International Journal of Clinical and Experimental Medical Sciences
2015; 1(2): 30-34
Published online July 1, 2015 (http://www.sciencepublishinggroup.com/j/ijcems)
doi: 10.11648/j.ijcems.20150102.16

Characterization of Three-Way Translocation [t(4;9;22)(p16;q34;q11.2)] in Chronic Myeloid Leukemia

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To cite this article:
Salil Vaniawala, Pratik Chavda, Ganesh Jori, Keur Patil, Pankaj Gadhia. Characterization of Three-Way Translocation [t(4;9;22)(p16;q34;q11.2)] in Chronic Myeloid Leukemia. *International Journal of Clinical and Experimental Medical Sciences*. Vol. 1, No. 2, 2015, pp. 30-34. doi: 10.11648/j.ijcems.20150102.16

Abstract: The Philadelphia (Ph) chromosome, consisting of the t(9;22)(q34;q11.2) is observed in 90% with chronic myeloid leukemia (CML), while variant translocations are observed in 5 to 10%. In variant translocations, three way translocations are rare. We report two cases of three way translocation involving chromosomes 4, 9 and 22. Bone-marrow was subjected to conventional cytogenetic and fluorescence in situ hybridization (FISH) and three way translocation was identified as 46,XX,t(4;9;22)(p16;q34;q11.2). Although other chromosomes are frequently involved in three-way translocation, chromosome 4 is very rare event. So far five cases have been reported in the literature with translocation involving 4p16. We present a six case of chronic myeloid leukemia having 4p16 breakpoints whose clinical interpretation is still unclear.

Keywords: Chronic Myeloid Leukemia, t(9;22), Three-Way Translocation Variant, FISH, Cytogenetic

1. Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder arising from neoplastic transformations in a pluripotent cell. The hallmark of CML is presence of Philadelphia chromosome (Ph), resulting from the t(9;22)(q34;q11.2) [1]. The crucial consequence of this translocation is the formation of a novel and chimeric BCR/ABL (break point cluster region-Abelson) gene in the breakpoint region of the derivative chromosome [2]. The BCR/ABL fusion gene generated encodes on oncogenic protein tyrosine kinase which causes the genesis of CML by activating multiple signaling pathways that are involved in cell cycle, adhesion and apoptosis [3].

In 70 – 85% of CML cases having t(9;22) Philadelphia positive, however, in 5 to 10% of CML cases, there is a variant Ph translocation with generally third chromosome involved with chromosome 9 and 22 [4]. The segment from third chromosome is usually translocated to band 9q34 whereas the 22q11→qter segment (including the 3' BCR region) moves to breakpoint of third chromosome [5].

The formation of the variant Ph translocation is controversial topic. A wide array of additional chromosome involved in translocation with the t(9;22) has been described in CML [6]. With regard to involvement of chromosome 4 that is rare, and only five previously reported cases showed a breakpoint at 4p16 [7]. In the present study we report the clinical, cytogenetic and FISH findings of two patients with complex translocation involving chromosome 4, 9, 22 at breakpoint 4p16.

2. Materials and Methods

The present study included patients from March, 2014 to February, 2015. After taking informed consent from the patients, we examined bone-marrow samples.

Of 732 cases, of which 378 (51.6%) were turned out as confirmed CML. Further analysis of variant translocations revealed only two cases of t(4;9;22)(p16;q34;q11.2) three-way complex translocation.

Conventional cytogenetic (CC) analysis was performed by our laboratory on all patients. Bone-marrow (BM) samples were cultured for 24-48 hours in Marrow Max medium without mitogen and with 10ug/ml colcemid solution. The chromosomal slides were prepared according to standard procedures. Standard Giemsa chromosome banding stain (GTG banding) (pretreatment with trypsin then Giemsa stain) was employed [8] on metaphase spread obtained. Depending upon availability, 20 – 25 metaphases per sample were analyzed. The karyotyped description followed [9] International System for Human Cytogenetic Nomenclature (ISCN) 2009 [9] recommendations.

Fluorescence In situ Hybridization (FISH) analysis was performed on prepared slides of fixed BM cells using BCR/ABL dual colour, dual, fusion kit (Vysis, Germany). Fluorescent signals were visualized under Axiomager Z2, Carl-Zeiss microscope.

3. Results

Conventional cytogenetic (CC) analysis of 732 patients (from March, 2014 to February, 2015) showed 378 cases with classical t(9;22)(q34;q11,2) and rest 354 cases showed normal karyotype. Interestingly 2 patients had a variant Ph chromosome translocation.

