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Original Article

Pathology Section

Diagnostic Utility of Bone Marrow Examination in Bicytopenia

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ABSTRACT

Introduction: Bicytopenias refer to condition in which either of the two cell lineages is reduced because of various diseases affecting the bone marrow. Bone marrow examination plays an important role aiding the peripheral smear and complete blood count findings in various haematological disorders. The present study has emphasised on the utility of bone marrow aspiration and bone marrow biopsies in cases of bicytopenias to assess the causative disease thus aiding for further clinical management.

Aim: To study the cytomorphology of bicytopenia in peripheral smear and bone marrow examination and to estimate the utility of bone marrow examination in bicytopenia in arriving at the diagnosis of the disease.

Materials and Methods: The present descriptive study was carried out in the Department of Pathology, Bangalore Medical College and Research Institute (BMCRI), Banglore, India, for a period of one year from April 2016 to April 2017. Total 80 confirmed cases of bicytopenias were included. Bone marrow aspiration, imprint smears and biopsy was performed for all cases. The parameters assessed after bone marrow aspiration and biopsy were on cellularity, myeloid to erythroid ratio, cytomorphological details of the cells. The data obtained was tabulated, descriptive statistics and Chi-square test was used for statistical analysis.

Results: Out of total 80 subjects, age group ranged from two to 79 years. The bicytopenias observed in peripheral smear examination has been anaemia and thrombocytopenia in 88.75% of cases, followed by anaemia and leukopenia in 8.75% of cases, lastly followed by leukopenia and thrombocytopenia in 2.5% of cases. Bone marrow aspiration performed showed predominantly normoblastic maturation followed by megaloblastic maturation and micronormoblastic maturation. The bone marrow aspiration yielded cellular marrow in 75 cases and diluted marrow in five cases. Imprint smears and bone marrow biopsy was performed in all the cases. Out of five diluted aspiration both imprint smears and bone marrow biopsy revealed normoblastic marrow in three cases, single case showed myelofibrosis and a single case of non Hodgkin's lymphoma.

Conclusion: Bone marrow aspiration helps to understand the cytomorphological details. In cases with diluted marrow or dry tap, imprint smears along with bone marrow biopsies help to arrive at the diagnosis. So, they complement each other along with haematological parameters and clinical details to aid for a better clinical management of the patients.

Keywords: Anaemia, Blood, Leukopenia, Thrombocytopenia

INTRODUCTION

The diseases of the blood are frequently seen in a diverse age group. Patients can present as a sole cytopenia or in a dual combination and in many instances, pancytopenia can be the presenting feature. Sometimes, only the complete blood count and peripheral smear examination cannot bring the clinician close to the diagnosis and in such situation bone marrow examination plays an integral role in the diagnosis of the underlying cause [1]. It also becomes an indispensable tool in evaluating the disease process and establishing the final diagnosis, to aid the clinician in the treatment of the condition [2,3].

Bicytopenia refers to a condition in which any two cell lineages of haematopoietic cell lines are reduced. They occur in a wide spectrum of diseases, ranging from benign viral infections to the graver malignancies [2]. The present study has included dual cytopenias since there is limited literature in evaluating the spectrum of aetiologies in bicytopenias. It is also trying to assess the most frequent combination of bicytopenias in present setup and if these independent parameters can be interrelated for the disease causation, so that the treatment could vary accordingly. Bone marrow examination plays an important role in arriving at the diagnosis of the disease [1-3].

The rationale of the study was to know which dual combination of bicytopenia is prevalent in present setup and the likely bone marrow findings in those cases that can help in arriving at the diagnosis of the disease, thus helping in clinical management. The aim was to study the morphology of bicytopenia in peripheral smear examination and to estimate the utility of bone marrow examination in cases of bicytopenias in arriving at the diagnosis of the disease.

MATERIALS AND METHODS

The present descriptive study, prospective in nature was carried out in the Department of Pathology, BMCRI, Bangalore, India, for a period of one year from April 2016 to April 2017. Ethical approval was obtained from Institutional Ethical Committee (BMCRI/PS/344/2015-16). Informed consent was taken before the procedure.

