

ORIGINAL ARTICLES

HISTOPATHOLOGICAL OUTCOME OF PANCYTOPENIA CASES
ON BONE MARROW TREPHINE BIOPSY

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ABSTRACT

Objective: To determine the histological outcome of pancytopenia cases on bone marrow trephine biopsy and to see the frequency of various causes of pancytopenia in our population.

Study Design: Descriptive study.

Place and Duration of Study: Pathology department, Combined Military Hospital (CMH), Kharian (Pakistan). One year (Jan 2015–Dec 2015).

Material and Methods: Two hundred bone marrow trephine biopsies were done in one year (2015), out of which 40 were done for evaluation of pancytopenia. The criteria for diagnosis of pancytopenia were; haemoglobin less than 10 g/dl, total leukocyte count (TLC) less than $4.0 \times 10^9/l$ and platelet count less than $100,000 \times 10^9/l$. Patients with pancytopenia secondary to drugs, chemotherapy and radiotherapy were excluded from the study. Trepine biopsies showing marked crushing and having inadequate material were also excluded from the study. Biopsies were processed, slides made and examined under light microscope by haematologist and histopathologist. Frequencies of various causes of pancytopenia diagnosed on histopathology were calculated. The findings were analyzed by using SPSS version 10.0.

Result: Out of 40 cases of pancytopenia, male to female ratio was 3:2. The age range was between 1 year to 75 years. Histopathological analysis of bone marrow trephine biopsies revealed megaloblastic anaemia as the most common cause of pancytopenia (30%), followed by aplastic anaemia (25%) and hypersplenism (15%).

Conclusion: Megaloblastic anaemia is the most common cause of pancytopenia in our population as compared to aplastic anaemia mentioned in most of the international studies. This indicates prevalence of nutritional deficiency in our population and megaloblastic anaemia must be kept at top of list while evaluating pancytopenia cases. Early diagnosis and treatment of megaloblastic anaemia will prevent any further complication of this disease.

Keywords: Aplastic anaemia, Bone marrow trephine biopsy, Megaloblastic anaemia, Pancytopenia.

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INTRODUCTION

Pancytopenia is described as deficiency of all cellular components of blood including red blood cells, white blood cells and platelets, resulting in anaemia, leukopenia and thrombocytopenia, respectively¹. Pancytopenia in peripheral blood can be a manifestation of primary or secondary disorders affecting the bone marrow. Patients may present with clinical manifestations related to anaemia including pallor, breathlessness and weakness, leukopenia resulting in infections and thrombocytopenia causing bleeding

manifestations². A good clinical and haematological work up is essential to determine the underlying cause of pancytopenia. Bone marrow examination including bone marrow aspiration and trephine biopsy is extremely important and rewarding in this regard. Bone marrow aspiration may be useful in determining the underlying cause in most of the cases, but bone marrow trephine biopsy has a greater value as it can be effective in case of dry tap on aspiration³. Trepine biopsy provides relatively large piece of bone marrow for examination and diagnosis of underlying pathology. In addition, ancillary studies like cytochemical stains, immunohistochemistry and molecular studies like fluorescent in situ hybridization (FISH) can

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be done on trephine biopsy material⁴. The underlying pathology causing pancytopenia determines the management and prognosis in these cases⁵. In this study, we present 40 cases of pancytopenia with their histological outcome on bone marrow trephine biopsy.

MATERIAL AND METHODS

This prospective, descriptive, hospital based study was carried out in Pathology Department of Combined Military Hospital, Kharian (Pakistan) over a period of 01 year from January 2015 to December 2015. The local ethical committee of the hospital approved the study protocol. During study period of 01 year, 200 bone marrow examinations were done, out of which 40 were done for evaluation of pancytopenia. The criteria for diagnosis of

marrow aspiration and making smears of aspirated material, trephine biopsy was performed in all cases to get an adequate tissue of bone marrow. Bone marrow trephine biopsies were decalcified in 5% nitric acid, fixed in 10% formalin and processed in automatic tissue processor (Auto tissue processor Leica 1020, Germany). Paraffin embedding was done and 4-5 micrometer thick sections were cut with rotary microtome (Leica, Germany) and stained with haematoxylin and eosin (H&E) stains. Special cytochemical stains including periodic acid schiff (PAS) stain, reticulin stain and immunohistochemistry markers were done, where required. The slides were seen by haematologist and histopathologist to make a diagnosis. All the data was entered and analyzed by using SPSS version 10.0.

