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A rare case of a three way complex variant positive Philadelphia translocation involving chromosome (9;11;22)(q34;p15;q11) in chronic myeloid leukemia: A case report

MUHAMMAD ASIF[1,2], ABRAR HUSSAIN[1] and MAHMOOD RASOOL[3]

[1]Department of Biotechnology and Informatics; [2]Office of Research Innovation and Commercialization, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Balochistan 87300, Pakistan; [3]Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Makkah 21589, Saudi Arabia

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Abstract. The t(9;22)(q34;q11) translocation is present in 90-95% of patients with chronic myeloid leukemia (CML). Variant complex translocations have been observed in 5-8% of CML patients, in which a third chromosome other than (9;22) is involved. Imatinib mesylate is the first line break-point cluster region-Abelson gene (BCR/ABL)-targeted oral therapy for CML, and may produce a complete response in 70-80% of CML patients in the chronic phase. In the present study, a bone marrow sample was used for conventional cytogenetic analysis, and the fluorescence *in situ* hybridization (FISH) test was used for BCR/ABL gene detection. A hematological analysis was also performed to determine the white blood cell (WBC) count, red blood cell count, hemoglobin levels, packed and mean cell volumes, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet values of the patient. The hematological analysis of the patient indicated the increased WBC of 186.5x10^3 cells/μl, and decreased hemoglobin levels of 11.1 g/dl. The FISH test revealed that 67% cells demonstrated BCR/ABL gene translocation. The patient was treated with 400 mg imatinib mesylate daily, and was monitored at various intervals over a 6-month period. The present study reports the rare case of a patient that demonstrates a three-way Philadelphia chromosome-positive translocation involving 46XY,t(9;11;22)(q34;p15;q11)[10], alongside CML in the chronic phase. The translocation was analyzed using cytogenetic and FISH tests.

Correspondence to: Mr. Muhammad Asif, Office of Research Innovation and Commercialization, Balochistan University of Information Technology, Engineering and Management Sciences, Iqbal Hall, Building 1, Takatu Campus, Airport Road, Baleli, Quetta, Balochistan 87300, Pakistan
E-mail: asifjallali@yahoo.com

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Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of pluripotent stem cells. CML is caused by reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11), termed the Philadelphia chromosome (Ph) (1,2). The translocation consists of the combination of the break-point cluster region (BCR) and Abelson (ABL) genes to form the fusion gene BCR/ABL, which produces a chimeric protein with deregulated tyrosine kinase activity. The tyrosine kinase activity is responsible for regulating the maintenance of adult tissues, aiding signaling pathways and regulating cell division (3-5). The fusion gene BCR/ABL is associated with increased levels of erythrocytes, monocytes, megakaryocytes, myelocytes and platelets in the peripheral blood and marked myeloid hyperplasia in the bone marrow (6).

The Ph-positive translocation that results from reciprocal translocation between chromosomes 9 and 22 (q34;q11) is present in 90-95% of CML cases. However variant or complex translocations involving one or more additional chromosomes compared with (9;22)(q34;q11) may be observed in 5-8% CML patients (1,7,8).

Gleevec imatinib mesylate (Novartis Pharmaceuticals, Basel, Switzerland) is an orally-designed selective BCR/ABL protein tyrosine kinase inhibitor, which may induce a complete cytogenetic response in 65-90% of CML patients (9,10). The therapy is the first line treatment for >90% of CML patients, and the key function of imatinib mesylate is to hinder proliferation and induce apoptosis in cells that express the BCR/ABL gene (7). There is evidence to suggest that imatinib mesylate may prolong survival and improve the quality of life for patients with CML (6,11,12).

The present study reports of the case of a 45-year-old male patient diagnosed with CML in the chronic phase of disease, who demonstrated a rare Ph-positive three-way complex variant translocation involving chromosome

46XY,t(9;11;22)(q34; p15; q11.2)[10]. Written informed consent was obtained from the patient.

