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EVALUATION OF BONE MARROW ASPIRATION IN PANCYTOPENIA

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Abstract:

Introduction:

Pancytopenia is the reduction of all the three formed elements of blood (erythrocytes, leucocytes & platelets), below the normal reference range leading to anemia, leucopenia and thrombocytopenia. Pancytopenia is not a disease itself but a triad of findings that may result from a number of diseases processes–primarily or secondarily involving the bone marrow. There are various mechanisms for developing pancytopenia, in which most important is the decrease in hematopoietic cell production (hypocellular marrow). Others includes ineffective erythropoiesis, increase peripheral utilization and destruction of cells & bone marrow with malignant infiltration (hypercellular or normocellular marrow). First step in the diagnosis of a disease is assessing the blood elements. Physical examination findings and peripheral blood picture provide important information in the work up of patients with pancytopenia and help in planning the investigations on bone marrow samples.

Material & Methods:

The present study "Evaluation of Bone Marrow Aspirations In Pancytopenia" was carried out in the department of pathology in a tertiary care hospital in central India, from September 2018 to October 2020 from the patients fulfilling the inclusion and exclusion criteria.

Result:

Megaloblastic anemia was the common cause of the pancytopenia, followed by nutritional anemia. Megaloblastic anemia was seen in 24 patients, accounting for 48% of the total cases and nutritional anemia was seen in 9 patients, accounting for 18% of the total cases.

Conclusion:

Megaloblastic anemia was the commonest cause which indicates the high prevalence of nutritional anemia in study area.

Key Words: Anemia, Pancytopenia

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Main Text

Introduction:

Pancytopenia is the reduction of all the three formed elements of blood (erythrocytes, leucocytes & platelets), below the normal reference range leading to anemia, leucopenia and thrombocytopenia.[1] Pancytopenia is not a disease itself but a triad of findings that may result from a number of diseases processes–primarily or secondarily involving the bone marrow.[2] There are various mechanisms for developing pancytopenia, in which most important is the decrease in hematopoietic cell production (hypocellular marrow). Others includes ineffective erythropoiesis, increase peripheral utilization and destruction of cells & bone marrow with malignant infiltration (hypercellular or normocellular marrow).[3,4] First step in the diagnosis of a disease is assessing the blood elements.[5] Physical examination findings and peripheral blood picture provide important information in the work up of patients with pancytopenia and help in planning the investigations on bone marrow samples.[6]

Bone marrow aspiration is extremely useful in evaluating the cause of pancytopenia by cellularity and cytology in order to prevent grave complications and mortality as the underlying pathology determines the management and prognosis of the patients.[7] Various factors encompassing geographic distribution and genetic disturbances may cause variation in the incidence of disorders causing pancytopenia.[8-10] India is a large and diverse country. People have different age groups, customs and dietary habits. The incidence of causes for pancytopenia is difficult and few studies have discussed about it in the Indian scenario.[8]

The present study has been undertaken to evaluate the various causes of pancytopenia and to evaluate bone marrow findings in cases of pancytopenia. Thus it would help in planning the diagnostic and therapeutic approach in patients with pancytopenia

Objective:

To evaluate bone marrow findings in cases of pancytopenia.

Material & Methods:

The present study “Evaluation of Bone Marrow Aspirations In Pancytopenia” was carried out in the department of pathology in a tertiary care hospital in central India, from September 2018 to October 2020 from the patients fulfilling the inclusion and exclusion criteria.

The cases were selected on the basis of clinical features and laboratory results. Bone marrow aspiration was carried out after getting a written consent from the patient or the guardian.

INCLUSION CRITERIA:

Presence of all three of the following:

Hemoglobin < 10gm/dl

Total leukocyte count (TLC) < 4000/microL

Platelet count < 1,50,000/ microL

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EXCLUSION CRITERIA:

Patients who have recently received blood transfusions
Patients on radiotherapy
Patients on cytotoxic drugs
Pregnant patients
Patients with psychiatric illness

FOLLOWING INVESTIGATIONS WERE CARRIED OUT:

1. Hemoglobin
2. RBC count
3. WBC count
4. Platelet count
5. HIV, HBsAg
6. Bleeding time and clotting time when required
7. Peripheral smear study
8. Bone marrow study

SAMPLE COLLECTION:

Two ml of blood was collected by venepuncture under aseptic precaution in a dry bulb containing ethylene di-amine tetra acetic acid (EDTA) anticoagulant. Samples were processed by an automated autoanalyzer and blood counts with other details were obtained.