Sample size calculation: Sample size was 80 cases with a margin of 5% error maintaining the power of study more than 80%.

Inclusion criteria: All cases of bicytopenias determined by autoanalyser and confirmed by peripheral smear examination, irrespective of age and sex of the individual were included in the study.

Exclusion criteria: All patients diagnosed as pancytopenias determined by autoanalyser and confirmed on peripheral smear examination.

Study Procedure

Relevant clinical history and physical examinations were performed and the findings were recorded. Haematological investigation was performed for all cases. Complete blood count with haemoglobin percentage, total leukocyte count, differential count, red blood indices, platelet count, reticulocyte count was documented.

Bone marrow aspiration was performed in posterior iliac spine under aseptic precautions for all the cases. Aspiration smears were

stained with Giemsa stain. Touch imprint smears were prepared by gentle touch and rolling of biopsy core on the slide. The biopsy core was fixed, processed in the histokinette; later paraffin embedded blocks were made. The sections were stained with Haematoxylin and Eosin (H&E) stain. Perl's stain to grade iron stores were used. Myeloperoxidase (MPO), Periodic Acid Schiff (PAS), reticulin stain were done wherever necessary. The following parameters were documented in the aspiration smears:

1. Cellularity
2. Myeloid to erythroid ratio
3. Cytomorphological details of the cells.

A well structured proforma was used for data collection. Information regarding age, sex, clinical details, indication for bone marrow examinations, final diagnosis of bone marrow aspiration, bone marrow imprint smears and bone marrow biopsy was documented.

STATISTICAL ANALYSIS

Data was presented in tables and descriptive statistics was used, variation using Chi-square of independence was used as appropriate. The level of significance was set at 5% (p-value<0.05). Data obtained was analysed using STATA software version 10.

RESULTS

Out of total 80 subjects, males to females ratio was 1.1:1 with slight male predominance. The age group ranged from two years to 79 years, with a mean age of occurrence of 40.5 years. The most common clinical history elicited was weakness, fatigue (80%) followed by pallor (15%) and fever (5%).

In the present study, the most common bicytopenias observed in peripheral blood smear were anaemia with thrombocytopenia followed by anaemia with leukopenia and thrombocytopenia with leukopenia as shown in [Table/Fig-1].

Bicytopenia in peripheral blood	Number of cases	Percentage
Anaemia with thrombocytopenia	71	88.75%
Anaemia with leukopenia	07	8.75%
Thrombocytopenia with leukopenia	02	2.5%

[Table/Fig-1]: Shows the distribution of bicytopenias in peripheral blood.

The bone marrow aspiration yielded cellular marrow in 75 cases and diluted marrow in five cases. Imprint smears and bone marrow biopsy was performed in all the cases. Out of five diluted aspiration both imprint smears and bone marrow biopsy revealed normoblastic marrow in three cases, single case showed myelofibrosis and a single case of non-Hodgkin's lymphoma as shown in [Table/Fig-2].

Bone marrow biopsy findings	Number of cases (n)
Normoblastic erythroid marrow	03
Myelofibrosis	01
Non Hodgkin's lymphoma	01

[Table/Fig-2]: Bone marrow biopsy findings in cases with diluted marrow.

The most common aetiology of bicytopenia in the present study in bone marrow examination was normoblastic erythroid hyperplasia, followed by megaloblastic anaemia and iron deficiency anaemia [Table/Fig-3].

Aetiology	Number of cases (n)	Percentage
Normoblastic erythroid hyperplasia	44	55%
Megaloblastic anaemia	28	35%
Iron deficiency anaemia	06	7.5%
Myelofibrosis	01	1.25%
Lymphoid neoplasia	01	1.25%

[Table/Fig-3]: Aetiological distribution of bicytopenias.

DISCUSSION

In present study, marrow examination was performed in both adults and children. Posterior iliac spine is the preferred site. Other sites include anterior iliac spine, sternum, iliac crest and tibial tuberosity. Usually, tibia is preferred in infants. Posterior iliac spine was the preferred site in the present study [1-3]. The absolute contraindication for bone marrow aspiration/biopsy is coagulation disorder. Other contraindications include skin infection, osteomyelitis, previous radiation therapy in the sampling area and a non cooperative patient [1-4].

