July 2018

Distribution of chromosomal abnormalities commonly observed in adult acute myeloid leukemia in Pakistan as predictors of prognosis

Muhammad Shariq Shaikh  
_Aga Khan University, muhammad.shariq@aku.edu_

Zeeshan Ansar Ahmed  
_Aga Khan University, zeeshan.ansar@aku.edu_

Mohammad Usman Shaikh  
_Aga Khan University, mohammad.usman@aku.edu_

Salman Naseem Adil  
_Aga Khan University, salman.adil@aku.edu_

Mohammad Khurshid  
_Aga Khan University, mohammad.khurshid@aku.edu_

_See next page for additional authors_

Follow this and additional works at: https://ecommons.aku.edu/pakistan_fhs_mc_pathol_microbiol

Part of the _Hematology Commons, Oncology Commons_, and the _Pathology Commons_

Recommended Citation

_Available at: https://ecommons.aku.edu/pakistan_fhs_mc_pathol_microbiol/745_
Authors
Distribution of Chromosomal Abnormalities Commonly Observed in Adult Acute Myeloid Leukemia in Pakistan as Predictors of Prognosis

Muhammad Shariq Shaikh1*, Zeeshan Ansar Ahmed1, Mohammad Usman Shaikh1, Salman Naseem Adil1, Mohammad Khurshid2, Tariq Moatter1, Anila Rashid1, Farheen Karim1, Ahmed Raheem1, Natasha Ali1

Abstract

Objectives: The heterogeneous response to treatment in acute myeloid leukemia (AML) can be attributed largely to the difference in cytogenetic features identified in between cases. Cytogenetic analysis in acute leukemia is now routinely used to assist patient management, particularly in terms of diagnosis, disease monitoring, prognosis and risk stratification. Knowing about cytogenetic profile at the time of diagnosis is important in order to take critical decisions in management of these patients. The study was conducted to determine the distribution of cytogenetic abnormalities in Pakistani adult patients with AML in order to have insights regarding behavior of the disease. Methods: A retrospective analysis of all the cases of AML (≥15 years old) diagnosed at Aga Khan University from January 2011 to December 2016 was performed. Cytogenetic analysis was made for all cases using the trypsin-Giemsa banding technique. Karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN) criteria. Results: A total of 321 patients were diagnosed with AML during the study period, of which 288 samples successfully yielded metaphase chromosomes. The male to female ratio was 1.7:1. A normal karyotype was present in 61% (n=176) of the cases whereas, 39% (n=112) had an abnormal karyotype. Of the abnormal cases, t (8;21) (q22;q22) and t (15;17) (q22;q12) were identified in 8.3% and 4.9% cases respectively. Adverse prognostic cytogenetic subgroups including complex karyotype, monosomy 7 and t(6;9)(p23;q34) were identified in 9%, 1% and 0.7% patients respectively. Conclusions: This largest cytogenetic data in adult AML from Pakistan showed comparable prevalence of favorable prognostic karyotype to international data. The prevalence of specific adverse prognostic karyotype was low.

Keywords: AML- cytogenetics- G-banding- metaphase- adult- Pakistan

Asian Pac J Cancer Prev, 19 (7), 1903-1906

Introduction

Acute myeloid leukemia (AML) is a malignant disorder characterized by clonal expansion and accumulation of precursor myeloid cells with a reduced capacity to differentiate into more mature cellular elements. It can occur at all ages but has its peak incidence in the seventh decade. The heterogeneity of AML in terms of morphology, immunological phenotype, cytogenetic and molecular abnormalities is reflected in substantially different response to treatment between cases.

Treatment related mortality and resistance to standard chemotherapy are the two chief determinants of risks in AML (Estey, 2013). Diagnostic karyotype in AML predicts disease resistance and allows risk-stratified treatment approaches to be followed. Comprising of 11% of all cases, “AML with recurrent genetic abnormalities” is a separate entity recognized by 2008 World Health Organization (WHO) classification (Vardiman et al., 2009). This definition has been retained in updated 2016 classification as well (Arber et al., 2016). It is the most influential independent prognostic factors in terms of treatment outcomes (Grimwade and Hills, 2009).

