Case Report

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Acute Myeloid Leukemia with Complex Hyperdiploid Karyotype: A Case Report

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ABSTRACT

Hyperdiploidy in acute myeloid leukemia (AML) forms a subset of complex karyotype, which needs to be cytogenetically characterized so as to accurately determine its prognostic factor. The following study, involves the case of 54 year old female patient diagnosed with denovo acute myeloid leukemia by cytomorphological and immune-phenotype studies.

Cytogenetic analysis revealed a high hyperdiploid karyotype with a modal chromosome number ranging from 51~54 with the gain of chromosomes 8, 9, 11, 12 and 19 and also monosomy for chromosome 5. It also revealed other chromosomal abnormalities such as formation of derivative chromosome 10, isochromosome 21 and presence of a marker chromosome.

Present case reinforces the importance of cytogenetic categorization of an adverse-risk complex aberrant karyotype to stratify patients to individual optimized treatment strategies other than standard treatment in order to achieve good treatment outcome.

Keywords: *Acute myeloid leukemia, Hyperdiploidy, Complex Karyotype*

Introduction

Accurate assessment of prognosis is central to the management of Acute Myeloid Leukemia (AML) for treatment decisions. Cytogenetics is considered the most important independent prognostic parameter in AML.[1]

Among the various cytogenetic risk classification system in AML, core- binding factor AML (CBF-AML) with t(8;21)(q22;q22) or inv(16)(p13.1q22)/t(16;16)(p13.1;q22) are classified in the favorable-risk, those with cytogenetically normal AML (CN-AML) in the intermediate-risk, and those with a complex karyotype and monosomal karyotype are placed in the adverse-risk categories. European Leukemia Net (ELN) classifies patients in adverse genetic risk category if they have karyotypes with three or more aberrations.[2]

UK National Cancer Research Institute Adult Leukemia Working Group (abbreviated as MRC for Medical Research Council), however requires four or more abnormalities to qualify as adverse prognosis.[3]

WHO 2016 classification defines disease entities by incorporating genetic information with morphology, immune-phenotype and clinical presentation.[4]

AMLs with modal chromosome number 49-48 harbor gain of one to two chromosomes in particular gains of chromosomes 4, 8, 11, 13, 21, and 22 and termed as low hyperdiploid AML In contrast, high hyperdiploidy with modal number 49-65 chromosomes and triploidy/tetraploidy (TT;>65 chro-mosomes) are rare.[5] AMLs with HH/TT constitute < 2% of all adult AML cases and mostly have complex karyotypes (CK), they are usually grouped as high risk. However, the types of chromosome abnormality present may modify the prognosis.[6]

Present case demonstrates complex aberrant karyotype with multiple unrelated cytogenetic abnormalities such as high hyperdiploidy, monosomy 5 and presence of three non-recurrent structural abnormalities which have led to partial gains and losses of chromosomal regions. These findings highlight the characterization of complexicity for better prognostic evaluation.

Case Report

A 54 years female was referred to Global Reference Laboratory in Mumbai, Maharashtra, India - Metropolis Healthcare Ltd. Mumbai, with a history of hypercellular bone marrow, suppressed erythropoetic activity and near total replacement by sheets of blasts on morphological evaluation. The patient was diagnosed of denovo acute myeloid leukemia with monocytic differentiation on bone marrow biopsy. The patient was diagnosed and classified as AML-M5b according to the French-American-British classification on account of the morphology and relative proportion of monoblasts to promonocytes.[7]

Flow cytometry revealed a population of blast cells which expressed CD13, CD33, HLA-DR, CD64, CD117, CD34, CD38. The overlying monocytic cells showed abnormal loss of CD4 expression and homogenous dim HLA-DR expression.

Cytogenetic analysis of unstimulated bone marrow cells was performed by direct, overnight and 48 hours culture. The medium used for culturing the cells was RPMI 1640 (Sigma, Schnelldorf, Germany) supplemented with 20% fetal bovine serum (GIBCO, Grand Island, 5 NY). Harvesting and GTG banding were performed as per standard procedure and metaphase chromosomes were G-Banded and karyotype description was according to ISCN 2016 nomenclature.

