A Sole t(10;19)(p11.2;p12) in AML-ETO negative AML-M2 paediatric patient: First novel case from India

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Abstract :

Acute myeloid leukemia (AML) is a heterogeneous group of disorders with regards to its pathology and molecular genetics features. The translocation t(8;21)(q22;q22), which results in the fusion of the AML and ETO genes, is a recurrent aberration in AML, preferentially correlated with FAB- M2, and has the highest incidence in childhood AML. Because of the favorable prognosis, the evidence of the t(8;21) or the AML1-ETO fusion gene is mandatory in most of the therapy trials, allowing the stratification of the patients to the correct risk group in terms of treatment. Here, we describe a novel case of sole translocation t(10;19)(p11.2;p13) in a AML1-ETO negative AML-M2 patient. In general, this translocation is previously observed with combination of complex translocations, but sole abnormality was not previously observed. This is a novel translocation and not observed previously as a sole abnormality in any AML case. Hence, the functional role of this translocation is still unknown. Short term bone marrow culture was carried out for conventional cytogenetics and karyotype was 46, XX,t(10;19)(p11.2;p12) in all 20 metaphases analyzed. To confirm this translocation FISH with Whole chromosome paint probe was applied and results confirmed the translocation between chromosome 10 and 19. To the best of our knowledge, this is the first novel case of sole t(10;19) in a paediatric AML-M2 patient with AML-ETO negative fusion. Patient expired within a week. Therefore, the present case challenges the view that the occurrences of sole and new novel translocation require more such cases to be studied in large cohort which is generally an indication for poor prognosis.

Key words : Acute Myeloid Leukemia, AML-ETO, FISH

Introduction :

Multiple recurrent chromosomal aberrations in acute myeloid leukemia (AML) have been identified by conventional cytogenetic analysis, and these findings have been used as the most important diagnostic and prognostic markers.[1] The t(8;21)(q22;q22) is considered a distinct AML subtype associated with a favorable prognosis and is found in approximately in 5% of cases of AML and in 10% of the prior AML with maturation (M2) category of the French American British classification.[2] However, approximately 3-4% of AML cases associated with t(8;21) have variant translocations,[3][4] and the clinicopathologic features of AML carrying variant t(8;21) are less well characterized.[5]

Molecularly, the AML1 gene located at 21q22 fuses to the ETO gene located at 8q22, generating a chimeric AML-ETO fusion gene on the der (8). Translocation (8;21) usually correlates with specific morphological features, which include large blast cells with Auer rods, often containing numerous azurophilic granules and very large pseudoChe´diak-Higashi granules as well as homogeneous pink-colored cytoplasm in neutrophils. The leukemic cells generally respond to chemotherapy, even after relapse. Thus, detection of the AML-ETO fusion gene allows patients to be assigned to the appropriate risk group for treatment.[2]

AML with t(8;21)(q22;q22) is a distinct AML subtype with characteristic morphology and clinical manifestations. In the clinical course, approximately 87% of AML patients with t(8;21) experience remission; however, relapse is frequent, and these patients have worse prognoses, with an overall survival of 50% at 5 years.[6] Approximately 35% of patients with t(8;21) also display loss of Y chromosome in males and loss of X chromosome in females. Another 20% of patients with t(8;21) show deletion of 9q12-q23. Trisomies for chromosomes 4 and 8 are observed in 6-10% of patients with t(8;21). Additional cytogenetic abnormalities, irrespective of their nature or complexity, do not appear to have a deleterious effect on remission, relative risk, and overall survival.[7][8]

To the best of our knowledge till date in Mitelman database of chromosome aberrations only 4 cases were

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observed with t(10;19) with different regions. The present case is of sole t(10;19) in AML-ETO negative AML-M2 patient. This is the first novel observation in an Indian patient.

Materials and Methods:

Case Details:

A 9 years old paediatric girl visited The Gujarat Cancer & Research Institute, Ahmedabad during December 2011. Patient was suffering from fever, vomiting, headache and general weakness since last 7 days. In laboratory investigations were Hemoglobin-4.1 gm/dl WBC.-54.4 x103 /cmm, Platelets.-6 x103 /cmm, Blast cells-55 %, Myelocyte-16 %, Polymorphs-4 %, Lymphocytes-25%. Bone marrow report revealed hypercellular marrow. Tumour blast cells were 63 %. The blasts were medium in size, with moderate Nuclear: Chromatin ratio, fine diffuse chromatin, 0-1 prominent nucleoli and moderate amount of cytoplasm. Few of them showed Auer rods in their cytoplasm. Eosinophils and Eosinophilic precursors constituted 15%. Both myeloid and erythroid precursors were markedly suppressed. Myeloid: Erythroid ratio altered. Megakarycytes were not seen. PAS negative and Sudan black B was positive. Peripheral smear showed mild leucocytosis with 55% blasts, severe thrombocytopenia. Overall findings suggested, AML with maturation [AML-M2E0].