Amongst various familiar cytogenetic abnormalities in AML, t (15;17) (q22;q12) in patients with acute promyelocytic leukemia (APL), t (8;21) (q22;q22) and inv (16) (p13q22)/t (16;16) (p13;q22) have been consistently found associated with better outcomes. Cure rates up to 60-70% have been documented in several assessments (Zhu et al., 2013). Conversely, abnormalities of 3q (abn(3q)), deletions of 5q (del(5q)), monosomies of chromosome 5 and/or 7 (-5/-7), t(9;22) or complex karyotype (more than three unrelated changes) are
associated with very poor prognoses. In fact, monosomal karyotype invariably portends resistant disease even after allogeneic bone marrow transplant (Kayser et al., 2012). The prognosis of normal karyotype, the commonest cytogenetic feature in AML, is highly variable ranging from cure to highly refractory disease. The influence of underlying mutations on outcome of such cases is well known (Schlenk et al., 2008).

The cytogenetic data of Pakistani adults with AML is scarce. Literature review retrieved a couple of small studies addressing the cytogenetic profile of Pakistani AML patients (Harani et al., 2006; Aziz and Qureshi, 2008). The current study aimed in determining the distribution of chromosomal abnormalities in Pakistani adult patients with AML in order to have an insight about the behavior of this condition.

Materials and Methods

Study area and subjects

This was a cross-sectional analysis performed at Aga Khan University Hospital in the Sections of Hematology and Molecular Pathology. Using non-probability consecutive sampling technique, all patients diagnosed as AML who were ≥15 years of age from January 2011 to December 2016 were included in the analysis. Cases which didn’t yield metaphase chromosome were excluded from the analysis.

Diagnosis

In all cases, the diagnosis of AML was confirmed by morphology and appropriate cytochemical staining. Immunophenotyping by either flow cytometry or immunohistochemistry was performed where possible by the use of standard methodologies.

Cytogenetic analysis

Analysis was performed on pretreatment bone marrow samples by the use of conventional G-banding techniques. Bone marrow samples were cultured using standard culture techniques followed by harvesting (incubation, centrifugation and addition of hypotonic solution). After addition of fixative (3:1 methanol to glacial acetic acid) and trypsin treatment, Giemsa staining was performed. Slides were examined under microscope and at least 20 mitosis were analyzed whenever possible.

Cytogenetic abnormalities

Chromosomal abnormalities were identified and described according to the International System for Human Cytogenetic Nomenclature (ISCN 2009, 2013, 2016). Cytogenetic abnormalities were classified into balanced, unbalanced and complex abnormalities. Complex karyotype was defined as presence of three or more clonal chromosomal abnormalities in the absence of established chromosomal abnormalities. Based on WHO 2016 update, three cytogenetic risk groups were defined as favorable, intermediate and unfavorable (Arber et al., 2016).

Data analysis

Age, gender and types of cytogenetic abnormalities were included for analysis and results were expressed as frequencies and percentages. Categorical variables were compared by the use of the Chi-square test or Fisher Exact test. Significance of mean age between two groups was calculated by Independent-Samples T-Test. A p-value of <0.05 was taken as significant.

Ethical issues

An ethical exemption to conduct this analysis was granted by the institutional ethical review board (3569-Pat-ERC-15). Written and informed consent was taken from all patients as per institutional policy before collecting bone marrow samples. Relevant counseling regarding prognostic impact of the detected abnormality was provided to all who followed up in outpatient department or in the wards during admissions.

Results

A total of 321 adults were diagnosed with AML during the study period. There were 201 males and 120 females (M:F=1.7:1). Thirty-three (10%) cases didn’t yield metaphase chromosomes and were excluded from the analysis. Of successful 288 cases with cytogenetic results, a normal karyotype was identified in 176 (61.1%) patients and abnormal karyotype in 112 (38.9%) patients (Table 1). The most prevalent favorable chromosomal abnormality was t(8;21) (q22;q22), which was present in 24 (8.3%) of 288 patients. Translocation (15;17) (q22;q12) occurred in 14 (4.9%) and inv(16)(p13q22) was present in 2 (0.7%) patients. Poor cytogenetic abnormalities including t(6;9) (p23;q34), trisomy 8, monosomy 7 and complex karyotype collectively were identified in 38 (13.2%) patients (Table 1). Thirty four (11.8%) patients had miscellaneous chromosomal abnormalities including deletions, additions, inversions, other translocations and marker chromosomes. Forty-two percent (n=122) patients were under 30 years of age however, prevalence of favorable or unfavorable

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>176 (61.1)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>112 (38.9)</td>
</tr>
<tr>
<td>Balanced</td>
<td>50 (17.4)</td>
</tr>
<tr>
<td>t (8;21) (q22;q22)</td>
<td>24 (8.3)</td>
</tr>
<tr>
<td>t (15;17) (q22;q12)</td>
<td>14 (4.9)</td>
</tr>
<tr>
<td>inv (16) (p13.1q22)</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>t (6;9) (p23;q34.1)</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Others</td>
<td>8 (2.8)</td>
</tr>
<tr>
<td>Unbalanced</td>
<td>36 (12.5)</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>7 (2.4)</td>
</tr>
<tr>
<td>Monosomy 7</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Others</td>
<td>26 (9.0)</td>
</tr>
<tr>
<td>Complex</td>
<td>26 (9)</td>
</tr>
</tbody>
</table>

Numbers and percentages presented are out of total cases with successful cytogenetic results; †Three or more clonal chromosomal abnormalities in the absence of aforementioned established chromosomal abnormalities.
cytogenetic abnormalities was not significantly different in the various age groups (p=0.3) (Table 2).

