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 and 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, immunosuppression and immune mediated mechanism. Drugs related bone marrow aplasia can occur either due to related mechanism and idiopathic or hypersensitivity mechanism. Drugs like chloramphenicol, benzene, myelosuppressive 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 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, haemodiluted 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 and in severe aplastic anemia.
serum creatinine. Microbiological investigations included serological tests like markers for 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 X-ray 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). At least 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, Fanconis anaemia etc, that cause AA. Hence the criteria laid down by Camitta et al. are preferred to IAAS 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 anaemia 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 fatigability 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 its 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 low 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 earlier 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).

<table>
<thead>
<tr>
<th>AGE GROUP</th>
<th>NO. OF CASES</th>
<th></th>
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<tbody>
<tr>
<td>0-6 years</td>
<td>6 cases (18.2%)</td>
<td></td>
</tr>
<tr>
<td>7-12 years</td>
<td>27 cases (81.8%)</td>
<td></td>
</tr>
</tbody>
</table>

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 400 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% to 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
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 in which 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 follow 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 van Gieson 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 Nilofer Hospital, Hyderabad which is a medical and surgical pediatric centre catering to its own and neighboring 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 for 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 worldwide 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 the age of 15 years in other studies and below 12 years in our study, the aetiopathogenesis of aplastic anemia and hypoplastic marrow can be further divided into 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.

Fancioni’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 fancioni’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 medicine 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%). N. Clausen et al. at New York University medical centre reported a case of AA associated with use of herbal medication in a 12 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 pathogenetic mechanisms 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 children 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 Ravalpindi medical 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.

<table>
<thead>
<tr>
<th>Aetiological factors</th>
<th>Number of cases</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>14</td>
<td>42.42%</td>
</tr>
<tr>
<td>Fancioni’s anemia</td>
<td>06</td>
<td>18.18%</td>
</tr>
<tr>
<td>Herbal medicines</td>
<td>05</td>
<td>15.15%</td>
</tr>
<tr>
<td>Viral Hepatitis</td>
<td>02</td>
<td>6.06%</td>
</tr>
<tr>
<td>Chicken pox infection</td>
<td>01</td>
<td>3.03%</td>
</tr>
<tr>
<td>Immunization</td>
<td>01</td>
<td>3.03%</td>
</tr>
<tr>
<td>Drugs</td>
<td>01</td>
<td>3.03%</td>
</tr>
<tr>
<td>Multifactorial aetiology</td>
<td>03</td>
<td>9.09%</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>
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 200 x – Giemsa stain: Pronounced fat spaces in a child of 6 years.

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

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

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

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

REFERENCES: