



A Novel Four-Way Translocation t(1;3;9;22) (p36;p14;q34;q11.2) involving Philadelphia Chromosome in a Patient of Chronic Myeloid Leukemia: A Case Report

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Abstract

90% of Chronic Myeloid Leukemia (CML) patients having Philadelphia (Ph) chromosome or t(9;22)(q34;q11.2), and the remaining 5–8% of CML patients demonstrate variant translocations, with the involvement of third, fourth, or fifth chromosome other than 9;22. Though, in very rare cases, the fourth chromosome is involved. Here, we found a novel case of four-way translocation involving 46,XY,t(1;3;9;22) (p36;p14;q34;q11.2) in CML-chronic phase. In the present study conventional cytogenetic result showed variant translocation t(9;22) along with chromosome 1p36 and 3p14 which was confirmed by Fluorescence in Situ Hybridization (FISH) technique. The patient is successfully treated with a dose of 400 mg/day Imatinib Mesylate (IM) and patient achieved Complete Hematological Response (CHR) within 4 months.

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Keywords: Chronic Myeloid Leukemia (CML); Variant Ph chromosome; Conventional Cytogenetics; Fluorescence in Situ Hybridization (FISH).

Introduction

In CML reciprocal translocation occurred by association of 3' sequences from the *ABL* (Ablason) on chromosome 9, with the 5' sequences of the truncated *BCR* (Breakpoint Cluster Region) on chromosome 22 [1,2]. The variant translocation was observed in 5–8% of cases with an involvement of additional chromosome [3].

As per literature variant Ph breakpoints were commonly occurring in the G-light bands, within the cytosine (C) and guanine

(G) richest regions of the genome [9]. GC content was related to chromatin condensation and transcription activity. Open chromatin is transcriptionally active and likely to undergo breakage and repair, and as a results in illegitimate recombination and translocation [4].

According to Atlas of Genetics and Cytogenetics in Oncology and Haematology, if a case is reported for the first time (not reported in literature) it is considered as novel case, if case is reported less than 20 times, it is considered rare case and if the case is reported more than 20 times it is considered as recur-



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rent abnormality. Database was last updated on 6 June, 2022 [5]. Breakpoint with 1p36 region was found to be in 22 CML cases and with 3p14 was found to be affected in 7 CML cases. But, with $t(1;3;9;22)(p36;p14;q34;q11.2)$ not previously found so, for the first time, we present a four-way Ph translocation in a CML patient with a new complex rearrangement between chromosomes 1 and 3 as well as 9 and 22.

Material and methods

Case details

A 42-year-old male was referred to The Gujarat Cancer & Research Institute, Ahmedabad, India with chief complaints of generalized weakness, weight loss and abdominal pain. The hematologic parameters were as follows: Hemoglobin 5.7 gm/dl, platelet count $451.00 \times 10^3/\text{cmm}$, and white blood cell count of $194.30 \times 10^3/\text{cmm}$ with $161.90 \times 10^3/\mu\text{l}$ absolute neutrophil count, $11 \times 10^3/\mu\text{l}$ absolute lymphocyte count, 5.70% lymphocytes, 5.40% monocytes, 5.40% eosinophils, 83.30% polymorphs and 0.2% basophiles. The bone marrow aspirate revealed hypercellularity with 8% blasts, 2% promyelocytes, 17% myelocytes, 8% metamyelocytes, 4% band cells, 36% polymorphs, 2% eosinophils, 12% basophils, 7% lymphocytes, 3% late normoblast, 1% inter normoblast and 1% early normoblast. The marrow showed increased myeloid precursors with an increased myeloid to erythroid ratio, and a myelo-poly peak thus suggesting CML-CP. The patient was treated with IM at 400 mg/day after the diagnosis. Patient achieved Complete Hematological Response (CHR) within 4 months.

Conventional cytogenetics

For cytogenetic study, bone marrow or blood samples were collected, and short-term culture were carried out in RPMI-1640 medium, Fetal Bovine Serum and Heparin. After overnight incubation, harvesting, washing, slide preparation and GTG banding was performed according to standard protocol. Metaphases were captured in automatic karyotyping system (AXIO Imager.Z2, Zeiss USA) and analysis was done using IKAROS software. Karyotyping description was done according to the International System of Cytogenetic Nomenclature (ISCN) 2016 guidelines [6].

