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Case Report

Identification of der(6)t(3;6)(q26; p21) in *RUNX$_1$/RUNX$_1$T$_1$* Negative AML - M2 Pediatric Patient by Fluorescence *In Situ* Hybridization Technique

Pina J Trivedi[1]*, Dharmesh M Patel[2], Darshita H Patel[2], Mahnaz M Kazi[2], Priya K Varma[2], Dhara C Ladani[2], Nehal A Patel[2] and Prabhudas S Patel[2]

[1]*Senior Scientific Officer, Gujarat Cancer and Research Institute, India*

[2]*Gujarat Cancer and Research Institute, India*

***Corresponding Author:** Pina J Trivedi, Senior Scientific Officer, Gujarat Cancer and Research Institute, India

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Abstract

Heterogeneity is an indicator for Acute Myeloid Leukemia with regards to pathology and molecular genetics features. Translocation (8;21)(q22;q22) is a common cytogenetic aberration which is found in AML-M2 subtype. Here, we present a novel der(6)t(3;6)(q26;p21) in AML - M2. Conventional cytogenetics and Fluorescence *in Situ* Hybridization (FISH) using dual color dual fusion probe analysis confirmed the der(6)t(3;6)(q26;p21). Patient didn't achieve remission and was expired within a year. Further studies are required to ascertain the expression of genes that play role in causing der(6)t(3;6)(q26;p21) to explore its prognostic value. For conventional cytogenetic study, Short term culture of bone marrow and Giemsa Trypsin Giemsa banding were carried out according to standard protocols. Cytogenetic nomenclature was carried out using ISCN 2016 guidelines. For *FISH RUNX$_1$/RUNX$_1$T$_1$* DCDF probe (Abbott Molecular USA) and Whole Chromosome Paint (WCP) FISH probes for chromosome 3 Spectrum Orange (SO) and chromosome 6 with Spectrum Green (SG) were applied on metaphase cells. Sole t (3;6) in AML is not reported till date. So, it is novel case. It would be interesting to compile similar observations from the study of chromosomal anomaly evolution in neoplasia.

Keywords: *RUNX$_1$/RUNX$_1$T$_1$*; Fluorescence; in Situ Hybridization; Acute Myeloid Leukemia; Chromosomes

Introduction

Acute Myeloid Leukemia (AML) is a malignant disorder of blood that is characterized by blocked or impaired differentiation of haemopoietic stem cells, resulting in an abnormal accumulation of immature precursors and suppression of growth and maturation of cells involved in normal hematopoiesis [1-3]. There is variation in clinical outcome of AML, ranging from survival of a few days to cure. Clinical and biological features are different at diagnosis and have been reported as for the prediction of clinical outcome [4,5]. Heterogeneity is the characteristic of AML and somatic mutations that disturb cellular growth, proliferation, and differentiation accumulate in hematopoietic progenitor cells. Cytogenetic study at the time of diagnosis is mandatory for diagnosis which again helpful in prognosis and treatment of AML [6,7].

AML is classified according to two classification system French-American-British Cooperative Group (FAB) which depends on morphology and cytochemical changes of cells [8]. Recurring balanced translocations, particularly t(8;21)(q22;q22), t(q22;q12), inv(16) (p13;q22), and 11q23 translocations, represent a substantial percentage of cytogenetic abnormalities in AML. These aberrations constitute 34% - 47% of pediatric and 21% - 28% of adult AML cytogenetic abnormalities. Additional abnormalities including trisomies, deletions, and complex karyotypes contribute to another large percentage of adult AML and show overlap with cases of myelodysplasia [9]. Less common balanced abnormalities representing distinct clinicopathologic entities in the classification are as follows: t(6;9)(p23;q34.1), inv(3)(q21.3;q26.2) or t(3;3) (q21.3;q26.2) and t(1;22) (p13.3;q13.1). Many other balanced abnormalities are found to recurrent in AML, but these tend to be uncommon and are not currently recognized to represent distinct clinicopathologic entities [10]. The t(8;21)(q22;q22) is one of the most frequent chromosomal abnormality associated with AML-M2 sub type.

