ORIGINAL ARTICLE

EPIDEMIOLOGY OF ACQUIRED APLASTIC ANAEMIA IN PAKISTAN

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Background: Acquired aplastic anaemia is a rare disease which results in morbidity and mortality at a young age. This study was carried out to determine the clinical presentation, haematological parameters and association factors of acquired aplastic anaemia in a cohort of Pakistani patients.

Methods: This was a cross-sectional study conducted at Haematology Department, Sheikh Zayed Hospital, Lahore over 7 years from June 2000 to July 2007. Eighty-two patients of acquired aplastic anaemia were enrolled in the study by non-probability purposive sampling. Their diagnosis was confirmed by complete blood count, bone marrow aspirate and trephine biopsy. The cohort was classified on the basis of severity and the epidemiological, clinical and haematological parameters were analysed.

Results: Of the 82 enrolled patients of acquired aplastic anaemia, 49 (59.8%) were males and 33 (40.2%) were females. Mean age of the patients was 27.93±18.7 years with a range of 1–80 years. The male to female ratio was 1.48:1. Bone marrow cellularity was less than 25% in 31 (38.0%) cases and between 25–30% in 51 (62%) of patients. Most of the cases were clinically severe aplastic anaemia (68%). In 62 (76%) of the cases no association factors predisposing to aplastic anaemia could be identified. Conclusion: Acquired Aplastic anaemia is a disease of all ages. In the second decade and the elderly predominantly severe clinical stages were seen. Males presented at a younger age while females presented at all ages with a somewhat similar incidence. No association factors of Aplastic Anaemia could be identified in majority of the patients.

Keywords: Aplastic anaemia, epidemiology, clinical staging

INTRODUCTION

Acquired aplastic anaemia (AA) is a rare disease which results in morbidity and mortality at a young age.1 The incidence ranges from two to six new cases per 1 million inhabitants per annum.1,2 The incidence of aplastic anaemia in the West is 2 per million with a recently reported 2.34 per million per year in Barcelona.3 It is about 2–3 fold higher in Asia.4 AA most commonly presents between 15 years and 25 years, but there is a second smaller peak in incidence after 60 years in West and USA.5 A study conducted at Aga Khan University Hospital, Karachi concluded that AA is found to occur mostly in young males and most common type was idiopathic severe AA.6 The Barcelona study reported severe and very severe aplastic anaemia to be the commonest stage of presentation.3

Recent evidence suggests an immune-mediated process underlying aplastic anaemia pathogenesis.7 Most cases are classified as idiopathic as no etiological agent can be identified. Nonetheless, several factors are associated with an increased risk. Aplastic anaemia has been etiologically associated with chemical such as pesticides and benzene, and drugs especially chloramphenicol in many studies.8 Five to ten percent of cases of aplastic anaemia follow an episode of seronegative hepatitis.9 The diagnostic criteria and clinical staging of AA is given in Table-1.10

This study was conducted to evaluate the demographic features, presenting features, association factors, haematological parameters and clinical stage in patients of acquired AA in a cohort of Pakistani patients and correlate them with other regional and international reports.

MATERIAL AND METHODS

This cross sectional study was carried out to describe the clinical presentation, haematological parameters and association factors of aplastic anaemia in patients coming to Shaikh Zayed Hospital, Lahore over a period of 7 years from June 2000 to July 2007. All cases of hypo-cellular bone marrow were identified. Fanconi’s Anaemia was excluded by physical examination for skeletal deformity and confirmation by cytogenetic studies in suspected cases. Hypo-plastic acute leukaemia and hypoplastic Myelodysplastic Syndrome (MDS) cases were excluded by identifying dysplasia in 10% cells of any cell line, blast count 1% or above in bone marrow nucleated cells, ring sideroblasts on Perl’s stain and Abnormal Localization of Immature Precursors (ALIP) or reticulosis in trephine biopsy.11 Post-chemotherapy and radiotherapy marrow aplasias, i.e., within 6 months of remission induction in leukaemias, lymphomas and solid tumours were also excluded. Eighty two patients were diagnosed to be suffering from acquired aplastic anaemia AA by the given diagnostic criteria (Table-1) during the study time. They were included by non-probability purposive sampling. Informed consent was taken from the patient and socio-demographic data like name, age, sex, occupation was
collected. Patient was specifically asked for exposure to toxins or chemicals at the place of work or residence near factory area. A detailed history was taken about fever; bleeding from any site including multiple bruises with minor trauma, purpura, epistaxis, gum bleed, hematomenesis, haemoptysis, haematochezia, malena, haematuria, menorrhagia, excessive bleeding from wounds or cuts or after a surgical procedure; breathlessness on mild exertion and easy fatigability. General physical examination was carried out including pallor; fever; bleeding manifestations in the skin (e.g., bruises and purpura), signs of bleeding from the nose, oral cavity, vagina, anal canal; accessible lymph adenopathy in the cervical, axillary and inguinal region. Hepatomegaly and splenomegaly were sought in abdominal examination and confirmed by abdominal ultrasound. The blood sample from the patients were tested for Complete Blood Counts (CBC) including Haemoglobin (Hb), Total leucocyte count (TLC), Platelet count, Mean cell volume (MCV). Blood film was stained by May-Grunwald-Giemsa stain and peripheral smear examination was carried out for differential leucocyte count (DLC), identification of any blasts, presence of dysplasia. Bone marrow aspirates were done from right posterior iliac crest with Islam Bone Marrow Aspirate Biopsy Needle®. May-Grunwald-Giemsa staining was performed on the aspirate. The bone marrow trephine biopsies were done by Islam Bone Marrow Trephine Biopsy Needle® from the same site and stained with Haematoxylin and Eosin stains. Slides were evaluated for cellularity, reticulosis and evidence of dysplasia. Cellularity assessment was based on visual examination and graded into two groups for all identified hypocellular samples 