A total of 20 cells were analyzed and 10 well-spread metaphases were photographed and karyotyped using ASI (Applied Spectral Imaging) software. The fixed cellular pellet was stored in a fixative solution (methanol: acetic acid 3:1) at -20°C and was used for other molecular cytogenetic techniques such as FISH (fluorescence in situ hybridization). FISH was performed using t (8;21) (q21.3; q22): LSI RUNX1/RUNX1T1 (AML1/ETO) dual colour, dual fusion translocation probe, Vysis (Abott Molecular, Des Plaines, IL; Wiesbaden-Delkenheim, Germany), PML RARA: LSI PML/RARA dual colour dual fusion translocation probe, Vysis (Abott), RARA: RARA break apart rearrangement probe, Vysis (Abott), inv (16): CFBβ break apart rearrangement probe, Vysis (Abott), t(11q23): LSI MLL dual colour, break apart rearrangement probe, Vysis (Abott), XL 5q31/5q33/ 5q35 deletion probe, MetaSystems GmbH, Altlussheim, Germany. FISH was performed on unstimulated cultured cells using optimized Vysis protocol. FISH analysis was done on an Olympus BX61 fluorescent microscope with appropriate filters using ASI (Applied Spectral Imaging) software.

Karyotypic analysis showed karyotype 52~54, XX, -5,+8,+9, der(10)t(10;?)(p11.2;?)del(10)(p11.2),+11,+12, +19,+iso(21)(q10),+mar (Fig. 1). Cytogenetic analysis revealed a high hyperdiploid karyotype with a modal chromosome number ranging from 51~54 with the gain of chromosomes 8, 9, 11, 12 and 19. It also revealed other chromosomal abnormalities such as monosomy for chromosome 5, formation of derivative chromosome 10, isochromosome 21 and presence of a marker chromosome.

FISH analysis indicated negative status for AML1/ ETO :t(8; 21) but revealed an extra copy of the ETO gene in 63% of the cells analyzed and extra copies of both ETO and AML1 genes in 12% of the cells analyzed (Fig. 2A and 2B). FISH analysis showed negative status for PML RARA: t(15; 17), RARA variants and CFBβ :inv (16). Extra copy of the MLL gene by FISH in 70% of the cells analyzed was a result of trisomy 11. Monosomy 5 observed by cytogenetic analysis was confirmed by FISH studies (Fig. 3).

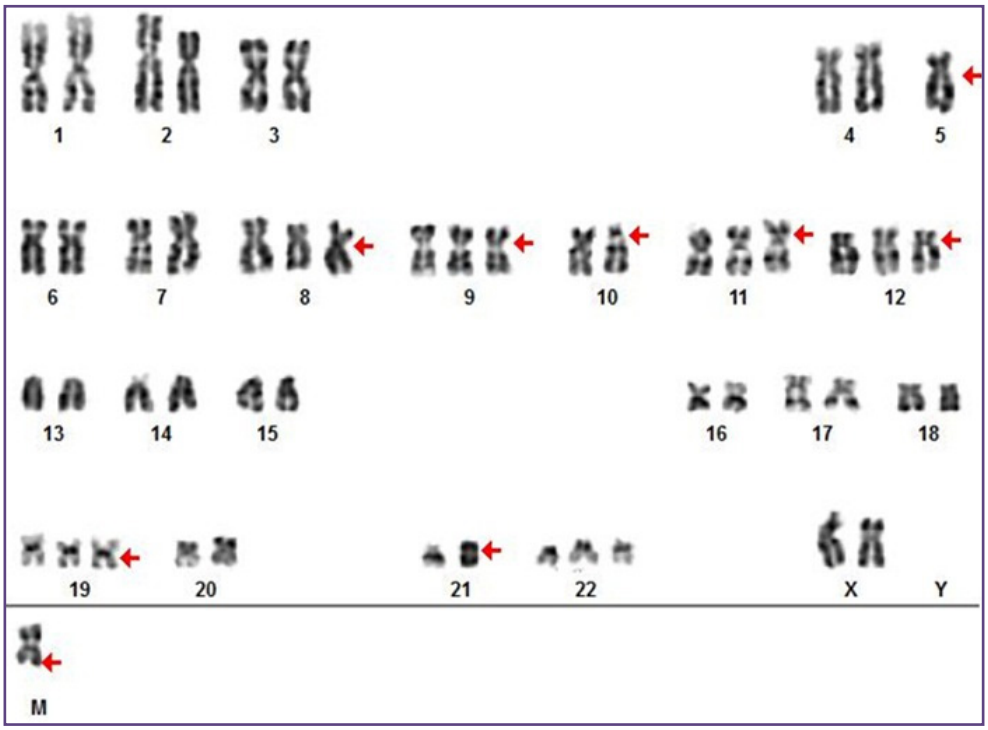

Fig 1: Cytogenetic analysis performed at 100X magnification revealed karyogram indicating hyperdiploid karyotype with additional chromosomal abnormalities. 52~54,XX,-5,+8,+9,der(10)t(10;?)(p11.2;?)del(10)(p11.2),+11,+12,+19,+iso(21)(q10), +mar