In this paper we describe a report of AML-M2 patient with cytogenetic and FISH studies. Bone marrow sample was used for above mentioned techniques. Cytogenetic results revealed that

there was a translocation between chromosome 3 and 6 in all 20 metaphases analysed. Patient's karyotype analysis revealed 46, XY, der (6)t (3;6) (q26;p21) [20]. Karyotype results and FISH results were compared using Whole Chromosome Paint (WCP) FISH probes and Locus Specific Identifier (LSI) *RUNX*$_1$/*RUNX*$_1$*T*$_1$ fusion Dual color dual fusion (DCDF) probe.

Till date in Mitelman database, 68,379 cases and 11,207 gene fusions for the chromosomal aberration have been reported [11]. Moreover, t(3;6)(q;p) with different regions have been observed in five cases in AML-M2 but there was not a single case describing der(6)t (3;6) (q26;p21) in AML-M2 reported from India. So, our case is the novel finding in Indian scenario.

Gene present on chromosome 3q26 is Ecotropic Viral Integration Site 1 (EVI1) and Myelodysplastic Syndrome 1 (MDS1-EVI1). Over expression of *EVI1* play an important role in leukemogenesis and is associated with treatment resistance and a poorer prognosis in patients with Myelodysplastic Syndrome (MDS), AML, Chronic Myeloid Leukemia (CML) and BCR-ABL negative myeloproliferative neoplasm [12,20,21]. Pim-1 proto-oncogene (PIM1) gene located on 6p21 plays a role in signal transduction in blood cells, which is involved in cell proliferation and survival, and provides a selective advantage in tumorigenesis in AML [13].

Case Details

We report a patient with AML-M2 who developed der(6)t(3;6) (q26;p21) prior to the course of therapy. A 3-years-old male child suffering from low grade fever, weakness and blood loss was referred to Gujarat Cancer and Research Institute, Ahmadabad, India. The laboratory investigations revealed White Blood Cell count 9 x 10^3/cmm, Red Blood Cell count 3.26 million/cmm, Haemoglobin-8.4 gm/dl, and Platelets 8 x 10^3/cmm, Myelocyte 8%, Polymorphs 38%, and Lymphocytes 46%. Bone marrow report showed hypercellular bone marrow aspirate with marked proliferation of blasts cells constituting 24%. These blasts were medium in size with high N:C ratio, coarse nuclear chromatin, prominent 1-2 nucleoli seen with moderate cytoplasm. Myeloid precursors (8%) and polymorphs (20%) seen with dysmyelopoiesis. Erythroid series showed dyserythropoiesis. The patient was treated with 6-thioguinine-40mg-Thio at 19 days of interval (2 cycles) and expired within a year of diagnosis

Immunophenotyping

A result of Immunophenotyping of bone marrow was 20% blasts 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 was seen. Final diagnosis was AML-M2.

Materials and Methods

Conventional Cytogenetics

Bone marrow sample was collected in sterile Sodium Heparinized vaccuate. For conventional cytogenetic study, short term culture was carried out as per standard protocol. Slides were banded using Giemsa Trypsin G banding technique. Good morphology metaphases were captured in Zeiss automatic karyotyping system and analysis using IKAROS software and karyotype description was done using ISCN 2016 guidelines.

Fluorescence in situ hybridization (FISH)

FISH was performed using *RUNX*$_1$/*RUNX*$_1$*T*$_1$ LSI probe. In LSI *RUNX*$_1$/*RUNX*$_1$*T*$_1$ probe, *RUNX*$_1$ gene was tagged with SG and *RUNX*$_1$*T*$_1$ gene tagged with SO. The WCP FISH was carried out to determine the nature of the translocation. The WCP FISH probes for chromosome 6 SO and chromosomes 3 with SG were applied on metaphase cells as per manufactures instructions. Twenty metaphases were analyzed, using Epi-fluorescence microscope (AXIO Imager.Z2, Zeiss, USA) equipped with appropriate filter sets. Image capturing, and processing were carried out using an ISIS FISH imaging system (MetaSystems, Germany). So, FISH result was nucish (*RUNX*$_1$/*RUNX*$_1$*T*$_1$) x2 [200].