Fluorescence in Situ Hybridization (FISH)

BCR/ABL probe

FISH was performed using Dual Colour Dual Fusion *BCR/ABL* Locus Specific Identifier (LSI) probes, according to manufacturer's (Zytovision) instructions. Images were captured on Epi-fluorescence microscope (AXIO Imager.Z2, Zeiss, USA) and analysis was done using ISIS software (Metasystems, Germany).

Whole Chromosome Painting (WCP) probe

The WCP FISH was carried out to determine the nature of the translocation. WCP probe for chromosome 3 and 22 (Abbott Molecular, USA) used in metaphases. WCP 3 spectrum green and WCP 22 spectrum orange probe was used.

Results

Conventional cytogenetics

Karyotyping was done before initiation of treatment, and the results were described as $46,XY,t(1;3;9;22)(p36;p14;q34;q11.2)$ [20] (Figure 1). The results were further characterized by molecular cytogenetic studies.

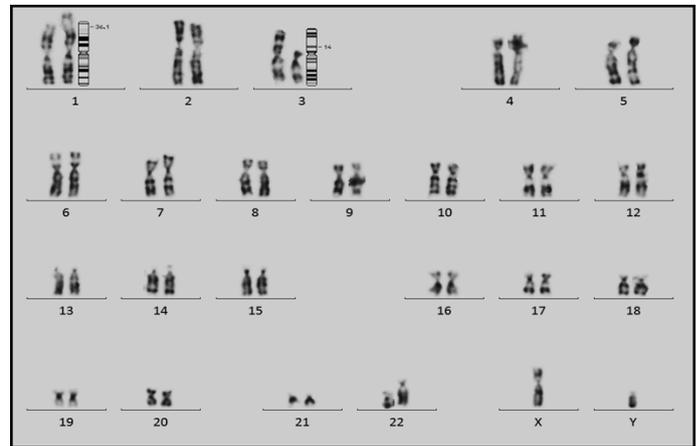


Figure 1: GTG banded Karyotype image showing $46,XY,t(1;3;9;22)(p36;p14;q34;q11.2)$.

Fluorescence in situ hybridization

BCR/ABL DCDF FISH

The FISH results with *BCR/ABL* DCDF revealed variant positive signal pattern, i.e. 2G2O1F and confirmed *BCR/ABL* fusion gene (Figure 2).

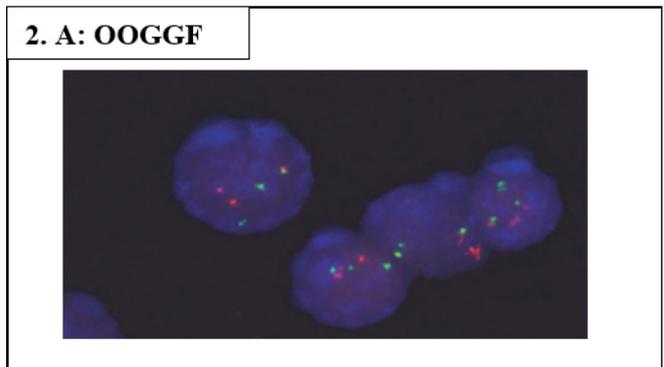


Figure 2: Representative FISH results with *BCR-ABL* DCDF FISH probe showing OOGGF signal Pattern.

3. WCP 22(SO) & WCP 3(SG)

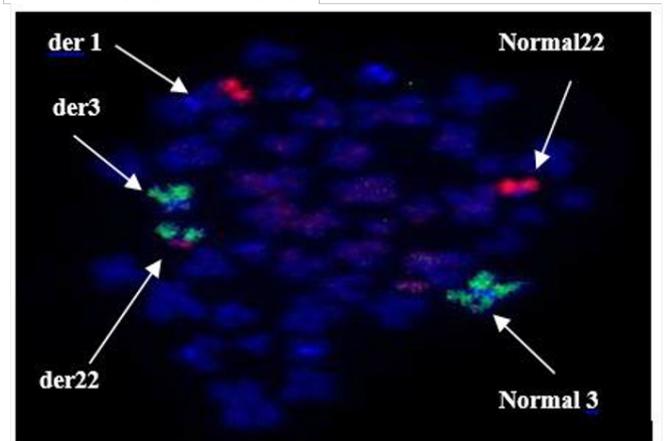


Figure 3: Whole chromosome painting FISH for chromosome 22 (spectrum orange) and for chromosome 3 (spectrum green) confirmed the translocation.

