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STUDY OF APLASTIC ANEMIA AT PAEDIATRIC REFERRAL CENTRE- A 24 MONTHS PROSPECTIVE STUDY

Pathology

KEYWORDS: Aplastic anemia, pancytopenia, pediatric age, bone marrow .

DR.SSS QUADRI	Associate professor of Pathology,Gandhi medical college
DR. Shyamala Srujana	Assistant professor of Pathology Gandhi medical college
DR.C.R.H.T. Pavani	Professor of Pathology,Niloufer Hospital (Rtd)

ABSTRACT

Aplastic Anemia is a serious blood disorder characterized by marked reduction in blood counts. Depending on the aetio pathogenesis and degree of severity of AA, the case management and prognosis vary widely. Bone marrow in AA patients also shows variable degree of cellularity and relative increase in fat spaces. Aetiology of AA is multifactorial. It may be drug induced, effect of environmental factors or genetic factors. Pathogenesis of aplastic anemia is widely varied ranging from autoimmunity to immune suppression. The therapeutic modalities of aplastic anemia are equally variable from supportive therapy,splenectomy, androgen and steroid therapy, immune-supression to the ultimate bone marrow transplantation,based on aetiopathogenesis. **AIMS OF THE STUDY:**To analyze the aetiology of aplastic anemia in children below 12 years of age and the significance of Camitta et.al, diagnostic criteria in the diagnosis of aplastic anemia in children. **MATERIALS AND METHODS:**The present study of aplastic anemia in paediatric age group i,e.below 12 years of age was conducted in Niloufer Hospital which is a paediatric centre for medical and surgical diseases for a period of 24 months ,from January 2000 to December 2001.Total number of 61 cases were found to be pancytopenia cases fulfilling the criteria laid down by Camitta et.al. All these patients were screened by conducting relevant investigations including biochemical, pathological, microbiological , radiological and also cytogenetic study.The bone marrow aspiration was done as a routine for diagnosis and management ,the cases wherein aspiration is scanty, haemo diluted or dry tap bone marrow biopsy was done. **RESULTS:** Total number of haematological cases for which B.M.A and B.M.Bx were performed during the period of 2 years was 367 cases. Of these, pancytopenia due to various causes were 61 (16.6%) and aplastic anemia was diagnosed in 33 (54.09%) out of these 61 cases . Bleeding time is moderately prolonged in severe aplastic anemia, coagulation parameters were within normal limits. Marked elevation of serum iron levels and saturated iron binding proteins are observed in severe aplastic anemia cases. **CONCLUSION:** The various aetiological factors leading to AA were investigated and documented. Drugs, herbal medicines and viruses were found to be causing AA of variable degrees. The prognosis is good in mild to moderate aplastic anemia with good clinical care bleak in severe aplastic anemia.

INTRODUCTION: PAUL EHRLICH first described aplastic anemia in 1888 from the autopsy of a pregnant woman with history of severe anemia, fever, ulcerated gums, menorrhagia associated with leukopenia. Later DARRYL. M. WILLIAMS described AA as a distinct clinical entity characterized by pancytopenia and thought to be the result of depressed bone marrow activity. Definition of AA has been revised/refined over a period of years by various authors. AA is simply defined as peripheral pancytopenia i.e, failure of haematopoiesis along the three cell lines. Bone marrow cellularity was not required for case definition. CAMITTA et.al, (1976-1979) proposed criteria for AA and classified AA into mild moderate and severe degree. These criteria were widely accepted and followed.Aetiology of aplastic anemia is varied ranging from drugs to auto immune mechanisms,but it is observed that most of the cases are due to idiopathic causes. Aetiological classification of AA includes inherited and acquired causes.Inherited causes of aplastic anemia are Fanconi anemia, familial aplastic anemias and Schwachman-Diamond syndrome.Acquired causes of aplastic anemia are secondary to drugs ,chemicals, toxins, irradiation, infection, immunological disorders,thymoma,pregnancy,paroxysmal nocturnal haemoglobinuria(PNH), preleukemia and idiopathic.

