Frequency of Common Chromosomal Abnormalities in Patients with Idiopathic Acquired Aplastic Anemia

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ABSTRACT

Objective: To determine the frequency of common chromosomal aberrations in local population of idiopathic acquired aplastic anemia at the time of diagnosis, using G-banding cytogenetic analysis.

Patients and Methods: This cross sectional study was conducted in Department of Haematology, Pakistan Institute of Medical Sciences, and Islamabad and Department of Genetics, Children Hospital, Lahore from June 2015 to July 2017. Sample size was calculated using WHO sample size calculator. A total of sixty cases of peripheral blood pancytopenia having clinical suspicion of acquired aplastic anemia and diagnosed on bone marrow examination with aplastic anemia were included in the study. Bone marrow or peripheral blood samples were also processed for cytogenetics by G-banding and karyotyping according to International System for Human Cytogenetic Nomenclature (ISCN) to determine frequency of chromosomal abnormalities in the patients of acquired aplastic anemia.

Results: Sixty cases diagnosed to have acquired aplastic anaemia using bone marrow examination as gold standard were included in the study based on inclusion criteria. Forty-five out of 60 patients (75%) had successful karyotyping whereas 15 out of 60 patients (25%) had inconclusive cytogenetics due to culture failure, inadequate metaphase cells and contamination. G-banding revealed normal karyotyping in 40 out of 45 patients (88.9%) while 5 out of 45 patients (11.1%) were found to have abnormal karyotyping. Chromosomal abnormalities revealed by abnormal karyotyping included three numerical abnormalities i.e. monosomy 7, trisomy 8, trisomy 14 and two structural abnormalities i.e. deletion of 11q, deletion of 13q. The frequency of chromosomal abnormalities in patients with acquired aplastic anemia in this study was found to be 11.1%.

Conclusion: Cytogenetic analysis may be beneficial in differentiating acquired AA from other haemopoietic disorders of bone marrow failure, which may be missed, based on cell morphology alone. It also guides in deciding appropriate mode of treatment earlier and predicting prognosis of the disease.

Key words: Aplastic anemia, Chromosomal, Cytogenetic Nomenclature


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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. Aplastic anemia is considered if at least two of the following defining criteria 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. Disease severity of aplastic anemia is based on the criteria given by Camitta et al. in 1975. 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 Camitta criteria is based on blood cell counts and bone marrow cellularity. Grading of disease is significant in management decisions but has less prognostic value in terms of response to immunosuppressive therapy.

Incidence of aplastic anaemia in West is around 2 per million populations in a year, that is twofold higher (3 to 4 per million population) in Asia. Aplastic anaemia can be congenital or acquired. 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. Some cases of congenital aplastic anemia may lack the characteristic phenotypic abnormalities. 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. 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. 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.

No definitive causative factor is found in majority of the cases of acquired aplastic anemia. Various drugs, chemicals, 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 aplastic anemia. Cytogenetic abnormalities have been described infrequently worldwide in few patients (12%) with otherwise typical aplastic anaemia at diagnosis. Common cytogenetic abnormalities related with idiopathic acquired aplastic anemia include trisomy 6, trisomy 8, trisomy 14, trisomy 15, monosomy 7, monosomy 19, del 5q and del 7q.

Figure 1: G-Banding Chromosomal analysis of peripheral blood cell culture reveals male karyotypewith a missing chromosome no.7 in 20 metaphase cells examined. This finding is consistent with diagnosis of Monosomy 7

Figure 2: G-Banding Chromosomal analysis of bone marrow cell culture reveals female karyotype with an extra chromosome no.8 in 20 metaphase cells examined. This finding is consistent with diagnosis of Trisomy 8
Monosomy 7 is associated with a high risk to develop haematological malignancies (MDS or AML) and has poor response to immunosuppressive therapy (IST) with poor prognosis. In contrast, trisomy 8 is associated with good response to IST and has better prognosis.

Cytogenetic analysis is attempted to detect cytogenetic abnormalities related with acquired aplastic anaemia, although it is difficult to perform because of inability to obtain sufficient metaphase cells for chromosomal analysis in a hypocellular bone marrow. In such situation, molecular cytogenetics by fluorescent in situ hybridization (FISH) can be attempted which is limited to few centers only and has a higher cost constraint.

