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Hematology

COMPARATIVE ETIOLOGICAL AND CLINICO-HEMATOLOGICAL EVALUATION OF PANCYTOPENIA AMONG ADULT AND PEDIATRIC PATIENTS

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ABSTRACT

Introduction:- Pancytopenia is a common clinico-hematological condition characterized by decrease in all three peripheral blood lineages(erythrocytes, leukocytes and platelets) below normal reference range. Cause of pancytopenia can be acquired or congenital. It is imperative to evaluate the causes of pancytopenia in adult and pediatric subjects as well as to understand the significant differences in their etiology and manifestations.

Aim And Objectives:-Etiological evaluation of pancytopenia in adult and pediatric patients on the basis of their clinical presentation.
To assess pancytopenia in adult and pediatric patients through their Peripheral blood and Bone Marrow Findings.
Statistically compare these findings in adult and pediatric patients.

Material And Method:-In this study 80 adult and 30 pediatric patients of pancytopenia were evaluated. Detailed clinical history,Hb,Total leukocyte count, platelet count and bone marrow studies were performed. Statistical analysis was done using independent sample T test.

Results:-The commonest presenting age in pediatric group was between 7-18 years and in adults was 19-71 years. Male to female ratio was 1.94:1.15.On bone marrow examination most common cause of pancytopenia among pediatric subjects was aplastic anemia(37.55%)followed by acute leukemia(25%),,megaloblastic anemia(12.5%),.Megaloblastic anemia was the commonest cause of pancytopenia among adult cases (37.5%) followed by acute leukemia(16.67%) and aplastic anemia(16.67%).

Conclusion:-Our study concludes that there is statistically significant difference between the values of haemoglobin.among adult and pediatric patients of pancytopenia but the p value of TLC and platelet were not significant implying that hemoglobin parameter vary with respect to range between the two groups but the value of TLC and platelets does not vary with respect to range. We also conclude that on bone marrow examination megaloblastic anemia was the commonest cause of pancytopenia in adults and aplastic anemia in pediatric patients.

KEYWORDS :

INTRODUCTION

Pancytopenia is a relatively common hematological entity. It is a striking feature of many serious and life-threatening illnesses, ranging from simple drug-induced bone marrow hypoplasia, megaloblastic anemia to fatal bone marrow aplasias and leukemias [1] Pancytopenia is defined as haemoglobin of < 9 gm/dl, WBC < 4,000/cmm, and platelets < 100,000/cmm. Severe pancytopenia is defined as absolute neutrophil count < 500/cmm, platelet count < 20,000/cmm, and corrected reticulocyte count < 1% [2].While in pediatric population, pancytopenia is when haemoglobin <10 g/dl, leukocyte count is $<4 \times 10^9$/L; and the platelet count is $<150 \times 10^9$/L . In pancytopenia the marrow is customarily hypocellular as a result of primary production defects, it can be due to diminution of hemopoitic cell production, ineffective haemopoiesis or may be due to peripheral devastation of cells. [3]. Pancytopenia is not a disease but a triad of findings with varying degree of clinical trends, etiology, treatment modalities and outcomes. Cause of pancytopenia can be acquired or congenital. The etiology of pancytopenia can be broadly categorized as a central type that involves disorders of production or a peripheral type that involves disorders of increased destruction.These causes could contribute to the pancytopenia independently or as a combination.Decreased production (central type): Pancytopenia due to decreased production is mostly secondary to nutritional deficiencies. Pancytopenia caused by bone marrow failure is known as aplastic anemia. Aplastic anemia could be idiopathic/autoimmune,[4]. Multiple complications such as thrombocytopenia leads to bruising, mucosal bleeding and neutropenia to sharply increased susceptibility to infection. Furthermore, multiple infections including Cytomegalovirus (CMV), Epstein Barr virus (EBV),Human Immunodeficiency Virus(HIV), Rubella, Influenza, Para-influenza, and Hepatitis-A virus (HAV) may also lead to pancytopenia. Single-line immune-mediated degradation of platelets, erythrocytes, or neutrophils is frequent, combinations there of are rare and often serious ([5,6] The various underlying mechanisms of Pancytopenia include decreased hematopoietic cell production due to destruction of marrow tissue, replacement of bone marrow by abnormal or malignant tissue, ineffective haematopoiesis with cell death in bone marrow, formation of defective cells which are rapidly removed from circulation, sequestration or destruction of cells by the action of antibodies, and trapping of normal cells in hypertrophied reticuloendothelial system [7,8]. As per the etiology, duration and degree of impairment, clinical manifestations of pancytopenia are pallor, fever, fatigue, dizziness, bleeding, anorexia, infection, hepatomegaly, splenomegaly, lymphadenopathy and even death [9]. Therefore, understanding the exact etiology of pancytopenia is important for effective treatment and prognosis. In understanding the etiology of pancytopenia, bone marrow examination plays a major role as it provides a reliable index of cellularity and often reveals bone marrow infiltration, primary/secondary malignant cells, acellular marrow, fibrosis and granulomas. Depending upon the etiology, bone marrow picture may vary from normocellular(non-specific changes) to hypercellular(replaced completely by malignant cells) [9].Since the causes of pancytopenia is not well defined in adults and pediatric population, it is imperative to perform detailed clinical examination, history taking and complete haematological work-up including bone marrow studies to understand the significant differences in their etiology and manifestations. Very few of such comparative studies were done previously. Hence, this study was designed to evaluate pancytopenia in adult and pediatric patients through haematological parameters and bone marrow studies and to statistically compare their outcome.

The present study was conducted with an aim to compare the etiological and clinico- haematological parameters of patients with pancytopenia. We evaluated the etiology of pancytopenia in adults and pediatric population through their peripheral blood smears and bone marrow findings. This will help to understand the underlying cause, pathology and o diagnostic approaches, which will further aid in timely management and prognosis of pancytopenia.

AIMS AND OBJECTIVES

1. Etiological evaluation of Pancytopenia in adult and pediatric patients on the basis of their clinical presentation.
2. To assess Pancytopenia in adult and pediatric patients through their Peripheral blood smears and Bone Marrow Findings.
3. Statistically compare these findings in adult and pediatric patients.

Review Of Literature

Peripheral pancytopenia is not a disease by itself; rather it describes simultaneous presence of anemia, leucopenia and thrombocytopenia resulting from a number of disease processes. Detailed clinical history and meticulous physical examination along with baseline hematological investigations, provides invaluable information in the complete workup of pancytopenic patients. Some of the common acquired causes of pancytopenia include HCV-infected patients which are prone to to develop peripheral cytopenia, which has been proposed to be a multifactorial process also influenced by antiviral medication, such as Ribavirin Myeloproliferative disorders also one of the most common cause of pancytopenia in India[3]. Pancytopenia is a diagnostic challenge with a wide spectrum of potential causes, overlapping signs and symptoms. Therefore, clinicians must be familiar with clinical scenarios that should prompt evaluation of whole-blood profile and a haematology referral, for early management of pancytopenia.

Epidemiology

Pancytopenia commonly presents in pediatrics (age group 11-18 years) and adults frequently in the 3rd and 4th decades, with 1.4 and 2.6 to 1 male to female predominance [10]. In elderly patients, conditions such as multiple myeloma,chronic leukemia and myelodysplastic syndrome are more prevalent, while in younger patients acute leukaemia,aplastic anemia ,megaloblastic anemia and viral infections (parvovirus B19) are seen more commonly.

Geographic and socio-cultural influences determine the major causes of pancytopenia. In developing countries such as india,pakistan and nepal megaloblastic anemia should always be kept as first differential diagnosis followed by aplastic anemia, and HIV infections [11,12] . Previous Indian studies have reported megaloblastic anemia as the most frequent cause, followed by aplastic anemia [13]. There are more number of cases in the East than the West, most likely due to the higher incidence of infections and drugs causing pancytopenia being used in developing countries.

Etiology

The etiology of pancytopenia has a wide age, sex and geographical variation which includes megaloblastic anemia, anemia of nutritional origin, aplastic anemia, blood cancer myelodysplastic syndromes (MDS), multiple myeloma, lymphoma, sepsis, alcoholic diseases, viral diseases like HIV and hepatitis, autoimmune diseases, endocrinological disorders and infiltrating diseases of the bone marrow (Gaucher's disease).[14-16]. These causes could contribute to the pancytopenia independently or as a combination.

Decreased Bone Marrow Function:[14]

- Acute Leukemia: The production of cell lines is also impaired when the bone marrow is infiltrated by tumour cells (lymphoma, leukemia, multiple myeloma)
- Aplastic anemia (bone marrow failure)(due to prolonged benzene exposure,germline mutations). Fanconi anemia (replication-dependent removal of inter-strand DNA cross-links)5
- Nutritional deficiencies (vitamin B12, folic acid): Severe deficiency may cause pancytopenia
- Inadequate dietry intake (as seen in eating disorders leading to Vit B12 deficiency and alcoholics) or malabsorption disease such as tropical sprue disease.
- Metastatic tumour cells infiltrating the bone marrow (eg, Advanced breast cancer neuroblastoma, rhabdomyosarcoma)
- Fulminant sepsis
- Myelodysplastic syndrome
- With the current Covid-19 pandemic, pancytopenia has been reported secondary to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Increased Destruction or Pooling of Blood Cells[15]

- Splenomegaly: Peripheral blood cells are trapped in the enlarged spleen
- Autoimmune conditions: systemic lupus erythematosus, rheumatoid arthritis
- Splenic sequestration: alcoholic liver cirrhosis, HIV, tuberculosis, malaria
- Paroxysmal nocturnal haemoglobinuria: complement-mediated destruction of blood cells
- Acquired hemophagocytic lymphohistiocytosis: decreased bone marrow activity due to cytokine storm and hemophagocytosis

The causes of pancytopenia can further be divided into congenital or acquired.