Table-1: Frequency of various histopathological diagnosis in patients of pancytopenia on bone marrow trephine biopsy examination.

S.No	Histological Diagnosis	No of cases = (n)	Percentage
1.	Megaloblastic Anaemia	12	30%
2.	Aplastic Anaemia	10	25%
3.	Hypersplenism	06	15%
4.	Non Hodgkin's Lymphoma	04	10%
5.	Acute Myeloid Leukaemia	02	05%
6.	Reactive Changes	02	05%
7.	Visceral Leishmaniasis	01	2.5%
8.	Gaucher's Disease	01	2.5%
9.	Myelodysplastic Syndrome	01	2.5%
10.	Tuberculosis	01	2.5%
	Total	40	100%

pancytopenia were; haemoglobin less than 10 g/dl, total leukocyte count (TLC) less than 4.0 x 10⁹/l and platelet count less than 100,000 x 10⁹/l. A proforma was designed to record the clinical history with special emphasis on history of drug intake, chemotherapy/radiotherapy, clinical examination findings and details of investigations. Patients with pancytopenia secondary to drugs, chemotherapy and radiotherapy were excluded from the study. All the bone marrow aspiration and trephine biopsies were done from posterior superior iliac spine (PSIS) using Salah needle and Jamshidi needle, respectively. After doing the bone

RESULTS

A total number of 200 bone marrow trephine biopsies were done during a period of 01 year (Jan 2015–Dec 2015). Out of these 200 bone marrow trephine biopsies, 40 (20%) were done for evaluation of pancytopenia. There were 24 (60%) male and 16 (40%) female patients, showing a male preponderance with a male to female ratio of 3:2. The age range was between 1 year to 75 years (mean age 38 years). Most of the patient (n=18,5%) were in 30-50 year age group and the least number (n=5,2%) of patients were from more than 70 years age group. Patients with pancytopenia presented with different clinical

features out of which fever was the most common complaint (62.5%) followed by generalized weakness (50%) and pallor (45%). Other clinical features included easy fatigability (40%), splenomegaly (25%), lymphadenopathy (15%), bleeding (12.5%), hepatomegaly (10%), anorexia (5%) and weight loss (5%).

Histopathological analysis of bone marrow trephine biopsy of 40 cases of pancytopenia

diagnosed on bone marrow examination⁶. Pancytopenia may occur in different age group and etiological factors in different age groups are different. In our study, megaloblastic anaemia (30%) was the commonest histopathological diagnosis in pancytopenia cases, followed by aplastic anaemia (25%). These results are comparable to the results of a study done by Tilak V, Jan R (1998) which revealed

Table. 2. Comparison of most common causes of pancytopenia in different studies.

S. No	Study	Country	Year	No of cases	Commonest Cause	Second Commonest Cause
1.	International agranulocytosis & Aplastic anaemia Group Study ¹²	Israel & Europe	1987	319	Aplastic anaemia (52.7%)	Myelodysplastic syndrome (4.5%)
2.	Keisu and Ost ¹³	Europe	1990	100	Aplastic anaemia (32%)	Neoplastic diseases (19%)
3.	Hossain et al ¹⁴	Bangladesh	1992	50	Aplastic anaemia (52%)	Chronic malaria & visceral leishmaniasis (24%)
4.	Verma and Dash ¹⁵	India	1992	202	Aplastic anaemia (40.6)	Megaloblastic anaemia (23.26%)
5.	Tilak and Jain ⁷	India	1999	77	Megaloblastic anaemia (68%)	Aplastic anaemia (7.7%)
6.	Kumar et al ⁸	India	1999	166	Aplastic anaemia (29.51%)	Megaloblastic anaemia (22.30%)
7.	Khodke et al ⁹	India	2000	50	Megaloblastic anaemia (44%)	Aplastic anaemia (14%)
8.	Bajracharya et al ¹⁶	Nepal	2005	10	Aplastic anaemia (60%)	Megaloblastic anaemia (40%)
9.	Shilpa and Pooja ¹⁷	India	2015	34	Megaloblastic anaemia (29.4%)	Aplastic anaemia (23.5%)
10.	Present Study	Pakistan	2015	40	Megaloblastic anaemia (30%)	Aplastic anaemia (25%)