Pathogenesis of aplastic anemia: Normal hematopoiesis occurs within the specialised physical and functional environment of bone marrow ans is the result of interaction between hematopoietic cells and surrounding micro-environment.Possible pathogenetic mechanisms for aplastic anemia includes stem cell defects, abnormal micro environment, immunosupression and immune mediated mechanism.Drugs related bone marrow aplasia can occur either dose related mechanism and idiosyncratic or hypersensitivity mechanism.Drugs like chloramphenicol,benzene,myelosupressive drugs were implicated in bone marrow suppression in turn causing aplastic anemia.Viral infections like Parvo virus B 19,EBV,HIV, CMV, Dengue virus,hepatitis virus are thought to mediate suppression of hematopoiesis by variety of mechanisms.Fanconi anemia is autosomal recessive disorder characterised by inability to repair damaged DNA.

The clinical presentation of patients with aplastic anemia is usually insidious, characterised by non specific symptoms like progressive weakness and fatigue due to anemia. Bleeding can be an initial manifestation in some cases of AA due to thrombocytopenia .Fever and infections are due to leucopenia.

AIMS OF THE STUDY:

1. To analyze the aetiology of aplastic anemia in children below 12 years of age .
2. The significance of Camitta et.al, diagnostic criteria in the diagnosis of aplastic anemia in children.
3. The clinical course of severe Aplastic Anemia.

MATERIALS AND METHODS: The present study was conducted at Niloufer hospital which is a 350 bedded pediatric centre. The study was undertaken from January 2000 to December 2001(24 months).The cases that presented with various haematological problems such as aleukemic leukemia,overwhelming infections, storage disorders beside metastatic carcinoma,lymphoma and mycobacterial infections causing pancytopenia were screened by conducting relevant investigations. Total number of 61 cases were found to be pancytopenia cases fulfilling the criteria laid down by Camitta et.al. There were cases with monocytopenia and bicytopenia which are not considered as true pancytopenia cases.There are several children who come foe follow up of post chemotherapy and radiotherapy in acute leukemias, neuroblastomas etc. These cases are not included in the present study. Total number of cases presented with haematological disorders for which bone marrow aspiration and bone marrow biopsy(when needed) was done were 367 cases in 24 months .

The routine investigations were done in all the 61 cases of pancytopenia included basic biochemical parameters like serum electrolytes (Na and K),Liver function tests-serum bilirubin, SGOT, SGPT, Alkaline phosphatase and Renal function tests-CUE,BUN,

serum creatinine. Microbiological investigations includes serological tests like markers foe Hepatitis-A, Hepatitis-B, EBV antibody panel .CMV antibody titre, Varicella antibody titre, Mantoux test and culture sensitivity of sputum , blood, and urine. The main pathological investigations like haemogram, BT, CT, reticulocyte counts, haemoglobin electrophoresis, sickling test, bone marrow aspiration and bone marrow biopsy were done and results were analyzed. Radiological investigations like Xray chest, abdomen, upper and lower limbs ,ultrasound examination of chest, abdomen and pelvis were done where ever required. For the diagnosis of familial cases cytogenetic study was also done and results were analyzed.

The criteria followed in the present study for diagnosing a case of pancytopenia are those suggested by Camitta et.al, (1976-1979). Atleast 2 of three criteria on the peripheral smear study are to be fulfilled.

A) Hb ≤ 10 gms% (or)Hct ≤ 30%
B) WBC≤ 3500 cells/mm3
C) Platelets≤ 50,000/mm3

Similarly for confirming the diagnosis of AA on B.M.A, imprint smears and paraffin embedded sections of B.M.Bx the following criteria laid down by Camitta et.al, are as follows:

A) Marked hypocellularity 25% of normal cellularity (or)
B) Marrow of 50% (25-50%) cellularity with 30% hematopoietic cells.

The criteria laid down by Camitta et.al, are applicable to our study in children .

In the present study we followed Camitta's criteria for the following reasons:

1.Firstly, in pediatric age group ,fibrosis is a rare feature causing pancytopenia.