Case 1:

The case number 1 was a 34-year old woman. Her blood analysis showed total white blood cell count (WBC) as 214 X 10^9 g/L, platelets were 606 X 10^9/L, hemoglobin content was 11.3 g/dl, 33% lymphocytes and 4% monocytes. She had 25% blast cells.

Cytogenetic study from bone-marrow revealed complex translocation having chromosome 46, XX, t(4;9;22) (p16;q34;q11.2) (Fig. 1).

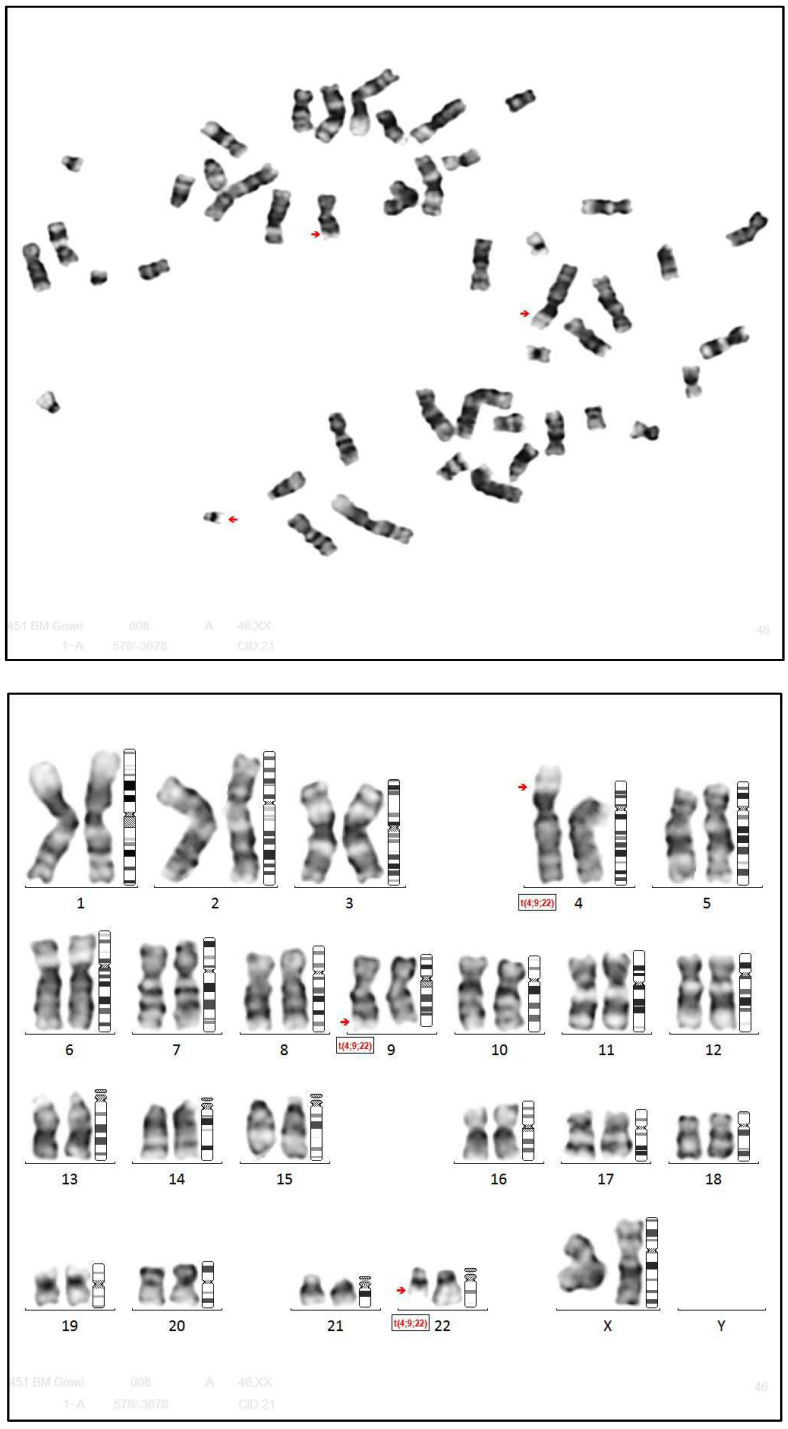

Figure 1. *Karyotype of a Female with Chronic Myeloid Leukemia with Three-Way Translocation, 46,XX,t(4;9;22)(p16;q34;q11.2).*

Case 2:

The second case was a 35-year old women showing WBC count as 148 $X10^9$ g/L. Her differential count showed 43% lymphocytes, 6% monocytes, platelets count was 535 X 10^9/L and hemoglobin content was 12.3 g/dl. She had 65% blast cells. The cytogenetic study revealed variant translocation having 46, XX, t (4; 9; 22) (p16;q34;q11.2) (Fig. 2).

