An evaluation of pancytopenia in peripheral BLOOD smears

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Abstract

Background: This study is designed to find out the pathological spectrum of pancytopenia in patients suffering from hematological disorders.

Aims: To identify the common hematological disorders producing pancytopenia.

Setting And Design The present study was done in the Department of pathology, Gandhi Medical College and associated Hamidia hospital,Bhopal M.P. A total of 3 consecutive prospective cases were studied,

Method and Material: Patient's Selection criteria our study included all the patients who were admitted in Hamidia hospital with a clinical suspicion of a hematological disorder and demonstrating pancytopenia in the peripheral blood smears. OPD patients on clinical suspicion of a hematological disorder by the consultant incharge were also included in the study group after obtaining their detailed history, clinical examination and all other relevant investigations. Patients with highly increased bleeding time and clotting time were deterred from the study.

Procedure to find out the cause of pancytopenia, bone marrow aspiration was done from Manubrium of the Sternum after injecting 2% xylocaine to the part in addition to sedation with diazepam in uncooperative patients and in small children. Bone marrow smears were prepared and stained with Leishman stain along with the simultaneous staining of the peripheral smears. A complete hemogram including Hb%, PCV, Red cell indices, platelet count, total leucocyte count and differential leucocyte count was also done by automated cell counter. Finally, the bone marrow and peripheral smears were examined manually under oil immersion.

Statistical analysis Bone marrow aspiration was done in 135 patients including children suspected to be suffering from some kind of hematological disorder. Out of the total patients who were advised bone marrow aspiration to arrive at conclusive diagnosis around 21% had pancytopenia under evaluation on peripheral smear examination. In adults, Megaloblastic anemia was the leading cause of pancytopenia followed by Hypo plastic marrow while in pediatric population Hypo plastic marrow topped the list followed by anemias.

Results: Pancytopenia is not an uncommon hematological diagnosis. The investigation of choice in all the cases of pancytopenia is Bone marrow aspiration which proved to be conclusive in almost 100% of the cases. Megaloblastic anemia in adults and Hypo plastic marrow in children were the leading causes producing pancytopenic blood picture.

Keywords: Pancytopenia, Bone Marrow Aspiration, Megaloblastic Anemia, Hypo plastic Marrow.

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Introduction

Term Pancytopenia is used in medical practice when all the cellular components in the blood are reduced below a critical level. Criteria for diagnosis is: Hemoglobin **<10gm%**, Total Leucocyte Count **<4000/cubic millimeter** and Platelet Count **<1 lac/ cubic millimeter** of blood. In order to establish the cause of pancytopenia, Bone Marrow Aspiration[7] is needed which can be done from Manubrium Sterni, Iliac Crests or Upper End of Tibia in case of children. Bone marrow[2,3,8,11,15,16] is a soft, gelatinous tissue that fills the bony cavities. Red bone marrow contains Stem cells, Progenitor cells, Precursor cells and Functional blood cells. In today's era, aspiration of bone marrow is an indispensable adjunct to the study of diseases of the blood and in certain circumstances is the only way by which a correct diagnosis can be made of hematological and some non-hematological disorders. Bone marrow can be obtained by needle aspiration, percutaneous trephine biopsy or surgical biopsy. Bone marrow aspiration is an outdoor procedure which is simple, safe and not much time consuming. It can be repeated many times, if enquired, without any additional patient preparation. Thus, bone marrow aspiration reduces or eliminates the need of hospitalization and decreases the patient morbidity, thereby benefitting the patient and the healthcare system as well. The quality and quantity of the aspirated material is important and is largely dependent on the skills of the aspirating hematopathologist and to a certain extent on the type of disorder under valuation. Needless to say that bone marrow aspiration is a gold standard as far as the diagnosis[6] of the cause of Pancytopenia is concerned, if applied using proper discretion. The rationale for this study is to evaluate and analyse Pancytopenia in

peripheral lood smears and the pathology behind its causation.

Method and Material

This study was conducted in the Department of Pathology, Gandhi Medical College, Bhopal, Madhya Pradesh, India. A total of 3 consecutive prospective cases of pancytopenia were studied. Patients admitted in Hamidia Hospital (Associated Hospital of Gandhi Medical College) with a clinical suspicion of hematological disorder and demonstrating pancytopenia in the peripheral blood smears were included in the study.

Methodology

Complete patient details including presenting complaints with duration, past history, information regarding occupation and exposure to any drug or radiation was collected. General physical as well as systemic examination was done. All routine investigations including Hemoglobin estimation, Total and Differential Leucocyte counts, Platelet count, serum glucose, blood urea, serum creatinine, serum bilirubin and urine examination were done. Bone marrow aspiration was planned for these patients.