2.Secondly, metastasis causing pancytopenia is not the common aetiology resulting in pancytopenias and AA in children.

3.For prognostic purpose and case management, Camitta et.al. criteria are found to be helpful in our study.

The present study is mainly intended to analyze the various other aetiological factors such as military TB, drugs, Fanconi's anemia etc, that cause AA. Hence the criteria laid down by Camitta et.al, are preferred to IAAAS criteria.

Detailed clinical history, family history pertaining to consanguinity, herbal medicine/indigenous intake, physical abnormalities etc recorded in the special format. Many children giving history of indigenous drug intake as treatment of choice for anemia and jaundice are noted. The various ayurvedic and herbal medication given could not be analyzed with regards to composition, dosage schedule, and drug toxicity so that their role in causing AA can not be established. Hence ,only the mention of drug intake is recorded.

In cases of post hepatitis induced AA, LFT was done and the levels were elevated.Viral serological profile was done in suspected cases and the aetiology was confirmed as hepatitis A in one case and hepatitis B in another .Physical examination of suspected Fanconi's anemia cases showed skeletal abnormalities like thumb abnormalities and polydactyly X rays showing phenotype abnormalities have been documented.

In all these 61 cases of pancytopenia , the clinical presentation varied widely. Some children presented with gross pallor, mild pedal edema, shortness of breath, easy fatiguability and loss of appetite.Some of the children were brought with complaints petechia, epistaxis, bleeding gums etc, while some children presented with overwhelming infections, transient arthralgia and bone tenderness. The various radiological and lab investigations in these 61 cases of pancytopenia enabled us to differentiate 33 cases of AA from the rest.In Niloufer hospital most of the cases treated with methyl prednisolone therapy while solitary case was treated with stanozolol.Stanozolol is given as a substitute for methyl prednisolone in some cases to observe it's response .Secondly ,in some children where the initial response is not encouraging ,stanozolol is given instead. The advantages of using above therapies are the relative less cost and fewer complications as compared to BMT, while the disadvantage is the long period of supportive care which can last for months, persisting thrombocytopenia and occasional relapse.in conclusion in situations where the compatible donor is not available or the patient is unfit for bone marrow transplantation for various reasons such as several blood transfusions ealier to the initial diagnosis or poverty, the conventional management is the treatment of choice in AA.

OBSERVATION: The present study of aplastic anemia in pediatric age group was carried out at Niloufer hospital for a period of 2 years. Total number of cases for which B.M.A and B.M.Bx were performed were 367 and of these cases pancytopenia due to various causes were 61 (16.67) cases , aplastic anemia was diagnosed in 33 (54.09%) out of these 61 cases. After analyzing the clinical presentation and carrying out various investigations mentioned, 33 cases were found to be fulfilling the criteria for aplastic anemia. In all these 33 cases the confirmation is done by bone marrow biopsy.

Age of the patients of aplastic anemia ranged from 11/2 years to 12 years of age. The peak was in the age group of 7-12 years(27 cases).

AGE GROUP	No.OF CASES
0-6 years	6 cases (18.2%)
7-12 years	27 cases (81.8%)

Study showed a definitive male preponderance. Of 33 cases , 20 were males.

Males	20 (60.6 %)
Females	13 (39.4%)

Among the 20 males of aplastic anemia , 17 cases were in the age group of 7-12 years.

In the study conducted , lowest Hb concentration was 2.6% gms%: the highest being 10.9 gms%. 18 children had less than 5 gms% Hb at the initial stage of investigation. Among 33 cases of AA, 19 children (57.6%) had haematocrit value less than 15 vol. Percentage of Hct value ranged from 4.8 vol to maximum of 30 vol in all these 33 cases.

Lowest WBC count recorded was 4oo cells/cumm and highest count was 8500 cells/cumm. 18 children were having less than 3500 cells/cumm Platelet count were low in all 33 cases. Highest count recorded was 70,000 cells/cumm and remarkably lowest was 5000 cells/cumm.

