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ORIGINAL ARTICLE

A Sep-wise Approach to Diagnose Pancytopenia: Experience from a Tertiary Care Hospital in AJ&K, Pakistan

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Abstract

Objective: To find out the occurrence of pancytopenia in relation to the age and sex of the patients. To establish a step-wise approach by correlating the clinical, haematological and bone marrow findings to reach a diagnosis, and to evaluate the cause of pancytopenia individually.

Material and Methods: This study was a prospective study over a period of one year conducted at a teaching hospital of Mirpur, AJ&K, with a catchment population of 1.6 million with a wide range of diversity in its terrain ranging from plains to mountains. This was the first ever study conducted on this topic in this region. Basic tests including blood complete picture followed by peripheral film, Reticulocyte percentage, Bone marrow examination including aspiration and trephine biopsy along with other supportive tests were used to reach a diagnosis in each patient.

Results: Out of a total of 119 patients, with age ranging from 2 months to 90 years, the mean age was 59 years with female predominance. Most of the patients presented with generalized weakness and fever. The commonest physical finding was pallor, followed by splenomegaly and hepatomegaly. Anaemia was the predominant finding in blood film examination. Bone marrow aspiration was conclusive in all cases. The commonest marrow finding was hypercellularity with megaloblastic erythropoiesis. The commonest cause of pancytopenia was megaloblastic anaemia (74.04%), followed by aplastic anaemia (18.26%).

Conclusion: The present study concludes that detailed primary haematological investigations along with bone marrow studies in cytopaenic patients are helpful for understanding the disease process and diagnosis to rule out the causes of cytopaenia.

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Introduction

A decrease in all three cell lines (Erythrocytes, Leukocytes and Thrombocytes) below the lower normal limit for that age and gender is known as Pancytopenia[1]. This may happen in many diseases affecting bone marrow which may lead to its hypofunction. Patients may present with symptoms resulting from a decrease in platelets leading to the bleeding manifestation of variable severity and symptoms like pallor, and lethargy because of a decrease in hemoglobin concentration[2]. As stated by Azaad et al., that pancytopenia is not a single disorder but a trio of findings, which may be an outcome of many diseases[3]. The set criteria in order to rule out pancytopenia is reduced hemoglobin below 11.5gm% in females while 13.5gm% in males, TLC below $4x10^9/l$ and Thrombocytes counts below $150x10^9/l$[4]. The presenting symptoms can be due to anemia (pallor, fatigue and

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dyspnoea), leukopenia (recurrent infections) thrombocytopenia leading to bruising and mucosal bleeding[5]. Since the life span of erythrocytes is 120 days which is longer than leukocytes and thrombocytes so anaemia appears late when compared with symptoms of Thrombocytopenia and infections due to reduction in platelets and leucocytes respectively[6]. The typical symptoms of anaemia are tiredness, fatigue, anorexia and lassitude. The patient may exhibit pallor of mild to a severe degrees along with koilonychia[7]. The causes of anaemia include Macrocytic anaemia which may be megaloblastic or non-megaloblastic, aplastic anaemia or anaemia of chronic disorder. It may be a result of hypersplenism due to malaria or Leishmania. The platelets are destroyed and decrease in their number is usually the first to appear. Thrombocytopenia manifests as mucocutaneous bleeding from the nose, gastrointestinal and Genito-urinary tract bleeding. The decrease in platelet count, below 20 × 10^9/l indicates severe marrow failure and may lead to bleeding manifestations[8]. Fever may be an initial presentation indicating Leukopenia. Patients with neutropenia may present with fever, pharyngitis or respiratory tract infections not responding to antibiotic[9,10].

Clinically, a child presenting with pancytopenia should undergo a bone marrow examination to rule out storage disorders or enlargement of the liver, lymph nodes and/or spleen resulting from any malignancy. For evaluation of Pancytopenia bone marrow studies are done. BM studies (BM aspiration and trephine biopsy) are taken as the gold standard for the diagnosis and treatment of most hematological disorders including pancytopenia[4].

