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Association of Various Etiological Factors with Idiopathic Acquired Aplastic Anemia

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Abstract

Objective: To determine demographic features, clinical features and association of various etiological factors in patients of idiopathic acquired aplastic anemia in local population at the time of diagnosis.

Patients and Methods: This cross sectional study was conducted in Department of Haematology, Pakistan Institute of Medical Sciences Islamabad and Department of Genetics, Children Hospital Lahore, from June 2015 to July 2017. Total 64 cases of peripheral blood pancytopenia having clinical suspicion of acquired aplastic anemia were enrolled in the study. Patients with congenital aplastic anaemia, patients with features of bone marrow dysplasia and abnormal infiltrates and patients with post chemotherapy and radiotherapy aplasia were identified and excluded from the study. Sixty patients diagnosed to have acquired aplastic anaemia on the basis of clinical features, peripheral blood and bone marrow findings, were included in this study as per inclusion criteria.

Results: Among 60 diagnosed cases of acquired aplastic anaemia, 34 were male and 26 were female with a male to female ratio of 1:1.30. Age distribution of study participants ranged from 1 to 84 years with a median age of 10 years. Most of the patients (73.3%) presented with non severe stage of disease. Different etiological factors reported to be implicated in acquired aplastic anaemia in the patients of this study were Viral infections (hepatitis B & hepatitis C), Mycobacterial infection, Fertilizers & Pesticides, Chemicals & Toxins, Ionizing radiations, Autoimmune diseases (Rheumatoid arthritis & Systemic lupus erythematosus), Transfusion associated GVHD, Pregnancy, Drugs (Analgesics & Hypoglycemics) and Cigarette smoking. In 14 out of 60 patients (23.3%) various etiological factors were found to be associated with idiopathic acquired aplastic anaemia.

Conclusion: Aplastic anaemia was seen in patients of all ages and both genders were equally affected. Non severe aplastic anaemia was most common stage of the disease. Pallor, bleeding and fever were common clinical features. Etiological factors could not be identified in majority of the patients, however various etiological factors have been reported to be associated with increased risk of acquired aplastic anaemia. Identification and treatment of underlying cause may be beneficial in the management of patients with acquired AA. It may also guides in prevention of the disease.

Introduction

Aplastic anaemia is an immune mediated haemopoietic stem cell disorder characterized by pancytopenia with a hypocellular bone marrow in the absence of abnormal infiltrate and no increase in bone marrow reticulin.[1] Aplastic anemia is considered if at least two of the following defining criteria (Table I) are fulfilled that includes haemoglobin level less than 10g/dl, neutrophil count less than 1.5 x 10^9/L, platelet count less than 50 x 10^9/L and bone marrow cellularity less than 25% or 25 to 50% with less than 30% residual haemopoietic cells.[2]

Table I: Diagnostic criteria for aplastic anemia

Haemoglobin level	< 10g/dl
Neutrophil count	< 1.5 x 10^9/L
Platelet count	< 50 x 10^9/L
Bone marrow cellularity	< 25 %

Disease severity of aplastic anemia is based on the criteria given by Camitta et al. in 1975.[3] According to these criteria the severity of aplastic anemia is graded into very severe aplastic anaemia (VSAA), severe aplastic anemia (SAA) and non severe aplastic anemia (NSAA). Severity of aplastic anaemia according to Camitta criteria is based on blood cell counts and bone marrow cellularity (Table II).[4] Grading of disease is significant in management decisions but has less prognostic value in terms of response to immunosuppressive therapy (IST).[5]

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Table II: Classification of aplastic anemia (Camitta criteria)

Very severe aplastic anaemia (VSAA)	Bone marrow cellularity < 25% Neutrophil count < 0.2 x 10^9/L Platelet count < 20 x 10^9/L Reticulocyte count < 20 x 10^9/L
Severe aplastic anaemia (SAA)	Bone marrow cellularity < 25% Neutrophil count < 0.5 x 10^9/L Platelet count < 20 x 10^9/L Reticulocyte count < 20 x 10^9/L
None severe aplastic anaemia (NSAA)	Patients not fulfilling the criteria of very severe AA or severe AA