Table 1: Congenital And Acquired Causes Of Pancytopenia

Congenital Pancytopenia	Fanconianemia Shwachman-diamond syndrome DyskeratosisCongenita Hemophagocytic Lymphohistiocytosis Pearson syndrome Cartilage-hair hypoplasia Congenital amegakaryocytic thrombocytopenia
Acquired pancytopenia	Leukemia Aplastic Anemia Paroxysmal Nocturnal Hemoglobinuria Infections Toxins, Drugs Immune disorders

Signs And Symptoms

The clinical presentation can be variable, with mild pancytopenia being asymptomatic to life-threatening emergencies in severe pancytopenia. Patients can present with manifestations of any of the decreased cell lines.

Anemia has well-known clinical consequences: malaise, sleepiness, irritability, exercise intolerance, shortness of breath, and pallor. A rapid drop in red blood cell hemoglobin due to acute hemolysis may abruptly trigger clinical symptoms that are reminiscent of a large blood volume loss due to hemorrhage. In contrast, an insidious- onset anemia is associated with relatively mild symptoms as the cardiopulmonary and biochemical compensatory mechanisms activate over time [16].

Leukopenia impairs the immune system's ability to fight infections. Neutropenia (low absolute neutrophil count [ANC]) is frequently encountered in clinical practice. Children with severe neutropenia (defined as ANC <500/μL [0.5 × 10^9/L]) face a high risk of life-threatening bacterial and fungal infections. Fever in a child who has neutropenia is a medical emergency, particularly in the context of acute neutropenia due to cancer chemotherapy or stem cell transplantation. Evidence-based guidelines for the management of febrile neutropenia in children have been developed by an international expert panel [17]. Affected children must be evaluated emergently under the guidance of a pediatric hematologist-oncologist. Blood cultures must be obtained and empiric broad-spectrum antibacterial therapy started immediately.

In the absence of trauma, a low platelet count (thrombocytopenia) does not lead to clinical symptoms until the count decreases below 10 to 20 × 10^3/μL (10 to 20 × 10^9/L). Children with severe thrombocytopenia develop spontaneous bruising, bleeding, and petechiae (small subcutaneous capillary hemorrhages). Menstruating females experience heavy and prolonged periods [17].

Physical examination may reveal pallor, petechiae, ulcers, rash. Patients with underlying liver disease can present with anorexia, nausea, or lethargy. Signs of underlying liver disease may be seen in patients with cirrhosis. Splenomegaly may be seen in patients with splenic sequestration. Lymphadenopathy can be seen in patients with infections and lymphoma. Attention must be paid to signs of nutritional deficiencies in patients with eating disorders and alcoholism [18]. The neurological examination is essential as it may show impairment of proprioception with a positive. Romberg test and ataxia, suggesting subacute combined degeneration of the spinal cord secondary to vitamin B12 (cobalamin) deficiency and macrocytic anemia [19].

DIAGNOSIS

Initial workup includes a complete blood count, along with reticulocyte count. This helps to determine if the pancytopenia is secondary to decreased production. The mean corpuscular volume points towards megaloblastic anemia. Peripheral blood smear helps to identify any abnormal cells such as blasts, dysplastic leukocytes, and immature cells. Infectious workup should be done as pancytopenia can be associated with infections such as HIV, malaria, and tuberculosis [20]. In pancytopenia cases secondary to an acute viral infection, no further workup is performed as most resolved rapidly [4]. Similarly, in severe infections with sepsis, the termination of infection and sepsis will correct the pancytopenia. If suspected, further evaluation for undiagnosed hepatitis and autoimmune conditions or malignancies should be pursued. Bone marrow aspiration and biopsy is done if no specific etiology is found in order to evaluate the status of bone marrow stem cells [20].

Treatment

Treatment is based on the underlying etiology for the pancytopenia. Nutritional deficiencies are corrected with supplements and diet. Any offending drug is discontinued. If any infection such as HIV, tuberculosis or autoimmune condition/ malignancy is diagnosed, the treatment is done accordingly. Aplastic anemia secondary to viral infections such as parvovirus is transient and symptomatic treatment suffice. For patients with severe aplastic anemia, treatment options include hematopoietic stem cell transplant and immunosuppression. Haematology referral is sought for these patients.

Supportive care for patients includes red blood cell transfusion for anemia to alleviate symptoms and perfuse vital structures. Platelet transfusion is indicated for thrombocytopenia of less than 10,000 per mcL to prevent spontaneous intracranial bleeding. Prompt initiation of broad-spectrum antibiotic therapy is recommended for patients with neutropenic fever or severe neutropenia with an absolute neutrophil count of less than 500 per ml due to the risk of septic death [20].

Previous Studies

An early study on children presenting with pancytopenia at a children's hospital in the United States was conducted by ***Meghann pine and Andrew W Walter (2010)***. A total of 64 children were identified with the diagnosis of pancytopenia. The most common diagnoses were infectious in origin (64%), followed by hematologic (28%), and miscellaneous (8%) etiologies. It was concluded that the most common etiology of pancytopenia in hospitalized children without cancer was infections. This differs from earlier reports in other countries, where megaloblasticanemia was found most often. This study provided guidance to the diagnoses which should be considered when evaluating a child with pancytopenia[21].

Naseem S et al (2011) conducted a study to evaluate the etiological and clinico- hematological profile in children with bicytopenia and pancytopenia.During the study period of 2 years, a total of 990 children were referred for bone marrow examination for different indications. Of these, 571 (57.7%) had either pancytopenia (17.7%) or bicytopenia (40%). Commonest form of bicytopenia was anemia and thrombocytopenia seen in 77.5% cases, followed by anemia and leukopenia in 17.3% and leukopenia and thrombocytopenia in 5.5% cases. Most common etiology was acute leukemia (66.9%) in bicytopenic children and aplastic anemia (33.8%) in pancytopenic children. Children with bicytopenia had a higher incidence of underlying malignancy (69.5% vs. 26.6%), splenomegaly (60.5% vs. 37.4%), lymphadenopathy (41.8% vs. 15.1%) and circulating blasts (64.6% vs. 20.1%) and a lower incidence of bleeding manifestations (12.1% vs. 26.6%) as compared to children with pancytopenia[22].

Pathak R et al (2012) conducted a study to identify the causes of pancytopenia based on bone marrow examination. Bone marrow examinations were performed in 503 cases for different indications over a period of one year. One hundred and two (20.27%) cases fulfilled the criteria of pancytopenia. Trephine biopsy was possible only in 48 cases. In 75% cases aspiration findings were similar to biopsy. Mean age of patients was 38.8 years. Maximum number of cases was seen in age group of 15-30 years. Hypoplastic anemia was the most common cause followed by hematological malignancies, megaloblastic anemia, leishmaniasis and Gaucher disease. Bone marrow examination alone was able to establish the diagnosis in 76.5% cases. In rest marrow findings were nonspecific and in 4.9% cases findings were normal. It was concluded that Bone marrow aspiration coupled with trephine biopsy can diagnose majority but not all the cases of pancytopenia. Hypoplastic anemia, hematological malignancies and megaloblastic anemia are the commonest causes of pancytopenia. Maximum diagnostic yield can be achieved by correlation with clinical findings, peripheral blood findings and with other laboratory and radiological parameters[23].

A parallel study to determine the distribution of bone marrow findings in cases of new-onset pancytopenia was conducted by ***Weinzierl EP et al (2012)***in a tertiary academic medical center in California. They evaluated 250 recent bone marrow aspirates and biopsies performed in the setting of new-onset pancytopenia in patients without previously diagnosed hematologic neoplastic disease. Of the 250 bone marrow studies, 193 were performed in adults and 57 were performed in children. In children, the most prevalent bone marrow finding was B-lymphoblastic leukemia, followed by nonspecific changes attributed clinically to a variety of factors including multifactorial, autoimmune, inflammatory, and infectious etiologies. In adults, hematologic neoplastic causes of pancytopenia were the most prevalent diagnoses, with the cases divided mostly between acute myeloid leukemia and myelodysplastic syndrome, with fewer numbers of cases of acute lymphoblastic leukemia, myeloproliferative neoplasms, and lymphomas. Many bone marrow findings demonstrated nonspecific changes that were attributed clinically to a variety of etiologies such as myelodysplastic syndrome, multifactorial causes, hypersplenism, drugs, and systemic disease. Overall, in both the pediatric and the adult population, new-onset pancytopenia was most commonly associated with neoplasia, although the neoplasm differed by age group[24].

Mohamed A El-Koumi et al (2013) investigated the relative frequency of pancytopenia in children with brucellosis. Sixty patients with brucellosis, were enrolled in the study. Complete blood count (CBC) and blood culture were performed for all cases. Bone marrow (BM) aspiration was considered only in those with pancytopenia. Out of 60 children with brucellosis, 50 (83%) ingested raw animal milk and 27 (45%) had a positive family history of brucellosis. This study concludes that although pancytopenia is an uncommon complication of brucellosis in children, it does occur. Therefore, brucellosis should be considered in the differential diagnosis of pancytopenia in children, particularly in endemic areas such as Saudi Arabia[25].

