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Original Research Article

Clinical Profile of Pancytopenia in Adults and Its Response to Therapy

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ABSTRACT

Background and objectives: To assess the clinical profile of Pancytopenia in adults and its response to therapy in KIMS, Karad. **Method:** 50 patients were taken in the study to assess the clinical profile of Pancytopenia in adults and its response to therapy. **Results:** Out of 50 patients studied most common cause was Megaloblastic Anaemia followed by Hypoplastic/Aplastic anaemia. **Conclusion:** Megaloblastic Anaemia due to Vit B12 and or Folate deficiency seems to reflect the higher prevalence of pancytopenia in Indian subjects. Other important causes of pancytopenia like myelofibrosis, leukemia, malaria should be kept in mind while planning investigations for the complete work up of pancytopenic patients. Early treatment can be planned depending upon the cause and severity of pancytopenia.

Key words: Pancytopenia, Vitamin B12 , Megaloblastic anaemia, myelofibrosis.

INTRODUCTION

Pancytopenia was not a discrete hematological entity even as late as 1919. The term was used almost synonymously for aplastic anaemia, it being the major cause of pancytopenia in the western countries. [1,2]

Pancytopenia is reduction in all three major classifications of formed elements of the blood erythrocytes, leucocytes and platelets. Pancytopenia, therefore exists in the adult when the hemoglobin (Hb) level is less than 13 gm/dl in males or 11 gm/dl in females; the leucocytes count is less than 4 x 10^9 /L and platelet count is less than 150x 10^9 . [3]

It is not a disease entity, but a triad of findings that may result through different mechanism like destruction of marrow tissue by toxins, radiation (aplastic or hypoplastic marrow), replacement by abnormal or malignant tissue like Hodgkin's and Non Hodgkins lymphoma, multiple myeloma, myelofibrosis or suppression of normal marrow growth and differentiation like megaloblastic anemia, systemic lupus errythematosus. [1]

It has been observed in Indian scenario that megaloblastosis- Vit B 12 and or folate deficiency is the commonest cause of pancytopenia [4,5,6] Other important conditions manifesting as pancytopenia are aplastic/hypoplastic anaemia, paroxysmal nocturnal hemoglobinuria (PNH), overwhelming infection, hypersplenism, etc.[1] However, the frequency with which each condition is associated with

pancytopenia differs considerably depending upon various factors including geographic distribution.[6]

Complete blood cell count and peripheral smear examination considered in the light of detailed history and proper physical examination are first steps towards diagnosing and analyzing pancytopenia, as it often gives an indication of the underlying pathophysiology process, the differential diagnosis and the need for further investigations.[6]

Bone marrow aspiration and core biopsy are useful and complementary to each other. Cellular details are better appreciated in an aspirate, which may not be possible in trephine biopsy. The marrow trephine biopsy is most useful in determining overall cellularity and the anatomic relation of cells to fat and connective tissue stroma. It is useful in evaluating diseases those characteristically produce focal rather than diffuse involvement of marrow e.g. metastatic tumour. [7,8]

A look at both, western and Indian literature shows that there are few comprehensive studies on pancytopenia. In India, the causes of pancytopenia are not well defined. This data, if available, would help in planning the diagnostic and therapeutic approach in patients with pancytopenia. [9]

Thus the study was undertaken to diagnose the cases of pancytopenia and to ascertain the cause for it, which would help initiating early and effective treatment.

MATERIALS AND METHODS

This prospective study was conducted at Krishna Institute of Medical Sciences, Karad, India

Study Design: Hospital based cross sectional study.

Study Setting: The study included 50 patients after proper consent being taken from them.

Study Period: October 2011- May 2013

Sampling Method and Sample Size: A total of 50 patients after meeting the inclusion criteria were included in the study.

Inclusion Criteria:

Anemia - Males< 13 gm %(as defined by WHO)

Females :< 11 gm%

Platelet count: < 150 x 10^9/L. (as defined by De gruchy)

Leucopenia :< 4.0 x 10^9/L or ANC: < 1000 / cumm (as defined by De gruchy).

Investigations:- Clinical profile will govern the investigation for a particular case.