For the evaluation of pancytopenia in children, BM is the most commonly advised investigation which is the comparatively safe invasive procedure as a reduced number of platelets and mild risk of bleeding will not be a contra-indication for this test. Bone marrow aspiration is conclusive in many cases however, if aspiration is diluted, or there is a dry tap then Trephine Biopsy is required. Dry tap is obtained as a result of the marrow being fibrotic or very densely cellular as in leukaemia[11]. Trephine biopsy also reveals architecture, the outline of distribution and the presence of any atypical infiltrate in the marrow[12]. It can be used for special stains and immunohistochemistry for further studies, especially in lymphomas[3].

Megaloblastic anaemia (MA), hypersplenism, Storage disorders, aplastic anaemia, tuberculosis, myelodysplastic syndrome (MDS), sub-leukemic leukaemia and multiple myeloma are some of the aetiologies presenting with reduced all three cell lines. The most frequent aetiologies resulting in pancytopenia on BM evaluation are aplastic anaemia/hypoplastic BM (29.05%), MA (23.64%), hematological cancers, i.e., AML (21.62%), and erythroid hyperplasia (19.6%) according to a study. Blasts are found on peripheral blood commonly but sometimes they may be missed if their number is low or there is a decrease in leukocytes[13]. BM cellularity may be either increased or decreased.

In children, acute lymphoblastic leukaemia can be misdiagnosed as AA[14,15]. The occurrence of diseases leading to pancytopenia differs in diverse inhabitants and areas, according to the pattern of distribution of age, nutritional status, environment and the occurrence of infections in that area[16]. Management and prognosis of pancytopenia is complex and should be individual patient oriented[9]. Workup for the evaluation of decrease in all three cell lines should comprise of comprehensive clinical history including medicine and exposure to chemicals[13]. Genetic disturbances and topographical distribution can be the factors which may have some effect. Arrangement and BM cellularity differ in association with aetiology. Supportive management comprises treatment of the anaemia including (B12/ FA) thrombocytopenia (Platelet Concentrates) and infections (antibiotics). Nutritional deficiencies like megaloblastic anaemia causing pan cytopenia are easily treatable and reversible by inducing the required therapy[17,18]. However, the prognosis depends upon the degree of pancytopenia, the age of the patient and aetiology. Hence, identifying the cause of the disease is essential for proper management and to improve long-term outcome[19,20]. The current study was executed to find out the occurrence of pancytopenia in relation to the age and sex of the patients. And to establish a step-wise approach by correlating the clinical, hematological and bone marrow findings to reach a diagnosis, and to evaluate the cause of pancytopenia individually.

Material and Method

The study was prospective and conducted over a period of 12 months from July 2020 to June 2021 at the Divisional Headquarters Teaching Hospital, Mirpur, Azad Jammu & Kashmir.

Both males and females of all age groups were included in the study. The participants were selected on the basis of their clinical presentation and laboratory findings which included Pancytopenia/bicytopaenia and the third parameter trending towards a lower range on blood complete picture report by automated hematology analyser. Patients with already diagnosed malignancy, CLD, and bleeding disorders were excluded from the study.

A total of 119 patients were included who exhibit pancytopenia on their blood complete picture. Their detailed clinical history regarding the nature and duration of ailments, weight loss, significant family history and drug history, if any, was taken. A history of any chronic disease like tuberculosis or diabetes was also noted. History of fatigue, fever, chills and rigours, dyspnoea, failure to thrive/ anorexia, myalgia, bone or joint pains, and active bleeding from any site is taken. Clinical features like pallor, bleeding in the form of bruising or petechiae, organomegaly and lymphadenopathy were noted.