Discussion

The prognosis in AML depends on both the clinical and cytogenetic/molecular features (Yunus et al., 2015; Zehra et al., 2016). Clinical features that predict the likelihood of achieving a complete remission and subsequent disease-free survival include: younger age, good performance status, absence of prior hematological disorder like myelodysplastic syndrome or myeloproliferative neoplasms, exposure to radiation or cytotoxic agents and other medical co-morbidities (Su et al., 2013). Karyotype is one of the main determinants of prognosis in AML and all patients must undergo cytogenetic analysis at the time of diagnosis. The updated 2016 WHO classification of hematological malignancies, continues to define AML by focusing on significant cytogenetic and molecular genetic subgroups. To the best of our knowledge, we have reported the largest cytogenetic data in Pakistani adults with AML.

Acute myeloid leukemia is around twice as common in males than females. Gender distribution in our analysis was comparable with published western data with a significantly higher proportion of males (p=0.007). The distribution of favorable and unfavorable cytogenetic abnormalities with respect to the gender was not significantly different (p=0.2).

Translocation (8;21)(q22;q22) and t(15;17)(q22;q12) constituted the only recurring abnormalities with a frequency above 3%. Both these abnormalities comprise 5-8% of AML (Byrd et al., 2002; Su et al., 2014). In our study, the former occurred at a frequency of 8.1% whereas the later at 4.9%. Other specific abnormalities such as t(6;9)(p23;q34) and inv(16)(p13q22) were seen less frequently. The cumulative prevalence of favorable cytogenetic abnormalities (13.9%) including t(8;21)(q22;q22), t(15;17)(q22;q12) and inv(16)(p13q22) was not significantly higher in the different age groups (p=0.3) (Table 2).

Complex karyotype emerged as the predominant unfavorable cytogenetic risk group in this study. The prevalence of monosomy 7, the only specific unfavorable abnormality in our study was very low (1%); the two large studies reported a frequency of around 7% (Byrd et al., 2002; Grimwade et al., 2010). Other unfavorable cytogenetic abnormalities including 3q (abn(3q)), deletions of 5q (del(5q)), monosomy 5 and t(9;22) were not identified in this analysis. Several other single chromosomal abnormalities were also identified but were too infrequent to be analyzed separately.

Besides being the largest cytogenetic data in Pakistani adults with AML, other strength of this study is use of conventional cytogenetic method for karyotype determination. Conventional cytogenetic provides status of all chromosomes and hence, it identifies all the changes present in a karyotype. Nonetheless, a fraction of cases are liable to omission due to its inherent low sensitivity. More sophisticated methods like fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) have higher yields but target only specific lesion in question and therefore, information about other possible findings is not provided.

Table 2. Age Wise Distribution of Karyotype in Various Prognostic Groups

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>Favorable</th>
<th>Intermediate</th>
<th>Unfavorable</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30</td>
<td>23 (18.9)</td>
<td>81 (66.4)</td>
<td>18 (14.8)</td>
<td>122 (42.4)</td>
</tr>
<tr>
<td>31-45</td>
<td>11 (13.9)</td>
<td>61 (77.2)</td>
<td>7 (8.9)</td>
<td>79 (27.4)</td>
</tr>
<tr>
<td>&gt;45</td>
<td>6 (6.9)</td>
<td>74 (85.1)</td>
<td>7 (8)</td>
<td>87 (30.2)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (13.9)</td>
<td>216 (75)</td>
<td>32 (11.1)</td>
<td>288 (100)</td>
</tr>
</tbody>
</table>

In conclusion, this study showed recurrent cytogenetic abnormalities in 14.6% Pakistani adults with AML. Favorable karyotypes, t(8;21)(q22;q22) followed by t(15;17)(q22;q12) were identified as the most prevalent specific chromosomal abnormalities; the cumulative prevalence however was not significantly different in various age groups. The complex karyotype constituted the predominant unfavorable karyotype.

References


This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.