Results

Conventional cytogenetic

Conventional chromosome analyses at diagnosis of GTG banded metaphase were carried out. Total 20 metaphases were karyotyped. All metaphases showed 46, XY, der(6) t (3;6) (q26;p21) [20] (Figure 1).

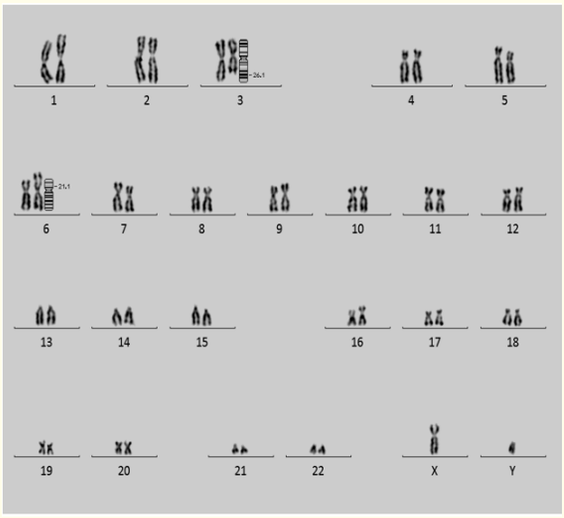

Figure 1: G banded karyotype results showed der (6)t (3;6) (q26;p21).

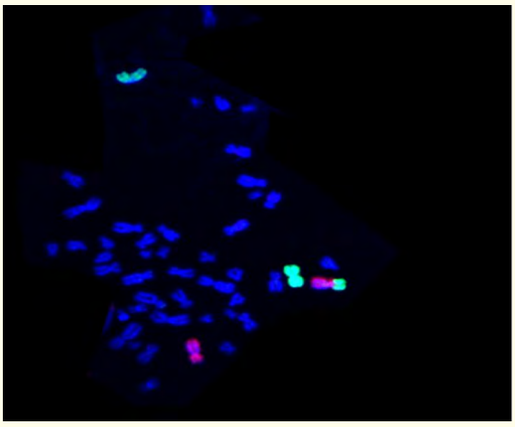

Figure 3: FISH using WCP FISH probes for chromosome 3 spectrum green and 6 spectrum orange FISH results confirmed der (6)t (3;6)(q26; p21), q arm of chromosome 3 was observed on p arm of derivative chromosome 6. One orange colour chromosome showed normal chromosome 6 and one green colour chromosome showed normal chromosome 3.

Fish

FISH results with *RUNX*$_1$/*RUNX*$_1$*T*$_1$ probes results revealed metaphases with 2O2G signals which showed that there was no fusion for *RUNX*$_1$/*RUNX*$_1$*T*$_1$ gene (Figure 2). FISH results with WCP probes showed q arm of chromosome 3 (SG) on p arm of chromosome 6 (SO) and one orange colour chromosome showed normal chromosome 6 and one green colour chromosome showed normal chromosome 3 (Figure 3).

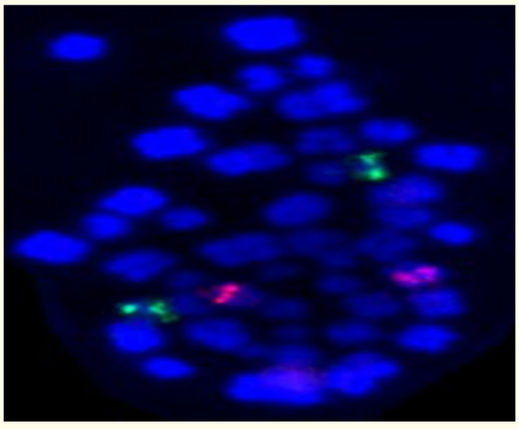

Figure 2: FISH using *RUNX*$_1$/*RUNX*$_1$*T*$_1$ probes. Two green signals of *RUNX1* gene situated on Chr#21 and two orange signals of *RUNX1T1* gene situated on Chr#8. FISH signal 2O2G pattern indicating negative for *RUNX*$_1$/*RUNX*$_1$*T*$_1$ translocation.