Blood samples were taken in EDTA containing Vacutainer CP vials following the instructions. These samples were run on ABACUS 380 Automated Hematology Analyzer. The parameters noted included:

- TLC, LYM%, MID%, GRA%
- TRBC, Hb%, PCV & RDW CV/SD
- Mean Corpuscular Volume, Mean Corpuscular Hb and Mean Corpuscular Hb Concentration
- Platelets, PCT, MPV, PDW CV/SD

Peripheral smears examination was performed on field-stained slides according to procedures mentioned by Waheed et al (2022). Reticulocyte percentage was also noted. Written consent was taken from all the participants before the bone marrow examination. The technique used for BM aspiration and trephine biopsy and the techniques of preparation, staining of slides and marrow sections used were followed according to Lewis. In adult patients, the site used for BM aspiration and trephine biopsy was the Posterior Superior Iliac spine (PSIS) while in children below 2 years Ant. The tibia was selected. The specimen was then fixed overnight in 10 per cent buffered saline and then after decalcification, it was processed in an automatic tissue processor followed by embedding and staining.

All the smears made by aspirates were stained by May-Grunwald Giemsa (MGG) stain while trephine biopsy sections were stained by routine Haematoxylin & Eosin (H&E). The findings of Aspirate were studied for cellularity, Type of maturation of all the three cell lines i.e., erythroid, myeloid, and megakaryocytes, presence of any parasite, abnormal cells, any increase in plasma cells, lymphocytes and histiocytes noted. The bone marrow aspiration slides containing adequate fragments were stained for iron stain and their iron status was noted. The smears with the presence of any atypical cells/blasts were then subjected to special stains like SBB (Sudan Black B), and PAS (Periodic Acid Schiff). Trephine biopsies sections were studied for cellularity, distribution of all cell lines and any infiltration by leukemia, lymphoma cells, granulomas or any metastasis/secondaries. Both biopsy and aspirate were compared to each other for establishing a final diagnosis and to evaluate the cause of pancytopenia. Additional tests were done if required, e.g., LFTs, RFTs, urine for Bence Jones proteins, serum calcium, and B12/ FA levels, among others.

All the data were analyzed in statistical software (SPSS-16). All the variables like age, gender, presenting complaints, Peripheral film and findings of BM and ultimate underlying cause of pancytopenia were entered. Cross-tabulation was done for different variables to find out the mean, median, and SD, among others. Frequency and percentage were computed as well. The steps followed to evaluate pancytopenia are shown in figure 1.