revealed megaloblastic anaemia as the most common cause of pancytopenia (30%), followed by aplastic anaemia (25%) and hypersplenism (15%). Summary of histological outcome of pancytopenia cases on trephine biopsy is given in table-1.

DISCUSSION

Pancytopenia is a serious haematological problem commonly encountered in clinical practice with features of anaemia, infections and bleeding diathesis. There are numerous underlying causes of pancytopenia, which can be

megaloblastic anaemia (68%) followed by aplastic anaemia⁷. On the other hand another study on pancytopenia cases carried out by Kumar et al (1999) revealed aplastic anaemia (29.5%) as the most common histopathological finding on bone marrow trephine biopsy followed by megaloblastic anaemia (20%)⁸. Another study carried out by Khodke et al (2000) also revealed comparable results with our study revealing megaloblastic anaemia (44%) followed by aplastic anaemia (14%)⁹. In another study, Jha A et al (2000) noted aplastic anaemia (29.5%) as most

common and megaloblastic anaemia (23.5%) as the second most common cause of pancytopenia¹⁰. In a study carried out in Pakistan, aplastic anaemia (35.29%) was the commonest histopathological finding in bone marrow trephine biopsies done in pancytopenia cases, followed by megaloblastic anaemia (17.64%)¹¹. A summary of comparison of histopathological outcome of bone marrow trephine biopsies in pancytopenia cases in different studies is given in table-2.

The increased incidence of megaloblastic anaemia and this being the most common cause of pancytopenia in our study indicates the high prevalence of nutritional deficiency in our country as compared to aplastic anaemia which is the commonest cause of pancytopenia reported from various studies throughout the world^{8,18}. Megaloblastic anaemia which occurs due to vitamin B12 and / or folate deficiency, is now a well-recognized and established cause of pancytopenia and there is increased health concern about its consequences world wide¹⁹. In our population, there is a large number of patients, who have vitamin B12 and folate deficiency and present with pancytopenia due to underlying megaloblastic anaemia. In our study, aplastic anaemia was the second most common histological finding on bone marrow trephine biopsies done in patients presenting with pancytopenia. This was contrary to many studies showing aplastic anaemia as the most common cause of pancytopenia^{8,12-16,18}. Aplastic anaemia can be idiopathic or secondary to some other cause. Aplastic anaemia being more common cause of pancytopenia in developed countries may be due to less nutritional deficiencies found in developed parts of the world, bringing megaloblastic anaemia lower down in list as compared to aplastic anaemia in pancytopenia cases^{12,13}. The third most common cause of pancytopenia revealed on bone marrow trephine biopsy was hypersplenism. In hypersplenism, pancytopenia is due to pooling of blood components including red blood cells, white blood cells and platelets in enlarged spleen,

resulting in decrease of all blood components in peripheral blood. Bone marrow in cases of pancytopenia due to hypersplenism is essentially unremarkable. As in our study, hypersplenism was third most common cause of pancytopenia in another study done at Abbottabad (Pakistan)¹¹. Rest of the causes of pancytopenia noted in our study with relatively lower frequency including non Hodgkin's lymphoma, acute leukaemia, visceral leishmaniasis and tuberculosis are comparable to these causes noted in other studies^{3,18,20}.

CONCLUSION

Pancytopenia is a common haematological manifestation which can be due to different underlying causes. Bone marrow examination is a very useful tool in determining the underlying cause of pancytopenia. Bone marrow trephine biopsy can provide more representative material for diagnosis even in cases of dry tap on aspiration and plays a vital role in diagnosis of pancytopenia. Megaloblastic anaemia is the most common cause of pancytopenia in our population, followed by aplastic anaemia. Early diagnosis and treatment of megaloblastic anaemia will prevent any further complication of this disease.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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