Granulomas/storage cells.

The cellularity was assessed by estimating the percentage of hemato-

poietic cells compared to fat spaces in the bone marrow. The categories were identified in the following way: 1), hypercellular: > 75% cells; 2), normocellular: 25-75% cells; 3), hypocellular: < 25% cells, depending upon the age of the patient.

Bone marrow biopsy

The microscopic examination of trephine biopsy is best for determining the overall cellularity and presence of infiltrates. Touch imprint preparations from the biopsy material are also useful for morphology. Trephine Biopsy Interpretation - Hematoxylin and eosin sections were studied as follows: adequacy of the biopsy - the ideal specimen should be 1 - 2 cm in length, should not be distorted and should have at least 5 well preserved intertrabecular marrow spaces for interpretation.

Results

As shown below are the results with further statistical analysis

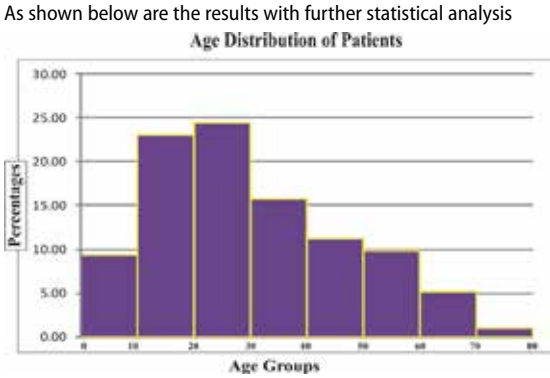

Figure 1 : Comparisons of age groups and percentage of patients.

AGE DISTRIBUTION		
AGE YEARS	NUMBER OF PATIENTS	%
0-10	27	9.44
11-20	66	23.08
21-30	70	24.48
31-40	45	15.73
41-50	32	11.19
51-60	28	9.79
61-70	15	5.24
71-80	3	1.05
Chi sq = 99.77, p < 0.0001		

Table 1 : Representing the numbers of patients of age distribution.

Bone marrow examination was most commonly performed in the age group of 21-30 years which comprised of 24.48%. There is highly unequal age distribution of patients. In 11-20 years of age group percentage of bone marrow performed was 23.04%(figure 1). At extremes of age (0-10 years and 71-80 years) least number of bone marrow examinations were performed (9.44% and 1.05%). Chi square for sex distribution comes out to be 99.77 and p value is significant.(<0.001) (table 1).

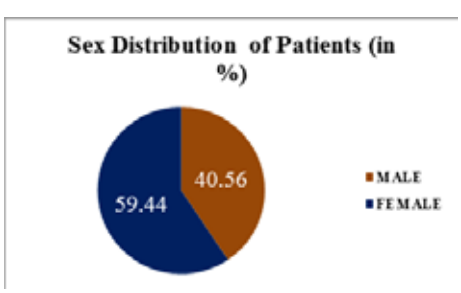

Figure 2 : Pie chart showing female predominance.

SEX DISTRIBUTION		
SEX	NUMBER OF PATIENTS	%
MALE	116	40.559
FEMALE	170	59.44
Chi sq = 10.20, p = 0.006		

Table2 : Characteristic distribution of number of patients.

It was also observed in the study that 59.4% of bone marrow examination were of female patients and 40.6% were of male patients. So, there is a significant difference between number of male and female patients(figure 2).

Findings of bone marrow examination revealed that the maximum number of cases were of megaloblastic anemia which constituted 22.38%. Nutritional anemia comprised 13.98% and cases of Iron deficiency anemia came out to be 12.24% of the total bone marrow examination performed. Dyserythropoietic marrow was seen in 8.39% patients, leishmaniasis, Plasmacytosis, Thalassemia, Multiple myeloma and Plasma cell dyscrasias in 1.05%. Pancytopenia, Chronic myeloid leukemia and Metastatic deposits in 4.55%, 3.15% and 2.45% respectively. Acute myeloid leukemia and Hypoplastic marrow comprised of 4.2% of cases. Aplastic anemia and Idiopathic thrombocytopenic purpura 2.1 and 1.75. Cases of myelofibrosis, Sideroblastic anemia, Myelodysplastic syndrome and Thrombocytopenia were very less in number (approximately 0.35%). Cases of erythroid hyperplasia, lymphoproliferative disorders, hypereosinophilic syndrome, storage disorder, acute lymphoblastic leukemia and mixed nutritional anemia were also seen in very less number.(Table 3).