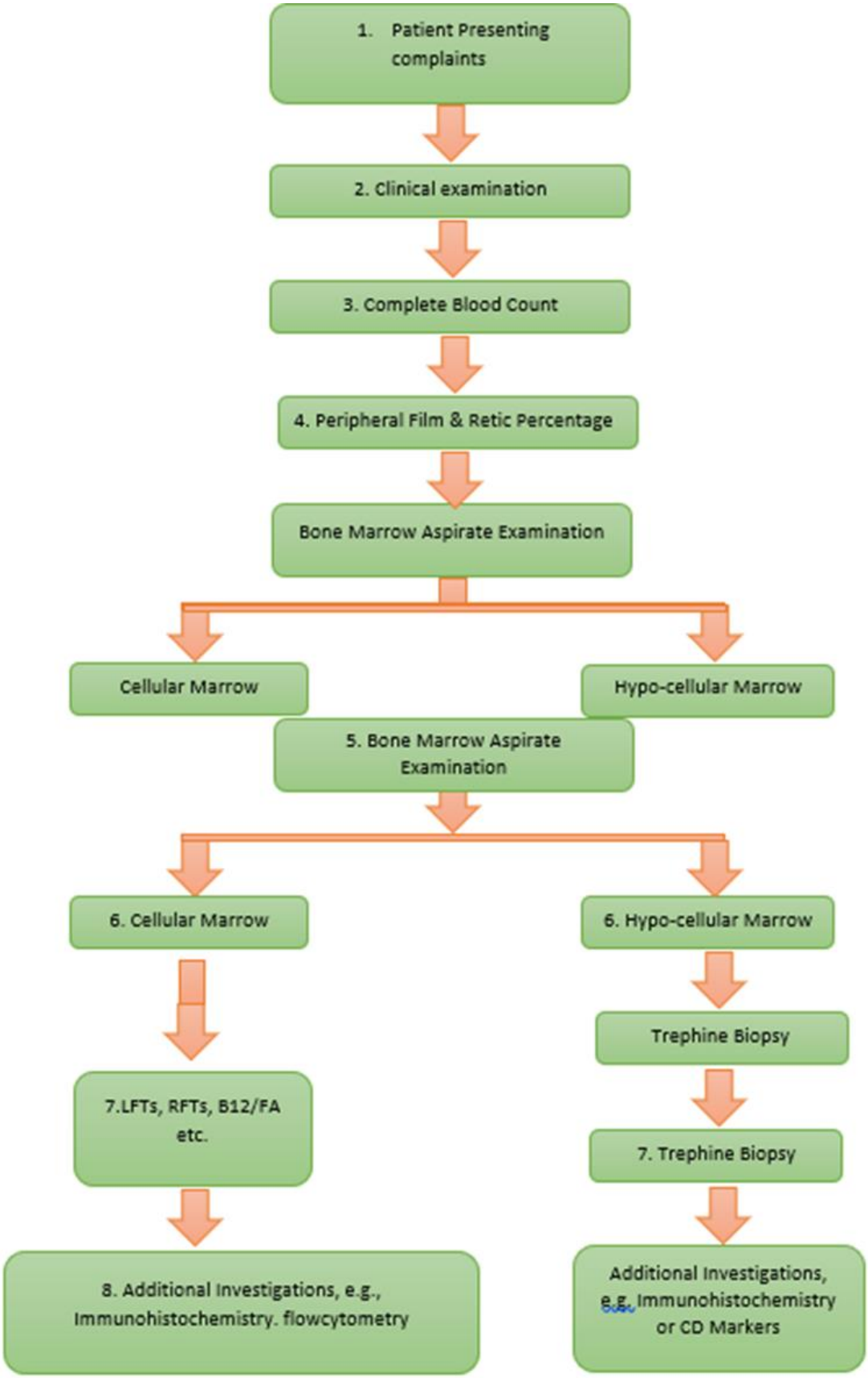

Figure 1: Steps to evaluate pancytopenia

Results

Out of a total of 119 patients, age ranging from 2 months to 90 years, the mean age was 59 years. There were 57male (47.89%) and 62 females (52.10%). The incidence of pancytopenia showed a slight preponderance in females than males. Most of the patients presented with generalized weakness and fever. The commonest physical finding was pallor, followed by splenomegaly and hepatomegaly. Anaemia was the predominant finding of the blood picture. Bone marrow aspiration was conclusive in all cases. The commonest marrow finding was hypercellularity with megaloblastic erythropoiesis. The commonest cause of pancytopenia was megaloblastic anemia (74.04%), followed by aplastic anemia (18.26%).

The majority 17 (14%) had infection related changes, 16 case (13%) had megaloblastic anaemia responsible for pancytopenia. Hypoplastic bone marrow were observed in 13 cases (13%) and Acute Myeloid Leukaemia in 12(10%). Another less common cause of pancytopenia was lymphoproliferative Disorder (8%). Reactive bone marrow was observed in 08 patients (7%). Acute Lymphoblastic Leukaemia, Lipid Storage Disorder and ITP, pure red cell Aplasia and Hyper splenism (3%). Myelofibrosis and MDS (2%). Multiple Myeloma, Hemophagocytic Syndrome, secondaries in the bone marrow and plasma cell dyscrasia all are (1%) (Fig. 2).