Reticulocyte count ranged from 0.1%- 2.5%. Most of the cases had reticulocyte count of less than 2%. None of the 33 cases had greater than 2% count at the initial diagnosis.

In some of our case of AA, Hb F was elevated. One case had Hb F levels of 15.7%.Bruce M.Camitta et.al, in their study of aplastic anemia reported elevated levels of Hb F in some patients. They have explained this on the presence of small population of cells with increased Hb F rather than to homogenous increase of Hb F in all red cells.

Most of the bone marrow aspirations showed scanty aspirates/insufficient material for opinion and few bone marrow aspirations were dry taps. Hence the bone marrow biopsy study was performed.20 out of 33 cases bone marrow aspirates revealed mild to moderate degree of cellularity and increased fat spaces. The maturation of all the cell lines was normal but decreased.There was

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relative increase in lymphocytes in these cases.In remaining 13 cases bone marrow aspiration was hemodiluted with few marrow particles and plenty of fat spaces. Imprint smears of 20 cases showed fat spaces were greater to the age related normal marrow and cellularity was relatively decreased . Bone marrow biopsy sections revealed variable cellularity with focal hypercellular areas and hypocellular areas associated with varying degrees of fat spaces. In the remaining 13 cases bone marrow section findings were consistent with SAA. Scoring for iron deposits was done by Perl's prussian blue method inwhich all 33 cases the score ranged from 0 to 2 at the time of initial diagnosis..Two of 13 cases diagnosed as SAA cases on folloe up showed increased levels of iron stores in the range of 4 to 5.There was no evidence of fibrosis in all the 33 cases demonstrated by reticulin and vanGeison stain.

Of 33 cases of AA in our study ,13 cases were classified as severe degree of AA cases and the remaining 20 cases as mild to moderate AA cases, following the criteria proposed by Camitta et.al. which we have followed in our study.

DISCUSSION :Study of aplastic anemia in pediatric age group was undertaken to observe the age and sex incidence, the various aetiopathogenic factors and the progress in children below 12 years of age.The study was done at Niloufer Hospital,Hyderabad which is amedical and surgical pediatric centre catering to it's own and neighbouring states. 367 children were subjected to Haematological investigations including bone marrow aspiration and bone marrow biopsy during the 24 month period for evaluation of gross anemia. Pancytopenia was detected in 61 cases (16.6%) and aplastic anemia was diagnosed by bone marrow aspiration and bone marrow biopsy in 33 out of 61 cases (54.09%). This study does not include children with hypoplastic marrow secondary to post- chemotherapy and post- radiotherapy and single cell line suppression disorders.The peak age incidence as observed in these documented aplastic anemia cases in our study ranged from 7- 12 years in 21 out of 33 cases. N.Clausen et.al,did a population based study of severe aplastic anemia in pediatric patients aged 0-14 years.He observed that there is no age discrimination foe development of severe aplastic anemia due to various aetiopathogenic causes. The sex incidence of aplastic anemia in our study was 1.53:1 amongst male and female children respectively.Similar observation was also quoted by N.Clausen et.al, from Denmark who did exclusive study of aplastic anemia in children and found male to female ratio as 2:1.

The cause of aplastic anemia is widely studied by various study groups world wide and it is observed by all of them that the aetiopathogenesis of AA in individuals from 0-50 years is multifactorial while over 60 years individuals have primary exhaustion of bone marrow elements resulting in aplastic anemia.Among children below thw age of 15 years in other studies and below 12 years in our study , the aetiopoathogenesis of aplastic anemia and hypoplastic marrow can be further divided in to primary AA and secondary AA due to chemotherapy and radiotherapy. Our present study is confined to primary multifactorial causes in aplastic anemia as our intention is to understand these primary aetiological factors.

Aetiological factors	Number of cases	%
Idiopathic	14	42.42%
Fanconi's anemia	06	18.18%
Herbal medicines	05	15.15%
Viral Hepatitis	02	6.06 %
Chicken pox infection	01	3.03%
Imuunization	01	3.03%
Drugs	01	3.03%
Multifactorial aetiology	03	9.09%
Total number of cases	33	

Fanconi's anemia is more common than it was thought earlier prior to the development of specific chromosome testing and thus chromosomal aberrations form a crucial diagnostic tool..In our study 6 cases presented with dysmorphic features, anemia and related infections. And chromosomal study in all the 6 cases was positive for aberrations confirming the diagnosis of fanconi's anemia.