A retrospective study was conducted by ***Anwar Zeb Jan et al (2013)*** at a tertiary care centre in Pakistan, to determine the various spectrum of pancytopenia with its frequency on the basis of bone marrow examination in children from 6 months to 14 years. Total 14642 patients admitted to the Pediatric Department from 2006-2012 were included in the study and out of them 205 (1.4%) patients were pancytopenic on their peripheral blood smear. Male outnumbered female with a ratio of 1.8:1. 42.5% of the patients were in the age group of 1 month to 5 years. Common etiological pattern identified were aplastic anemia 58(28.3%), hematological malignancies 49 (23.9%), megaloblastic anemia 40 (19.5%), idiopathic thrombocytopenic purpura 16 (7.8%), iron deficiency anemia 9 (4.4%), haemolytic anemia 7 (3.41%), Visceral leishmaniasis 6 (2.93%), hypersplenism 5 (2.44%), malaria 5 (2.44%), anemia of chronic disorder 4 (1.95%), Myelodysplastic syndrome 3 (1.46%), Niemen pick disease 2 (0.97%) and Gaucher disease in 1(0.49%). Common clinical presentations were fever, pallor, body aches, petechial hemorrhages and epistaxis. It was concluded that Pancytopenia is one of the importance occurrences in pediatric patients. Acute leukemia and bone marrow failure are the most common causes yet megaloblastic anemia and infections are the treatable and reversible causes of pancytopenia[3,26].

A prospective study was conducted by ***Sweta et al (2014)*** at a tertiary care centre in the capital city of India to estimate the frequency of different diseases producing pancytopenia. The study included 100 consecutive patients with pancytopenia. Blood samples of the patients were analyzed for red cells, white cells and platelets morphology along with presence and absence of immature cells and abnormal cells. In bone marrow examination, morphology of all cell lineage, cellularity, parasite and abnormal cells were scrutinized. Trephine biopsy was done where ever indicated. They found that the most common cause of pancytopenia was megaloblastic anemia (66%) followed by aplastic anemia (18%), malaria (6%), kala-azar (4%), acute myeloid leukemia (2%), multiple myeloma (2%), myelodysplastic syndrome (1%), and tuberculosis (1%). The study concluded that detailed primary haematological investigations along with bone marrow examination in cytopenic patients is helpful for understanding disease process, to diagnose or to rule out the causes of pancytopenia. It is also useful in planning further investigations and management[27].

A cross-sectional prospective study was conducted by ***Chand R et al (2018)*** to study the etiology, clinical profile and bone marrow morphology of pancytopenia in children at a tertiary care centre in Kumaun region of India. The study included 42 children of aged 1 to 15 year with pancytopenia. The commonest physical finding was pallor, followed by splenomegaly and hepatomegaly. They found that the commonest cause for pancytopenia was megaloblastic anemia. Among the non-haematological causes, kalaazar 5 (11.9%) was the leading cause in this study.

The study reported that detailed primary haematological investigations along with bone marrow examination in pancytopenic patients is helpful for diagnosis and management. This study also suggested that megaloblastic anaemia, dimorphic anaemia and kala-azar should also be included in differential diagnosis of pancytopenia in this geographical area of India[28].

To study the etiology and clinico-hematological profile in adults, an observational study was conducted by Vargas-Carretero CJ et al (2019) at a tertiary care centre in Mexico. Of 109 cases included, the mean age at diagnosis was 49.4 years, with a slightly higher female incidence (53.2%). The most common causes of pancytopenia were: myelodysplastic syndromes (20.2%), megaloblastic anemia (18.3%) and acute lymphoblastic leukemia (12.8%).The authors concluded that in studying a patient with pancytopenia, a good clinical correlation is of utmost importance to evaluate each specific case and plan for further evaluations. Additionally, identifying the sociocultural context in which the patients develop helps narrowing the possible etiology of pancytopenia, and therefore hasten the diagnostic process[29].

A comparative evaluation of pancytopenia/bicytopenia in adult and pediatric patients through hematological parameters and bone marrow studies was done by ***Shams A et al (2018***) at a tertiary care centre in India. The study enrolled 120 patients, among which 80 were adult and 40 pediatric patients were evaluated. They found that the commonest presenting age in children was between 12-16 years of age and 19-40 years in adults. Male to female ratio in adults was 1.94:1, while that of paediatric patients was 1:0.74. On bone marrow examination, most common cause of pancytopenia/bicytopenia among paediatric subjects was aplastic anemia (32.5%) followed by megaloblastic anemia(20%), acute leukemia (17.5%)a, erythroid hyperplasia (15%). Megaloblastic anemia was the commonest cause of pancytopenia/ bicytopenia among adult cases (37.5%) followed by erythroid hyperplasia (20%) and aplastic anemia (10%). The study concluded that there is statistically significant difference between the values of Hb, TLC, Platelet and MCV among adult and pediatric patients of pancytopenia; implying that these hematologic parameters vary with respect to mean values and range between the two groups. Further, nutritional deficiency was found to be a common cause of pancytopenia in both the age groups[30].

In a recent study by***Gayathri BN et al (2020)***the clinical presentations of pancytopenia due to various causes were co-related with hematological parameters, to evaluate the cause of pancytopenia. Total 104 patients in the age groups of 2 to 80 years were enrolled in the study. Most of the patients presented with weakness and fever. The commonest physical finding was pallor, followed by splenomegaly and hepatomegaly. Dimorphic anemia was the predominant blood picture. Bone marrow aspiration was conclusive in all cases. The commonest marrow finding was hypercellularity with megaloblastic erythropoiesis. The commonest cause for pancytopenia was megaloblastic anemia (74.04%), followed by aplastic anemia (18.26%).This study concludes that detailed primary hematological investigations along with bone marrow aspiration in cytopenic patients are helpful for understanding disease process and to diagnose or to rule out the causes of cytopenia. These are also helpful in planning further investigations and management[31].

Cytopenia in pediatric population was studied by ***De B et al (2020)*** with an aim to evaluate the etiological and clinico-haematological profile in children at a central Indian medical college and rural hospital from December 2017 to December 2018. 100 patients in paediatric age group having pancytopenia/bicytopenia on peripheral smear were included in the study. They found that the main presenting features of children with both pancytopenia and bicytopenia was fever and the commonest non- malignant conditions causing bicytopenia was megaloblastic anaemia and aplastic anaemia in pancytopenia and the commonest malignant condition which was associated with bicytopenia and pancytopenia was acute leukaemia. The study concluded that primary haematological investigations along with bone marrow aspiration in cytopenic patients will be helpful for understanding the disease process; diagnosis, or will help to rule out the causes of, cytopenia; and in planning further investigations and management of cytopenic patients[32].

Dasgupta S et al (2020)conducted a study to delineate etiological factors leading to pancytopenia in a Tertiary Care Hospital of West Bengal from Eastern Region of India. Among 248 patients studied, 156 (62.9%) were males and 92 (37.09%) were females. The mean age of the patients was 33 years. Aplastic anemia was the most common cause of pancytopenia that was observed in 83 cases (33.47%) followed by megaloblastic anemia in 52 cases (20.97%), leishmaniasis in 34 patients (13.71%), hypersplenism also in 34 patients (13.71%), and tuberculosis and other connective tissue disorders in 18 cases (7.26%). The occurrence of aplastic anemia was statistically significant in pediatric (≤15 years) age group. It was concluded that Aplastic anemia was found to be the most common cause of pancytopenia in this study, which is in contrast to studies conducted from other regions of India. Delineation of etiologies of pancytopenia in various regions can help in defining diagnostic and therapeutic strategies, which is expected to contribute toward the better management of such patients.[33]

MATERIALS AND METHODS

- This study was conducted in the department of pathology in Subharti Medical College and associated Hospital, Meerut on patients presented with Pancytopenia.
- It was a prospective observational study over a period of 2 years including IPD and OPD Patients from July 2019 to June 2021.
- A total of 110 patients of pancytopenia were included in this study.
- Patients between 7 -18 years were considered as pediatric patient and those who were more than 18 years considered as Adult patients.
- Complete detailed clinical history and further investigation were done to evaluate the cause of pancytopenia.

Inclusion Criteria

- Patient with all the three following findings were included in this study:-

1. Haemoglobin Adult parameter<13.5g/dl in males, <12g/dl in females.Pediatric parameter 7yr-18yr <11.5g/dl
2. Total leucocyte count<4000/cumm
3. Platelet count<1,50,000/cumm

Exclusion Criteria

1. Patients on myelotoxic chemotherapy.
2. Patients who received blood and blood products.

- Blood samples were collected by venepuncture under aseptic precaution in EDTA(ethylene di-amine tetra acetic acid) anticoagulant and processed by automated autoanalyzer.

Following Investigations Were Done-

- Haemoglobin, RBC count
- WBC count, Platelet count
- Peripheral smear study
- Peripheral smears were made within 2 hours. Bone marrow aspiration was done subsequently using Salah needle in cases where it is indicated in aseptic precautions after written consent from patient or guardian. Bone marrow aspiration was stained by leishman stain.
- Peripheral blood films were prepared in a clean glass slide wiped free of dust using cotton. Slides should be 7.5 x 2.5 cm in size and thickness of 1 mm. A drop of blood was placed at 1cm from one corner of a slide in the central line and a spreader slide was placed at a 30 degree angle in front of the drop. The slide was then moved backwards so that it touches the drop. The drop spreads quickly along the line of contact. Then the blood was spread along the slide and the spreader slide should not be lifted till last drop of blood was spread. Ideal smear should have a length of 3 cm and film should finishes 1cm before the end 42 of the slide. This forms the monolayer where the cells are widely spaced so that cell counts can be made. Blood film made was allowed to air dry. The thickness of the blood film can be regulated by varying the spreader angle or by changing the spreading speed and pressure. For anemic blood, wider angle was used to achieve correct thickness. For ideal thickness there should be some overlap of red blood cells throughout the smear length. White blood cells and platelets should be present throughout the blood film.[34]

Staining Of Blood And Bone Marrow Films

Romanowsky stains are employed universally for staining blood films. Romanowsky dyes consists of two components 1. Azure B (Trimethyl thionin) 2. Eosin Y (tetrabromo-fluorescein)[35,36]. In Romanowsky group, Jenner is the 43 simplest and Giemsa is the complex dye. Routinely employed stain is the Leishman stain. pH of the buffer recommended is 6.8. In order to obtain uniform pH, 1 L of water is mixed with 50 ml of Sorensen's phosphate buffer for diluting the stain and for washing the slide.[37,38]

Leishman's Stain

Leishman stain was prepared by mixing 0.2 gm of Leishman powder with 100 ml of methanol in the conical flask. Warm the solution at 50degree celsius for 15 minutes and then allow it to cool. Filtered solution can be used immediately but the staining quality can be improved on standing for few hours.