Discussion

AML is a heterogeneous disease with regard to its biology and its clinical course. About 55% of AML cases, showed cytogenetic abnormalities [14]. FAB subtypes showed specific chromosome abnormalities for specific subtypes. If molecular analysis could be done of these aberrations, then it will help in identifying genes involved in leukemogenesis and in the precise regulation of proliferation and differentiation in the hematopoietic system. Translocations are the best-studied chromosomal abnormalities. Due to translocation the function or activity of oncogenes located at or near the translocation breakpoint is altered [8].

In this study, we report the cytogenetic features of a patient with AML associated with der(6)t(3;6)(q26;p21). The der(6) t(3;6)(q26;p21) translocation is the novel in cases of AML. There is limited literature on t (3;6). Very few cases of such translocations from Asian countries are reported.

In myeloid malignancies (AML, MDS, CML and other well as other myeloproliferative disorders) involvement of 3q26 in balanced rearrangements is highly suggestive of EVI1and/ orMDS1/EVI1 rearrangements. In Myeloid malignancies 3q26 are mainly observed in young age at diagnosis trilineage dysplasia, dysmegakaryopoiesis and prior treatment with alkylating agents. Patients with these karyotypes are not responding to therapy, even

when the most active anti leukemic therapeutic options are used. According to Bruce., *et al*. 3q26 rearrangements are associated with poorer prognosis similar to this result our patient didn't achieve remission and expired within 1 year [11,19,20]. Voutsadakis., *et al*. 2003, suggested that the prognosis of AML with the t (3;12) (q26;p13) is poor, with a survival ranging in a few months [15].

MDS/AML with t(3;21) (q26.2;q22) is commonly a therapy-related disease associated with poor outcome. By analogy to other recurring chromosomal rearrangements in human leukemia that have been studied at the molecular level, the consistency of the breakpoints in the t (3;21) implies that specific genes in bands 3q26 and 21q22 participate in these rearrangements. It is possible that abnormality results in a specific genetic mutation critical to the malignant process. Candidate genes on chromosome 3 near band 3q26 include the transferrin receptor gene (TFRC) and the homolog of the Friend murine leukemia virus integration site 3 (FZM3) [15].

Two breakpoints 3′ of the *BCL6* exon 1, and the *H4* histone gene was substituted for the 5′ regulatory elements of *BCL6* on 3q27 of two cases carrying t(3;6) and observed in two cases and the study suggested that *H4* gene expression is tightly coupled to DNA replication, and an immediate mechanism for deregulated expression of *BCL6*, leading to the development of non-Hodgkin's lymphoma [17].

There are reports which suggest that balanced constitutional chromosome 3 translocation leads to tumorigenesis of clear cell Renal Cell Carcinoma (RCC) without *VHL* inactivation. To date, nine different chromosome 3 translocations have been associated with familial or multicentric clear cell RCC; and in three cases chromosome 6 was also involved [18]. Generally, t(3;6) (q21;p21) was observed in patients with AML with increased numbers of basophils and abnormal megakaryocytes in the bone marrow cells [19]. PIM1 gene located on 6p21 regulated by hematopoietic cytokine receptors; synergy with c-MYC in cell proliferation and in apoptosis induction through an enhancement of the activation of caspase-3-like proteases; Cdc25A (cell Cycle Phosphatase) is a substrate for Pim-1 [13].

Conclusion

In conclusion, we have presented a case of AML-M2 subtype with novel case of der (6) t(3;6) (q26;p21). This is indicator of poor prognosis. Further studies are required to ascertain the expression of genes that play a role in der(6) t(3;6)(q26;p21) to explore its prognostic value and therapeutic approach.

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