Enigmatic Inv(9): A Case Report on Rare Findings in Hematological Malignancies

Sangeetha Vijay,1 Geetha Narayanan,2 Santhi Sarojam,1 Suresh Kumar Raveendran,1 and Sreedharan Hariharan1,*

1Regional Cancer Centre, Division of Cancer Research, Medical College PO, Thiruvananthapuram-695 011, Kerala, India
2Regional Cancer Centre, Division of Medical Oncology, Medical College PO, Thiruvananthapuram-695 011, Kerala, India
*Corresponding author: Sreedharan Hariharan, Regional Cancer Centre, Division of Cancer Research, Medical College PO, Thiruvananthapuram-695 011, Kerala, India. Tel: +91-712522204, Fax: +471-2447454, E-mail: drshariharan@gmail.com

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Abstract

Introduction: Inversion of chromosome 9 had been widely discussed among geneticists and evolutionary biologists because of its significant impact on various hereditary disorders and in the evolution of man. The role of such inversions in human disease evolution is an area hitherto unclear.

Case Presentation: We present the case of a chronic myeloid leukemia (CML) patient who showed intermittent relapse on treatment, with a rare appearance of clones with dual inversion (9) breakpoints [inv(9)(p22q34); inv(9)(p11q21)]. We also present the first report of inv(9)(p11,q13) as the sole abnormality in a patient with chronic myeloproliferative disorder (CMPD). Both the patients registered in 2012 and were from Kerala, India.

Conclusions: Both the cases discussed in our study have inv(9) as the sole abnormality and are found to confer a relatively poor prognosis.

Keywords: Chromosome 9, Inversion, Chronic Myelogenous Leukemia, BCR-ABL Positive, Chronic Myeloproliferative Disorder

1. Introduction

An inversion is a structural aberration of the chromosomes due to an intrachromosomal break and subsequent rearrangement. The pericentric inversion of chromosome 9 or inv(9) is commonly seen in normal humans and its frequency is estimated to be from 1 to 3% in the general population. The most common inversion seen in humans is on chromosome 9, at inv(9)(p12q13). The disease association of inv(9)(p12q13) has been reported in various human diseases, particularly couples with repeated spontaneous abortions, bad obstetric history, infertility, and congenital anomalies. The abnormal phenotype was found to be expressed due to unbalanced inversions at different breakpoint regions. There are very few Indian reports to estimate the frequency and clinical impact of inv(9) in the population. Among them, one study (1) showed a high frequency of inv(9)(p12q13) (64.9%) among patients with genetic disorders, which also infers its decisive role in disease development, especially in the case of de novo inversions.

In spite of the reports on the significant impact of inv9 in genetic disorders and evolutionary patterns, there are very few reports on the inv(9) variations in hematological malignancies. There are, however, contradictory reports about inv(9) being regarded as a constitutional abnormality with familial inheritance (2) as well as an acquired chromosomal abnormality in hematological malignancies. Most of the studies perceive inv(9) as a constitutional abnormality with a minor molecular implication on prognosis (3).

In this report, we are presenting a novel occurrence of two types of inv(9) breakpoints in a CML patient showing intermittent relapse on treatment, and the first report of a CMPD case showing inv(9)(p11,q13).

2. Case Presentation

The first case is a 33-yr-old male, who presented to the out-patient clinic of the medical oncology division of regional cancer centre, Trivandrum in January 2012. The patient came from Kerala, India. Approval for this study was obtained from our institutional review board [HEC No. 41/2008 dated December3,2008]. After getting informed consent from the patient, bone marrow samples (up to 2 mL) were collected to perform the chromosome and FISH analysis, along with routine Cytopathological investigations. Also, peripheral blood (5 mL) was collected for the molecular analysis by venipuncture. The initial complete blood count results showed an Hb level
of 13.5 g/dL; a platelet count of 276,000 cmm; and a total count (TC) of 95,500 cmm with 45% segmental neutrophils, 5% lymphocytes, 4% basophils, 20% myelocytes, 10% promyelocytes, 10% metamyelocytes and band forms, and 3% myeloblasts. The bone marrow samples were processed by direct short-term culture methods without mitogen stimulation. Mitoses were harvested after hypotonic treatment with 0.075 M KCl, and slides were prepared using conventional technique. GGT-banding was performed using trypsin solution and 20 to 35 GGT banded metaphases were analyzed for patient using Cytovision software (USA). Chromosome identification and karyotype analysis was carried out according to the international system for human cytogenetic nomenclature and response was analyzed based on European leukemia net recommendations (2009). Bone marrow aspiration and biopsy reports were consistent with CML-chronic phase. The initial chromosome analysis showed the 46,XY,inv(9)(p?q22.3),t(9;22)(q34;q11.2)?der(16) karyotype in all the 20 metaphase cells. FISH analysis was performed on bone marrow samples using VYSIS® LSI® BCR/ABL dual color, dual fusion (DC, DF) FISH probe as per manufacturer’s instructions. Two red and two green signals indicates normal cells without the fusion gene, and one green, one red and two red-yellow-green fusion signals indicates t(9;22)/BCR-ABL. In this patient, FISH analysis confirmed the presence of the BCR-ABL fusion gene. The patient was treated with imatinib mesylate (IM) (400 mg, oral tablets) after initial diagnosis and a complete cytogenetic response was achieved within 6 months, and the karyotype evolved with a new breakpoint of inv(9) as 46, XY, inv(9)(p12q13). In the karyotype analysis after 9 months, 30% metaphases showed 46, XY, t(9;22)(q34;q11), inv(9)(p12q13) and 70% metaphases with 46, XY, inv(9)(p12q13) pattern (without Ph). On continuation of therapy with the same drug dose, all the clones were normal with 46, XY pattern, (inv(9) and Ph not seen) by the 12th month. But after 18 months of therapy, Ph positive clones (20%) reappeared with the presence of inv(9)(p12 q13) in all the metaphases (Figure 1). On continuation of therapy, the Ph positive clones dropped down to 5% after 36 months and were undetectable by the 48th month. The karyotype obtained at the 48th month showed the following admixed breakpoint of inv(9) in the karyotype: 46, XY, inv(9)(p12q13)[60%] /46, XY, inv(9)(p22q34)[40%]. From this, it can be assumed that the patient had different clones for the three types of breakpoints, of which inv(9)(p?q22.3) was found associated with the clones positive for Philadelphia chromosome. These clones must have diminished during the initial phase of therapy and were replaced by normal clones with inv(9)(p12q13) in all the 20 metaphases analyzed, and which can be suspected of being a constitutional ab-normality. Although the patient showed an optimal response to the drug, the intermittent relapse and loss of consistency of disease-free survival may be attributed to the presence of inv(9) subpopulations along with (Table 1). Allele specific PCR (ASO-PCR) (4) was performed to detect eight major BCR-ABL kinase domain mutations (T315I, F317L, G250E, Y253H, Y253F, E255V, E255K and M244V) from DNA isolated from the peripheral blood sample of the CML patient at diagnosis and on intermittent relapse using Phenol Chloroform extraction method. The involvement of eight major BCR-ABL kinase domain mutations in causing the intermittent relapse was ruled out by ASO-PCR analysis. The second case is a 75-year-old male patient with obvious splenomegaly, a history of breathlessness, loss of appetite and weight loss, who presented to the center in April 2012. The patient was from Kerala, India. Initial bone marrow aspiration and biopsy diagnostic reports were consistent with Ph negative chronic myeloproliferative disorder. Together, the hematological profile showed Hb -8.3 gm%, platelet count of 70,000 mm3, and total count (TC) of 79,500 mm3 with 10% lymphocytes, 2% eosinophils, abnormal cells, 38% and 50% polymorphs. The chromosomal analysis showed 46,XY,inv(9)(p1q13) pattern in all the 20 metaphase cells analyzed (Figure 2). The absence of BCR-ABL fusion gene was confirmed by FISH.