Procedure Of Leishman Staining

1. Smear is allowed to dry.
2. Leishman stain is poured on the smear for 2 min.
3. Buffer stain is added just double the volume of stain.
4. Keep for 10 minutes.
5. Wash with running tap water for 2 minute.
6. Kept the smear for drying.
7. Clean with xylene.
8. Mounting in DPX medium.

Analysis Of Peripheral Smears

Peripheral smear examination was done systematically under low, high and oil immersion. In red blood cells, morphological changes like anisopoikilocytosis, polychromasia, nucleated blood cells and Rouleaux formation if present was analyzed. Differential white blood cell count was done and noted for any atypical and dysplastic changes. Platelet count and morphology was analyzed. Peripheral smear examination was done to note the presence of parasites if any.

Examination Of Bone Marrow Aspiration

- Stained films with marrow particles was assessed for the degree of marrow cellularity as increased, normal or reduced in a low- power objective(×10).
- On low-power examination, megakaryocytes and clumps of non haemopoietic cells were looked for, mainly concentrated towards the tail of the film. In megakaryocytes, number, morphology and maturation pattern were examined in high power objective. Also examined morphology and content of clumps if present. Looked for macrophages if any and its morphology.
- Myeloid erythroid ratio was calculated in a cellular area of the film where the cells are well spread and stained.
- The differential count for 200-500 cells were done and categorized into erythroid, myeloid, lymphoid and plasma cells. The morphology of these cells was also analyzed.

Statistical Analysis

Data analysis was done using appropriate statistical tests by statistical package for social sciences (SPSS) version 19.0. Also p values were calculated using independent sample T test.

RESULTS

A total of 110 patients of both sex attending outpatient and inpatient Departments of C.S.S.H in all age groups over a period of two years were included to assess the cause of pancytopenia. The results were observed and analysed.

Table 2.- represent the distribution of pediatric and adult according to gender. There were 25% females and 29.03% males in pediatric age group. While in adult group, percentage of females were75% and male were 70.97%.

Table 2. Distribution Of Study Participants In Two Groups According To Sex.

		Group				Total	P Value
		Paediatric		Adult			
		Number	%	Number	%		
Sex	Female	12	25.00	36	75.00	48	0.67
	Male	18	29.03	44	70.97	62	
Total		30	27.27	80	72.73	110	

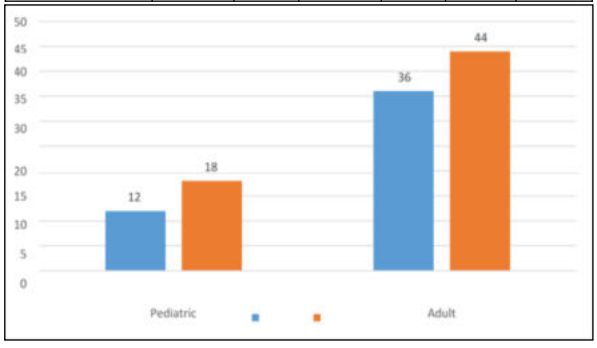


Fig 1: Bar Diagram Showing Distribution Of Study Participants In Two Groups According To Sex.

Table 3- represents sex wise distribution of anemia in both adult and pediatric patients. Majority of them were males in both adults and pediatric subgroups.

Table 3:- Proportion Of Anemic Male And Females In The Two Groups

		Anemia				Total
		Present		Absent		
		Number	%	Number	%	
Pediatric	Female	12	100.0	0	0.0	12
	Male	18	100.0	0	0.0	18
Adult	Female	36	100.0	0	0.0	36
	Male	44	100.0	0	0.0	44

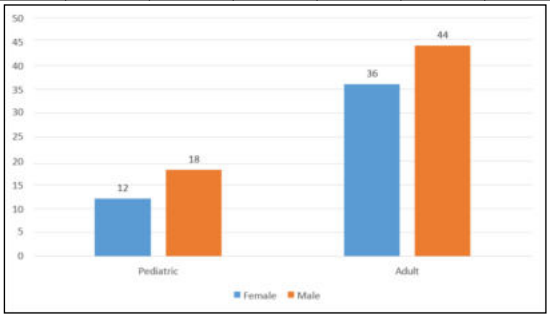


Figure 2: Bar Diagram Showing Proportion Of Anemic Males And Females In The Two Groups

Table 4- states the association between haemoglobin categories in pediatric and adult groups.Inpediatric group majority (11 patients) of them were having haemoglobin in 4-6g/dl range.While in adults(29 patients) most of them were having haemoglobin >10g/dl.

Table -4 Distribution Of HB Categories In Two Groups

Hb_categories (g/dl)	Pediatric		Adult		p value
	Number	%	Number	%	
1-3	10	55.6	8	44.4	0.002
4-6	11	36.7	19	63.3	
7-10	6	20	24	80	
>10	3	9.4	29	90.6	

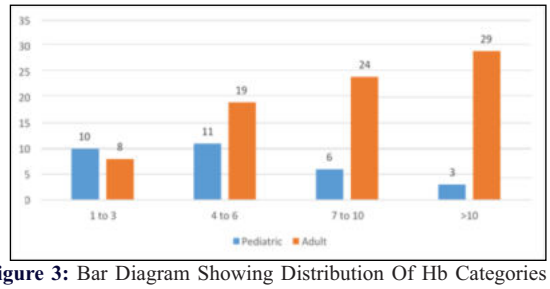


Figure 3: Bar Diagram Showing Distribution Of Hb Categories In Two Groups.

Table 5- represents the distribution of TLC categories according to gender and sex.In adult and pediatric categories most of the male patients were in 2500-4000 range.While in female, majority of adult patients were in category 1000-2499range and in pediatric patients most of were in category 2500-4000 range.

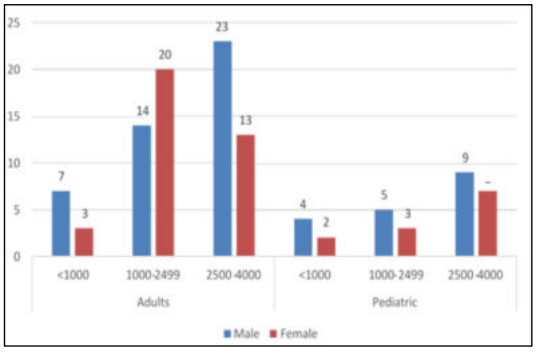


Figure 4: Bar Diagram Showing Distribution Of TLC Categories According To Gender In Both Groups

Table 5. Distribution Of TLC Categories According To Gender In Both Groups

Groups	TLC	Sex				Total	P Value
		Male		Female			
		Number	%	Number	%		
Adults	<1000	7	15.91	3	8.33	10	0.09
	1000-2499	14	31.82	20	55.56	34	
	2500-4000	23	52.27	13	36.11	36	
Pediatric	<1000	4	22.22	2	16.67	6	0.09
	1000-2499	5	27.78	3	25.00	8	
	2500-4000	9	50.00	7	58.33	16	

Table 6- represents the platelet count distribution according to gender in both males and females. In males most of patients were in less than 50,000 range in both adults and pediatric categories. Similarly in females, majority of patients were in less than 50,000 range in pediatric categories and in adult were in 50000-99999 range.

Table 6. Distribution Of Platelet Categories According To Gender In Both Groups

Groups	Platelet Count	Sex				Total	P Value
		Male		Female			
		Number	%	Number	%		
Adults	less than 50,000	22	50.0	14	38.9	36	0.61
	50,000-99999	20	45.5	20	55.6	40	
	1,00,000 - 1,50,000	2	4.5	2	5.6	4	
Pediatric	less than 50,000	13	72.2	7	58.3	20	0.73
	50,000-99999	4	22.2	4	33.3	8	
	1,00,000 - 1,50,000	1	5.6	1	8.3	2	

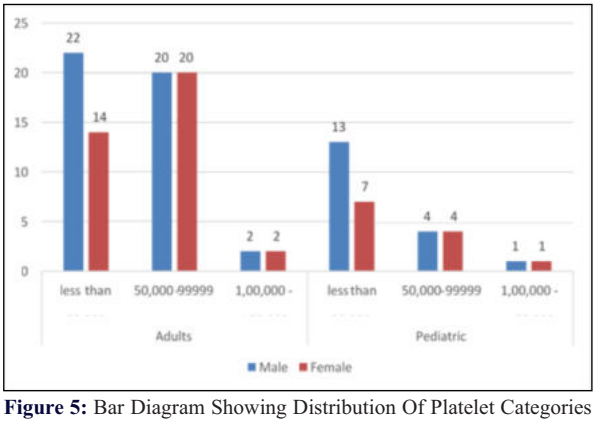


Figure 5: Bar Diagram Showing Distribution Of Platelet Categories According To Gender In Both Groups

Table 7 - represents distribution of patients according to symptoms in adult and pediatric group. Most of patients had fever (25 patients) in males in both adult and pediatric patients. But majority of symptom in females was generalized weakness in adult and fever in pediatric patients.