In our study, the four most common causes of Pancytopenia were compared in relation to haematological parameters. It was found that decreased level of Hb was observed predominantly in Megaloblastic anaemia followed by AML, Infection Related Changes and Hypocellular BM. A decrease in leukocyte count was predominantly observed in Hypocellular BM cases followed by Megaloblastic anaemia, Infection Related Changes and AML. Thrombocytopenia was significantly seen in AML, Hypocellular BM, Megaloblastic Anaemia and Infection Related Changes respectively (Fig. 3). Hypocellular bone marrow (BM) was found more frequently in AML followed by Infection-related changes, megaloblastic anaemia and hypocellular BM. Moderately cellular marrow was observed predominantly in hypocellular BM, megaloblastic anaemia, Infection-related changes and AML respectively (Fig. 4)

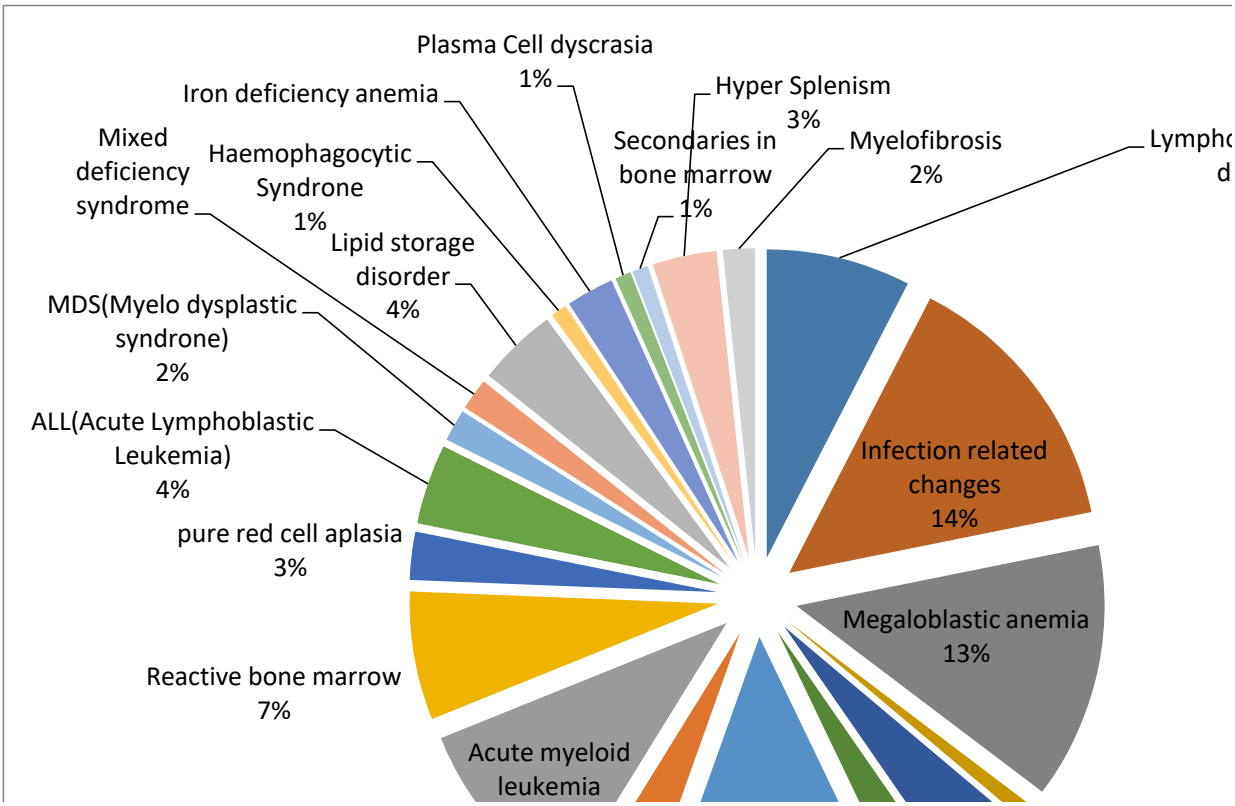

Figure 2: Causes of pancytopenia

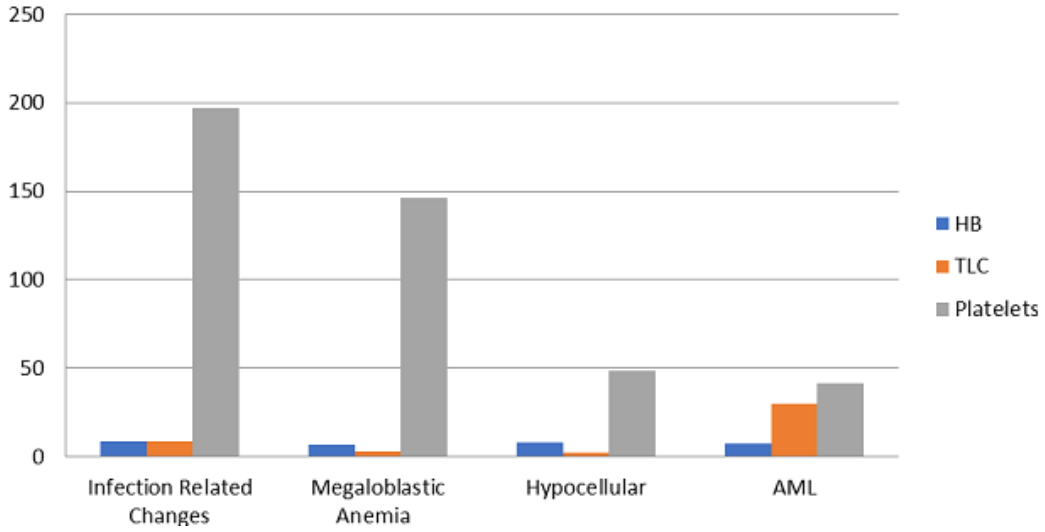

Figure 3. Comparison of hematological parameters among four leading causes of pancytopenia

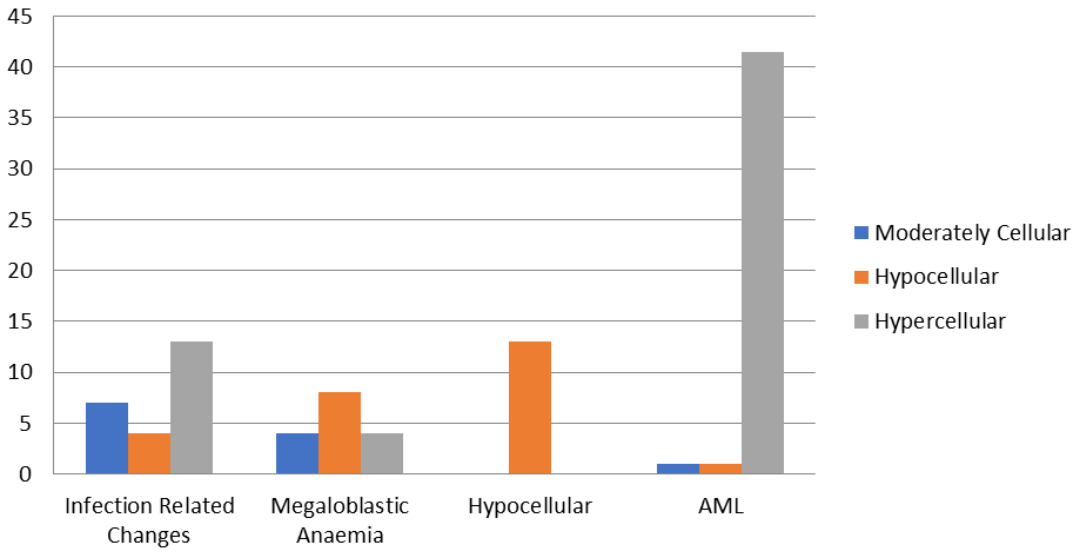

Figure 4. Comparison of bone marrow cellularity among four leading causes of pancytopenia