Aplastic anaemia can be congenital or acquired.[6] Presence of somatic abnormalities and characteristic sensitivity of haemopoietic cells to chromosomal breakage on exposure to clastogenic agents such as diepoxybutane (DEB) and mitomycin c (MMC) is suggestive of congenital aplastic anemia.[7] Some cases of congenital aplastic anemia may lack the characteristic phenotypic abnormalities.[8] Constitutional mutations result in increased genomic instability and reduced cell survival in congenital aplastic anaemia that leads to increased chromosomal DNA damage by DNA crosslinking agents due to aberration in BRCA pathway.[9] BRCA gene (a tumor suppressor gene) is involved in DNA damage response pathway; cells lacking BRCA protein are susceptible to chromosomal breakage after exposure to DEB or MMC.[10] Acquired aplastic anaemia is associated with various etiological factors that include different drugs, chemicals, ionizing radiations, pregnancy, autoimmune diseases, graft versus host disease and viral infections.[11] No definitive causative factor is found in majority of the cases of acquired aplastic anaemia.[12] Various drugs, chemicals, toxins, viral infections such as Hepatitis virus, Varicella Virus, Parvo virus, CMV, EBV, HIV and other factors such as Ionizing radiations, Smoking, Graft versus host disease etc. are associated with increased risk of aplastic anemia. Different etiological factors reported to be implicated in acquired aplastic anaemia are listed in Table III.

Table III: Etiological factors of acquired aplastic anemia

Drugs	Antibiotics: Chloramphenicol, Sulphonamide, Cotrimoxazole Anti-inflammatory drugs: Diclofenac, Neproxin, Piroxicam Anticonvulsants: Phenytoin, Carbamazepine Antidepressants: Phenothiazides, Dothiepin Antithyroid drugs: Thiouracil, Carbimazole Antimalarials: Chloroquine Hypoglycemics: Chlorpropamide, Tolbutamide Other drugs: Mebendazole, Thiazides, Allopurinol, Ticlodipine
Chemicals	Benzene (Industrial chemicals), Pesticides (Pentachlorophenol), Insecticides (Organochlorines, Organophosphates, DDT), Fertilizers, Lubricating oils, Hair dyes, Glycol ether
Viral infections	Hepatitis virus, Varicella Virus, Parvo virus, CMV, EBV, HIV
Autoimmune diseases	SLE, Rheumatoid arthritis, Celiac disease, Eosinophilic fasciitis
Other factors	Ionizing radiations, Smoking, Graft versus host disease, Pregnancy, Exposure of farmers to ducks and geese

Incidence of aplastic anaemia in West is around 2 per million population in a year, that is twofold higher (3 to 4 per million population) in Asia.[13] In Southeast Asia, the incidence is 5 to 6 per million population.[14] An incidence of 7.4 per million population is reported from a study in China.[15] A study from Japan in 2015 has estimated the incidence of 8.2 per million nationwide population.[16] Recently, incidence of aplastic anaemia in Pakistan has been estimated to be around 3.5 patients per million population by collecting data from different tertiary care facilities of the country (personal communication) but the figures are likely to be higher since majority of the population lives in rural areas with limited access to advanced health care facilities.[17] There is biphasic age distribution, aplastic anaemia is commonly seen in younger population (10 to 25 years) and elderly population (above 60 years). Both males and females are equally affected.

The objective of this study was to determine demographic features, clinical features and association of various etiological factors in patients of idiopathic

acquired aplastic anemia in local population at the time of diagnosis.

Methodology

This cross-sectional study was conducted in Department of Genetics, Department of Haematology, Children Hospital, Lahore and Department of Haematology, Pakistan Institute of Medical Sciences (PIMS), Islamabad from June 2015 to July 2017. Sample size was calculated using WHO sample size calculator and sampling was done by convenient random sampling technique. Total 64 participants (both male and female patients of all age groups) who presented to the Department of Haematology with peripheral blood pancytopenia having a clinical suspicion of acquired aplastic anemia, with features of bone marrow aplasia were enrolled in the study. Patients with congenital aplastic anaemia, patients with features of bone marrow dysplasia and abnormal infiltrates and patients with post chemotherapy and radiotherapy aplasia were identified and excluded from the study. The study was approved by ethical board of each participating hospital. Informed consent was taken from the patients or their parents. Sixty patients diagnosed to have acquired aplastic anaemia on the basis of clinical features, peripheral blood and bone marrow findings, were included in this study as per inclusion criteria. Diagnosis of aplastic anemia was confirmed by considering bone marrow findings as gold standard according to defining criteria and sampling was done by convenient random sampling technique.

Informed consent was taken from patients or their parents and sociodemographic profile and clinical data of the patients was collected. A detailed history was taken about fever, bleeding from any site including multiple bruises with minor trauma, purpura, epistaxis, gums bleed, heamatemesis, haemoptysis, haematochezia, malena, haematuria, menorrhagia, excessive bleeding from wounds or cuts or after a surgical procedure, breathlessness on mild exertion, palpatation and easy fatigability. Patient was specifically asked for exposure to toxins or chemicals at the place of work or residence near industrial area or agricultural land.