Table 7 . Distribution Of Symptoms Among Males And Females In Two Study Groups

Symptoms	Group							
	Adult				Pediatric			
	Males		Females		Males		Females	
	Number	%	Number	%	Number	%	Number	%
Fever	14	31.8	11	30.6	11	61.1	7	58.3
Generalized Weakness	20	45.5	19	52.8	4	22.2	3	25.0
Bleeding Manifestation	7	15.9	4	11.1	2	11.1	1	8.3
Pain Abdomen	3	6.8	2	5.6	1	5.6	1	8.3

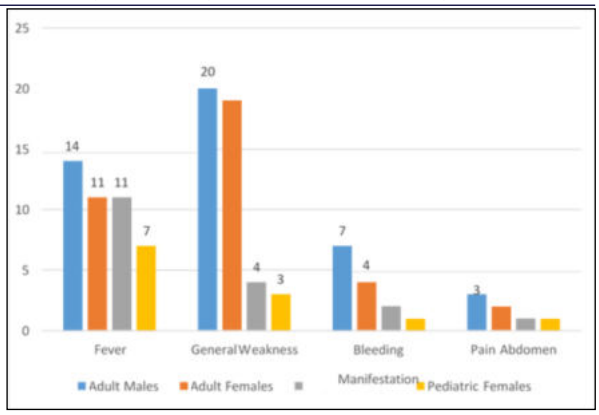


Figure 6: Bar Diagram Showing Distribution Of Symptoms Among Males And Females In Two Study Groups

Table 8 -represents distribution of patients according to sign both males and females in adult and pediatric. Most of patients had pallor both in males and females in adult and pediatric patients.

Table 8. Distribution Of Signs Among Males And Females In Two Study Groups

Signs	Group							
	Adult				Pediatric			
	Males		Females		Males		Females	
	Number	%	Number	%	Number	%	Number	%
Hepatomegaly	13	29.5	8	25.0	4	22.2	3	25.0
Splenomegaly	9	20.5	7	19.4	3	16.7	2	16.7
pallor	22	50.0	21	58.33	11	61.1	7	58.3

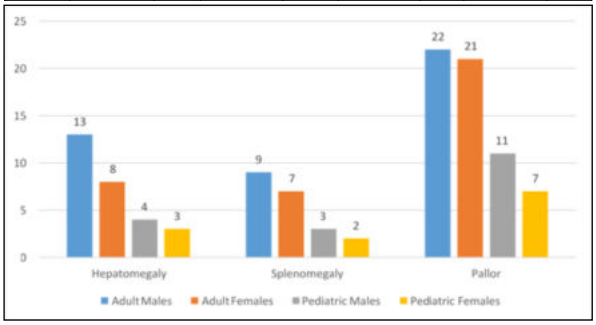


Figure 7: Bar Diagram Showing Distribution Of Signs Among Males And Females In Two Study Groups

Table 9- represents bone marrow cellularity in both males and females.In males(10 patients) majority of patients had hypocellular marrow. While in females most of patients had normocellular marrow.

Table 9. Distribution Of Cellularity Of Bone Marrow Among Males And Females Of Both Groups

Cellularity of Marrow	Group							
	Adult				Pediatric			
	Male		Female		Male		Female	
	Number	%	Number	%	Number	%	Number	%
Normocellular	2	2.5	5	2.5	1	3.33	1	3.33
Hypocellular	8	10	2	10	2	6.67	1	6.67
Hypercellular	1	1.25	3	1.25	1	3.33	1	3.33
Inadequate	2	2.5	1	2.5	0	0.00	1	0.00

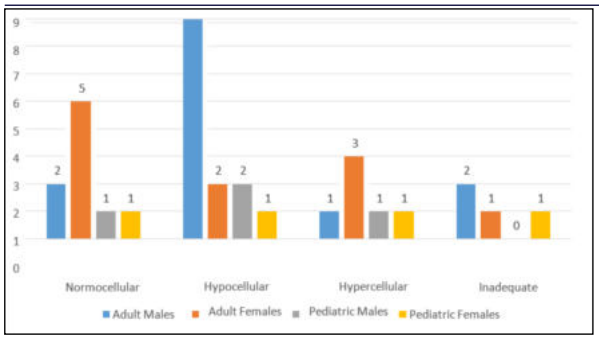


Figure 8. Bar Diagram Showing Distribution Of Bone Marrow Cellularity In Two Study Groups

Table 10-shows the bone marrow aspiration findings in pediatric patients.Most patients were having aplastic anaemia(37.5%) followed by acute leukemia.

Table 10:- Distribution Of BMA Findings Among Pediatric Subjects

Diagnosis	Number of Cases	Percentage
Aplastic Anemia	3	37.5
Acute Leukemia	2	25
Megaloblastic Anemia	1	12.5
Ineffective Erythropoiesis	1	12.5
Inadeqaute	1	12.5
Total	8	100

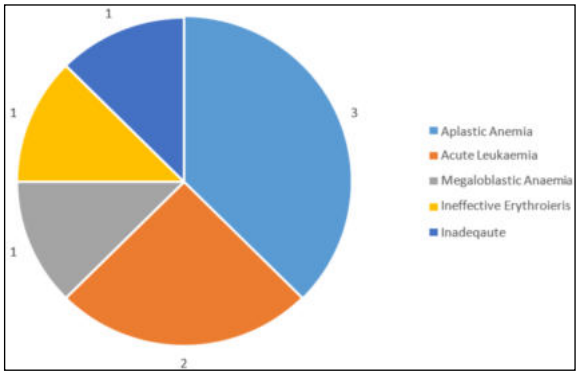


Fig 9:- Pie Chart Showing Distribution Of BMA Findings Among Pediatric Subjects

Table 11 shows the bone marrow aspiration findings in adult .Most patients were having megaloblastic anemia anaemia (20.83)followed by aplastic anaemia (16.67 %) and acute leukaemia(16.67%).

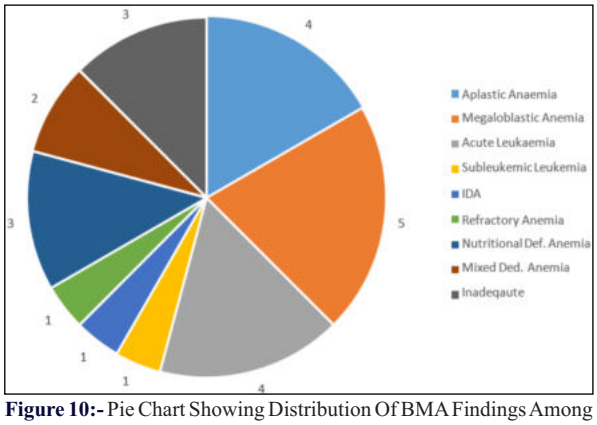
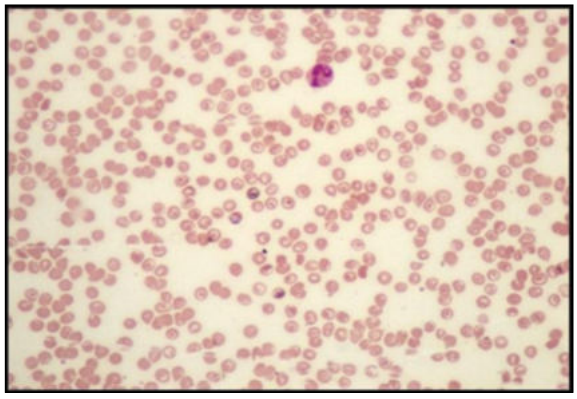


Figure 10:- Pie Chart Showing Distribution Of BMA Findings Among Adult Subjects

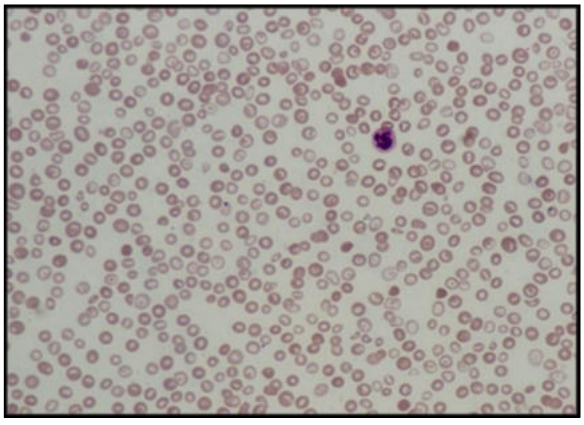
Table 11. Distribution Of BMA Findings Among Adult Subjects

Diagnosis	Number of Cases	Percentage
Aplastic Anemia	4	16.67
MegaloblasticAnemia	5	20.83
Acute Leukemia	4	16.67
SubleukemicLeukemia	1	4.17
IDA	1	4.17
Refractory Anemia	1	4.17
Nutritional Def. Anemia	3	12.50
Mixed Deficiency Anemia	2	8.33
Inadeqaute	3	12.50
Total	24	100

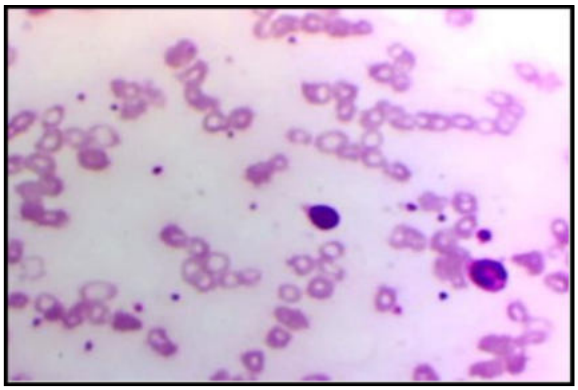
Photomicrographs



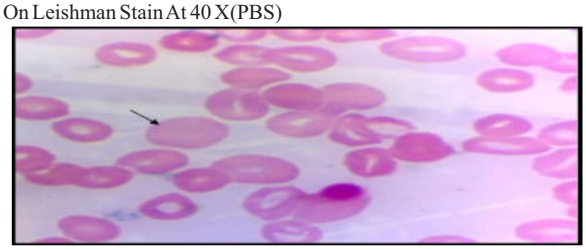
Photomicrograph 1- Exhibiting Pancytopenia Picture On Leishman Stain At 10X(PBS).



