

## Sole Translocation (6;9)(p23;q34) in AML-ETO Negative AML-M2 Patient

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Received date: November 28, 2017; Accepted date: December 21, 2017; Published date: December 28, 2017

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### Abstract

Acute myeloid leukemia (AML) is a heterogeneous group of disorders with regards to its pathology and molecular genetics features. Translocation (6;9)(p23;q34) is a cytogenetic aberration that can be found in specific subtypes of both AML and myelodysplastic syndrome. This translocation is associated with an unfavorable prognosis. The t(6;9) is found in myeloproliferative disorders with typical clinical characteristics. This translocation results in highly consistent abnormalities at the molecular level. Here, we describe a case of sole translocation (6;9)(p23;q34) in AML-ETO negative AML-M2 patient with conventional cytogenetic and Fluorescence *In Situ* Hybridization (FISH) study results.

**Keywords** Acute myeloid leukemia; Chromosome; Fluorescence *in situ* hybridization; Karyotype; Translocation

### Introduction

Acute myeloid leukemia (AML) is a heterogeneous bone marrow malignancy. Diagnosis of AML is established combination of different methodologies, including cytology, cytochemistry, immunophenotyping and cytogenetic and molecular biology, as well as clinical features. It is reported that 59% of pediatric patients and 52% of adult patients have chromosomal abnormalities. Detection of recurrent chromosomal translocations occurring in AML is mandatory for correct classification. These chromosomal aberrations are also useful for prognostic stratification of patients among different therapeutic options and also for targeting a specific molecular abnormality [1].

The t(6;9) is often the sole abnormality present in the neoplastic cells, which argues for a direct involvement of the translocation in the leukemic process. The occurrence of defined chromosomal translocations in specific subtypes of leukemia strongly suggests that these translocations play an important role in the process of leukemogenesis. Translocations may activate nearby cellular genes involved in the control of proliferation and/or differentiation. The segments of chromosome 6 and 9 that are translocated in t(6;9) AML are rather small. The 6p- and 9q+ chromosomes are not easily distinguishable from their normal counterparts in a cytogenetic analysis. Depending on the quality of chromosome preparations, the translocation can be missed [2].

In myeloid leukemia three translocation breakpoints have been cloned and analyzed at the molecular level, First t is the t(9;22) and second is the t(15;17) and third translocation is the t(6;9)(p23;q34), found in a specific subtypes of AML. This leukemia is characterized by a poor prognosis, affects young adults, and is classified mostly as M2 or M4 and rarely as M1 (according to the French-American-British classification of AML) [3]. In adult AML, translocations with a poor prognosis are more rare; these include t(6;9)(p23;q34), with one fusion transcript between DEK and NUP214 (CAN) genes, which occur in 1% of adult AML patients [1].

Here, we present a report of cytogenetic and FISH study of a patient with morphologically AML-M2 subtype. Short term bone marrow culture was carried out for cytogenetic study and results revealed a translocation between chromosome 6 and 9 in all 20 metaphases analyzed. Patient's karyotype result was 46, XX, t(6;9)(p23;q34). Karyotype results were confirmed with Whole Chromosome Paint (WCP) FISH probes and LSI AML-ETO Dual colour Dual fusion (DCDF) fusion probe. There is limited literature on t(6;9) and only a few cases of such translocations from Asian countries with only 1 case from India are reported [3].

### Case Details

A 15 years old girl visited at the institute during November 2014. Patient was suffering from fever, weakness, abdominal pain and giddiness from last 2 months. The laboratory investigations were peripheral blood report showed, Hemoglobin- 3.1 g/dl Whole Blood Count (WBC)  $2.4 \times 10^3/\text{cmm}$ , Platelets-  $8 \times 10^3/\text{cmm}$ , Blastcells- 66%, Polymorphs- 6% Lymphocytes- 23% Monocytes- 1%. Bone marrow report showed, normocellular marrow with altered M:E ratio erythropoiesis normoblastic, megakaryocytes not seen, PAS stain negative and Sudan Black B positive, Erythroid series showed normoblastic maturation. There was proliferation of blast cells (66%). These cells had moderate azurophilic cytoplasm, Open chromatin with prominent nucleoli. Thin Auer rods had been seen. Peripheral smear showed RBC normocytic normochromic. WBC 54% blast cells. Platelets severely reduced. Depending on all three results diagnosis was AML-M2.