General physical examination was carried out to check for pallor, fever, tachycardia and bleeding manifestations in the skin (bruises and purpura), signs of bleeding from the nose, oral cavity, vagina, anal canal. Systemic examination was performed to find positive signs of anemia, haemorrhage, infections, lymphadenopathy, hepatomegaly, splenomegaly, inflammatory conditions and especially any dysmorphic features to exclude congenital AA.

Biochemical and radiological findings, Complete blood counts (CBC) including Haemoglobin (Hb), Total leucocyte count (TLC), Platelet count, Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH) were recorded. Reticulocyte blood smear was stained with supravital (brilliant cresyl blue) stain and slides were examined for reticulocyte count. Peripheral blood smear was stained by wright stain and slides were examined for differential leukocyte count (DLC), identification of any blasts, dysplasia or atypical cells.

Bone marrow (aspiration and trephine) biopsy was done to confirm diagnosis of aplastic anemia. Bone marrow aspiration was performed with Islam Bone Marrow Aspirate Biopsy Needle from posterior superior iliac crest and smears were stained by Giemsa stain. Bone marrow trephine biopsy was performed with Islam Bone Marrow Trephine Biopsy Needle from the same site and smears were stained with Haematoxylin & Eosin stains. Bone marrow smears were examined for cellularity, evidence of dysplasia of any abnormal infiltrates. Aspirates were also stained with Pearl's (Prussian blue) iron stain for assessment of iron stores and identification of ring sideroblasts.

Serology for hepatitis B surface antigen (HBsAg) and hepatitis C antibody (anti HCV) were done to detect viral hepatitis. Anti double stranded DNA (anti dsDNA) antibodies, Anti rhematoid factor (RF) antibodies and Anti tissue transglutaminase antibodies (IgG & IgM) were also done in suspected cases to rule out underlying autoimmune diseases associated with acquired AA. LAP score, Ham's test, Sucrose haemolysis test and urine examination for haemoglobinuria and haemosiderinuria by Prussian blue staining of urinary sediment were done in suspected cases to rule out paroxysmal nocturnal haemoglobinuria (PNH).

Cytogenetic analysis was done in selected cases to exclude congenital aplastic anaemia. Blood and Bone marrow samples were collected under aseptic measures in sterile sample collection tubes (green top and purple top vacutainers) containing sodium heparin and EDTA anticoagulants respectively to prevent coagulation.

Collected data was recorded and analyzed using Statistical Package for Social Sciences (SPSS) Version 20. Nominal data of variables including pallor, fever, bleeding, splenomegaly. Hepatomegaly, lymphadenopathy, bone marrow features including cellularity and iron stores were expressed as frequency percentage. Median was calculated for Age of the study participants and association of various etiological factors was found in the study patients diagnosed to have acquired aplastic anemia.

Results

Age distribution of 64 study participants ranged from 1 to 84 years with a median age of 10 years. Most of the study participants were children as 52 patients (81%) were in age group of 1 - 18 years whereas 12 patients (19%) were in age group of 19 - 84 years. Four out of 64 cases were excluded from the study as per exclusion criteria, among those two cases (3.1%) were diagnosed to have Myelodysplastic syndrome based on peripheral blood and bone marrow findings while two cases (3.1%) were diagnosed as Congenital aplastic anemia on chromosomal breakage analysis. Sixty out of 64 cases (93.8%) diagnosed to have acquired aplastic anemia on the basis of clinical, peripheral blood and bone marrow findings, were included in this study as per inclusion criteria. Diagnosis of aplastic anemia was confirmed by considering bone marrow findings as gold standard. Among 60 diagnosed patients of acquired aplastic anemia, 34 were male and 26 were female with a male to female ratio of 1:1.30. Most of the patients presented with non severe stage of disease. Among 60 diagnosed cases 44 patients (73.3%) had non severe aplastic anaemia (NSAA), 13 patients (21.7%) had severe aplastic anaemia (SAA) and 3 patients (5%) had very severe aplastic anaemia VSAA (Table IV).

Table IV: Severity distribution of aplastic anemia among study patients

Severity	NSAA	SAA	VSAA
Male	27	5	2
Female	17	8	1
Total	44	13	3
Percentage	73.3%	21.7%	5%

Patients of acquired aplastic anaemia in this study commonly presented with pallor, fever, bleeding, lymphadenopathy, hepatomegaly and splenomegaly. On analysis of clinical presentation, pallor was observed in 60 patients (100%), fever was recorded in 18 patients (30%) while bleeding manifestations were present in 16 patients (27%). Lymphadenopathy was noted in 6 patients (10%) with infections. Splenomegaly was found in 2 patients (3.3%) with SLE and enteric fever. Hepatomegaly was also observed in 2 patients (3.3%) with hepatitis B and C. Clinical parameters of 60 diagnosed patients with acquired aplastic anaemia in this study are shown in Figure 1.