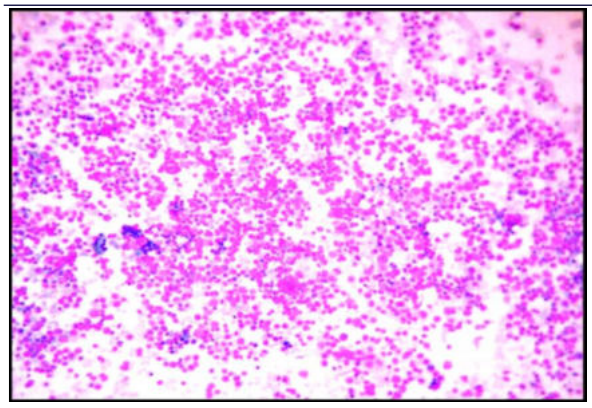
Photomicrograph 2- Exhibiting Dimorphic Anemia On Leishman Stain At 10X(PBS)



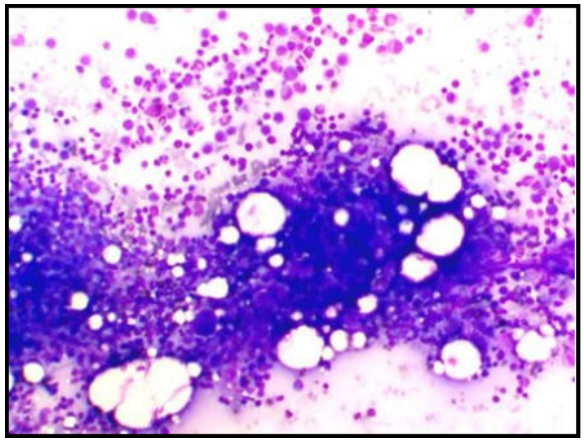
Photomicrograph 3- Exhibiting Microcytic Hypochromic Anemia On Leishman Stain At 40 X(PBS)



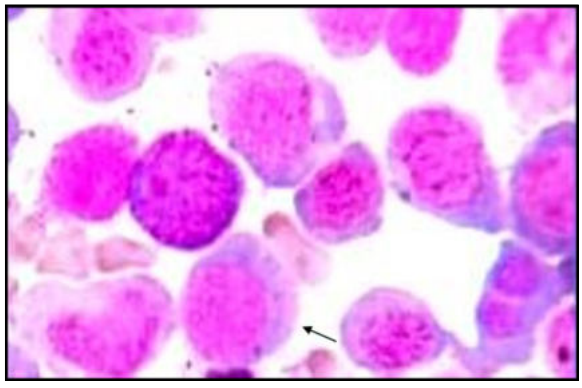
Photomicrograph 4- Exhibiting Macro-ovalocytes In Macrocytic Anemia On Leishman Stain At 100x (PBS)



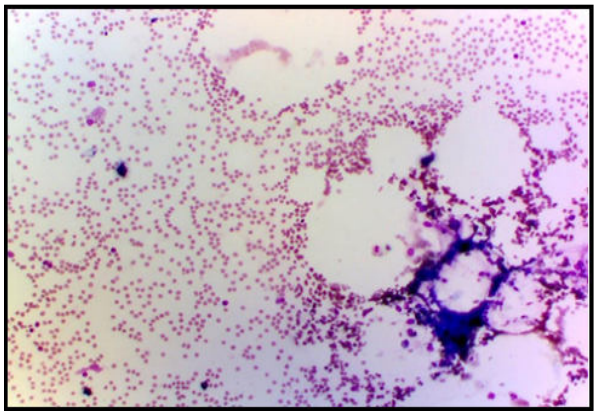
Photomicrograph 5- Exhibiting Megaloblastic Anemia On MGG At 10x (BMA)



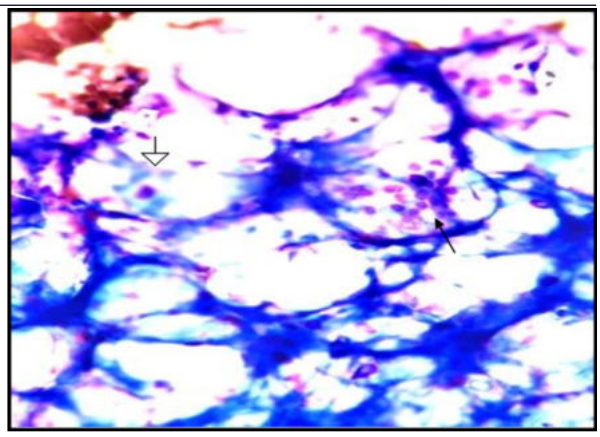
Photomicrograph 6- Exhibiting Cellular Fragments And Cell Trails In Megaloblastic Anemia On MGG At 40X(BMA)



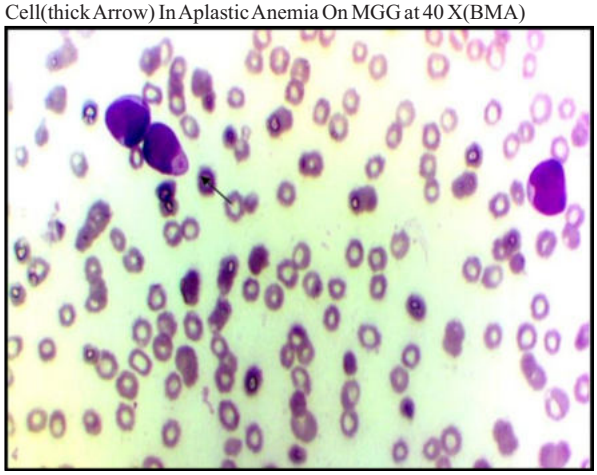
Photomicrograph 7 - Exhibiting Megaloblast In Megaloblstic Anaemia At 100 X (BMA)



Photomicrograph 8- Exhibiting Aplastic Anaemia With Hypocellularity At 10 X (BMA)



Photomicrograph 9– Exhibiting Lymphocytes(thin Arrow) And Mast Cell(thick Arrow) In Aplastic Anemia On MGG at 40 X(BMA)



Photomicrograph 10- Exhibiting Blasts In Subacute Leukemia On Leshman Stain At 40X(PBS)

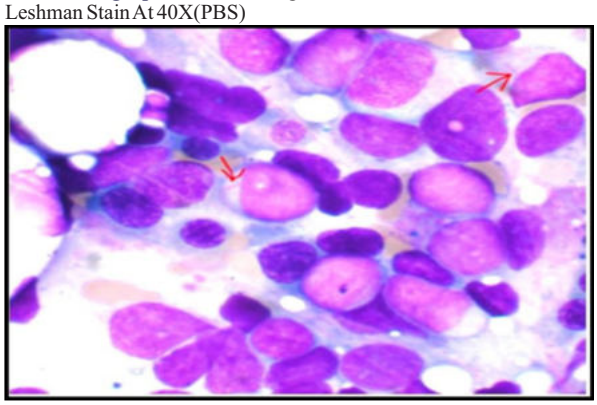
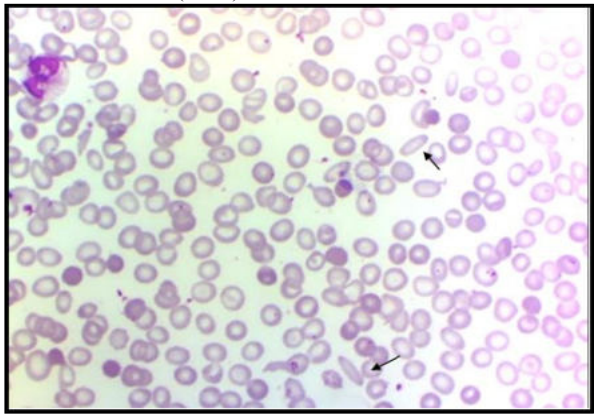
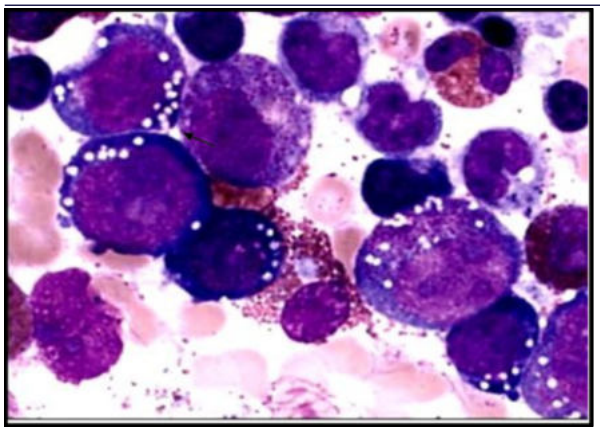


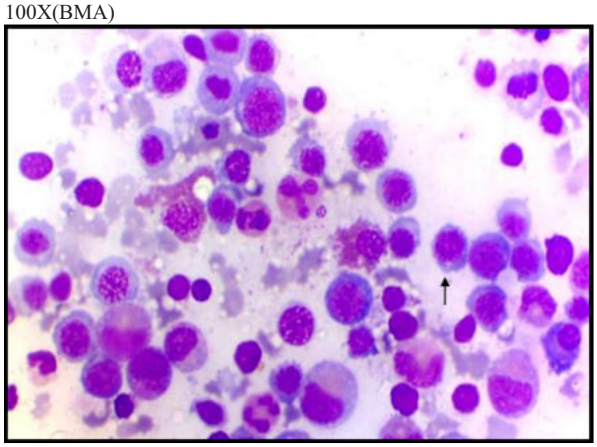
Figure11: Photomicrograph Exhibiting Auer Rods In Acute Leukaemia At 100X (BMA)