The age group in the present study ranged from 2-79 years, with a mean age group of 40.5 years. Athar R et al., in their study showed the mean age of occurrence as 35 years [5]. Saira PI et al., reported the age of occurrence as 38.9 years [6]. The variation in the mean age between the different studies can be attributed to the differing geographical areas and varied indications for the bone marrow study. Majority of patients in present study were in the age group of 31-40 years. Athar R et al., in their study showed the age range between 21-30 years [5]. Ekwere TA et al., showed similar findings [7].

The male to female ratio in present study has been 1.1:1, with slight male predominance. Athar R et al., showed female predominance of 1:2, while Niazi M et al., showed male preponderance with the ratio being 1.5:1 [5,8]. Singh I et al., also showed similar findings [9].

Patients presented with fatigue, weakness in 80% of cases, pallor in 15% of cases and fever was documented in 5% of cases in the present study. Singh I et al., in their study showed the most common presenting complain as pallor, followed by body weakness and headache, lastly by fever [9]. These presenting complaints usually vary from person to person as the haematological disorders are wider and have different pathogenesis which is also influenced by the person's genetic makeup and individual immunity.

Majority of the patients presented with anaemia with thrombocytopenia in 88.75% of cases, followed by anaemia with leukopenia in 8.75% of cases, lastly followed by leukopenia with thrombocytopenia in 2.5% of cases. Saadia HD et al., showed consistent findings [2]. Anaemia therefore represents a global problem, which is so commonly encountered, though the difficulty in arriving at the type of anaemia, may at times be cumbersome. Nutritional anaemias are predominantly present. The haematological and biochemical assessment namely complete blood count, peripheral smear examination, iron profile and vitamin B12 levels can aid in the prompt diagnosis and early treatment. Thrombocytopenia accompanying anaemia has been a majority bicytopenia observed in the present study. It is usually associated with megaloblastic anaemias. Megaloblastic anaemia has been known to induce dysmegakaryopoiesis. Dysmegakaryopoiesis is characterised by alterations in megakaryocyte maturation, leading to dysplastic features in megakaryocytes. These dysplastic features can be in form of multiple separated nuclei, micro megakaryocytes or hypo granular form. The non dysplastic features seen in megakaryocytes can be in form of immature forms, cytoplasmic vacuolisation or budding. These immature forms are unable to mature into platelets, leading to thrombocytopenia [10].

The present study encountered megaloblastic anaemia to be predominantly associated with thrombocytopenia in 28 cases. There has been an attempt made to study the causal relation between anaemia and thrombocytopenia, thus anaemia can be attributed to cause thrombocytopenia, rather than the chance association of thrombocytopenia with anaemia [11].

In present study, the second most combination was anaemia and leukopenia seen in 8.75% of cases. The bone marrow picture was normoblastic type of maturation in five out of seven cases, single cases of each with micronormoblastic pattern of maturation and megaloblastic pattern of maturation. The predominance of

normoblastic pattern of maturation in majority of cases with the anaemia and leukopenia combination points out that these could have been induced by viral infection and this would have led to leukopenia with associated anaemia. The findings of present study with few other studies have been tabulated in [Table/Fig-4] [1,2].

Findings	Singh A et al., [1]	Saadia HD et al., [2]	Present study
Mean age (years)	30.7	28.1	40.5
Male:Female	1.6:1	1.5:1	1.1:1
Common bicytopenia in peripheral blood	Anaemia with thrombocytopenia	Anaemia with thrombocytopenia	Anaemia with thrombocytopenia
Bone marrow diagnosis	Megaloblastic anaemia	Megaloblastic anaemia	Normoblastic erythroid hyperplasia

[Table/Fig-4]: Comparison between the studies.