1. Hemogram Including
   Hb
   TLC.
   DLC.
   Platelet count
2. Reticulocyte Count
3. Packed cell volume
4. ESR
5. Blood cell indices (MCV/MCH/ MCHC)
6. Bone marrow aspiration / Biopsy
7. Wherever indicated
   Vit B 12 levels

Methodology

Following investigation were done:

Complete Haemogram:

3 ml of blood was collected by venipuncture under aseptic precaution in a dry bulb containing Ethylene di amine tetra acetic acid (EDTA) anticoagulant. The sample was then put in Automated Haematology analyzer (Lab life Noble 3) and all Haemogram parameters, packed cell volume, red cell indices were noted (except Reticulocyte count).

Reticulocyte count was assessed manually under oil immersion using Brilliant cresyl blue (Biolab, for counterstain and Buffer).

Erythrocyte sedimentation rate: Westergren method

Peripheral smear was stained by leishman stain for all the cases and examined in detail.

Erythrocyte sedimentation rate:

Westergren method:

Using standard Westergren method tube, 3.8% Sodium citrate anticoagulated blood was drawn upto mark 0.The tube was then set upright in a stand in which a spring clip, pressing on the top, holds the point firmly against a piece of rubber at the lower end. The reading was noted at the end of 1 hr.

Normal value – Male -0-15 mm/hr

Female – 0-20 mm/hr

Packed cell volume:

Normal Value – Male- 41.5- 50.4 %

Female- 36- 45%

Total white cell count:

Normal value – 4000-11000 cells / mm^3

Total red cell count:

Normal value – 4.5 – 5.5 million/ cu mm

Platelet count:

Normal value: 1,50,000- 4,50,000 /cu mm

Reticulocyte count:

A small amount of new methylene blue stain was filtered in to a test tube. Two drops of the filtrate and 2 drops of well mixed blood specimen were transferred into a small test tube with the help of two separate Pastuer pipettes. New Methylene blue stain and the blood specimen were mixed and the test tube was covered with a cork or aluminium foil to prevent evaporation. The test tube was left undisturbed for 15 min in the incubators at 37 degree C. After 15 min the contents of the tube were removed and one small drop of the mixture was transferred to a clean grease free slide. A thin smear was prepared with the help of a spreader slide. Smear was air dried. The smear was first examined under the low power objective for scanning and a thin portion of the smear where the red cells were evenly distributed was located. Reticulocytes were identified by the fine, deep violet filaments arranged in a network and fine dot like structure.

A smaller circular piece of black paper in which a hole of 5 mm diameter has been made with a puncher was placed in the eye piece .10 consecutive fields were counted and the % was calculated using the formula

Reticulocyte count (%) = no of reticulocytes counted x 100/ no of red cell counted.

N value – 0.5 -2.5%

Red cell indices:

MCV(FL) = haematocrit (%) x 10 / RBC count in millions

Normal value: 79 -93 FL

MCH (pg) = Hb (g/dl) x 10 / RBC Counts in millions

Normal value: 26.7 – 31.9pg.

MCHC (%) = Hb (g/dl) x 100/ haematocrit (%)

Normal value: 32.3- 35.9g %.

Bone marrow aspiration:

Bone marrow aspiration was performed in all the patients using Salah needle after obtaining written consent for the procedure either from the patient or the guardian.

The aspiration site was prepared, cleaned with an antiseptic (spirit and betadine), scrubbed and draped exposing only the aspiration site. Skin and the area down to the periosteum was infiltrated with 2 ml of local anaesthetic (2% lignocaine) using 5 ml syringe.

The needle with the stylet in place was introduced into the site (preferably sternum) by gentle screwing motion, after adjusting the guard to appropriate length. The outer plate of bone was pierced with a gentle boring motion. As the marrow cavity was entered, sensation of giving in was experienced. Then the stylet was removed and 0.2 ml of marrow material aspirated with the help of 10 ml disposable syringe.

The aspirate was transferred to a set of slides and films were prepared by

crushing the marrow particles. The needle was withdrawn and the puncture site was sealed with tincture benzoin swab.

Slides were fixed in methanol for 15 min, dried and later stained with Giemsa/Leishman stain and marrow aspiration smears were examined for

1. Cellularity
2. Erythropoesis
3. Myelopoesis
4. Megakaryopoesis
5. Others – plasma cells, Lymphocytes, mast cells
6. Parasites
7. Abnormal cells

Special stains were done when required.
KIMS Lab is under external quality control of Biolab

Statistical Analysis

Statistical analysis was done using Z test, Student unpaired t test, mean standard deviation in which siginificance was found by p value<0.01 (highly significant).