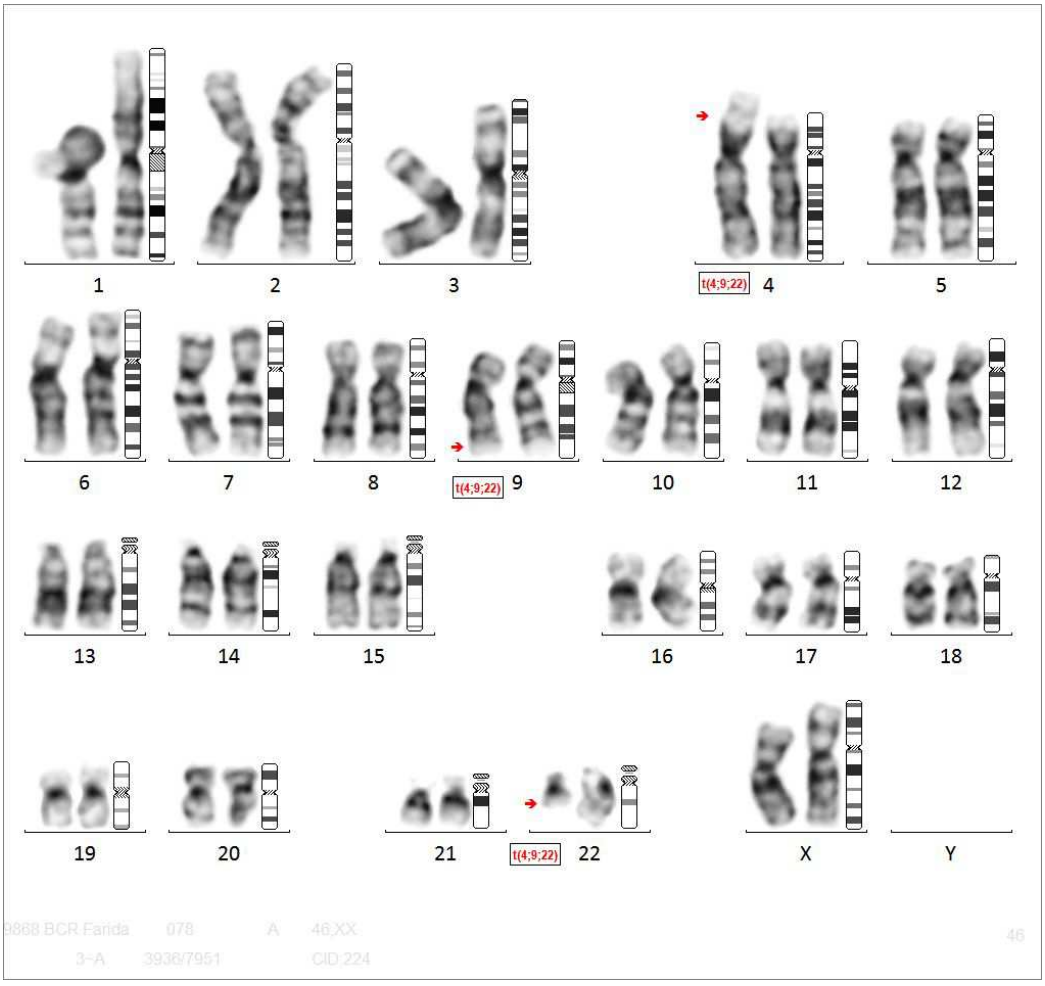

Figure 2. *Karyotype of a Female with Chronic Myeloid Leukemia with Three-Way Translocation, 46,XX,t(4; 9;22)(p16;q34;q11.2).*

4. Discussion

Most CML patients show the Philadelphia (Ph) chromosome arising from reciprocal t(9;22), but 5 to 10% present variants of this translocation involving different breakpoints besides 9q34 and 22q11.2 [10]. The formation of variant Ph translocation is a controversial topic and recognized for more than 25 years. The clinical course and influence of these variants on long term outcome are not well understood.

In the present study, we analyzed 732 cases of clinically suspected CML, of which 378 were confirmed CML with classical translocation of t(9;22) and Philadelphia positive. Among 378 confirmed cases of CML, we found two cases of three-way translocation involving chromosome 4 and breakpoint band p16. However, involvement of chromosome 4 is rare, and previous only five cases showed a break at 4p16 (Table1) [11-15].