BCR/ABL DCDF

Whole Chromosome Paint (WCP) FISH

The WCP FISH for chromosome 22 Spectrum Orange (SO)

and chromosome 3 Spectrum Green (SG) was used (**Figure 3**). WCP FISH results showed that, p arm of chromosome 3 was observed on q arm of chromosome 22 showing translocation between 3 and 22. Orange signal which was tagged for chromosome 22, observed on p arm of chromosome 1 showing translocation between 1 and 22. One green colour A group chromosome was normal chromosome 3 and one orange colour G group chromosome was normal chromosome 22 (**Figure 3**). So, FISH result confirmed the translocation between 1;3;9;22.

Discussion

The accurate and decisive diagnosis of CML should be confirmed by the presence of Ph chromosome/*BCR-ABL* fusion gene as a result of $t(9;22)(q34;q11.2)$. This genetic abnormality identified by cytogenetic method, and it has important role in diagnosis of CML. Conventional cytogenetic analysis using GTG banding remain as gold standard method for CML diagnosis and treatment monitoring [7]. Except Ph chromosome, cytogenetic analysis is also able to identify Additional Chromosomal Abnormalities (ACAs), including complex or variant translocations, which are associated with CML evolution [8]. For molecular confirmation of cytogenetic findings, FISH technique is routinely used. It can be done even on interphase cells from the PB or BM. The sensitivity of this technique ranges varies from 0.1-1% [8,9].

The mechanism involved in the formation of variant Ph translocation remains indefinable. According to the literature, there are two different mechanisms: a one-step mechanism in which chromosome breakage occurs simultaneously on 3 or 4 different chromosomes in 3-way or 4-way translocation, respectively, and a 2-step mechanism involving two consecutive translocations in which a standard $t(9;22)$ translocation is followed by a second translocation involving additional chromosomes [10,11]. These mechanisms may have prognostic importance in that a single-step rearrangement may confer a prognosis like the classical Ph translocation, where a multi-step mechanism represents clonal evolutions associated with a worse prognosis [12]. In our patient, simultaneous breakage occurred on the third and four chromosomes along with chromosome 9 and 22. As present study patient had a single-step variant Ph rearrangement, the prognosis of our patients did not differ from classical Ph patients.

IM potentially hinders *BCR-ABL* protein tyrosine kinase. It also inhibits the tyrosine kinase activities of the Platelet Derived Growth Factor (PDGF) receptor β and c-Kit. IM is recommended for Ph-positive chromosome-associated abnormalities. Therefore, it would be also efficient in three-way, four-way, and five-way complex variant translocations [10].

1p36 contains PRDM16 (PR domain containing 16) gene which plays a downstream regulatory role in mediating Transforming Growth Factor β (TGF β) Signaling [13]. PRDM16 is also required for cell-cycle regulation and self-renewal in neural stem cells [14]. 3p14 contain *FHIT* (Fragile Histidine Triad) gene which is more frequently missing in cancers of individuals with familial mutations causing deficiency in DNA repair genes. The *FHIT* locus is involved in translocations and deletions in some fraction of many types of cancer, likely due to the recombinogenic of the fragile region within *FHIT* and subsequent selective growth or survival advantage of cells with reduced *FHIT* protein expression [15]. Chromosome 3 is not frequently involved in complex translocations in CML.

The clinical significance of identifying variant translocations has remain uncertain over the years and mechanisms of the generation were not fully clear [14]. Studies that are more recent revealed that patients with a variant Ph had the same prognosis as those with a standard Ph so; patients with variant translocations do not comprise a “warning” category in the IM era [13, 15]. Similarly, in the present study patient responded well to treatment and achieved hematological response within 4 months.

Conclusion

In the TKIs era, it seems that variant Ph translocations have no allusion on the prognosis, disease evolution and treatment response. Here, in the present study patient showed good response to the IM therapy, which affirmed the previous studies. The present study also expressed that the combined conventional cytogenetic, FISH, and clinical studies can bring to light the frequency of this event and correlation with prognosis.

Conflict of interest disclosure statement

The authors declare that they have no conflict of interests.

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