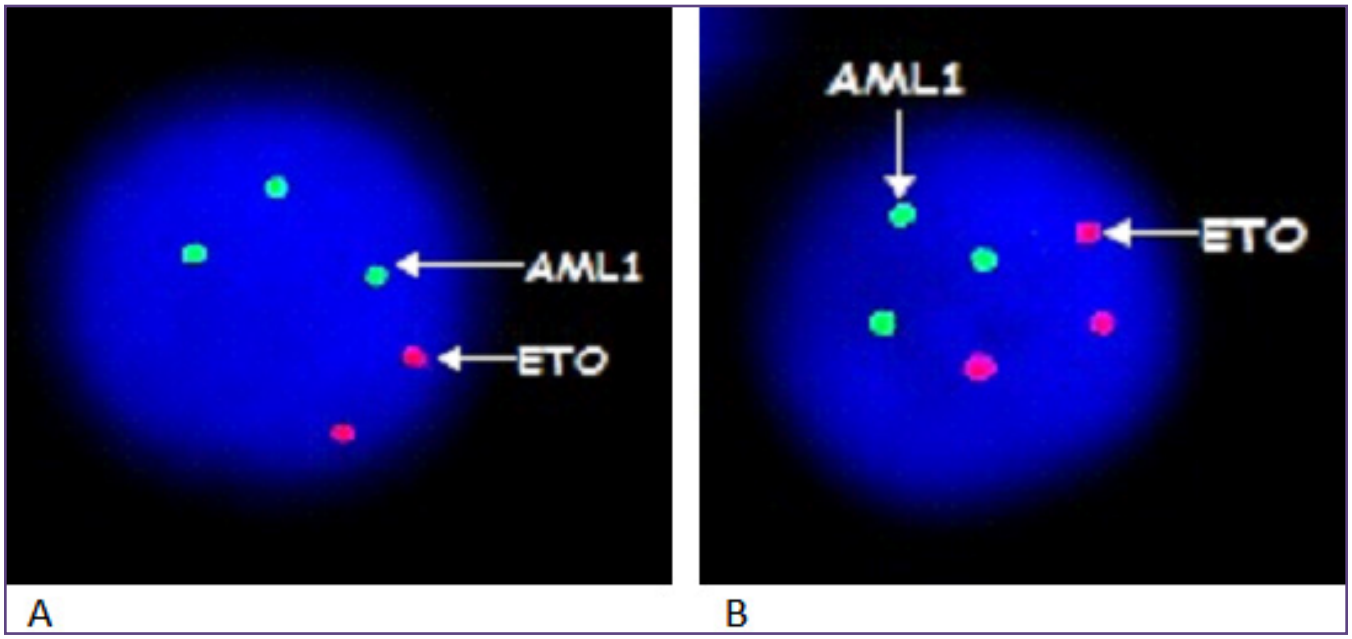

Fig. 2: FISH signals using fluorochrome labeled probes at 100X magnification showed (A) 3 green and 2 orange signals indicative of an extra copy of ETO gene (B) 3 green and 3 orange signals indicating extra copies of ETO and AML1 genes respectively (100X).