RESULTS

Most common age of presentation was in 2-3rd decade with overall ratio of 1:1 in male to female. (Table1)

48% presented with generalised weakness followed by breathlessness & fever in 14% cases. (Table2)

Pallor was present in more than half of the cases followed by knuckle pigmentation in 26 % cases. (Table3)

Anisopoikilocytosis was present in 56% cases followed by normocytic normochromic in 36% cases. (Table4)

36% Cases were hypoplastic on aspiration followed by megaloblastic in 34 % cases. (Table5)

58% cases were treated with iron +Vit.B12+ Folate supplements. 20% cases required blood transfusion. (Table6)

After applying Z test of difference between two sample means there is a highly significant difference between mean values of Hb, TLC, Platelet, and PCV before and after discharge (p<0.01). All statistics done with the help of Instat software. (Table 7)

Megaloblastic anaemia was the diagnosis in 42 % cases followed by Hypoplastic anaemia in 32% cases (Table 8)

Table1: Age & Sex wise Distribution.

Age in years	Male	Female	Total
< 20	3(12%)	4(16%)	7(14%)
20-30	8(32%)	5(20%)	13(26%)
30-40	2(8%)	2(8%)	4(8%)
40-50	2(8%)	5(20%)	7(14%)
50-60	3(12%)	4(16%)	7(14%)
60-70	4(16%)	4(16%)	8(16%)
>70	3(12%)	1(4%)	4(8%)
Total	25(50%)	25(50%)	50(100%)
Mean ± SD	42.20±11.02	41.40±12.48	43.60±20.31

Table2: Clinical Features

:Clinical Features	No.	Percentage (%)
General weakness	24	48%
Breathlessness	7	14%
Fever	7	14%
Giddiness	1	2%
Oedema	2	4%
Others	9	18%
Total	50	100%

Table3: Clinical Findings

Clinical findings	No.	Percentage (%)
Pallor	25	50%
Pallor,icterus	5	10%
Pallor, knuckle pigmentation	13	26%
Pallor, hepatomegaly	4	8%
Others	3	6%
TOTAL	50	100%

Table 4: RBC morphology

Morphology	No.	Percentage (%)
Anisopoikilocytosis, micro +,macro+	28	56%
Normocytic normochromic	18	36%
Anisopoikilocytosis, Tear drop cells	1	2%
Others	3	6%
Total	50	100%

Table 5: Bone Marrow Aspiration.

Bone Marrow aspiration	No.	Percentage (%)
Acute myeloid leukemia with Auer rods	1	2%
Acute Lymphoid leukemia	1	2%
Hypoplastic anaemia(Bm biopsy)	18	36%
Megaloblastic anaemia	17	34%
Not willing	6	12%
Not done	3	6%
Others	4	8%
Total	50	100%

Table 6: Treatment Modalities.

Treatment	No.	Percentage (%)
Iron supplements +B12+folate	29	58%
Antimalarials+ iron supplements	1	2%
PCV transfusion+iron supplements	10	20%
Haematologist referral for mgmt +iron supplements	3	6%
Stoppage (AZT+MTX)+Iron supplements/B12	5	10%
Others	2	4%
Total	50	100%

Table 8: Diagnosis.

Diagnosis	No.	Percentage (%)
Hypoplastic Anaemia	16	32%
Megaloblastic Anaemia	21	42%
Acute Lymphoid leukemia	1	2%
Acute myeloid leukemia	1	2%
AZT Toxicity	1	2%
Complicated Falciparum malaria	1	2%
Hodgkins lymphoma	1	2%
Hyperspleenism	2	4%
MTX Toxicity	4	8%
Myelofibrosis	1	2%
Plasma cell dyscrasia	1	2%
Total	50	100%

Table 7: Comparison of parameters before admission & after Discharge.

	Before discharge	After discharge	Z test value	'p' value and result
	Mean ± SD	Mean ± SD		
Hb %	5.69±2.31	7.77±1.53	5.33	p<0.01, highly significant
TLC	2662±1384.7	9410±2762.4	15.44	p<0.01, highly significant
Platelet	59755.35±88937	64653.92±89515	3.89	p<0.01, highly significant
PCV	29.65±6.90	36.63±7.07	4.77	p<0.01, highly significant

DISCUSSION

Bone Marrow Examination:

The most important in our study was bone marrow examination either for confirming the diagnosis or for excluding a primary marrow involvement and suggesting alternate etiology. We performed bone marrow aspiration and biopsy if required in patients. In the remaining 12 % of Patients cause of pancytopenia was obvious [Falciparum Malaria, Chemotherapy (dose related)] so bone marrow examination was avoided. Both aspiration and or biopsy were done simultaneously from the same punctured site but from different plane.