Photomicrograph 12– Exhibiting Pencil Cells In Iron Deficiency Anemia At 40 X(PBS)



Photomicrograph 13- Exhibiting Vacuoles In Erythroid Precursors And Dyspoietic Changes In Nutritional Deficiency Anemia At 100X(BMA)



Photomicrograph 14- Exhibiting Erythroid Hyperplasia In Nutritional Deficiency Anemia Anemia At 40X(BMA)

DISCUSSION

Pancytopenia is not an illness itself; rather, it is the result of a number of disease processes that result in anemia, leukopenia, and thrombocytopenia in an individual's peripheral blood. In adults, pancytopenia is defined as a condition when haemoglobin level is <13.5g/dl for males and less than <11.5g/dl for females, total leucocyte count(TLC)<4 x 10^9/L and platelet count <150 x10^9/L.[2]While in pediatric population pancytopenia is when haemoglobin is < 10g/dl, total leucocyte count(TLC) <4 x10^9/L and platelet count < 150 x 10^9/L. Even though pancytopenia is a frequent haematological issue, a thorough review of the literature identified just a few research that seek to pinpoint its origins. [1] Because one of the important factors that influences the cause of pancytopenia is geographical distribution, studies from various regions of different countries can be useful in understanding the underlying disease processes that lead to this manifestation, which can then help to define the required intervention.
In our study, a total of 110 patients of pancytopenia were studied, there were 80 adults (72.7%) and 30 paediatric patients (27.3%) who presented with pancytopenia. Age and sex wise distribution was done. Sign and symptoms like fever, generalized weakness, bleeding manifestations, abdominal, pain, pallor, hepatomegaly and splenomegaly were assessed. Hematological parameters like haemoglobin, total leucocyte count, platelet count and bone marrow aspiration findings were also assessed and compared with that of previous studies. The number of cases taken in our study was found to be higher than majority of the studies conducted in India under the same title. Tilak V et al. in 1999 conducted the study with 77 cases. Khodke K et al. in 2001and Phurailatpam Madhubala Devi et al. in 2008 conducted the study with 50 cases, Soma Yadav et al. in 2013 with 60 cases and S Pudasaini et al. in 2012 with 57 cases. [39-43]

In contrast, two studies conducted in 2001 and 2002 by Kumar R et al and Khunger JM et al took higher number of cases than our study (166 and 200).[43,44] Adult patients in our study were 72.7% which was in concordance with the study done by Jha A et al where they encountered 72% of adult patients. [45] Also, 67% of adult patients were seen in study done by Asbah Shams et al. [30] Pediatric patients in our study accounts for 27.3% which was comparable to the study conducted by Asbah Shams et al with 33% paediatric patients. [30] There was male predominance in our study whether it be in adult cases or in pediatric cases which was in concordance to studies done by Khode K et al and Jha A et al. [40,45]. The studies regarding pancytopenia including both adults and children has been done in many countries across many years. Rehmani et al., Jain et al. in India, Santra et al. in Singapore, Hamid et al. in Yemen, Keisu et al. in Sweden, Savage et al. In Zimbabwe andin South Africa Reteif et al had incorporated both adult and paediatric patients in their study regarding clinical, haematological investigations of pancytopenia. [46-52]

In our study, majority of adults had haemoglobin more than 10 gm/dl (90.6%) and children had haemoglobin ranging from 4-6 gm/dl (55.6%).

Table 12: Comparison Of HB In Current Study Versus Previous Literature

	Adult	Pediatric	Comment
Current Study (Included adult and pediatric patients)	Majority of patients had Hb>10 g/dl (29 out of 80 patients)	Majority of patients had Hb 4-6 g/dl (11 out of 30 patients)	Current study included adult patients >18 years and pediatric<18 years
Asbhah shams et al. 2018(30). (Included adult and pediatric patients)	Majority of patients were in the range of 4-7g/dl (47 out of 80 patients)	Majority of patients were in the range of 4-7g/dl (19 out of 40)	o This study had included patients Hemoglobin< 13.5 gm/dl in males or 11.5 gm/dl in females Which coincides with inclusion criteria of present study.
Aggarwal P et al 2018(included adult patients only)	Majority of patients (58.75%) had haemoglobin ranging from 4-7 g/dl	_	o This study included adult patients above 18 years and Hb was in the range :-1.4 - 10.8 g/dl.
Kumar et al.2021(included adult patients only)	Majority of patients (53%) had Hb in the range 6-9gm/dl	_	o This study included patients in the range of 18-75 years Hb was in the range:- 1.8-9.9 gm/dl.
Naseem et al(22) 2021(included pediatric patients only)	_	Sample size in this study were 990 & 486 patients had haemoglobin less than 10g/dl	o Children less than 12 years were taken.
Anita .P.Javalgi et al 2013(included pediatric patients)	_	Majority of cases were in range of 5-8g/dl	Patients in this study were in range of 15-65 years

Majority of adult as well as children had total leucocyte count ranging from 2500- 4000.Majority of adults had platelet count ranging from50,000-99,999 / µL and children had less than 50,000/ µL. These findings are in concordance with the study done by Asbah Shams et al[30]. where maximum patients had haemoglobin ranging between 4-7 gm/dl in both adults and paediatrics, total leucocyte count < 4000/µL and platelet count less than 50,000 / µL.[53]

The most common presenting complaints in patients with pancytopenia in our study among adults were generalized weakness [Females (52.8%) &Males(45.5%)] followed by fever [Males (31.8%) & Females (30.6%)]. In case of children, the most common presenting complaints were fever [Males (61.1%) & Females (58.3%)] followed by generalized weakness [Female 25%)&Male (22.2%)] Bhatnagar et al's research also found generalized weakness as the most common

presenting complaint in their patients (97.8%) followed by dyspnoea (75%)[54]. In another study by Rames Chand et al., most common presenting complaints were also generalized weakness (90.4 %) and weight loss (80.95%) followed by fever (69.04 %). This is well in concordance with our study. Kumar D B et al, Chandra et al, Desalphine et al reported generalized weakness as the second most common presenting complaint in their studies[28,44].

The most common physical finding in our study were pallor among adults[Males(50%)& Females (58.3%)] as well as in Children [Males (61.1%) & Females (58.3%)], followed by Hepatomegaly in adults [Males(29.5%)& Females (25%)], as well as in Children[Males (22.2%) & Females (25%)]. In a study of Ramesh Chand et al. Pallor (100%) was the most prevalent physical finding, followed by splenomegaly (80.95%) and hepatomegaly (71.42 %). (28). In Bhatnagar et al research also, the most prominent signs were pallor (98.3%) and splenomegaly (25.5%)[54]. Gomber et al. found hepatomegaly (66 %) and splenomegaly (21%) as a most prominent signs in their study [55].

Bone marrow examination has a great diagnostic value in patients of pancytopenia. In our study out of 110 cases, bone marrow aspiration were performed in 32 cases, which showed 3 distinct type of cellularity. Hypocellular bone marrow was observed in 10 cases of adults [Males(10%) & Females(2.5%)] and 3 cases of children[Males (6.67%) & Female (3.33%)] followed by normocellular bone marrow and hypercellular rmarrow. According to Ramesh Chand et al. the marrow was cellular in 71.4 % of patients with megaloblastic anemia as the most common cause, whereas it was hypocellular in 28.5 % of cases with aplastic anemia the most common cause [28]. Jha et al investigated the causes of pancytopenia in 148 individuals [45] The most frequent etiology identified by them was hypoplastic bone marrow (38.1%) in children and megaloblastic anemia (30.2%) in adults. Santra et al. had 60cases of cellular marrow and 50 cases of hypocellular marrow in their study[48].

Pancytopenia is a well-known diagnostic issue for clinicians due to the wide range of potential etiologies, published materials addressing the differential diagnosis have been few. Weinzierl and Arber recently reviewed the causes of new onset pancytopenia in adults and children, classifying them as congenital and acquired bone marrow failure syndromes, marrow infiltrative processes, peripheral destruction processes, autoimmune diseases, infections, and conditions resulting in ineffective haematopoiesis[24].

In the present study, Aplastic Anemia has been accounted as one of the common cause for pancytopenia in children (37.5%). Among the Asian continent, Rehmani et al[28]. conducted study among 244 adults and children where aplastic anemia accounted as the major reason for the pancytopenia (27%) [46]. Santra et al. in their study among 111 adults and children found that with 20.7%[45], Aplastic Anemia was found to be a major finding [48]. Jha et al. with 148 adults and children found that aplastic anemia was the major finding with 29% [45]. Retief at al. in his study found that Aplastic Anemia was the common finding among 195 adults and children bearing 11.3% [52]. Hossain et al. found that aplastic anemia was the major cause among tested 50 adults [56]. Hematopoietic stem cells are found in the bone marrow. These stem cells have the ability to proliferate, develop, and produce red, white, or platelet-like blood cells. A precipitating event in aplastic anemia is thought to cause immune- mediated death of hematopoietic stem cells. Certain immune system cells (T-lymphocytes) are thought to target and kill hematopoietic stem cells, the most primitive cells capable of turning into blood cells.