3. Discussion

A careful review of literature and Mitelman’s database revealed that among the various neoplasms/malignancies, concomitant inv(9) was the most frequently reported in different subtypes of acute myeloid leukemias, and its detection is considered important in hematologic malignancies (5). According to a Korean study (2), the inv(9) variation is relatively higher (7.8%) in CML patients than in acute leukemia patients. However, there have been very few studies on inv(9) detection in CML or CMPD, which indicates that this variation is either overlooked or underestimated.

In chronic myeloid leukemia, most of the cases reported have inv(9) along with different additional abnormalities, with Ph or without Ph. However, an optimal response was reported by most of the authors (6) as opposed to a few reports stating it was a poor prognostic indicator (7). The breakpoint (p22 q34) of inv(9) has been reported in only two other CML cases (8), known to be a rare and recurrent secondary chromosomal abnormality in Ph positive CML. Though contradictions exist, this breakpoint is said to be associated with poor prognosis (7). The presence of cytogenetic abnormalities in Ph-negative cells is considered a warning signal. Only one CML case with inv(9) is reported to show a complete cytogenetic response, but the type of breakpoint is undefined (6). Since there were
no BCR-ABL kinase domain mutations, the intermittent relapse in the CML patient of our study must be attributed to the presence of different clones with inv(9). This warrants a detailed molecular analysis on the physiopathological effects imparted by each type of breakpoint in inv(9), rather than considering inv(9) in general.
To the best of our knowledge, there were only two reports of CMPD patients showing the presence of inv(9). In one report, the breakpoint is not mentioned, and in the other, the breakpoint (p23q13) with an additional translocation [Karyotype:46,XY,t(5;14)(q33;q32),inv(9)(p23q13)c] is reported. Thus, we will be adding to the existing information, the presence of inv(9)(p11 q13) among CMPD cases. The prognosis of this patient was poor and the patient expired. Thus, taken together, both the cases discussed in our study had inv(9) as the sole abnormality and are found to confer a relatively poor prognosis.

Inversions are known to cause positional changes of the polymorphic regions and might influence gene expression. They are thought to cause partial amplifications or deletions of the constitutive heterochromatin, resulting in a loss or an increase of gene function. Kaiser has explained the de novo versus familial inversions in phenotypically abnormal probands, and detailed possible explanations to be the down-regulation of gene expression due to localization of the inversion breakpoint within cistrons, and further, a shift of genetically active DNA into the neighborhood of heterochromatin, resulting in heterochromatinization and thus an inactivation of previously euchromatic segments (9).

Chromosome 9 contains non-coding heterochromatin regions which form the inv(9) breakpoints, also called “gene deserts.” And, according to Francis Collins, director of the National Institute of Health and a leader in the original Human Genome Projects, they are “like the seat of the soul of the genome”. Nature reports a study showing these gene deserts as hotspots in diseases (10). We expect that upcoming studies would scale up the new approach and use other methods to annotate additional non-coding DNA variants identified through genome-wide association studies (GWAS): Studies linked to human disease, and which must answer to the enigmatic inv(9).
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Footnotes

Authors’ Contribution: Acquisition, analysis, and interpretation of data and drafting of the manuscript: Sangeetha Vijay; drafting of the manuscript: Santhi Sarojam, Suresh Kumar Raveendran; administrative, technical, and material support: Geetha Narayanan; study concept and design and study supervision: Sreedharan Hariharan; critical revision of the manuscript for important intellectual content: Sreedharan Hariharan and Geetha Narayanan.

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