Bone Marrow aspirate specimen was superior for morphological details over biopsy while biopsy specimen provided a more reliable index of cellularity.

Biopsy was successful in obtaining a sample and was often diagnostic in conditions where aspiration was inconclusive.

Bone marrow aspiration was not very helpful in those with aplastic anaemia even if fragments were aspirated. In these cases biopsy was needed in determining the overall cellularity and helped us in reaching upto diagnosis. However in megaloblastic anaemia biopsy did not provide any additional information compared to aspiration. So if megaloblastic anaemia was suspected clinically or on peripheral smear or blood indices alone marrow aspiration was done.

In the present study the commonest cause of pancytopenia was megaloblastic anaemia. Out of 50 cases of pancytopenia, 21 cases (42%) were of megaloblastic anaemia. Pancytopenia in megaloblastic anaemia from various series- 68%,44%,47%,25.4%,64% and 39% reported by Tilak V. et al, Sarode et al, Mikibi et al, kale et al, Ng SC et al and Sen et al respectively.[6,10-14]

As facilities for estimating serum B12 was not available it was done from laboratory outside in relevant patients in whom there was suspicion after clinically, haematological and Bone marrow aspiration. Megaloblastic anemia due to Vit B 12 and or Folate and iron deficiency is particularly nutritional in Indian subjects.[6]

Pancytopenia due to megaloblastosis is albeit, transient and easily reversible with treatment. Thus megaloblastic anaemia should always be considered in the evaluation of pancytopenia in Indian setting.

In the present study, second commonest cause of pancytopenia was Hypoplastic/aplastic anemia. Out of 50 cases, 16 cases (32%) were hypoplastic/aplastic anemia group. Out of 50 cases five cases (10%) had toxicity of Chemotherapy drugs (Methotrexate, Azathioprine) as a cause of pancytopenia. In such cases bone marrow was not done as cause was obvious and it was dose related. Pancytopenia due to drug toxicity are of 2 types a) Regular side effect- dose related b) idiosyncratic. In our case it was dose dependent as it got reversed after stoppage of culprit drugs (Methotrexate, Azathioprine) so drug toxicity was kept as a separate entity and not included in Hypoplastic Anemia / Aplastic Anemia.

International agranulocytosis and aplastic anaemia study group, Imbert M et al and Keisu M et al has reported the incidence of aplastic anaemia varying from 10% to 52.7% of all pancytopenic patients.[15] Thus the commonest cause of pancytopenia reported from various parts of world has been aplastic anaemia. This is in sharp contrast with the results of the present study where the commonest cause of pancytopenia was megaloblastic anaemia. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects. Similar are the observations of Tilak V. et al, who has reported 68% cases of megaloblastic anaemia in contrast to 7.7% cases of aplastic anaemia in a study of 77 pancytopenia cases.[6] Also Khodke K et al has reported 44% cases of megaloblastic anaemia against 14% cases of aplastic anaemia in a study of 50 cases of pancytopenia.[4]

Out of 50 cases of pancytopenia 16 cases belonged to Hypoplastic/aplastic anaemia group. We couldn't find an etiological factor responsible for hypoplasia/aplasia being labeled as "Idiopathic". Sen R et al and Tilak V et.al have reported 6% and 7.7% of hypoplastic/ aplastic anaemia in their studies of 191 and 77 cases of pancytopenia. Kumar R et al found 49 cases of aplastic anaemia in a study of 166 patients no etiological factor could be implicated in 36 (73.5%) cases which were labelled 'idiopathic'.[9]

In patients of aplastic anaemia it is difficult to find out the etiology as it may represent a common end result of different toxic mechanism. Also it is difficult to determine whether a particular agent is a cause because of low incidence of aplasia after exposure, inaccurate estimates of the exposed population and simultaneous exposure to other agents. Many of the patients have poor knowledge about the drugs they have been taking, giving an improper history. Investigation of the etiology is further obfuscated by the delay of upto 6 months between exposure to toxin and the development of pancytopenia. Thus in 50-70% cases it is not possible to identify any likely cause which are then labeled as "idiopathic".[3] Our findings were comparable with the available literature.