Clinical significance of cytogenetic abnormalities may also be that hypo plastic MDS or hypo plastic AML may present as aplastic anaemia and it can be difficult to distinguish two conditions on basis of morphology alone, treatment option and response to treatment is also different in such condition.

Cytogenetic analysis is limited to a few centers in Pakistan. Incidence of aplastic anemia is relatively more common in Southeast Asian countries. No study has been conducted before, to find association of cytogenetic abnormalities with acquired AA in Pakistan. The objective of this study was to determine frequency of common chromosomal aberrations among the patients of idiopathic acquired aplastic anemia in local population at the time of diagnosis by G-banding cytogenetic analysis.

Figure 3: G-Banding Chromosomal analysis of peripheral blood cell culture reveals female karyotype with an extra chromosome no.14 in 16 metaphase cells examined. This finding is consistent with diagnosis of Trisomy 14.

Figure 4: G-Banding chromosomal analysis of bone marrow cell culture reveals male karyotype with deletion of q-arm of chromosome no.11 in 16 metaphase cells examined. (del 11q)

Figure 5: G-Banding chromosomal analysis of peripheral blood cell culture reveals female karyotype with deletion of q-arm of chromosome no.13 in 16 metaphase cells examined. (del 13q)

Patients and Methods

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. In 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 included 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 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.
Sample size was calculated according to WHO sample
size calculator with 95% confidence interval, prevalence
of 12% cases of AA with chromosomal abnormalities and
8% margin of error and sample size calculated was 60
and sampling was done by convenient sampling
technique.

Demographic profile and clinical data of the patients was
taken. Systemic examination was performed to find
positive signs of anemia, haemorrhage, infections,
lymphadenopathy, hepatomegaly, splenomegaly and
especially any dysmorphic features to exclude congenital
AA. Biochemical and radiological findings, CBC,
reticulocyte count, peripheral blood smear and bone
marrow (aspiration and trephine biopsy was done to
confirm diagnosis of aplastic anemia Cytogenetics or
chromosomal analysis was done by Giemsa Trypsin
banding prior to induction of immunosuppressive therapy.
Blood or Bone marrow samples were collected under
aseptic measures in sterile sample collection tubes (green
top vacutainers) containing sodium heparin anticoagulant
to prevent coagulation. Samples were transported to
genetics lab. at room temperature within 24 hours for
cytogenetic analysis where they were processed for cell
culture, culture harvesting, slide preparation and slide
staining. Karyotyping was done according to (ISCN)
International System for Human Cytogenetic
Nomenclature21 metaphase and karyotype images were
seen using cytovision system (microscope, camera,
monitor, computer, software Macktype 5.6) for
chromosome analysis. At least 16 to 20 metaphase cells
were analyzed at each microscopic examination for a
successful result. Cytogenetic analysis was labelled
inconclusive if metaphase cells were less than 16. A
cytogenetic abnormality was considered to exist when 2
or more cells had the same structural or numerical
chromosomal abnormality (86). Data was recorded and
analyzed using Statistical Package for Social Sciences
(SPSS) Version 20. Median was calculated for Age of the
study participants and Frequency (%) of chromosomal
abnormalities was calculated in the study patients
diagnosed to have acquired aplastic anemia.

## Results

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
anaemia on chromosomal breakage analysis. Sixty out of
64 cases (93.8%) diagnosed to have acquired aplastic
anaemia 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 patients of acquired aplastic anemia,
34 were male and 26 were female. Age distribution of the
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.

Forty five out of 60 patients (75%) had successful
karyotyping among those 23 were male and 22 were
female whereas 15 out of 60 patients (25%) had
inconclusive cytogenetics.