Aplastic anemia appears to be two to three times more common in Asia than in Europe and North America, where annual incidence rates are around 2.0 per million per year [24]. While the prevalence of Aplastic anemia has been studied in various countries, the majority of these investigations were done more than 20 years ago and included both adult and paediatric cases. Jeong et al. published data on Aplastic anemia patients of less than 15 years old. A total of 828 patients were registered between 1991 and 2005. The rate of childhood Aplastic anemia was 5.16 per million per year. In 780 individuals, the severity of the illness was determined; 456 (58%) and 328 (42%) patients had severe and non-severe Aplastic anemia, respectively. A total of 44 individuals were found to have constitutional anemia, with 40 of them having Fanconianemia. The decreased frequency of constitutional anemia in Korea might be due to a lack of concern for diagnosis. Hepatitis-associated and drug-associated. Aplastic anemia were found in 15 (1.9%) and 10 (1.3%) of acquired aplastic anemia patients, respectively. Total of 1655 individuals with acquired Aplastic anemia younger than 15 years old were recorded in the Japanese Society of Paediatric Haematology database between 1988 and 2011. The rate was 4.79 per million per year. The ratio of severe to non-severe was 56% to 44%. A total of 178 individuals (10.8%) had hepatitis-associated aplastic anemia, whereas the majority of the others were diagnosed with Idiopathic aplastic anemia[57].

Ashwini B R et al. conducted a study retrospectively between January 2011 to June 2012, and prospectively from July 2012 to December 2013 to know the incidence of the Aplastic anaemia and categorize the patients into Aplastic anaemia of varying severity aiding in their management protocol. A total of 554 bone marrow aspirations were done during the study period. Bone marrow aspiration was done in 85 cases to evaluate pancytopenia. Aplastic anaemia was diagnosed in 15 cases and aplastic anaemia was found to be 1.5 times commoner in males than in females. [58]

Megaloblastic anaemia came out as the common cause of pancytopenia of adults(20.83%) in our study. Majority of the studies done nationally and internationally reported megaloblastic anaemia being the major cause for pancytopenia among adults and children. Lavigne et al. in Djibouti (South Africa) conducted a study among 81 adults where the major finding was megaloblastic anaemia (40%) [29]. Nafil et al. conducted a study among 118 adults in which megaloblastic anaemia (32.2%) was the major cause of pancytopenia. [59] Azad et al. among 25 adults found that in 28% of the patients had megaloblastic anaemia. [60] Yokuş et al. in a study among 137 adults came up with megaloblastic anaemia as the major finding for the causation of pancytopenia (17%)[61]. Ramesh Chand et al. conducted a cross sectional prospective study among 42 children where the commonest cause for pancytopenia was megaloblastic anaemia (19.04%). [28] Dr Preeti Negotia et al. conducted a prospective study among 70 cases of pancytopenia where megaloblastic anaemia (41.4%) was most common cause of pancytopenia irrespective of age and sex. [62] A higher percentage of megaloblastic anaemia (68%) was found in a study conducted by V Tilak et al. among 205 adults. Another study was in concordance with our study as 72% megaloblastic anaemia came out as the commonest cause of pancytopenia in India, in the clinico haematological study done by Jitender Mohan Khunger et al among 200 cases. Khodke et al. among 50 adults reported 44% of megaloblastic anaemia as the commonest cause of pancytopenia in India.[39,44]

Aplastic anaemia being one of the major cause of pancytopenia in our study can also be linked to the fact that aplastic anaemia is more prevalent in Asia. Many studies have investigated the cause of Aplastic anaemia in different regions in Asia and discussed its relevant cause. Possible causes included drug and chemical exposure, as well as indigenous viruses. Medical medicines, on the other hand, are a rare cause of Aplastic anaemia in Japan and Korea. A population-based, case-control research in Thailand likewise found that the proportion of patients due to drug exposure was just 5%, leading to the conclusion that drug usage did not explain Thailand's unusually high Aplastic anaemia prevalence. Further more, despite the fact that East Asia is a hepatitis-endemic region, the incidence of hepatitis-associated Aplastic anaemia in Asia is lower than in Europe[48].

In the case of megaloblastic anaemia finding in our study, both folate and, in particular, vitamin B12 deficiency are more common than previously thought, and several factors may be to blame, including decreased B12 absorption in older adults and the fact that depleting this vitamin's liver deposits takes less time than previously thought[47,48,49]. This suggests that B12 deficiency may be the primary cause of megaloblastic anaemia in developing countries, as evidenced by a paper published in 2014 in an Indian population, in which the authors discovered that B12 deficiency was responsible for the majority of cases of macrocytic anaemia.

Furthermore, an excess of folic acid in contrast to B12 appears to set off a cascade of more severe neurological and mental symptoms, thus correct diagnosis is critical.[47,50] Furthermore, B12 insufficiency might be nutritional, since only 3.9 percent of individuals with macrocytosis exhibited parietal cell autoantibodies, according to a research. [51] Despite this, a research showed no link between vitamin levels, either folate or cobalamin, and anaemia severity. This implies that additional variables have a role in its development, emphasising the need for

more research on the subject.

In case of children, After aplastic anemia, acute Leukaemia came out as the major malignant condition presented with pancytopenia (25%). Acute Leukaemia(11.7%) was also reported to be the most frequent malignant disease presenting as Pancytopenia by Anwar Zeb Jan et al. [53] Our findings are also in concordance with a research conducted by Memon et al. that included 230 patients [63] of which 40 cases presented with pancytopenia were malignant conditions. Acute Leukaemia was the most prevalent haematological malignancy presenting as pancytopenia, accounting for 8.69 % of the cases. According to Afzal Khan, the malignant disease is one of the most prevalent causes of pancytopenia, with 11.3% of patients reporting it.

In a retrospective study of 109 paediatric patients with pancytopenia, Bhatnagar et al. discovered that acute leukaemia was the most prevalent etiological cause in 21% of the children [54]. Gupta et al. examined 105 children with pancytopenia, ranging in age from 1.5 to 18 years. Aplastic anaemia (43%) was the most prevalent cause of pancytopenia in their research, followed by acute leukaemia (25 %) [64]. The aetiology of pancytopenia in acute leukaemia is unknown, although it is thought to be connected to a combination of normal haematopoiesis suppression and leukemic cell replacement in the bone marrow, resulting in pancytopenia and immunosuppression. Several genetic disorders, on the other hand, have been linked to an elevated risk of paediatric Acute leukaemia[65].

In the present study, megaloblastic anaemia followed by aplastic anaemia were found to be the most common causes of pancytopenia in adults and paediatric patients respectively. This is an indicator of high prevalence of nutritional deficiency in the adult age group, which should be diagnosed and treated at an early stage in order to reduce the economic disease burden and prevent unnecessary investigations. This requires raising awareness about the correct eating habits and nutritional values of various food items through educational programmes. Proper diagnosis can be made by using detailed clinical history, physical examination and haematological assessment including bone marrow examination.

Table 13 : Comparison Between Current Study And Previous Studies Of Pancytopenia

Author	Year	Place	Study population	No of cases	Duration of study	Most common cause
Present study	2021	India	Pediatric and adults	110	24 months	Aplastic anaemia in pediatric (37.5%). Megaloblastic in adults(20.83 %)
Vargas-Carretro et al	2019	America	Adults	109	48 months	Megaloblastic anaemia(18.3%)
Asbhah shams et al.	2018	India	Pediatric and adults	120	24 months	Aplastic anaemia in pediatric (32.5%) Megaloblastic anaemia in adults (37.5%)
Yokus et al	2016	Turkey	Adults	137	24months	Megaloblastic anaemia (17%)
Rawat et al	2016	India	Pediatric	50	12 months	Megaloblastic anaemia
Atipo-Tsibaetal	2016	Africa	Adults	65	-	Aplastic anaemia (29.2%)
Anjana et al	2016	India	Adult & Pediatric	132	24 months	Megaloblastic anaemia (50.7%)
Azad et al	2015	China	Adults	25	24 months	Megaloblastic anaemia (28%)
Bae et al	2015	South korea	Adults	798	48 months	Myelodysplastic syndrome (72%)

CONCLUSION & SUMMARY

In this study, we found a statistically significant difference in the various hematological indices (Hb, TLC, and Platelet,) between adult and pediatric pancytopenia patients.

Following results were obtained.

- There was male preponderance with male : female ratio 1.93:1.15.
- Majority of adult patients(both males and females) had haemoglobin more than 10g/dl(90.6%) while most of pediatric patients (both males and females)had haemoglobin in the range of 4-6g/dl(36.7%).
- The mean values of TLC and platelet were slightly higher among adult patients of pancytopenia than that of pediatric ones.
- In adults, majority of females had platelet count in the range of 50,000- 99,999 per microlitre (55.6%) and males had platelet count in the range of less than 50,000 per microlitre (50%) while in pediatric patients(both males and females) bulk of the patients lie in the range less than 50,0000 per microlitre (58.6%).
- Symptom wise in adults (both males and females) most of the patients had generalized weakness as presenting complaint while majority of pediatric patients had fever as presenting complaint.
- Most common clinical sign in adults as well as in pediatric patients was pallor.
- On bone marrow aspiration this study showed that Megaloblastic anaemia was the most common cause of pancytopenia in adults and aplastic anaemia in pediatric patients.
- Other causes included refractory anemia, nutritional deficiency anemia and mixed deficiency anemia. Causes such as megaloblastic anaemia, and nutritional deficiency anemia, are reversible. As a large proportion of pancytopenia is of reversible aetiology, early and accurate diagnosis may be life-saving.
- Our study also compared the bone marrow cellularity in both males and females. The majority of male patients were shown to be hypocellular and in females, the majority of cases were normocellular.

Pancytopenia is a common clinical entity that we see in day to day practice. There are various causes of pancytopenia which include both neoplastic and non neoplastic entities. Since most of the cases of pancytopenia are due to nutritional deficiencies they are remediable and reversible hence there diagnosis is of paramount importance. Proper diagnostic evaluation requires detailed clinical history, physical examination and haematological assessment including bone marrow examination. We here by conclude that bone marrow examinations are an important diagnostic tool in haematology which help to evaluate various causes of pancytopenia and to plan further investigation and management of patients. Taking this into consideration, researching the epidemiology of pancytopenia across the world might aid in guiding diagnostic work-up, therefore expediting therapy and improving prognosis.

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