### Immunophenotype

Immunophenotyping results of bone marrow showed, 64% blasts were gated using CD45PerCP vs. side scatter. The blasts mainly expressed myeloid markers MPO, CD13, CD33 and CD117 along with CD34 and HLADR. Final diagnosis was AML-M2.

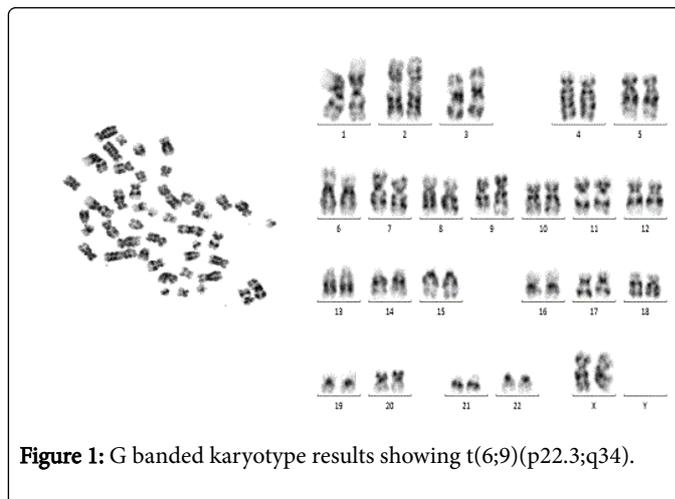
## Materials and Methods

Aseptically collect 4-6 ml of blood/bone marrow in green-top sodium heparin vacutainer tubes. Immediately invert the tubes several times to mix the sample and prevent clotting. Allow RPMI 1640 culture growth media & FBS to warm to 37°C temperatures for 1 to 2 h. Assess the quality of specimen for volume, lack of clotting, WBC and differential count. Prepare complete media as per the requirement. Aliquot 8 ml of RPMI 1640 complete media in sterile culture tube using glass pipette for blood culturing. Add 10 µl working colcemid in to each tube. Take blood sample in tube containing complete media. Incubate the culture at 37°C for 18 h. Harvest the sample. After 17 h incubation, Harvesting was carried out. First of all centrifuge all the tubes at 3000 rpm for 10 min. carefully pour off majority supernatant, break cell pellet and immediately resuspend cell pellet with 7 mL 0.56% (0.075 M) KCl solution. Incubate at 37°C for 27 min in a water bath and allow cells to swell. After completion of hypotonic treatment add 1mL freshly made chilled fixative drop by drop with constant agitation. Again incubate the tubes at 4°C for 30 min in a refrigerator. Centrifuge the tubes at 3000 rpm for 7 min to give three successive washes of fresh and chilled fixative. Resuspend cell pellet with appropriate amount of fixative to make a proper dilution. Apply few drops of cell suspension at the centre of the cleaned, chilled and wet slides. Make sure to label properly and dry it on a slide warmer at approximately 20°C. Allow it to air dry. Keep the slide in hot air oven for 1 h at 90°C for ageing purpose. Take the slides and cool at R.T. Dip the slides in Trypsin-EDTA solution (2:1) solution for 3-5 s. Transfer the slides in PBS Buffer (pH 7). Keep the slides in Giemsa stain for 5 min. Wash the slides in TDW. Observe under the microscope and check for the optimum banding on first slide. Well spreaded good morphology metaphases were captured in Zeiss automatic karyotyping scanning system-Metafer and analyzed using IKAROS software (Metasystem GmbH, altulsseheim, Germany) and karyotype were described using ISCN guidelines.