Serum Uric Acid-0.84 mg/dl, Serum Albumin-2.12gm/dl, Serum Bilirubin, Total Bilirubin-0.25 mg/dl, SGPT-ALT (SGPT)-3IU/L, Electrolytes- Sodium (NA)-134.7 mmol/L, Potassium (K)-3.35 mmol/L, Chloride (Cl)-103 mmol/L, S.Creatinine (Crea)-0.35 mg/dl. Lactate Dehydrogenate -.623 U/L.

Immunophenotype:

Immunophenotyping results showed, CD 19 :00 % , CD 79a :00 % , CD 5 :03 % , CD 3 :00 % , CD 7 :69 % , CD 13 :68 % , CD 33 :97 % , CD 117 :82 % , MPO :63 % , CD 34 :87 % , HLADR :66 % , CD 10 :00 % , Tdt :00 % , CD 22 :00 % . In Bone marrow, 72% blasts were gated using CD45 PerCP vs. Side scatter. The blasts mainly expressed myeloid markers MPO, CD13, CD33 and CD117 along with CD34 and HLADR. Aberrant expression of CD7.

Magnetic Resonance Imaging of whole spine with screening of bilateral hip joints was carried out. MRI lumbo-sacral spine was performed in body coil by taking T1W, T2W, FLAIR, STIR and post contrast study in multiple planes. There was normal alignment and curvature of spine. Vertebral bodies revealed normal marrow signal intensity. IV discs appeared normal. There was no significant disk bulge or herniation seen. Cord ends at L1 level. Spinal cord revealed normal signal intensity. No evidence of any focal or diffuses areas of altered signal intensity seen. No evidence of ligamentum flavum and facetal hypertrophy. Pre and paravertebral soft tissues appeared normal. On screening of the bilateral hip joints, presence of effusion was noted on right hip joint with normal marrow intensity signal of bone. Bilateral articular cartilage appeared normal. Results suggested that there was effusion in right hip joint and normal spine. Chest X-Ray (PA View) was normal.

After two weeks, laboratory investigations were Hemoglobin-10.2 gm/dl, WBC-0.5 x103 /cmm, Platelets-9 x103 /cmm, Polymorphs-3.2 % Eosinophils-.0.3 %, Lymphocytes-93.2 %, Monocytes-2.8%, Basophils-0.5 %. Patient expired within a week.

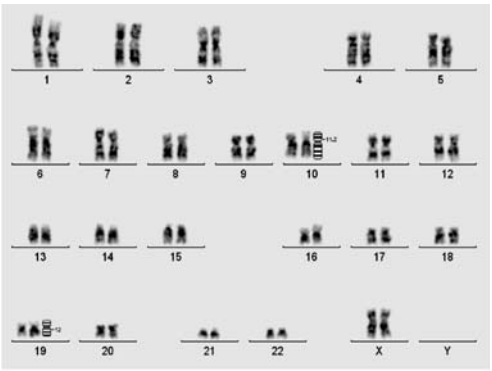
Methods:

Bone marrow sample was collected ascetically in sodium Heparinized vaccuate. For conventional cytogenetic study, short term culture and GTG banding was carried out according to standard protocol. Well spreaded good morphology metaphases were captured in Zeiss automatic karyotyping system and analysis using IKAROS software and karyotype description was done using ISCN 2009 guidelines. For Fluorescence in Situ Hybridization (FISH) Whole Chromosome Paint FISH probes for chromosome 10 spectrum Orange (SO) and chromosome 19 with spectrum Green (SG) was applied on metaphase cells. FISH for AML-ETO DCDF probe was also performed (Abott Molecular, USA).

Results:

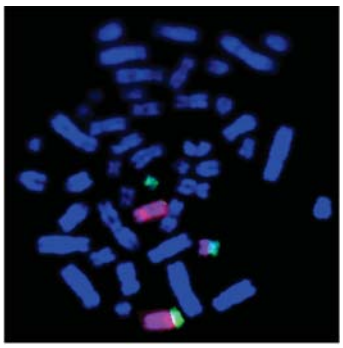
Conventional chromosome analysis at diagnosis detected an abnormal female chromosome complement in 10 metaphases. The karyotype was 46,XX,t(10;19)(p11.2;p12)[20] (Fig.1).