	DIAGNOSIS	NO. OF PATIENTs	%
1	NORMAL	12	4.195
2	NUTRITIONAL ANEMIA	40	
3	PANCYTOPENIA	13	4.55
4	CML	9	3.15
5	METASTATIC DEPOSITS	7	2.45
6	DYSERYTHROPOIETIC MARROW	24	8.39
7	MEGALOBLASTIC ANEMIA	64	22.38
8	MONOCLONAL GAMMOPATHY	4	1.4
9	ERYTHROID HYPERPLASIA	2	0.7
10	LYMPHOPROLIFERATIVE DISORDER	2	0.7
11	ITP	5	1.75
12	AML	12	4.2
13	HYPER EOSINOPHILIC SYNDROME	2	0.7
14	REACTIVE HYPERPLASIA	4	1.4
15	APLASTIC ANEMIA	6	2.1
16	IRON DEFICIENCY ANEMIA	35	12.24
17	CLL	3	1.05
18	HYPOPLASTIC MARROW	12	4.2
19	STORAGE DISORDER	2	0.7
20	LEISH MANIASIS	3	1.05
21	PLASMACYTOSIS	3	1.05
22	MYELOID HYPERPLASIA	4	1.4
23	THALASSEMIA	3	1.05
24	MYELOFIBROSIS	1	0.35

25	SIDEROBLASTIC ANEMIA	1	0.35
26	ALL	2	0.7
27	MYELO DYSPLASTIC SYNDROME	1	0.35
28	AGRANULOCYTOSIS	3	1.05
29	MIXED NUTRITIONAL ANEMIA	2	0.7
30	MULTIPLE MYELOMA	3	1.05
31	PLASMA CELL DYSCRASIAS	3	1.05
32	THROMBOCYTOPENIA	1	0.35

Table 3 : Various disorders found in bone marrow examination.

Discussion

Pancytopenia is defined as simultaneous presence of anemia, leucopenia and thrombocytopenia. So, it exists when hemoglobin (Hb) is less then 13.5g/dl in males or 11.5g/dl in females; the leucocytes count is less then $4x10^3$ /l and the platelets count is less than $150x10^3$ /l.[7] Pancytopenia may become apparent only during times of stress or increased demand (e.g., bleeding or infection) and Initially, mild impairment in marrow function may go undetected. It may be caused due to decrease in hematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective hematopoiesis with cell death, defective cell formation which are removed from the circulation, antibody mediated sequestration or destruction of cells and trapping of cells in a hypertrophied and over active reticuloendothelial system.[8]

In the present study, Megaloblastic anemia (22.38%) was the commonest cause of pancytopenia, followed by Nutritional anemia (13.98%), Aplastic anemia (2.1%), Malignant diseases (2.45%), and others (59.09%)(Table 3). Others included uncommon causes like Malaria, Hypereosinophilic syndrome, Lymphoproliferative and storage disorders. The diagnosis of Megaloblastic anemia in our study was established by characteristic bone marrow findings. The high prevalence of Nutritional anemia in India has been cited for the increased frequency of Megaloblastic anemia. Megaloblastic anemia as a common cause of pancytopenia was also seen in studies done by *Tariq Aziz et al[9], Iqbal et al[10] , Qazi et al[11]*. Increased incidence of Megaloblastic anemia in these studies was probably due to high prevalence of nutritional anemia in the non-industrialized world. Nutritional factors, recurrent infection and deficiencies of vitamin B12 and folate seem to be associated strongly with megaloblastic anemia.[12] Vitamin B12 deficiency is difficult to pick up clinically as it presents with vague complaints of decreased mental and/or physical work capacity, reduced attention span, memory loss, irritability and low mood.[13]Evidence in previous studies suggests that myeloperoxidase index measurement may assist differentiation of megaloblastic from aplastic anemia.[14]

Khodke et al (2000) observed megaloblastic anemia (44%), followed by hypoplastic anemia (14%) as the common causes of pancytopenia. [15] Studies done by *Mobina Ahsan Sodhy (2005)* concluded megaloblastic anemia (35.9%) followed by hypersplenism (16.3%) as the common causes. *Jha A et al [16] (2008)* found hypoplastic bone marrow (29%) followed by megaloblastic anemia (23.64%) as the common cause. The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anemia, which is in sharp contrast to results in the present study; here the commonest cause was megaloblastic anemia. This seems to reflect the higher prevalence of nutritional anemia in Indian subjects.

Studies done in Philippines[17] and Nepal[18] reported that males were affected with Aplastic anemia much more frequently then females, which might be a result of higher incidence of occupational exposure to chemicals and pesticides as a common etiological agent for Aplastic anemia in these countries. The third common cause of pancytopenia in the study by *Modood-ul-Mannan* in northern Pakistan was aplastic anemia (12.6% patients) while in other similar studies it varied from 38% to 41%.[19, 15,20]

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