Bone Marrow Aspiration Procedure.[1]

Patient and his attendants were told about the entire procedure and a written consent was taken. Complete patient preparation (xylocaine sensitivity testing, cleaning and draping) was done prior to the bone marrow aspiration. The skin over the sternum was cleaned with 70% ethy alcohol. The skin, subcutaneous tissue and the periosteum overlying the manubrium was infiltrated with 1-1.5 ml of 2% xylocaine. Two minutes were given to achieve the effect of anaesthesia. In case of small children and uncooperative patients, sedation with diazepam was used. The site of puncture of the manubrium was opposite to the second intercostal space and slightly to one side of the midline. The guard on the aspiration needle was adjusted and with the boring movement, needle (salah needle) was passed perpendicularly into the cavity. After piercing the s4kin and the subcutaneous tissue when the needle point reaches the periosteum,the needle was pushed with a boring motion into the cavity and the termination point was achieved when there was loss of resistance. Stilette was removed and a 10ml dispovan syringe was attached to the needle to suck the marrow contents. Not more than 0.3ml of marrow fluid was sucked in a single aspiration. Immediately, 6-8 good marrow smears were made and dried quickly with the help of a hair drier. Simultaneously, 2-3 peripheral blood smears were also made. The slides were numbered with a diamond pencil. Two marrow smears and one peripheral blood smear were taken for leishman staining while the rest of the unstained smears, after being fixed in methanol were wrapped in an aluminium foil and kept in a dry place for future use.

Leishman Staining[2,3]: Leishman stain comes under the broad group of Romanowsky's stains.

Principle of Romanowsky's Stains: These stains are not single stains but compound stains formed by the interaction of medicinal (not pure) methylene blue and eosin. With ageing or exposure to acids, alkalies or ultraviolet light, a number of oxidation products (methylene azures) are formed from methylene blue. By this process, a series of loosely combined chemical bodies (methylene blue eosinate, methylene azure eosinate etc.) are formed which give contrast colour staining. In practice, absolutely pure dyes are expensive and it is sufficient to ensure that the stain contain atleast 80% of an appropriate dye. A pH to the alkaline side of neutrality accentuates the azure component at the expense of the eosin and vice versa. A pH of 6.8-7.0 is recommended for general use.

Stain Preparation: 0.2 grams of dry leishman powder is added to 100 ml of acetone-free methyl alcohol in a 200-250 ml capacity conical flask. Both the contents are mixed well and warmed at 50 degree centigrade for 15 minutes with occasional shaking. The flask is allowed to cool and the contents are filtered. The stain is kept for 7 days to mature.

Buffered water: It consists of stock solution A and B which are prepared as follows - **Stock solution A** – 2.72 grams of potassium dihydrogen phosphate powder is dissolved in 100 ml of distilled water to get a concentration of 2.72 gram percent.

Stock solution B: 0.8 grams of sodium hydrogen powder is dissolved in 100 ml of distilled water to get a concentration of 0.8 gram percent. in the second step, 50 ml of stock solution A and 23.7 ml of stock solution B are mixed together to get the final stock solution.in step three, working solution is prepared by adding 2 ml of final stock buffer to 98 ml of distilled water, with the final pH between 6.8 to 7.0.

Staining of slides: Bone marrow smears and the peripheral blood smear were placed on a staining rack and leishman stain was put drop by drop on the film so as to cover it completely. After 2 minutes, double the volume of buffered water was added and the two are mixed together with the help of a dropper. After 20 minutes, slide of peripheral smear was washed under the running tap water and the scum was drained off while bone marrow smears were washed after 30 minutes. Back side of the slides was wiped off with a clean and dry filter paper. The slides were kept in a vertical position to drain and dry. The slides were now ready for the microscopic examination.

Reporting of Bone Marrow Smears: Bone marrow as well as peripheral smears were first scanned with 4X (scanner view)lens followed by the examination under low power(10X), high power(40X) and oil immersion lenses (100X) respectively. The final reports were dispatched in the prescribed format only.

Ethics: Bone marrow aspiration was done under all aseptic precautions and the samples were processed according to the established laboratory protocol before generating final reports to the patients. Informed consent regarding the procedure was taken prior to the aspiration. It was told to the patients that the information shared by them and the results thereafter will be used for medical research.