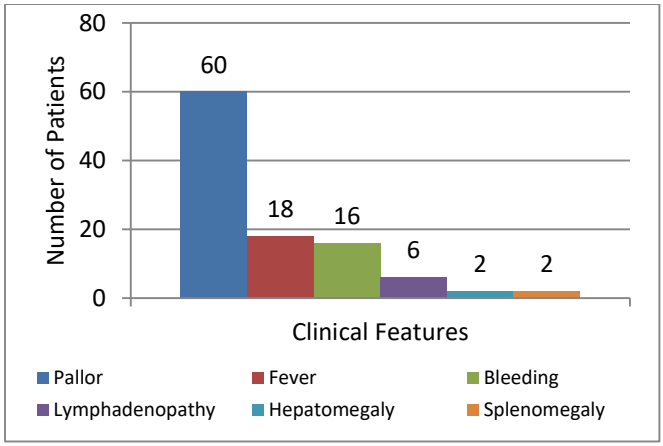

Figure 1. Clinical parameters in diagnosed cases of acquired aplastic anaemia

Various risk factors were found to be associated with acquired AA in this study. In 46 cases (76.7%) of AA no definitive cause or risk factor was identified whereas 14 cases (23.3%) were associated with different etiological factors. Two patients had history of viral hepatitis and were diagnosed to have hepatitis B and hepatitis C in the past. One patient had history of pulmonary tuberculosis who later developed septicemia due to miliary tuberculosis. Two patients had history of exposure to industrial chemicals and toxins as one of them had been working in paint industry for last 12 years while other had been working in leather factory for past 10 years. One patient was farmer by profession and had history of exposure to fertilizers and pesticides for last 13 years. One patient was chain smoker and had history of smoking 10–15 cigarettes per day for last 20 years. One patient was radio technologist in radiology department and had history of exposure to ionizing radiations for past 14 years. Two patient had history of autoimmune

disease, one of them was diagnosed to have Systemic Lupus Erythematosus 6 years ago while other was diagnosed to have Rheumatoid Arthritis for last 4 years. One patient had history of repeated transfusions and later underwent HLA matched bone marrow transplant for thalassemia who developed GVHD 2 years ago. One patient had history of un prescribed excessive use of analgesics for body aches due to heavy mechanical work in cargo department for past 11 years who later developed analgesic nephropathy. One patient had history of prolonged self-medication with various antibiotics and oral hypoglycemics for recurrent infections due to diabetes mellitus for past 10 years. One female patient developed aplastic anaemia during pregnancy which remitted spontaneously after caesarean. Association of various etiological factors with acquired AA in the patients included in this study is shown in Table V.

Table V. Association of risk factors with acquired aplastic anaemia among study patients

Risk Factors	Patients (N=14)
Viral infections	2
Mycobacterial infection	1
Fertilizers & Pesticides	1
Chemicals & Toxins	2
Ionizing radiations	1
Autoimmune disease	2
Transfusion associated GVHD	1
Pregnancy	1
Smoking	1
Drugs	2

All patients of acquired aplastic anemia were from different regions of the country. Geographic distribution of study patients is shown in Figure 2. Majority of them were from Islamabad and Lahore districts followed by Multan, Faisalabad, Sailkot, Gujrat, Abbottabad and Swat districts. Most of the patients diagnosed to have acquired aplastic anaemia in this study were residents of industrial areas and agricultural lands who were exposed to industrial chemicals, toxins, fertilizers and pesticides, therefore they were at higher risk of developing acquired AA associated with environmental factors.