Out of 50 cases five cases (10%) had toxicity of Chemotherapy drugs (Methotrexate, Azathioprine) as a cause of pancytopenia . In such cases bone marrow aspiration was not done as cause was obvious and it was dose related.

Third common cause in the present study was Leukemia. Out of 50 cases of pancytopenia 2 cases (4%) were leukemia. Out of the 2 cases one was Acute Myeloid leukemia and Acute Lymphoid leukemia. Kumar R et al, Kale P. et al and Niazi M et al have reported 12%, 14.5% and 13.6% cases of acute leukemia as a cause for pancytopenia in 166, 70 and 89 cases studied respectively.[9,12]

Out of 50 cases 2 cases (4%) had hypersplenism, Kumar R et al in the study of 166 patients found that 11.4% patients of pancytopenia were due to hypersplenism . Moderate splenomegaly was present in both

of them. There was no obvious cause for pancytopenia except Hypersplenism and bone marrow was normocellular.

In our study there was one case of pancytopenia due to myelofibrosis. Patient was 45 years old and presented with weakness. Peripheral smear showed the characteristic tear drop cells. Aspiration was dry tap. But bone marrow biopsy revealed increased reticulin fibrosis. Tilak V.et al and Kumar et.al have reported one and two cases of myelofibrosis in their study of 77 and 166 cases of pancytopenia respectively.[6,9]

Tilak V.et al, Kumar R.et al have also reported three and five cases of malaria in their respective studies. [6,9] However we had a single case of Falciparum malaria in the present study. The patient was put on antimalarial treatment immediately after diagnosis. Further marrow studies were not done as the cause of pancytopenia was obvious.

During Hospital stay Patients with Megaloblastic anaemia, Falciparum Malaria, Chemotherapy toxicity (dose related) showed complete clinical as well as haematological improvement as compared to others.

Thus it is suggested that in a patient of anaemia or prolonged illness, appearance of pyrexia and or bleeding manifestations should put the physician on high alert, as there are very high chances of the patient landing up into pancytopenia in which case further investigations should be carried out to look for the possible cause.

Since the severity of pancytopenia and the underlying pathology determines the management and prognosis of these patients, treatment should commence at the earliest possible to prevent the unacceptable serious complications of pancytopenia.

If all the elements of the complete blood cell count are considered in clinical context include findings of physical examination, they can provide invaluable information guiding to the possible causes for pancytopenia and helping in planning the work up of tests needed for definite diagnosis.

CONCLUSION

In this prospective study which was undertaken in KIMS Institute during a study period of May 2011-October 2013 a total of 50 cases of pancytopenia by applying definitive inclusion criteria were studied. The causes of pancytopenia were ascertained and the data was analyzed on the basis of etiology, clinical and hematological findings. Statistical analysis was done using Student Unpaired t Test and Mean standard deviations. Following results were obtained.

The commonest age group for presentation of pancytopenia was in 2-3rd decade with a male to female overall ratio being 1:1. Mean age of male and female were 42.20 and 41.40 yrs respectively. Hence we conclude that males and females were age and sex matched.

In our study we found that pallor (50%) and generalized weakness (48%) are the most common sign and symptom present in patients with pancytopenia.

We also found that Anisopoikilocytosis (56%) and Normocytic normochromic (36%) picture are commonly seen in patients with pancytopenia.

In our study, Megaloblastic Anemia (42%) due to Vit B12 and or Folate deficiency seems to reflect the higher prevalence of pancytopenia in Indian subjects putting hypoplastic/aplastic anemia (36%) in second position, which is the leading cause for pancytopenia in western countries.

In our study total 58% patients received iron supplements, Vit B12 and Folate and 20% patients received Blood transfusions which lead to clinical and hematological improvement in patients with megaloblastic anemia.

Bone marrow examination has a definite role in ascertaining the cause for pancytopenia. However in some cases where the cause for pancytopenia was obvious like Chemotherapy toxicity (dose related), Bone marrow examination can be avoided reducing the discomfort and the cost for patient care and risk of complications.

Pancytopenia due to megaloblastic anaemia is albeit, transient and easily reversible with appropriate treatment. Thus megaloblastic anaemia should always be considered in the evaluation of pancytopenia in Indian settings.

In our study, majority of the cases (megaloblastic anemia, complicated Falciparum malaria and dose related chemotherapy toxicity) had a treatable cause and so carried better prognosis.

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