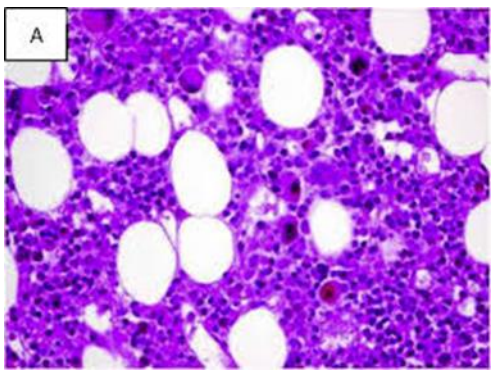

Figure 5: Myelodysplastic Syndrome

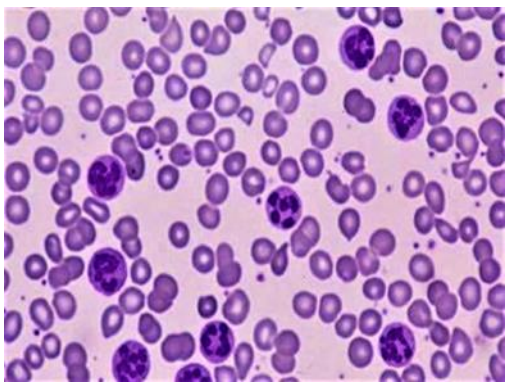

Figure 6: Megaloblastic Anemia

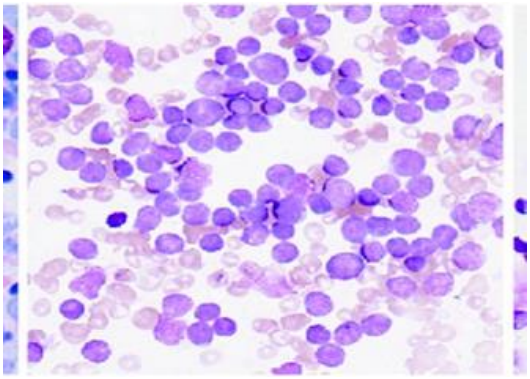

Figure 7: Acute lymphocytic leukemia

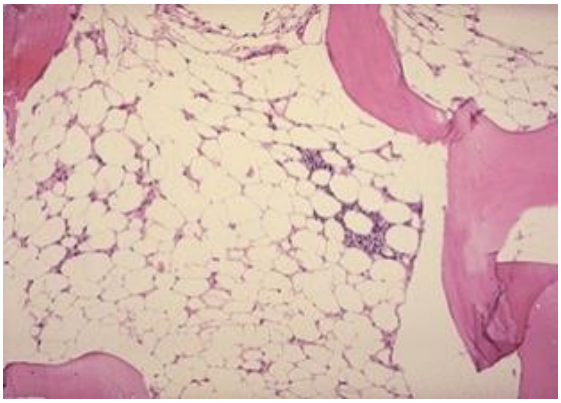

Figure 8: Lipid Storage Disease

Discussion

In the current study, 119 patients with pancytopenia were included. Age and gender-wise frequency, presenting complaints, complete blood count, BM aspiration smears and trephine sections were considered in all patients, and comparisons were made with those available in the literature. It is thought that various local, cultural, socioeconomic factors including dietary parameters play a significant role in the pathogenesis of pancytopenia. The age of the participants ranges from 6 months to 90 years, with 45years of age as the mean age. The supreme number of participants were belonging to the age group of 6 months to 10 years (17.9%). Gayathri et al., [21] reported mean age of 45 years presenting with cytopenia while in a study by Sharma et al., [8] the supreme occurrence of pancytopenia was seen in the 31-40 years (22%) age group, followed by 11-20 years (17%). Likewise, the supreme number of pancytopenia was observed in the age group of 12-30 years by Khodke et al.[5] However, Nazi and Razi[10] in their research found that the common age group of pancytopenia was 21 – 30 years of age group.