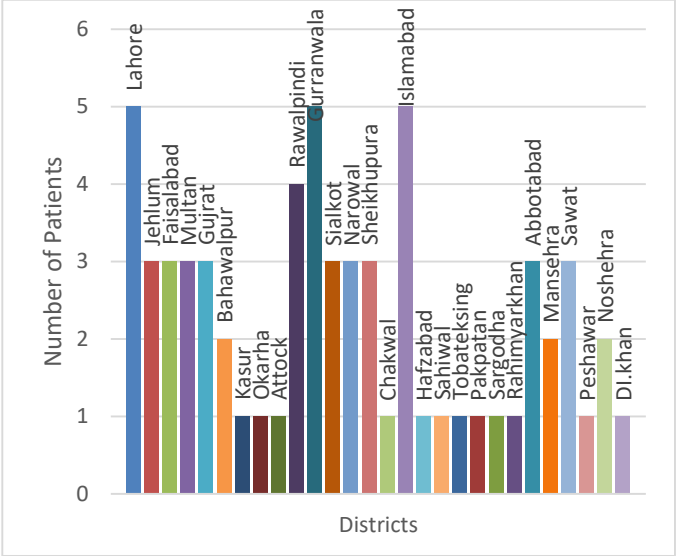

Figure 2. Geographic distribution of study participants

Discussion

Aplastic anaemia is haematological disorder of bone marrow failure characterized by T cell mediated destruction of haemopoietic cells.[18] In the current study, predominant clinical stage of disease was non severe aplastic anaemia (NSAA). Studied from Karachi and other regions of the world showed severe aplastic anaemia (SAA) in most of the cases of aplastic anaemia.[19] Patients with very severe aplastic anaemia (VSAA) has very poor prognosis as compared other categories. In this study most frequent clinical features were pallor, bleeding and fever. Common clinical signs observed were lymphadenopathy, hepatomegaly and splenomegaly in the current study. Similar clinical findings were reported in the study conducted by Gupta et al. in India who reported pallor, petechiae, bleeding from gums, epistaxis, haematuria, menorrhagia, lymphadenopathy and hepatomegaly as most common features while upper respiratory tract infection and viral hepatitis were most common infections in the study patients.[20]

Most cases of aplastic anaemia are classified as idiopathic because no etiological factors can be identified. Nevertheless, several causative factors are found to be associated with increased risk of the disease in various studies conducted worldwide.[21] Southeast Asia is an industrial and agricultural region, large population working in industries and fields without adequate protective measures are exposed to industrial chemicals, toxins, fertilizers and insecticides have increased risk of

developing acquired AA associated with environmental factors. In highly populated urban areas of Southeast Asia tertiary health facilities are insufficient, therefore acquired AA associated with infections and autoimmune diseases is more common.[22] In underdeveloped rural areas of Southeast Asia with poor primary health facilities where analgesics and antibiotics are available over the counter without any medical prescription, the incidence of drug associated acquired AA is higher. Environmental pollution is more common in Southeast Asia due to limited number of environment friendly vehicles and poor industrial waste management, that causes depletion of ozone layer resulting in increased exposure to ultra violet rays which are associated with acquired AA.[23] Improper nuclear power plants waste disposal adjacent to residential areas, agricultural lands and rivers, contaminates crops and water with ionizing radiations which increases risk of acquired AA associated with ionizing radiations in this region.[24] High population growth rate in this region may also be related to pregnancy associated acquired AA. Higher incidence of acquired aplastic anaemia in Southeast Asia is therefore related to increased risk of exposure to etiological factors as compared to rest of the world.[25] In majority of the patients (83.3%) in this study no etiological factors could be identified. Industrial chemicals & toxins, Fertilizers and Viral hepatitis were common risk factors found to be implicated in the etiology of AA. These findings are consistent with the study conducted by Ayesha Ehsan et al. at Sheikh Zayed Postgraduate institute, Lahore, Pakistan who reported 80% cases of apastic anaemia with no defined etiology.[26] Pestiside exposure and Hepatitis B&C infections were found to be common cause of acquired AA. Similar findings are reported in various other studies conducted in Thialand and India.[27]

Prognosis of the patients with acquired aplastic has improved with better supportive care of anaemia, infections and bleeding.[28] Estimated 10 years survival is 68% in patients receiving IST and 73% in patients with HSCT (Haemopoietic stem cell transplant). Mode of treatment in patients with aplastic anaemia depends on age of the patients, children with HLA matched sibling donor are preferably treated with HSCT whereas adults without HLA matched sibling donor are generally treated with IST along with supportive therapy.[29]

Approximately 25% patients of AA have inadequate response to therapy. HSCT is the curative treatment option, however may be at additional risk of developing graft versus host disease (GVHD) and potential for graft rejection.[30]

Conclusion

Aplastic anaemia was seen in patients of all ages with almost similar gender incidence. Non severe aplastic anaemia was most common stage of the disease. Pallor, bleeding and fever were common clinical features. Etiological factors could not be identified in majority of the patients, however various etiological factors have been reported to be associated with increased risk of acquired aplastic anaemia. Identification and treatment of underlying cause may be beneficial in the management of patients with acquired AA. It may also guides in prevention of the disease.

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