PERIPHERAL SMEAR STUDY

The peripheral smear was studied after staining with Leishman's stain. Special stains like periodic acid schiff reagent stain, myeloperoxidase, sudan black and perl's iron stain were used wherever indicated.

Smears were examined under microscope for following features

• RBC morphology- type, morphological anemia, immature RBC's, any inclusions.

• WBC morphology – for differential count, morphology of each cell, immature cells.
• Platelet count and its morphology.
• Any parasites.

BONE MARROW ASPIRATION:

A written consent from the patient or the patent's guardians were obtained prior to the procedure.

Needle used: Salah needle

The aspiration site was posterior iliac crest.

PROCEDURE:

Patient was given left or right lateral decubitus position.

The posterior superior iliac spine was located.

The site was prepared, cleaned with antiseptic betadine and spirit. The surrounding area was draped exposing only the aspiration area.

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Intramuscular atropine was given and xylocaine skin sensitivity test was done prior to the procedure.
Skin, subcutaneous tissue and periosteum overlying the selected site was infiltrated with a local anesthetic such as 2-5 ml 2% lignocaine.
Waited until anesthesia has been achieved.
Bone marrow aspiration was performed using salah needle.

With a boring movement bone marrow aspiration needle with a sty-let in place was passed perpendicularly into the cavity of the ilium at the center of the oval posterior superior iliac spine.
When the bone had been penetrated sty-let was removed.
2 ml syringe was attached and marrow contents were sucked up for making films.
About 0.2 to 0.5 ml of fluid was aspirated.
Aspiration needle was then removed and pressure was applied to the site with cotton pads until bleeding stopped.
Preparing the films from bone marrow aspirates: The films were made 3-5 cm in length of aspirated marrow using a smooth-edged glass spreader of not more than 2cm width. The marrow fragments were dragged behind the spreader by leaving behind a trail of cells.
After procedure was completed pressure bandage was done and patient was instructed to check the site frequently and to report in case of any bleeding.
These aspiration smears were dried and stained with Leishman or MGG stain

BONE MARROW BIOPSY
Biopsy, whenever required was done along with aspiration in the same setting. Bone marrow biopsy needle inserted at bone with stylet then stylet was withdrawn from the needle and the cap was closed, and then was further pushed into the cavity by rotating movements for about 0.5-1cm. The needle was then withdrawn in the reverse rotating direction. A wire probe was inserted at the needle hub on to a sterile gauge. The specimen was fixed in 10% formalin overnight and decalcified in 6% EDTA for 72 hrs. It is then processed similar to histopathological sample and H and E section were studied.

Statistical analysis:

The statistical analysis was performed through SPSS for windows (version 16.0). The Chi square test procedure tabulates a variable into categories and computes chi square statistics. This test compares the observed and expected frequencies in each category to test either that all categories contain the same proportion of values or that each category contains a user specified portion of values.

Result:

Fifty patients with haematological diagnosis of pancytopenia were studied during the period of September 2018 to October 2020, in the department of pathology at tertiary care teaching hospital in central India.

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Table No.1: Age And Sex Distribution In Patients With Pancytopenia

Age	Female	Male	Total cases	Percentage
18-20	0	1	1	2
21-30	3	5	8	16
31-40	4	8	12	24
41-50	4	10	14	28
51-60	5	8	13	26
61-70	0	2	2	4
Total	16	34	50	100

X2= 3.362, p<10, df=5

Most of the patients were in the age group of 41-60 years (54%) and least occurrence was seen in the age group of 18-20(2%). There was a male predominance and the male to female ratio was 2.1:1

Table No.2: Clinical features in Patients with Pancytopenia

Symptom	No. of cases	Percentage	Chi square
Fever	40	40	X2= 16.877, p <0.000
Generalized weakness	45	90	X2= 11.408, p< 0.001
Breathlessness	4	8	X2= 19.048, p < 0.338
Bone pain	2	4	X2= 24.008, p <0.8761
Weight loss	1	2	X2=11.408, p < 0.542
Dyspnea	10	20	X2= 17.88. p < 0.006
Bleeding	6	12	X2= 12.888, p <0.776
Pallor	48	96	X2= 16.7761, p <0.000
Hepatomegaly	10	20	X2= 1.765, p<0.005

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Splenomegaly	8	16	X2=12.1886, p < 0.547
Lymphadenopathy	9	18	X2= 13.018, p < 0.887

Pallor and weakness was the commonest symptom (96% and 90%), followed by breathlessness in 8% of cases and the least was weight loss seen in 2% of the cases

Table No.3: Bone Marrow cellularity in Patients with Pancytopenia

Cellularity	No. of cases	Percentage
Hypercellular	41	82
Hypocellular	4	8
Normocellular	5	10
Total cases	50	100

X2= 18.777, p < 0.000

Majority of the patients had hypercellular marrow (82%) and the least number of patients had hypocellular marrow (8%).

