A Case of AML-M2 with Sole Interstitial Deletion in 9q Without AML1–ETO/Inv 16 Rearrangement and FLT3/NPM1 Mutations

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Abstract Conventional/molecular cytogenetics is important in identification of genomic abnormalities, for prognostication and in risk stratification of de novo patients with acute myeloid leukemias (AML). Here we present an AML M2 case showing the sole karyotypic abnormality, the rare interstitial deletion in the long arm of chromosome 9 with the loss of segment q12–q13.

Keywords AML-M2 · Del 9q · CD7 · Conventional cytogenetics

Introduction

Acute myeloid leukemias (AML) are a heterogeneous group of disorders [1, 2]. Conventional/molecular cytogenetic analysis plays an integral part of the diagnostic workup of AML patients having a direct impact on the choice of treatment and patient prognosis [3]. Interstitial deletion of the long arm of chromosome 9 (9q-) is a recurrent uncommon karyotypic abnormality described in very, very few patients world wide [4–6] including India [7] and has been described as the primary sole karyotypic abnormality/ or in association with recurrent abnormalities such as 8;21 translocation in AML M2 or very rarely in association with

15;17 translocation in AML M3. Del 9q has also been described as a secondary abnormality. Interstitial del 9q is seen rarely in MDS, biphenotypic T lymphoid, myeloid leukemias associated with M1, M2, M4, M6 FAB subtypes and has suggested the possible involvement of a pleuripotent stem cell with trilineage myelodysplasia [4–7]. Association of CD34+, CD7+ with T lymphoid/myeloid biphenotypic leukemia and interstitial del of 9q has been described [4]. This case is being presented for rarity of the abnormality in this part of the world.

Case Report

A 39 year old male presented with symptoms of fever and cough for over 15 days, mild shortness of breath but no orthopnea, hemoptysis, anorexia and loss of weight. The patient had no significant past history. The patient was diagnosed elsewhere as having leukemia and came to our Institute for further evaluation and management. The patient had pallor, but no icterus, lymphadenopathy or hepatosplenomegaly. Other systemic examination was unremarkable. Chest X ray showed right pneumonitis. There was no CNS involvement.

Complete blood picture showed hemoglobin of 9.2 gm/dl, leucocytosis with total leucocyte count of $61.4 \times 10^3/\mu\text{l}$ with prominence of circulating blasts (84 %). Blasts were 3–4 times the size of mature lymphocytes with moderate amount of cytoplasm. Few of them showed Auer rods. The nuclei showed 2–3 nucleoli (Fig. 1). Platelets were decreased ($63 \times 10^3/\mu\text{l}$). No dysplasia was noted in the maturing myeloid precursors. Bone marrow showed 85 % blasts and maturing myeloid precursors. Erythroid precursors and megakaryocytes were markedly reduced.

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Fig. 1 Peripheral smear of an AML-M2 case showing blasts with Auer rods (Leishman stain, oil immersion X100)

Immunophenotyping on bone marrow sample revealed blasts showing bright positivity for the expression of the myeloid series CD13, CD33, CD34, CD45, CD117, HLADR, with aberrant expression of CD7. The scatter parameters and antigen expression profile as studied by flowcytometry were suggestive of AML M2 with aberrant expression of CD7 (Fig. 2).

Confirmation for AML1–ETO/inv 16 rearrangements was assessed by fluorescence in situ hybridization in interphase cells of peripheral blood using Vysis' locus specific identifier AML1/ETO dual color, dual fusion translocation probe and LSI CBFB dual color, break apart

rearrangement probe respectively. The denaturation and hybridization procedure were performed as per Vysis protocol and counterstained with 4,6-diamino-2-phenyl-indole (DAPI) stain. Fluorescent signals from 200 nuclei were visualized under the Olympus BX41 microscope equipped with the CCD camera. FISH analysis indicated lack of AML1–ETO as well as inversion 16 rearrangement in all the cells examined.

Conventional cytogenetic workup was performed to identify the possible genomic abnormalities. Short term (direct, 24 and 48 h) unstimulated cultures of peripheral blood were set up in RPMI 1640 medium containing 15 % FBS followed by GTG Banding. Twenty metaphases were analyzed for possible chromosomal abnormalities. Metaphases were analyzed and karyotyped using Applied Spectral Imaging Ltd Software, Migdal Hatmek, Israel. Karyotyping was done according to ISCN 2009 [8]. Clonal chromosomal abnormality was considered when 2 cells had a gain of chromosome or a structural abnormality like a translocation or 3 cells had a loss of chromosome. Cytogenetic analysis indicated the karyotype to be abnormal and showed evidence of interstitial deletion in the long arm of chromosome 9 with loss of the segment q12–q13 in all the metaphases examined. The karyotype rendered was 46,XY,del(9)(q12 q13) (Fig. 3).

Molecular workup for FLT3 and NPM1 gene mutations outsourced showed absence of FLT3 ITD/D835Y and NPM1 mutations.

