Biotechnol Res 2016; Vol 2(4):166-168
eISSN 2395-6763

ORIGINAL RESEARCH

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

Ketan K VAGHASIA[1], Vidhi M BHATT[1], Parth S SHAH[1], Nidhi D SHAH[1], Mandava V RAO[1,2]*, Sandip C SHAH[1]
[1]Supratech Genopath Laboratory and Research Institute, Ahmedabad, Gujarat
[2]School of Sciences, Gujarat University, Ahmedabad, Gujarat
*Corresponding Author email: manvrao@gmail.com

• *Received: 10 August 2016 • Revised: 06 September 2016 • Accepted: 21 September 2016 • Published: 06 October 2016* •

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 heterogenous 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 prognostification (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 to72 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^{o}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

with Giemsa stain. The stained slides were subjected to Carl Zeiss Automated MetaSystems for karyotyping. Bone marrow samples were processed without mitogen, phytohemagglutinin (PHA). Twenty five metaphases plates for each sample were utilized for identifying chromosomal aberrations using ISCN Nomenclature (Shaffer, LG. 2013). The karyotype data were correlated with FAB classification. The percentage of each type of disease was calculated.

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 M_2, M_3 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

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

*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).

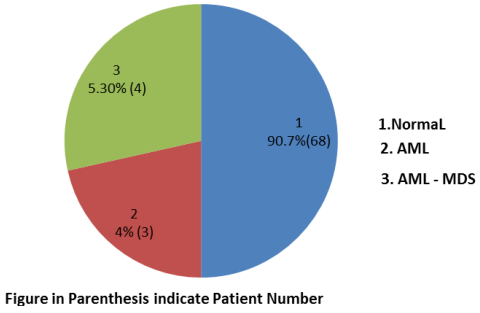

Figure 1: Pie chart showing percent of AML and others

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.

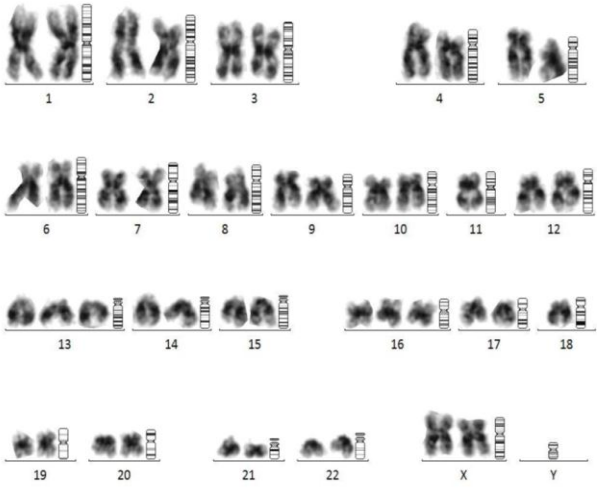

Figure 2: Complex Karyotype of AML with MDS related changes (46,XX,+13,+16,-11,-18)

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 M_2 having large blasts of 85% with Auer rods as well as M_3 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 Myelodysplastic Syndromes (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 referral AML cases, 9.3% had AML and AML with MDS related changes with complex karyotype having poor prognosis.

Acknowledgment

The authors are thankful to all staff of Supratech Genopath Laboratory for their technical help and support.

References

Appelbaum, F.R. (2004). Acute myeloid leukemia in adults. In: Clinical Oncology, Abeloff M, Armitage J, & Kastan M. (Eds), 3rd edition. Elsevier, Philadelphia, pp. 2825-2848.

Betz, B.L and Hess, J.L. (2010). Acute myeloid leukemia diagnosis in the 21st century. Arch. Pathol. Lab. Med. 134(10):1427-1433.

Byrd,,J.C., Mrozek,K., Dodge,R.K., Carroll, A.J. (2002). Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 100(13):4325-4336.

Caramazza, D., Ketterling, R.P., Knudson, R.A. et al. (2010) Trisomy 11: Prevalence among 22 403 unique patient cytogenetic studies and clinical correlates. Leukemia. 24; 1092-1094.

Grimward, D., Hills, R.K., Moorman A.V et al (2010). Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials.Blood. 22;116(3):354-365.

Dick, F.R., Armitage, J.O., and Burns, C.P. (1982). Diagnostic concurrence in the subclassification of adult acute leukemia using French-American-British criteria. Cancer. 1;49(5):916-920.

Jaffe, E.S., Harris, N.L., Herald, S. and James, W.V. (2001). WHO pathology and genetics. WHO Pub. Geneva. pp. 77-97.

Kumar, C.C. (2011). Genetic Abnormalities and Challenges in the Treatment of Acute Myeloid Leukemia. Genes. Cancer. 2(2): 95–107.

Licht, J.D. (2006). Reconstructing a disease: what essential features of the retinoic acid receptor fusion oncoproteins generate acute promyelocytic leukemia? Cancer Cell. 9:73-74

Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.M. and Hungerford, D.A. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. Experimental Cell Research, 20; 613-615.

Shaffer, L.G., Jorden, J.M and Schmid, M. (2013). An International System for Human Cytogenetic Nomenclature (ISCN), Karger, Basel.

Shipley, J.L. and Butera, J.N. (2009). Acute myelogenous leukemia. Exp. Hematol. 37:649-658.

Stolzel, F., Mohr, B., Kramer, M., et al. (2016). Karyotype complexity and prognosis in acute myeloid leukemia.Blood Cancer Journal 6, e386

Sun, T. (2008). Flow Cytometry and Immunochemistry for Hematologic Neoplasms. Williams and Wilkins Philadelphia.

Swerdlow, S.H., Campo, E., Harris, N.L. et al. (2008). WHO Classification of tumors of haemopoitic and lymphoid tissues. 4th edn. Vol. 2, IARC Press Lyon.

Wang, M.L. and Bailey, N.B. (2015). Acute myeloid leukemia genetics: Risk stratification and implications for therapy. Arch. Pathol. Lab. Med. 139(10):1215-1223