Results

This study revealed that "Pancytopenia under evaluation" is the cause for which Bone Marrow Aspiration is resorted to in around 21% of cases. Bone Marrow Aspiration is the only adjunct to arrive at definitive diagnosis in all such cases. It is the investigation of choice. Megaloblastic anemia followed by Hypo plastic Marrow are the main and major hematological disorders producing pancytopenic blood picture in adults while Hypo plastic Marrow holds top position in case of children followed by Megaloblastic and Dimorphic anemias .clinically, the patients having pancytopenic blood picture, present with the complaints of Fever, Fatigue, Pallor, Recurrent Infections and Bleeding from various sites. The occurrence and severity of complaints depend on the degree of pancytopenia in individual patients.

Discussion

Pancytopenia[10] is a hematological condition in which there is simultaneous occurrence of Anemia, Leucopenia and Thrrombocytopenia. In this study, our criteria for diagnosis was: Hemoglobin **<10gm%**, Total Leucocyte Count **<4000/cubic millimeter** and Platelet Count **<1 lac/ cubic millimeter** of blood while in the study by Khodke K etal[9], the criteria for diagnosis was: Hemoglobin **<10gm%**, Total Leucocyte Count **<3500/cubic millimeter** and Platelet Count **<1 lac/ cubic millimeter** of blood. In this study, Bone Marrow Aspiration was done from Manubrium sterni while in the study by Khodke K et.al, it was done from iliac crest or tibial tuberosity. In this study, Megaloblastic anemia in adults and Hypoplastic Marrow in children are the most frequent causes of pancytopenia in peripheral blood smears. In the studies by Bhatnagar SK etal[1], Memons etal[12] and Gupta V etal[4]the most common causes of pancytopenia in children were Megaloblastic anemia and Aplastic anemia respectively. Imbert Met al[5], Sarage DG et al[13] and Tilak V et al[14] conducted their studies on adult population and the most common isorder causing pancytopenia in their studies were Malignant Myeloid Disorders and Megaloblastic anemia respectively.

Statistics

Table 1: Indications for Bone Marrow Examination

Indication	Cases	
	No.	%
Anemia Under Evaluation	62	46.0
Pancytopenia Under evaluation	28	20.7
Suspected Leukemia	14	10.4
Thrombocytopenia	12	8.9
Hepatosplenomegaly Under evaluation	04	3.0
Pyrexia Under Evaluation	02	1.5
Others	13	10.0
Total	135	100.0

Table 2: Hematological Disorders behind Pancytopenic Blood Picture in Adults

Hematological disorder	Percentage
Megaloblastic anemia	61.0
Hypoplastic marrow	12.0
Acute myeloid leukemia	9.0
Dimorphic anemia	9.0
Lymphoproliferative disorder	6.0
Idiopathic thrombocytopenic purpura	3.0
Total	100

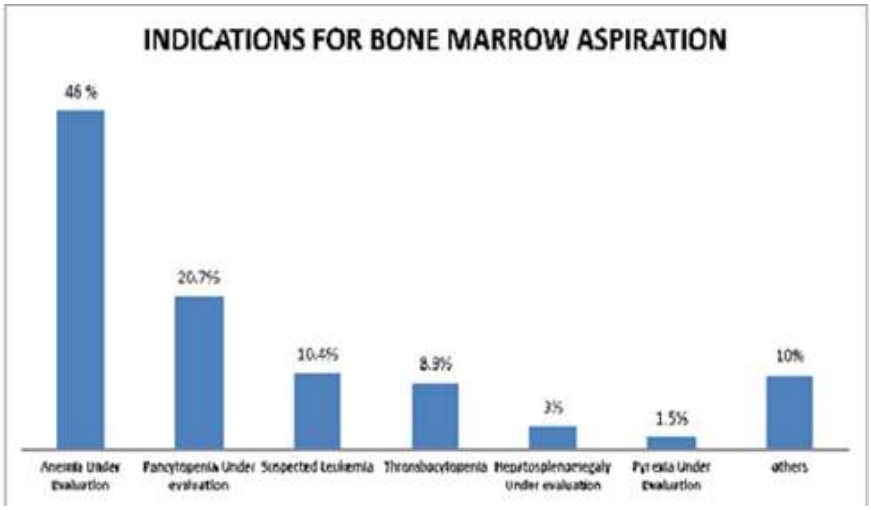
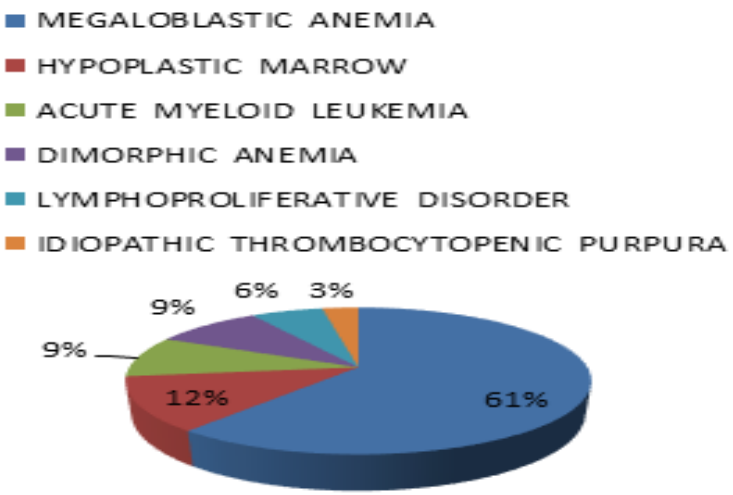


