Karyotypic Analysis of Acute Myeloid Leukemia (AML) in 75 Referral Cases: One Year Report

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ABSTRACT
We have analyzed 75 referral cases of Acute Myeloid Leukemia (AML) registered in our Research Institute, Supratech Genopath Laboratory of Ahmedabad. Karyotype analysis of bone marrow and blood samples revealed 7 cases (9.3%) were AML positive. Out of these cases, 3 patients (4%) have recurrent chromosomal anomalies of t(8;21) and t(15;17) and other 4 (5.3%) had AML with MDS related changes in which one karyotype had 4 chromosomal abnormalities viz, two each of trisomy and monosomy respectively. This group has poor prognosis.

KEY WORDS: Patients, AML, AML-MDS Changes, Chromosomal Anomalies

Introduction
Acute Myeloid Leukemia (AML) is one of the heterogeneous groups of leukemia exhibiting clonal expansion of myeloid blast cells in bone marrow and blood including tissue. It can be detected by morphology as well as cytochemistry including cytogenetics, molecular and Fluorescence in situ hybridization (FISH) techniques. Out of morphological and cytochemical methods, chromosomal analysis is a better method to classify these diseases (Byrd et al., 2002; Wong and Bailey. 2015; Grimwade et al. 2016). However, FISH method is restricted for some leukemias only. The purpose of the study is to identify heterogeneous groups of AML using chromosomal anomalies based on WHO classification (Swedlow et al., 2008) and compared to that of FAB (Dick et al., 1982) and illustrate the importance of cytogenetic studies to face diagnosis and prognostication (Shipley and Butera, 2009; Betz and Hess, 2010). The AML may also occur because progression of myelodysplastic syndrome or chronic bone marrow stem cell disorders; Hence AML with MDS has particularly poor prognosis (Kumar. 2016; Appelbaum, 2004; Licht, 2006). So, this study was done in referral cases registered at our Institute of Supratech Genopath Laboratory last year (2015-2016).

Material and Methods
Patients
Seventy five cases ranging in age from 1 to 72 years of suspected of AML were included in our studies. Their bone marrow and blood samples were collected in sodium heparin vials as per the instruction and then utilized for karyotype analysis. A volume of 0.5 ml of each sample was used for chromosome preparation using the method of Moorhead et al. (1960). The samples were incubated at 37°C (post colchicine addition 30µl at 69th hour) for 72 hrs. Then, they were harvested after treating with hypotonic solution. The metaphase slides were prepared with fixative and stained
Results

Cytogenetic Analysis

From a total of 75 cases, seven patients exhibited AML positive. Others (68) had normal karyotype of either 46,XX(47) or 46,XY(21). Three AML cases showed recurrent chromosomal rearrangements ie. t(8;21) and t(15;17). These classified AML patients had M₂, M₃ types of FAB classification with 85% and 90% blasts. Four had abnormal karyotypes related to aneuploid condition. The FAB classification indicated variable blast counts (26%). These AML-MDS related changes revealed various blood cell morphology also (Table-1).

Table 1: Analysis of referral AML Patients

<table>
<thead>
<tr>
<th>Age(Years)</th>
<th>Karyotype (WHO)</th>
<th>FAB Type</th>
<th>AML/Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-72</td>
<td>46,XX, (47); 46,XY (21)</td>
<td>- Normal</td>
<td></td>
</tr>
<tr>
<td>72*</td>
<td>46,XX,+13,+16,-11,-18 (01)</td>
<td>-</td>
<td>AML with MDS</td>
</tr>
<tr>
<td>16</td>
<td>47,XY,+11 (01)</td>
<td>M6</td>
<td>AML with MDS</td>
</tr>
<tr>
<td>16</td>
<td>47,XX,+11 (01)</td>
<td>M1,M2,M4</td>
<td>AML with MDS</td>
</tr>
<tr>
<td>01</td>
<td>47,XY,+21 (01)</td>
<td>M7</td>
<td>AML with MDS</td>
</tr>
<tr>
<td>25</td>
<td>46,XX,t(15;17)(q24;q21) (01)</td>
<td>-</td>
<td>M3 AML</td>
</tr>
<tr>
<td>12 and 18</td>
<td>46,XX,t(8;21)(q22;q22) (02)</td>
<td>-</td>
<td>M2 AML</td>
</tr>
</tbody>
</table>

*Complex Karyotype; Figures in Parenthesis indicate case numbers.