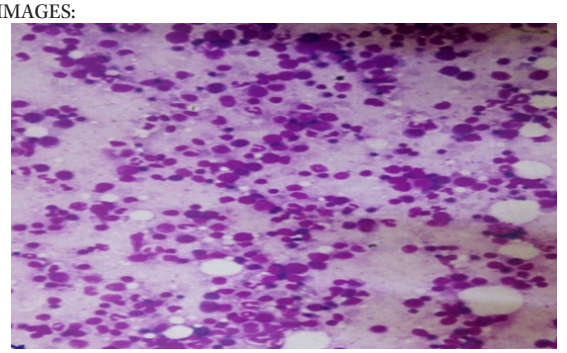
Most of the cases in our study had a history of indigenous/herbal medicine intake for various complaints like fever, fatigue, pallor and jaundice. We could not analyse the various ayurvedic and herbal medication used with regard to composition, dosage schedule and drug toxicity so that their role in causing aplastic anemia can be established.In our study of 33 cases of aplastic anemia , history of indigenous/ herbal medication was elicited in 5 cases(15.15%).Nelson et.al, at New York university medical centre reported a case of AA associated with use of herbal medication in a12 year boy. On analysis , they found that the herbal medicine contained phenylbutazone , which has been strongly associated with production of AA.

Most of the viruses have been implicated as the causative agent. Patients with viral illness develop transient pancytopenias, possibly due to direct effect of viruses on the bone marrow. Multiple and various pathogeneticmechanisms have been proposed to explain virus induced bone marrow failure. In our study of 33 cases of aplastic anemia, we had 2 (6.06%) cases of pure viral hepatitis associated AA. These childen giving history of hepatitis and positive biochemical parameters for hepatitis, on further investigation were found to be serologically positive for Hepatitis –A in one and Hepatitis –B in the other case. Hassan .K.et.al carried out a 4 year study of AA cases at Ravalpindimedical college,Pakistan. Out of 43 cases of SAA diagnosed by them there were 3 suspected cases of hepatitis induced AA. We had a single case (3.03%) of AA secondary to chicken pox virus infection (3 months) earlier in a 12 year old boy..During follow up, the patient developed AML-M3 leukemia and subsequently expired within 4 months after developing AA. There were 3 cases (9.09%)of aplastic anemia occurring in association with herbal medicine intake and viral infections such as mumps in one case and hepatitis in two others. In our study of 33 cases of AA ,we had a single case (3.03%) of aplastic anemia in a 12 year male child, who used to take NSAIDS/Phenylbutazone for his arthralgia and myalgia.

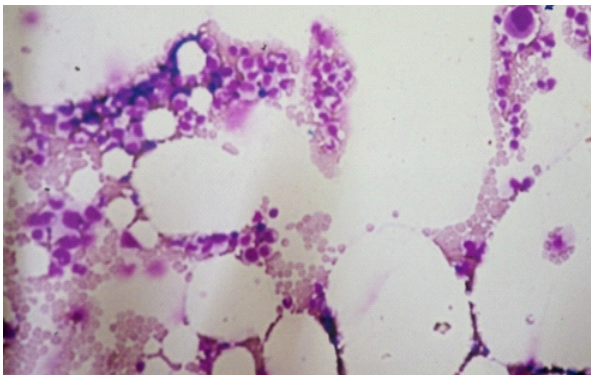
Of 33 documented cases of aplastic anemia in our study, 14 cases (42.42%) did not have any of the preceeding factors mentioned so far to cause aplastic anemia and these 14 cases are labelled as idiopathic origin.