The present study showed normoblastic erythroid hyperplasia predominantly, followed by megaloblastic maturation and lastly micronormoblastic maturation. The study showed by Singh I et al., showed predominance of normoblastic pattern followed by megaloblastic marrow [9]. Nigam RK et al., in their study conducted in Central India showed predominantly megaloblastic marrow [12]. Singh A et al., Anjum MU et al., also showed megaloblastic pattern of maturation as the highest occurrence [1,13]. The present study encountered diluted marrow in five cases. Bone marrow biopsy and imprint smear helped in this regard to arrive at a definitive diagnosis. While three cases showed a normoblastic erythroid marrow, deducing that an error in technique or altered pressure during the aspiration can be sited as a reason, authors came across a single case of hypoplastic marrow and a single case of non Hodgkin's lymphoma. Thus, imprint smears are also important in cases of dry tap or diluted aspirate to study the cytomorphological details of the cells. It should be a standard practice to evaluate imprint smears along with aspiration smears especially in these conditions. This furthermore lays importance on the bone marrow trephine biopsy, which not only will aid in a diagnosis, at times will help the pathologist to arrive at a definitive diagnosis when the aspiration fails to help us.

The diagnostic accuracy of bone marrow aspiration in previous studies were documented between 93-97% [7,14]. The observation in the present study was well with the range (93.75%), inferring that bone marrow aspiration has become an important diagnostic tool. This finding has been supported by previous studies [Table/Fig-5] [7,14].

Study	Number of cases	Diagnostic accuracy
Ekwere TA et al., [7]	62	58 (93.54%)
Egesie OJ et al., [14]	185	179 (96.8%)
Present study	80	75 (93.75%)

[Table/Fig-5]: Diagnostic accuracy of bone marrow aspiration.

In the present study, 79 (98.75%) of 80 cases showed non malignant cases of haematological disorders and 01 (1.25%) case showed haematological malignancies. The non malignant disorders comprised predominantly of nutritional anaemias. The haematological malignancies comprised of single case of non Hodgkin's lymphoma. Singh I et al., in their study, showed nonmalignant haematological disorders in 53.6% and haematological malignancies in 46.4% of cases [9]. This wide range of difference in the two studies can be attributed to the fact that authors included only cases with bicytopenia in their workup. This only suggests that the variation in the indication can also give a change in the yield of the study.

The bone marrow examination performed on children in the present study was nine cases. Majority of them presented with anaemia and thrombocytopenia, seen in 8 (88.8%) out of nine cases, while only a 1 (11.2%) case showed anaemia with leukopenia. Nutritional anaemias have been the most common aetiology. Micronormoblastic pattern of maturation suggesting iron deficiency anaemia has been seen in three out of nine cases, marrow with megaloblastic maturation in three out of nine cases signifying megaloblastic anaemias and the rest three cases showed normoblastic maturation. The study conducted by Saadia HD et al., showed that the most common bicytopenias in children was anaemia and thrombocytopenia [2]. The cause for this was acute lymphoblastic leukaemia followed by nutritional deficiency anaemia. However, the present study encountered only nutritional deficiency anaemia as the causative aetiology. The nutritional anaemia mainly in form of iron deficiency anaemia, should alert the pathologist to look for a parasitic infection, which is common in the Indian setup. An elaborate clinical history can thus aid in speedy and accurate diagnosis along with good morphological skills [15,16]. In the present study, imprint smears were performed for all cases and it significantly helped in arriving at the diagnosis when aspiration failed. Hence its inclusion as a standard practice will be of immense importance. Bone marrow examination can thus be performed on both adults and children. Bone marrow study in form of aspiration, imprint smears and biopsy, thus forms an invaluable asset in the diagnosis and aids in the clinical management of the patients [5,16,17].

Limitation(s)

The study encountered very limited insight on malignant diseases presenting as bicytopenias.

CONCLUSION(S)

Bone marrow study forms a cost effective tool in interpretation of bicytopenia. Bone marrow aspirations helps to know the cytomorphological details, thus plays a better role than trephine biopsies in studying the individual cells and is valuable in sub classifying anaemias and leukaemias. Imprint smears are very useful in cases of dry tap or diluted tap aiding to a diagnosis. However, bone marrow biopsies give us an overall insight in the architectural details, helpful in detecting fibrosis in myelofibrosis and pattern of infiltration in malignancies. These tools are harmonious in nature, complementing each other to arrive at a diagnosis. However, their results should be interpreted with the complete blood counts and peripheral smear examination along with the clinical history and presentation to aid for further clinical management.

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