i) Less than 25% cellularity 

ii) 25–50% cellularity 

The aspirates were stained with Perls’ stain for assessment of iron stores and identification of ring sideroblasts. Serology for hepatitis B surface antigen (HBsAg), and Antibody to Hepatitis C (anti HCV) was also done. Cytogenetic analysis was carried out in selected cases to exclude possibility of hypoplastic MDS and Fanconi’s anaemia. LAP score, ham’s test, sucrose lysis test and urine for haemosiderin were carried out in suspected cases to exclude paroxysmal nocturnal haemoglobinuria. 

The collected data was entered into SPSS version 13 for analysis. Nominal data of variables including pallor, fever, bleeding, splenomegaly. Hepatomegaly, lymphadenopathy; bone marrow features including cellularity, and iron stores were expressed as frequency percentage. The variables in CBC including Hb, TLC, Platelet count, absolute neutrophil count ANC, absolute lymphocyte count ALC and MCV were recorded as Mean±SD. 

### Table 1: Classification of Acquired Aplastic Anaemia

<table>
<thead>
<tr>
<th>Classification</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severe Aplastic Anaemia (SAA):</strong></td>
<td>Bone marrow cellularity of &lt;25% or 25–50% with &lt;30% residual haemopoietic cells plus two out of three of the following:</td>
</tr>
<tr>
<td></td>
<td>- neutrophil count &lt;0.5×10^9/L,</td>
</tr>
<tr>
<td></td>
<td>- platelet count &lt;20×10^9/L</td>
</tr>
<tr>
<td></td>
<td>- absolute reticulocyte count &lt;2×10^12/L</td>
</tr>
<tr>
<td><strong>Very Severe Aplastic Anaemia (VSAA):</strong></td>
<td>As for severe AA but neutrophil count &lt;0.2×10^9/L</td>
</tr>
<tr>
<td><strong>Non-Severe Aplastic Anaemia (NSAA):</strong></td>
<td>Patients with a hypocellular marrow, but not fulfilling the criteria for severe or very severe AA with two of three of the following:</td>
</tr>
<tr>
<td></td>
<td>- neutrophil count &lt;1.5×10^9/L,</td>
</tr>
<tr>
<td></td>
<td>- platelet count &lt;100×10^9/L</td>
</tr>
<tr>
<td></td>
<td>- haemoglobin &lt;10 g/dL</td>
</tr>
</tbody>
</table>

### RESULTS

Out of 82 diagnosed patients according to AA criteria, there were 49 (59.8%) males and 33 (40.2%) females in the cohort. Mean age of the patients was 27.9±18.7 years with a range of 1–80 years. Mean age in male patients was 28.9 years (SD=19.57) and in female patients was 26.48 (SD=17.54) years. The age of onset presented a definite decline with progressive age (Figure-1). Most of the patients (n=32), i.e., 39% presented in second decade. The male to female ratio of 1.48:1 was observed. However as shown in Figure-2 the female population had a somewhat similar age of onset in various decades with a small peak in the second only. This was in contrast to the male population which showed a steep peak in the second decade followed by a steady decline.

On analysis of clinical presentation history of progressive anaemia was present in (n=59) 72% cases; fever was present in (n=30) 36, 6% cases; history of bleeding manifestations in (n=43) 52.4% patients. Lymphadenopathy was seen in (n=3) 3.7% cases. Hepatomegaly was seen in (n=1) 1.2% cases; splenomegaly was not seen in any cases.