Table No.4: Type of anemia wise distribution of Patients with Pancytopenia

Type of anemia	No. of cases	Percentage
Aplastic anemia	4	10
Megaloblastic anemia	24	60
Dimorphic anemia	9	22.5
Leukemia	3	7.5
Total cases	40	100

Table No. 5: Pancytopenia with Hypercellular and Normocellular Marrow

Etiology	Total no. cases		Hypercellular		Normocellular	
	No.	Percentage	No.	Percentage	No.	Percentage
Megaloblastic anemia	24	52.17	21	51.21	3	60
Dimorphic anemia	9	19.56	8	19.51	1	20

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Hypersplenism	5	10.8	4	9.75	1	20
Leukemia	3	6.52	3	7.31	0	0
Myelodysplastic syndrome	1	2.17	1	2.43	0	0
Dengue	1	2.17	1	2.43	0	0
Hemolytic anemia	1	2.17	1	2.43	0	0
Malaria	1	2.17	1	2.43	0	0
Multiple myeloma	1	2.17	1	2.43	0	0
Total	46	100	41	100	5	100

X2= 3.913, p <0.16, df=7

There were 41 cases of hypercellular marrow and 5 cases with normocellular marrow. The most common etiology noted was megaloblastic anemia (52%), followed by dimorphic anemia (19%), hypersplenism (10%), leukemia and others. Normocellular marrow was seen in megaloblastic anemia (60%), nutritional anemia (20%) and hypersplenism(20%).

Discussion:

Pancytopenia is commonly seen in various clinical settings all over the world. The recognition of the cause of the pancytopenia is important in order to come to the accurate diagnosis and proper treatment planning. A total of 50 cases were studied from September 2018 to October 2020, at tertiary care hospital in central India.

Age of the patients in the present study ranged from 18-70 years with the mean age of 45 years and there was male predominance with the M:F ratio being 2.1:1. Most common age group affected in Tilak et al[11]was between 5-20 years, in Khodke et al[12] it was 12-30 years, in Khunger et al[7] it was in the third decade and in Yadav et al[13] it was in the range of 41-50 years. In the present study, it was in the range of 41-50 years, which is in accordance with Yadav et al.[13]

In the present study, pancytopenia was found more in males as compared to females, the ratio being 2.1:1. This is similar to studies done by Tilak et al[11] (1.4:1) and Khodke et al(1.3:1).[12] In a study done by Khunger et al[7] and Yadav et al[13] pancytopenia was predominantly seen in males with the M:F ratio being 1.2:1 and 1.76:1 respectively. The present study also found a male predominancy in pancytopenia cases.

The present study shows megaloblastic anemia as the most common cause of pancytopenia with 24 cases, constituting 48% of all the pancytopenia cases, and this co-incides with the studies

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conducted by Khunger et al[7] and Tilak et al[11] where in the megaloblastic anemia constituted 72% and 68% of all the pancytopenia cases. Recenty, Gayathri et al[14] Reddy et al[15] and Varma et al[16] found 74%, 38% and 39% of the megaloblastic anemia cases in their studies.

Present study revealed Dimorphic anemia in 18% of the total pancytopenia cases, similar to the studies done by Yadav et al[13] where the incidence was 17%.

Aplastic anemia contributed to about 8% of the total pancytopenia cases in the present study. It was the second most common cause in the studies done by Tilak et al[11] Khunger et al[7]where it constituted to about 7% and 29% of the total pancytopenia cases respectively. Our findings were similar to the studies conducted by Graham et al[16] and Varma et al[17] where aplastic anemia contributed to total of 6% and 8% of all the pancytopenia cases.

Leukemia was also one of the etiological factor contributing to pancytopenia, here in we found three cases of leukemia contributing to total of 6% of all the pancytopenia cases. It was similar to Khodke et al[12] and Tilak et al[11] with one case in their study. Khunger et al[7] reported 10 cases, constituting 5% of the total pancytopenia cases.

Conclusion:

Megaloblastic anemia was the commonest cause which indicates the high prevalence of nutritional anemia in study area. The other common causes were dimorphic anemia and aplastic anemia. However, uncommon and rare causes such as multiple myeloma, dengue fever, hemolytic anemia and malaria infection should be kept in mind while planning investigation for complete work up of cytopenic patients.

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