Table 1. *CML Patients Karyotyped Reported in Literature for t(4;9;22) with 4p16 locus.*

Case	Karyotype	Locus	Clinical evaluation	Ref.No
1	46,XY,t(4;9;22)(p16;q34;q11.2)	4p16	CML: patients entered BC, additional structural Changes were seen	11
2	46,XX,t(4;9;22)(p16;q34;q11.2)	4p16	CML: Karyotyped reported when she was in CP	12
3	46,XY,t(4;9;22)(p16;q34;q11.2)	4p16	Karyotyped reported in CP	13
4	46,XY,t(4;9;22)(p16;q34;q11.2)	4p16	Karyotyped reported with BC	14
5	46,XX,t(4;9;22)(p16;q34;q11.2)	4p16	Karyotyped reported with CP	15
6	46,XX,t(4;9;22)(p16;q34;q11.2)	4p16	Karyotyped reported with CP	Present case
7	46,XX,t(4;9;22)(p16;q34;q11.2)	4p16	Karyotyped reported with BC (65%)	Present case

BC: Blast Crisis, CP: Chronic Phase

As such masked translocations are difficult to visualize with routine cytogenetics. Few studies suggested that patients with masked and variant Ph translocations have adverse prognosis [16, 17], but others have suggested no prognostic effect [18, 19]. It is known that variant Ph breakpoints usually occur in the G-light bands within the cytosine (C) and guanine (G) richest regions of the genome.

It is well established that breakpoint 4p16 is rare in CML variant and as per Mitelman database [7] only five cases have been reported. In the present study, we reported 2 female cases from Western India. Case number 1 was reported to us with chronic phase and case number 2 with blast phase, both showed three-way translocation and chromosome complement of 46,XX,t(4;9;22)(p16;q34;q11.2) with conventional cytogenetics. While D-FISH results indicated that three way translocation was one step mechanism where 1 fusion, 2 green and 2 orange (1F,2G,2O) signals were recorded (Figure 3).

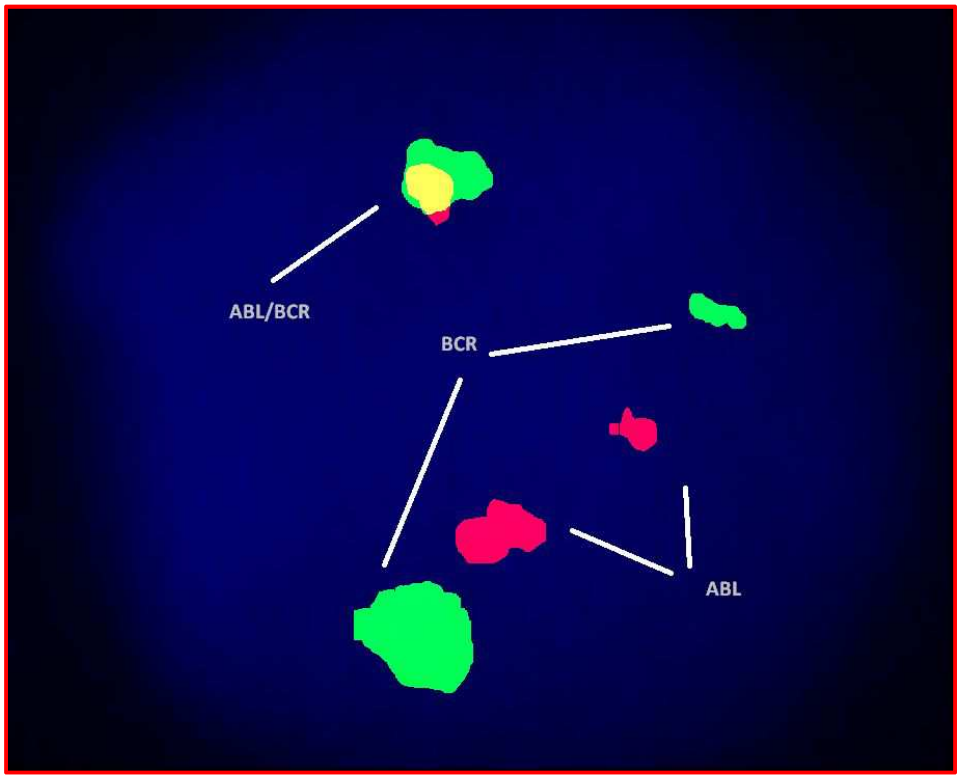

Figure 3. *FISH Interphase Cell Showing 1 Fusion, 2 Green and 2 Orange Confirming Ph Positive Variant Chromosome Compliment.*

It is reported in the literature that different mechanisms involved in the formation of variant translocation may have different clinical implications. The clinical significance of variant t(9;22) translocation is not clear but a study carried out by Albano et al.[20] have shown that the rearranged regions are characterized by an elevated content of miRNAs, Alu repeats, GC and known genes.

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