### Table 1: Severity distribution of aplastic anemia
among study patients

<table>
<thead>
<tr>
<th>Severity</th>
<th>NSAA</th>
<th>SAA</th>
<th>VSAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>73.3%</td>
<td>21.7%</td>
<td>5%</td>
</tr>
</tbody>
</table>

### Table 2: Frequency breakup of Cytogenetics
results among study patients

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>Percentage</td>
<td>89.9%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>
### Table 3: Categories of Chromosomal abnormalities in acquired aplastic anemia

<table>
<thead>
<tr>
<th>Numerical abnormalities (N=3)</th>
<th>Structural abnormalities (N=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomy 7</td>
<td>Deletion 11q</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>Deletion 13q</td>
</tr>
<tr>
<td>Trisomy 14</td>
<td></td>
</tr>
</tbody>
</table>

Inconclusive cytogenetics can be due to cell culture failure, inadequate metaphase cells or contamination. G-banding revealed normal cytogenetics results in 40 out of 45 patients (89.9%) among those 24 were male and 16 were female, while five out of 45 patients (11.1%) had cytogenetic abnormalities among those two were male and three were female, table 2. Majority of the patients of acquired aplastic anemia (n=40) included in this study had normal cytogenetics. In total 44 patients had NSAA, 13 SAA and 3 patients had VSAA (Table 1). Among five patients with chromosomal abnormalities, three patients had severe aplastic anaemia (SAA) while two patients were diagnosed to have non-severe aplastic anaemia (NSAA). Chromosomal abnormalities revealed by G-banding in five patients of acquired aplastic anemia were numerical and structural. Three out of five patients had numerical chromosomal abnormalities among those one was male and two were female having monosomy 7, trisomy 8 and trisomy 14 respectively whereas two out of five patients had structural chromosomal abnormalities among those one was male and one was female having deletion of 11q and deletion of 13q respectively. All five patients of acquired aplastic anemia found to have abnormal cytogenetics in this study were in age group of 1 - 18 years. The frequency breakup of chromosomal abnormalities is depicted in table 3.

**Discussion**

Aplastic anaemia is haematological disorder of bone marrow failure characterized by T cell mediated destruction of haemopoietic cells. In the current study, cytogenetic abnormalities were observed in 11.1% patients of acquired aplastic anemia. Trisomy was the commonest numerical cytogenetic abnormality seen in two patients followed by monosomy in one patient. Deletions were the structural cytogenetic abnormalities found in two patients. Clinical and haematological profile of the patients with abnormal cytogenetics were similar to those with normal cytogenetics. Some of these characteristic cytogenetic abnormalities are also seen in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), however the morphological diagnosis was consistent with aplastic anemia. It may be difficult to distinguish two conditions on the basis of cell morphology alone therefore in this situation abnormal karyotype may suggest diagnosis of hypoplastic MDS or hypoplastic AML. Cytogenetic analysis can play a significant role in management of patients with cytogenetic interpretation, which can reduce risk of misdiagnosing hypoplastic MDS and hypoplastic AML as aplastic anemia, monosomy and trisomy are mostly seen in aplastic anemia, deletions favor diagnosis of MDS whereas translocations and inversions are commonly found in AML. Cytogenetic analysis in this way may be most beneficial in differentiating aplastic anemia from other haemopoietic disorders of bone marrow hypoplasia/aplasia, therefore guides in suggesting appropriate mode of treatment that helps in avoiding possibility of mistreating the patients. Some authorities exclude diagnosis of aplastic anaemia in patients with abnormal cytogenetics regardless of bone marrow morphology, however in some research centers patients with abnormal cytogenetics are diagnosed as aplastic anemia on morphological grounds. Response to therapy and survival in patients of aplastic anaemia with normal and abnormal cytogenetics was also compared in some studies. Patients without cytogenetic abnormalities have good response to immunosuppressive therapy (IST) and better survival therefore cytogenetic analysis helps in predicting prognosis of aplastic anemia. Patients with persistent cytogenetic abnormalities after treatment are at higher risk of developing haematological malignancies (AML or MDS). Patients of MDS presents with clinical
manifestations of bone marrow failure which are related to anaemia, neutropenia and thrombocytopenia. Immunosuppressive therapy (IST) improves pancytopenia but response is relatively poor in patients of MDS. In few patients, acquired aplastic anaemia may develop into acute leukemia. Patients of AML presents with clinical features of bone marrow failure and organ infiltration by leukemic cells. Chemotherapy regimens have limited role in treating patients with AML. 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.

**Conclusion**

Cytogenetic analysis may be beneficial in differentiating acquired AA from other haemopoietic disorders of bone marrow failure, which may be missed, based on cell morphology alone. It also guides in deciding appropriate mode of treatment earlier and predicting prognosis of the disease.

## References