In our study, outstanding female predominance (female to male) was observed. Sharma et al.,[8] reported male to female ratio as 1.64:1 which is approximately the same as in the study by Jha et al.,[11] while the study of Khodke et al.,[5] found male to female ratio of 2:1. Gayathri et al .,[21] reported pancytopenia more in males (54.81%) than females (45.19%), with male-to-female (M: F) ratio of 1.2: 1.

In our study, the commonest clinical sign was pallor seen in all the cases. Weakness and fatigue were the most common symptom seen in 80.5% of cases. Other clinical presentations were fever, weight loss, bleeding manifestation, dyspnoea and lymphadenopathy. In the study by Sharma et al., [8] most patients presented with generalized weakness, fever and bony tenderness. Most common presenting complaint reported by Gayathri et al., [21]was generalized weakness (100%), followed by dyspnoea (43.26%). The most common physical finding was pallor (100%), followed by splenomegaly (35.57%) and hepatomegaly (26.92%).

In the present study, amongst 39 cases, 17.9% were diagnosed as megaloblastic anemia making it the most common cause of pancytopenia. Infectious related changes (17.9%) were second most evident etiology. Hyper plastic anemia (15.3%), Hypo plastic anemia (10.2%), aplastic anemia (7.6%), acute myeloid leukemia (7.6%), lympho proliferative disorder (5.12%), mixed deficiency anemia (5.12%), lipid storage disease (5.12%), acute lymphoid leukemia (2.56%), hypocellular bone marrow (2.56%), myelo dysplastic syndrome (2.56%), Gaucher disease (2.56%).

Data have been reported from different provinces of Pakistan pertaining to our study.[22] In almost all those studies, pancytopenia was the main presentation and so was the case in this study. Among the mixed deficiencies anemia (microcytic and macrocytic) was seen in two cases and aplastic anemia was seen in five cases only. Sharma et al.,[8] reported megaloblastic anemia (60%), aplastic anemia (16%), subleukemic ALL (11%), subleukemic AML (4%), Kala-azar (3%), multiple myeloma (2%), MDS (1%) and metastasis (1%). Similarly Tilak and Jain,[12] Khodke et al[5] and Khunger et al.,[13] who in their studies found megaloblastic anemia in 68%, 44% and 72% respectively as the most common cause of pancytopenia. Megaloblastic anemia was the common cause for pancytopenia, followed by aplastic anemia by Gayathri et al.,[21] as well. Similar results were reported by Bhatnagar et al.,[8] Aplastic anemia was the most common cause of pancytopenia (43%), followed by acute leukemia (25%). Infections were the third most common cause of pancytopenia, of which kala-azar was the most common.

Megaloblastic anemia was seen in 6.7% of the patients [9]. 3 cases of AML-M2 and 1 case of ALL-L2 were reported by Gayathri et al., [8] while Khodke K et al., [5] reported a single case of AML-M2 out of 50 cases of pancytopenia. Kumar R et al., [23] reported 5 cases of ALL, 13 cases of AML, 2 cases of hairy cell leukaemia out of 166 cases of pancytopenia, over a 6-year study period[5]

In our study one child having Gaucher disease presented with pancytopenia similarly Sharma et al., [8] reported a single case of storage disorder (Niemann-pick disease), in a 15-year-old boy, who presented with hepatomegaly, splenomegaly and pancytopenia.

Conclusion

The present study concluded that detailed primary hematological investigations along with bone marrow studies in cytopenic patients are helpful for understanding the disease processes and diagnosing or ruling out the causes of cytopenia. These are also helpful in planning further investigations and management. The liaison between hematologist and treating physician may improve the overall management of these patients. Following the stepwise approach will help clinicians at secondary health care facilities to reach a diagnosis in a systematic way which in turn will lead to a decrease in the workload/burden at tertiary care settings. This will in turn save time, avoid unnecessary investigations and reduce the morbidity in the patients.

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