Figure 1: GTG banded karyotype results showing t(10;19)(p11.2;p12)



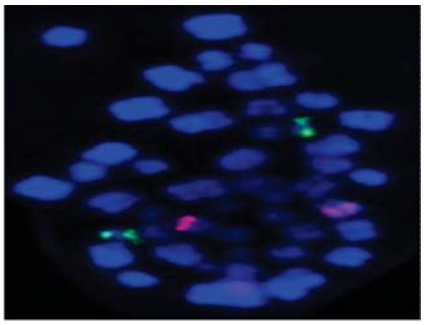
The Metaphase FISH was carried out using WCP FISH probes for chromosome 10 spectrum orange and 19 spectrum green(Abbott Molecular/Vysis, Des Plaines, IL). FISH results showed that q arm of chromosome 19 was observed on p arm of chromosome 10 and p arm of chromosome 10 was observed on p arm of chromosome 19 showing balanced translocation between chromosome 10 and chromosome 19. One orange color chromosome showed normal chromosome 10 and one green color chromosome showed normal chromosome 19 (Fig.2). FISH results confirmed t(10;19) in all metaphases. FISH with AML-ETO showed normal signal pattern i.e OOGG, indicating negative results (Fig.3).

Figure: 2 FISH using WCP FISH probes for chromosome 10 spectrum orange and 19 spectrum green.



FISH results confirmed t(10;19)(p11.2;p12), q arm of chromosome 19 was observed on short arm of derivative chromosome 10 and short arm of derivative chromosome 10 was observed on q arm of chromosome 19 showing balanced translocation 10 and 19. One orange color chromosome showed normal chromosome 10 and one green color chromosome showed normal chromosome 19. (Figure 2)

Figure: 3 FISH using AML1-ETO FISH probes.



Two green signals of AML1 gene situated on Chr#21 and two orange signals of ETO gene situated on Chr#8. FISH results 2O2G signal pattern indicating negative for AML-ETO translocation. (Figture 3)

Discussion:

AML is a heterogeneous disease with regard to its biology and its clinical course. AML constitutes about 20% of all childhood leukemias.[9] Chromosomal translocations resulting in specific fusion genes are a hallmark of the leukemias. While studies of the fusion proteins have yielded extensive information on the biology and clinicopathology of the leukemias, less is understood about the structure and etiology of chromosomal fusions.[9] The translocation t(8;21)(q22;q22) is the most common chromosomal aberration and occurs in 10% to 12% of AML. This translocation is detected in 40% of cases of the M2 subtype.[10] In the present study this paediatric patient with AML-M2 subtype, karyotype results showed sole t(10;19). Sole cytogenetic abnormalities have received attention, because the term sole refers not only to the fact that these are the first cytogenetic changes to occur in neoplastic cells, but also because of their causal role in tumorigenesis. [11]

Involvement of chromosome 19 in AML-M2 is a rare event. Generally, trisomy 19 as a sole abnormality is a rare event that may be associated with myeloid malignancies, and it was previously described in various hematologic malignancies, a case of adenocarcinoma, and a case of astrocytic tumor. The presence of an extra copy of chromosome 19 has been correlated with myeloid blast crisis of CML.[11] In addition, an additional chromosome 19 or 19q also detected in 13.2-33.3% of patients with acute megakaryoblastic leukemia (AML-M7) and it occurs as a secondary anomaly in all cases[12] whereas in present study patients BM report showed AML-M2 subtype. Also trisomy 19 is occurred as the

sole anomaly in refractory anaemia with excess blasts (RAEB) and AML-M0 subtype.[11] Based on a review of the existing literatures, there is no report describing the occurrence of $t^{(10;19)}$ as a sole anomaly in cases with hematologic disorders worldwide.[13]

However, it is still unclear which gene(s) located on chromosome 19 might have a functional role in the development of hematologic malignancies. Patient expired within a week of diagnosis which confers poor prognosis. Unfortunately, this is only a one case so exact role of this fusion genes are still unclear.

Conclusion

In summary, the rarity of such cases makes difficult to determine the role of such translocation in the prognosis of patients with AML-ETO negative in AML-M2 subtype. However, results of clinical outcome in other reported cases of complicon might be taken as evidence that its occurrence is associated with inferior clinical prognosis. Genomic mapping and sequencing of chromosomal fusion junctions of specific types has led to speculations on the possible mechanisms of formation of these fusion junctions.

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