Procedure up to slide preparation is same as conventional cytogenetic protocol for Fluorescence *In Situ* Hybridization (FISH). FISH for AML-ETO Locus Specific Identifier probe and WCP FISH probes for chromosome 6 spectrum Orange and chromosome 9 with spectrum Green were applied on metaphase cells as per protocol provided by manufacturers.

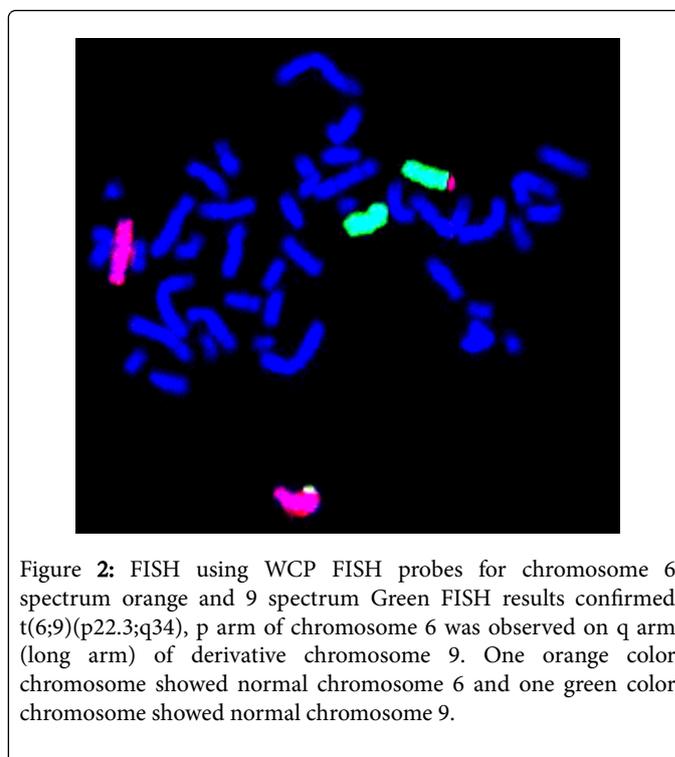
## Results

Conventional chromosome analysis at diagnosis of GTG banded metaphases were carried out. Total 20 metaphases were karyotyped. All metaphases showed abnormal female chromosome complement. The karyotype result was 46, XX, t(6;9)(p22.3;q34) (Figure 1).



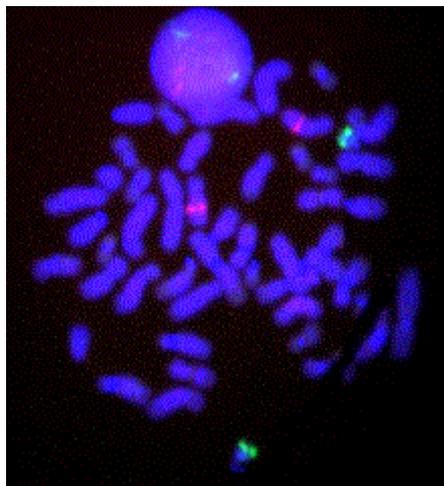
**Figure 1:** G banded karyotype results showing t(6;9)(p22.3;q34).

In FISH results, Metaphase FISH was carried out using WCP FISH probes for chromosome 6 spectrum orange and 9 spectrum green (Abbott Molecular/Vysis, Des Plaines, IL). FISH result showed that p arm of chromosome 6 was observed on q arm of chromosome 9 and q arm of chromosome 6 was observed on p arm of chromosome 9 showing balanced translocation between 6 and 9. One orange color C group chromosome was normal chromosome 6 and one green color C group chromosome was normal chromosome 9 (Figure 2). FISH results confirmed t(6;9) in all metaphases.



**Figure 2:** FISH using WCP FISH probes for chromosome 6 spectrum orange and 9 spectrum Green FISH results confirmed t(6;9)(p22.3;q34), p arm of chromosome 6 was observed on q arm (long arm) of derivative chromosome 9. One orange color chromosome showed normal chromosome 6 and one green color chromosome showed normal chromosome 9.