Analysis of laboratory data in 82 patients of aplastic anaemia is presented in Table-2. Bone marrow biopsy and trephine examination revealed that bone marrow cellularity was less than 25 percent in (38.0%) and between 25–30 percent in (62%) of patients. Erythropoiesis, myelopoiesis and megakaryopoiesis were found to be markedly depressed in all of patients but there was no evidence of dysplasia in the bone marrow. Iron stores were present in 76 patients. They were increased in 68 patients, normal in 8 and absent in 6 cases. Most of the cases presented at the SAA stage (68%). NSAA was seen in 20% of the patients and VSAA was present in 12% cases. VSAA was seen predominantly in the second decade (25% of cases falling between the age of 10–20 years) and the elderly aged above 60 years also showed a more severe spectrum of disease with 55% SAA and 18% VSAA. The age group from 25–59 years showed no cases of
VSAA. They consisted of 73% SAA and 27% NSAA. In the age group below 25 years 17% cases were VSAA, 69% were SAA, and 15% cases were NSAA (Figure-1).

About 80% of these cases (n=65) of AA were considered idiopathic as no cause or association factor was identified; 6% (n=5) cases were associated with positivity of hepatitis markers (HBsAg positive in 2 cases, Anti HCV positive in 5 cases). Two cases were attributed to chloramphenicol of which one child was exposed to chloramphenicol eye drops. Ten cases were declared to be related to possible toxic exposure; from pesticides in the agriculture sector (n=7), chemicals in the dye industry (n=2) and residence adjacent to factory area (n=1).

Comparison of the clinical stage with previous studies 

Studies from India and Thailand reported that the male to female ratio was close to 1:1.5. 

From Pakistan previous studies had reported a higher male to female ratio. Hanif et al reported male preponderance with a male to female ratio 3.4:1 and most of their patients were also found in second decade of life. However, Hassan K reported that males were more commonly affected with a male: female ratio of 3.3:1. Adil et al reported in their study that most of patients were below 30 years of age and 74% were males and 26% were females. An Indian study done by Gupta V et al concluded that males outnumbered females by 5:1. 

In the studied cohort, progressive anaemia was the most common clinical finding, followed by fever in frequency. The most frequent infection was upper respiratory tract infections seen in 27 cases. History of fever was present for more than one month in 22 patients. Bleeding from gums was a predominant symptom seen in 43 patients followed by petechiae/bruises in 38, epistaxis in 14, haematuria in 6 cases. History of menorrhagia was present in all menstruating female patients. Pallor was the predominant clinical sign observed in the present study. Gupta et al similarly reported pallor to be the most important clinical sign observed in 100% of their patients.

The predominant clinical stage identified was SAA. Studies from Barcelona and Karachi, Pakistan also give comparable results. VSAA has a very poor prognosis with a life expectancy much poorer than the other categories. It was found only in the second decade and elderly.

No etiological agents could be identified in majority of the patients. Ten patients gave positive history for possible toxin exposure from pesticides in the agriculture sector, chemicals in the dye industry and residence near factory area. No pregnant females were identified in the cohort. Hassan K studied 43 cases of severe AA and reported that etiologically 58.1% had idiopathic AA.  Studies from India and Thailand implicated pesticide exposure as a common etiological agent for AA. We found 5 patients (6%) with antibody to hepatitis C and HBsAg at the time of diagnosis of AA. Adil et al has reported 15.9% aplastic anaemia patients to have either hepatitis B virus markers or antibody to hepatitis C at the time of diagnosis.
Brown et al. reported that no causative agent for hepatitis was found in majority of the patients tested.16

CONCLUSION
Aplastic anaemia afflicted all ages and both sexes. It was seen in the severer forms in the second decade and the elderly. Males presented in the early age group predominantly while females presented at all ages with a similar incidence. SAA was the most common clinical stage. Pallor and bleeding were common clinical features. No association factors of AA could be identified in most of the patients.

Tabel-3: Comparison of age and sex distribution of the present study with study from Bangkok12

<table>
<thead>
<tr>
<th>Study</th>
<th>Male</th>
<th>Female</th>
<th>1–25 years</th>
<th>26–39 years</th>
<th>40–59 years</th>
<th>60+ years</th>
</tr>
</thead>
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<td>43</td>
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<td>18</td>
<td>12</td>
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<tr>
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<td>51</td>
<td>16</td>
<td>28</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
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<td>60</td>
<td>21</td>
<td>9</td>
<td>23</td>
<td>47</td>
</tr>
<tr>
<td>Lahore</td>
<td>60</td>
<td>40</td>
<td>59</td>
<td>18</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

Figures express percentage of cases

REFERENCES

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