The pie chart indicated graphical representation of these cases (Fig. 1). Complex karyotype showed monosomy and trisomy conditions of chromosomal aberrations. Monosomy related to -11 and -18, trisomy of chromosomes +13 and +16 indicating chromosomal changes were observed in this case (Fig. 2).

![Figure 1: Pie chart showing percent of AML and others](image)

Discussion

Our study reports the cytogenetic analysis of 75 cases referred for AML checkup. Their blood and bone marrow samples were subjected to routine cytogenetic analysis. Only seven (9.3%) cases seemed to be AML positive. Three (4%) cases were cytogenetically AML as these have t(8;21) and one has t(15;17) anomalies.

![Figure 2: Complex Karyotype of AML with MDS related changes (46,XX,+13,+16,-11,-18)](image)

No case was identified for inv (16) or t(16;16) anomaly according to WHO classification, (Wang and Bailey, 2015). These cytogenetic markers are well established for identification of AML from other heterogenous subgroups. These recurrent cytogenetic abnormalities i.e. t(8;21)(q22;q22), AML/ETO and t(15;17)(q22;q12): PML/RARA found in our study are correlated to FAB classification of M₂ having large blasts of 85% with Auer rods as well as M₃ having promyelocytic leukemia with abnormal granulocytes between myeloblasts and monocytes followed...
by bleeding and coagulopathies respectively. It seems that many AML patients are of higher range of these chromosomal translocation (Jaffe et al. 2001). In the present study, too 4 patients (5.3%) had specific chromosomal aberrations in relation to their gain and loss and were considered as AML with myelodysplastic syndrome (MDS) related cancer or myelodysplastic/myeloproliferative disorders or dysplastic with sufficient degree of morphology of myeloid lineages in bone marrow (Sun, 2008). Wang and Bailey (2015) in contrast to the recurrent chromosomal rearrangements, patients may have tendency to have a pattern of unbalanced chromosomal changes as noticed in our report and also balanced abnormalities as well as complex karyotypes. They have presented such cases with adverse prognosis having -5, -7, del (5q), 11q23 abnormalities related to AML with MDS as unbalanced anomalies. Further these others also reported balanced abnormalities having t(11;16) (q23:p13.3), t(3;5)(q25;p34) etc. However, complex karyotypes with poor prognosis included 3 or more cytogenetic abnormalities who live only one year were not reported (Wang and Bailey. 2015). Betz and Hess (2010) mentioned that complex karyotypes are monosomal karyotype in which at least 2 autosomal, monosomies or an autosomal monosomy in the presence of 1 or more structural anomalies. Trisomy of 11, 13, 21 were reported earlier as unbalanced AML with MDS who analyzed karyotypically 21,403 AML patients (Caramazza et al, 2010 ; Stozel et al, 2016) to support our data.

In our one year study, we found trisomy of +12, +13, +21 in 3 patients under unbalanced AML-MDS related changes and one case whose age was 72 years, had complex karyotype with 4 abnormalities of +13, +16, -11, -18 in support of Betz and Hess, (2010) observation. Thus, our report documented that 9.3% referral cases were AML positive with recurrent chromosomal translocation (4%) and AML with MDS (5.3%) related changes. In the latter group, one had depicted two each of trisomy and monosomy condition of complex karyotype.

**Conclusion**

Out of seventy five live referral AML cases, 9.3% had AML and AML with MDS related changes with complex karyotype having poor prognosis.

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**References**