FISH for Locus Specific Identifier (LSI) AML-ETO probe was also carried out. In LSI AML-ETO probe, AML gene was tagged with spectrum green and ETO gene tagged with spectrum orange. In metaphase results 2O2G signals were obtained which indicated that there was no AML-ETO (Figure 3).



**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.

## Discussion

AML is a heterogeneous disease with regard to its biology and its clinical course. In about 55% of AML cases, chromosome aberrations are detectable by cytogenetics [4]. Defined karyotypic aberrations are associated with specific subtypes of leukemia. Detailed molecular characterization of these aberrations may identify genes involved in leukemogenesis and in the precise regulation of proliferation and differentiation in the hematopoietic system. Translocations are the best-studied chromosomal abnormalities. As the result of a translocation, the function or activity of oncogenes located at or near the translocation breakpoint is altered [1].

The t(6;9)(p23;q34) is a rare recurrent abnormality. AML with t(6;9)(p23;q34) has unique clinical and laboratory features and its prognosis is poor in most cases [4]. The t(6;9)-positive AML is classified as a separate clinical entity because of its early onset and poor prognosis. The hallmark of t(6;9) AML is the expression of the DEK-CAN fusion protein. The leukemogenic potential of DEK-CAN has been in question, because it was shown to be unable to block the differentiation of hematopoietic progenitors [5]. The t(6;9) is the sole anomaly in 85% of 195 cases with available data, and in 83% of cases in the largest study [6]; recurrent, although rare, additional anomalies are the following: +8 (in 6 of 126 cases, 5%), +13 (in 3 of 126 cases, 2%), +21. A -7/del(7q) was found once, a t(9;22)(q24;q11) once. A three way complex t(6;9;Var) has been found in 3 instances [7]. The t(6;9) may be secondary to toxic exposure; in some instances. In the t(6;9), long-term (Sca1+/c-Kit+/lin-/Flk2-) hematopoietic stem cells (LT-HSC) appear to be the leukemia-initiating cells, while leukemia-maintaining cells represent a larger and phenotypically heterogeneous cell population [8]. The t(6;9) is found in about 1% of AMLs (0.9% in the series of 69 cases, with a repartition of 1.4% in children AMLs, and 0.7% in adult cases; median age was 23 years (range 2-66 years), with 30 children out of 69 cases (43%), a younger age than in AML in general. From 199 cases herein reviewed, the sex ratio is balanced: 1M/1F (100 male

patients and 99 female patients) [6]. The t(6;9)(p21-22;q34) may be seen in either AML M2 or less frequently in M4 or MDS and acute myelofibrosis often in association with excess basophils. The t(6;9) is reported mostly in young adults. The prognosis of patients carrying the t(6;9) is unfavourable. Overall survival in patients with t(6;9)(p23;q34) in AML is the unfavourable risk cytogenetics subgroup [7].

The t(6;9) is often the sole abnormality present in the neoplastic cells, which argues for a direct involvement of the translocation in the leukemic process. The occurrence of defined chromosomal translocations in specific subtypes of leukemia strongly suggests that these translocations play an important role in the process of leukemogenesis. Translocations may activate nearby cellular genes involved in the control of proliferation and/or differentiation. The segments of chromosome 6 & 9 that are translocated in t(6;9) AML are very small and are not easily distinguishable by cytogenetic techniques and required expertise. Our patient was expired within a week of diagnosis which indicates poor prognosis.

## Conclusion

t(6;9) has been included as a separate entity in the revised 2008 World Health Organization of AML. It is therefore important to be aware of this relatively rare abnormality because these patients respond poorly to chemotherapy alone and may require the inclusion of allogenic stem cell transplantation early in the treatment plan. Clustering of the breakpoints on chromosomes 6 and 9 in combination with disruption of the CAN transcription unit argue for a causative role of the t(6;9) in leukemia. Further analysis is needed to define the exact nature of the CAN disruption and its effect on the biological activity of the putative CAN protein.

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