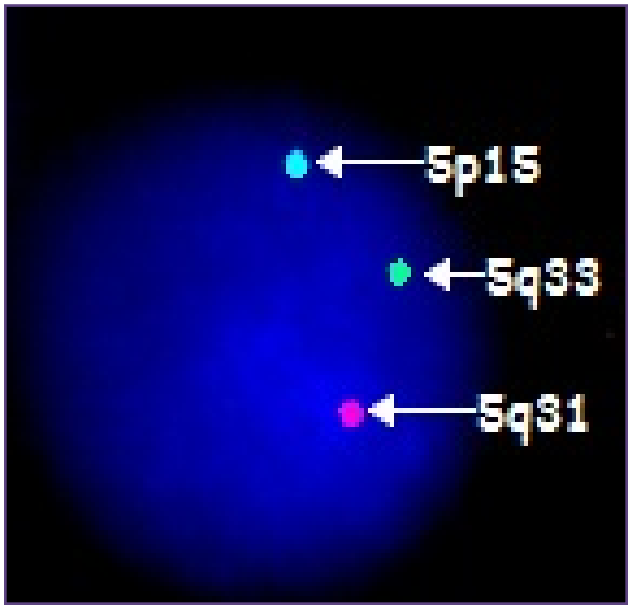

Fig. 3: FISH signals using fluorochrome labeled probes at 100X magnification showing 1 green, 1 orange and 1 aqua signals indicating monosomy for chromosome 5 (100X).

Discussion

Present case demonstrates complex aberrant karyotype with multiple unrelated cytogenetic abnormalities such as high hyperdiploidy, monosomy 5 and presence of three non-recurrent structural abnormalities which have led to partial gains and losses of chromosomal regions.

FISH studies ruled out presence of cryptic translocations for recurrent markers such as PML/RARA, RARA variants, RUNX1/RUNX1T1, inversion 16 and MLL gene rearrangements.

As there are >4 independent chromosomal aberrations the case classifies into adverse genetic risk group based on the recommendations of the European Leukemia Net (ELN) and also the UK National Cancer Research Institute Adult Leukaemia Working Group.[2,8]

Present case falls into the unfavorable category as against cases of hyperdiploidy without structural abnormality-atypical complex karyotype, that fall into the intermediate category.[9]

Along with hyperdiploidy the complexicity was increased by monosomy, presence of a derivative chromosome 10 resulting in partial monosomy for 10 p11.2 to pter region, presence of isochromosome 21 and presence of unidentifiable marker chromosome.

Gain of chromosome 8 was observed which is the most common gain in hyperdiploid karyotype reported. Gain of chromosome 19 observed is less frequently reported. Sex chromosomal gains were not present.

Monosomy 5 was observed which is a common marker known to confer an intermediate risk effect in AML without hyperdiploidy. A study by Clinton et al., on 1563 hyperdiploid karyotype identified a total of 97 patients

with specific chromosomal abnormalities associated with an adverse outcome which included monosomy 5 and 5q deletion Another study carried out by Lazarevic et al (2015) performed a study on 33 high hyperdiploid and triploid/ tetraploid cases which harbored adverse abnormalities, and found out that 39% (13 cases) of the cases had monosomy 5.[10]

Monosomy 5 is a common marker known to confer an intermediate risk effect in AML without hyperdiploidy. Present case is characterized as monosomal karyotype defined by the presence of monosomy for chromosome 5 and this abnormality additionally enforces the negative prognostic impact reported to be an independent prognostic factor with poor prognosis.[11]

Marker chromosomes are frequently common in adverse-risk karyotypes and associated with a dismal prognosis in AML patients [12] and presence of marker chromosome adds up to the complexicity and in effect would lead to adverse prognosis. Isochromosome 21 was detected which leads to morphologically identical genetic information in both arms reported in acute lymphoblastic leukemia and acute myeloid leukemia.[13] Hyperdiploidy with complex events have shown to confer an adverse prognosis by Clinton and Lazarevic et al., and also precise classification of hyperdiploid needs to be done as chromosome numbers >65 and absence of adverse aberrations seem to translate into a more favorable prognosis.[6,10] The impact of gene dosage or copy number variations due to hyperdiploidy, isochromosome formation, monosomy 5, partial monosomy of chromosome 10p region and extra marker chromosome in present case needs further attention reinforcing the need for collection of clinical data on such rare complex cumulative events and can be a clinical indicator for the pathophysiological manifestations of the disease stage and for prognosis. Several treatment strategies have been tried for AML patients with a complex karyotype which include usage of a combination of chemotherapeutic drugs as well as employment of allogenic stem cell transplantation therapy. Study by M-C Be'ne et al., have shown that AML patients with near-tetraploidy might benefit from therapy with intermediate or high doses of cytosine-arabinoside combined with anthracyclines for CR induction and/ or consolidation, followed by autologous stem cell transplantation in first CR.[14]

Conclusion

Present case reinforces the importance of cytogenetic categorization of an adverse-risk complex aberrant karyotype to stratify patients to individual optimized treatment strategies other than standard treatment in order to achieve good treatment outcome.

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