Table 3: Spectrum of Hematological Disorders Causing Pancytopenia in Children

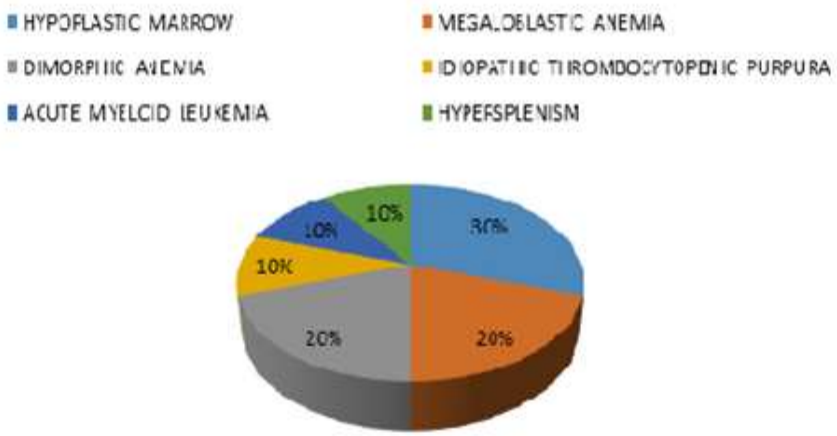
Hematological disorder	Percentage
Hypoplastic marrow	30
Megaloblastic anemia	20
Dimorphic anemia	20
Idiopathic thrombocytopenic purpura	10
Acute myeloid leukemia	10
Hypersplenism	10



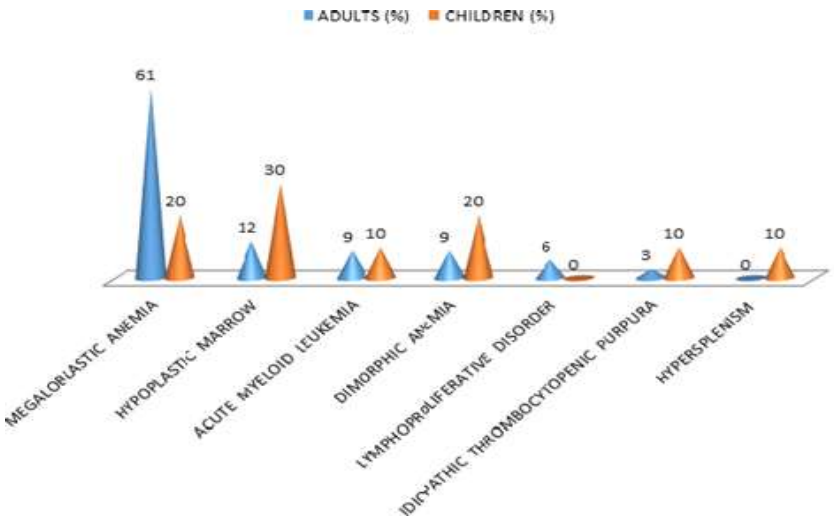
Hematological Disorders behind Pancytopenia in Adults

Table 4: Relative frequency of disorders producing pancytopenia in adults and children

	Adults (%)	Children (%)
Megaloblastic anemia	61	20
Hypoplastic marrow	12	30
Acute myeloid leukemia	9	10
Dimorphic anemia	9	20
Lymphoproliferative disorder	6	00
Idiopathic thrombocytopenic purpura	3	10
Hypersplenism	0	10
Total	100	100



Specteum of hematological Disorders causing pancytopenia in Children



Frequency of disorders causing pancytopenia in adults and children

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