The morphological and immunophenotyping features were consistent with AML-M2 associated with aberrant

Fig. 2 The gated population of neoplastic cells showing positivity for CD13, CD33, CD34, CD45, CD117, HLA DR, with aberrant expression of CD7

Fig. 3 Karyotype shows the abnormality—46,XY,del(9)(q12-q13)

expression of CD7, and a sole karyotypic abnormality 46,XY,del(9)(q12q13). The patient is on induction therapy with Daunorubicin and Cytosine arabinoside and is being followed up for further management.

Discussion

Conventional cytogenetics is important in identification of genomic abnormalities, for prognostication and in risk stratification of de novo patients with AML. Abnormal karyotypes have been described in 50 % of adult AML patients, with t(8;21)(q22;q22) approximately in 6 % adults with AML-M2 and associated with good prognosis [1–3]. Del(9q), described in AML is a rare uncommon karyotypic abnormality reported in very few patients in literature world wide. One publication described its occurrence in 31 cases [4] while another study described del 9q as a diagnostic karyotypic abnormality in 81 patients [5]. Del(9q) has been reported either as a sole abnormality (in 21 patients); in association with t(8;21) (15–35 % AML patients [6]) and in association with other cytogenetic abnormalities. Interstitial del 9q has also been described as secondary abnormality in AML M2 with t(8;21)(q22;q22)/trisomy 21 [5, 6]. One publication in Indian population described an increased incidence of deletion of 9q (q22q34)(5.1 %) in AML patients [9] while 2 case reports indicated the occurrence of del(9) (q21q22) in association with t(8;21) [7] and del 9q13q21 as a part of a complex karyotype including t(8;21)(q22;q22) [10].

Evidences indicated differences in the length of the chromosomal segment of 9q that is lost and varies between q13–q21 [4], q12–q22 [4] q21–q22 [4, 7]. Two studies indicated that the regions 9q21–22 (90 %) [5], and 9q13–9q21 [4, 7] are more commonly deleted segments in chromosome 9q, suggesting that these region may carry critical genes of importance and deletion of single or multiple tumour suppressor genes located in this region may underlie the pathogenesis of del 9q [4, 5, 7].

Molecular characterization of del 9q, indicated the deletion of 2.4 Mb region containing 11 genes, 7 of which were down regulated in del(9q) AML [11]. TLE1 and TLE4 genes, were implicated as potential tumor suppressors, as loss of expression in AML1–ETO expressing cells led to increased proliferation and cell survival. Haploinsufficiency of these TLEs overcomes the negative survival and antiproliferative effects of AML1–ETO on myeloid progenitors, allowing preleukemic stem cells to differentiate into AML [12].

Cytologic and morphologic associations of blast cells showing del 9q in various studies include frequent, agranular leukemic blasts with larger vacuoles on Giemsa staining and localized positivity for myeloperoxidase (MPO), presence of giant MPO positive granules (pseudo chediak Higashi) with a characteristic bone marrow picture with dysgranulopoiesis and dyserythropoiesis [4, 5, 7].

In the present case conventional cytogenetics as well as FISH analysis confirmed the absence of translocation 8;21/AML1–ETO fusion as well as inversion 16 rearrangement. Instead the cytogenetics showed the rare interstitial deletion

in the long arm of chromosome 9 as the sole karyotypic abnormality with deletion of the segment q12–q13 at the time of diagnosis. To the best of our knowledge this the second case with del 9q being reported from India, showing the loss of a very small chromosomal segment of long arm of chromosome 9, the region q12–q13. Loss of such a small segment has not been reported earlier and possibly the genes in this region (not yet defined) may be critical for leukemogenesis in the absence of AML1 ETO rearrangement suggesting the operation of another pathway.

AML normal cytogenetics involves testing for prognostic indicators FLT3, NPM1, CEBPA mutations [1–3]. Data on status of FLT3/NPM1/CEBPA mutations in the context of del 9q as the sole abnormality/in association with t(8;21)/other karyotypic abnormalities with reference to loss of various segments in del 9q is sparse. In an upfront attempt to prognosticate the case, molecular studies on FLT3/NPM1 were performed. We demonstrate lack of FLT3-ITD/D835Y and NPM1 mutations in our AML M2 case with del 9q 12–q13 as the sole abnormality at diagnosis. However one report demonstrated strong association of del 9q with NPM1 mutations and CEBPA loss-of-function mutations in the context of a noncomplex karyotypic AML with del(9q) (41 %). Loss of a critical segment of 9q, most likely in 9q22, and disruption of CEBPA function possibly contribute to the pathogenesis of del(9q) AML [13, 14].

The prognostic impact of del 9q at diagnosis, as the sole abnormality/in relation to other abnormalities such as t(8;21)/as a secondary abnormality and in association with molecular markers is variable [4–7]. Del(9q) in AML as the sole primary abnormality has been shown to be associated with poor prognosis [7] and we are yet to see the outcome.

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Conflict of interest None.

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