STUDY/REGION	AUTHOR	TOTAL NO.OF CASES	NO. OF IDIOPATHIC CASES	PERCENTAGE
SWEDEN	L.E.Bottinger et. Al	80	58	72.50
Denmark study	N.Clausen	39	18	46.15
Nordic countries	N.Clausen et,al	101	72	71.28
Pakistan study	Hasan et,al	43	25	58.13
Our study		33	14	42.42

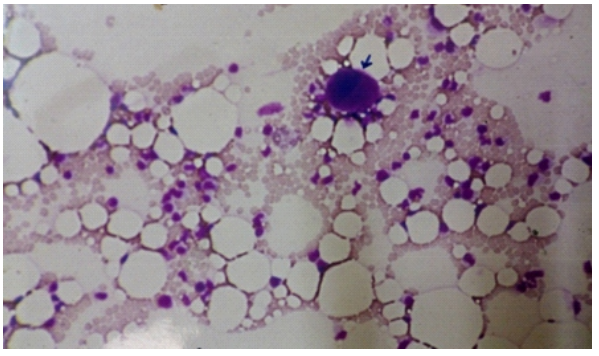
IMAGES:



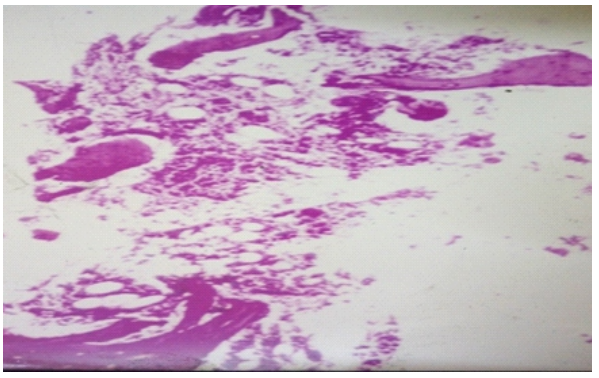
PICTURE 1: B.M.A X 200 x- Leishman stain – mild AA: few fat spaces in a child of 2 years



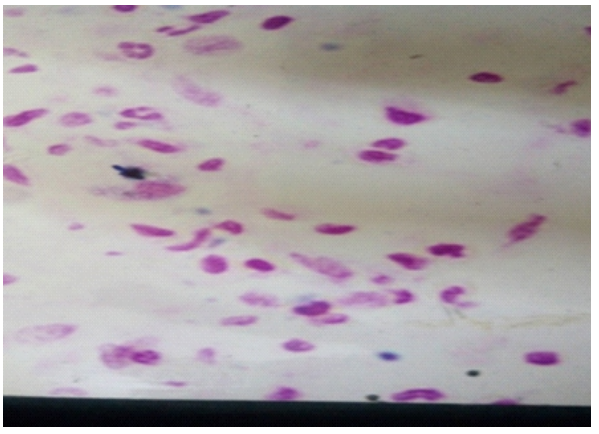
PICTURE 2:B.M.A.X 200x –Giemsa stain : Pronounced fat spaces in a child of 6 years.



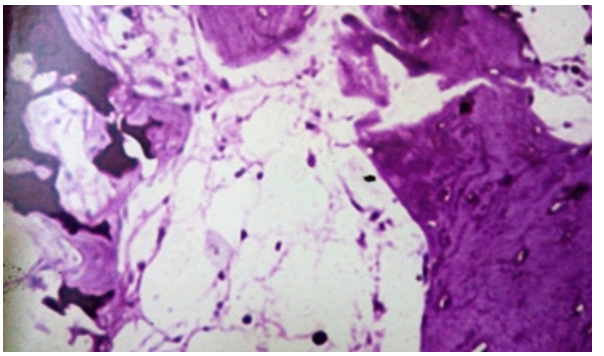
PICTURE 3 :B.M.A.X 200x Leishman stain : Pronounced fat spaces and decreased cellularity in a 5 year old child.



PICTURE 4 :B.M.A X 100x- H & E stain: Mild AA.



PICTURE 5 :B.M.A X 400x- Perl's stain: Severe AA.



PICTURE 6 :B.M.A X 400x- Geimsa stain: Severe AA, showing scattered myelopoietic elements.

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