Case report

A 45-year-old male patient was diagnosed with CML at the Sundayman Civil Hospital (Quetta, Balochistan, Pakistan) on August 5, 2008. The patient was referred to the hospital due to a 15 week history of weight loss, sleep disturbance, fever, anxiety, depression, dry skin, sweating, swelling on body and high cholesterol levels. The hematological parameters of the patient were as follows: White blood cell (WBC) count, 186.5x10^3 cells/μl (normal range, 4-11x10^3 cells/μl); hemoglobin, 11.1 g/dl (normal range, 14-18 g/dl); platelet (PLT) count, 235x10^3 PLTs/μl (normal range, 150-400x10^3 PLTs/μl); neutrophils, 66% (normal range, 40-75%); lymphocytes, 2% (normal range, 20-45%); eosinophils, 1% (normal range, 1-6%); monocytes, 6% (normal range, 2-10%); myelocytes, 15% (normal range, 0%); metamyelocytes, 4% (normal range, 0%); promyelocytes, 6% (normal range, 0%); and basophils, 0% (normal range,0-1%). Due to the high WBC count and low hemoglobin levels identified, the patient was subsequently administered ongoing treatment with imatinib mesylate (400 mg/day).

For complete blood cell analysis, a hematological analyzer (Celltac F MEK-8222; Nihon Kohden Corporation, Tokyo, Japan) was used to assess the total leukocyte, WBC, neutrophil, lymphocyte, eosinophil, monocyte, myelocyte, promyelocyte, basophil and platelet counts. CML patients tend to exhibit high WBC and platelet counts. The aforementioned assessments were used to determine if the patient was suffering from leukemia. The patient was advised to undergo additional tests.

A cytogenetic analysis was performed using the GTG-banding technique. Bone marrow specimens were examined on direct or short-term (24 h) cultures and >10 metaphases were analyzed. The karyotypes were classified using the International System for Human Cytogenetic Nomenclature (13,14).

FISH was performed using directly labeled dual color locus-specific indicator/centromere enumeration probes, according to the manufacturer's protocol (Oncor; Ventana Medical Systems, Tucson, AZ, USA) in order to detect BCR/ABL translocation. In total, 500 metaphase or interphase cells were counted in order to calculate the percentage of BCR/ABL cells. The main steps in the methodology included the hypotonic treatment and fixation of direct demecolcine-treated cultures, dehydration, codenaturation, overnight hybridization, washing and the mounting of slides using 4',6-diamidino-2-phenylindole (DAPI)-antifade (15,16). The slides were viewed using an Axioskop 2 microscope (Zeiss AG, Oberkochen, Germany) with a HBO 100 mercury lamp (Osram, Munich, Germany). The slides were analyzed through a fluorescent microscope (BMX60; Olympus, Tokyo, Japan) with DAPI, rhodamine, fluorescein isothiocyanate and triple band pass filters (Chromo Technology, Bellows Falls, VT, USA). The slides showed fluorescent red dots (rhodamine) that corresponded to the ABL (9q34.1) gene and green dots (fluorescein) that corresponded to the BCR (22q11) gene. Therefore, a cell exhibiting two isolated orange and green dots was counted as normal, or without translocation. A cell exhibiting one isolated orange dot, one isolated green dot and one fused orange and green signal was considered to be presenting the irregular translocation of CML. Morphological and hematological analyses were performed on the bone marrow of the CML patient every 6 months for a year. Follow-up revealed a reduction in fatigue, anxiety and swelling of the body on June 5th, 2009. In total the patient was followed-up for 84 months.

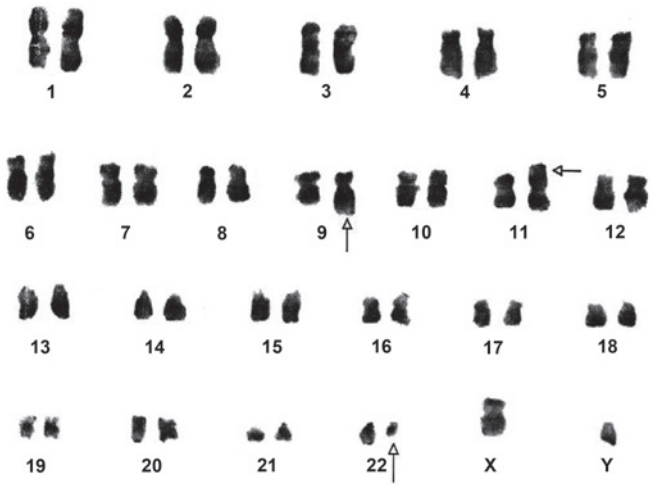

Figure 1. Cytogenetic analysis showing the karyotype of a chronic myeloid leukemia patient with the 46XY,t(9;11;22)(q34;p15;q11.2)[10] translocation. Derivative chromosomes are highlighted by arrows.

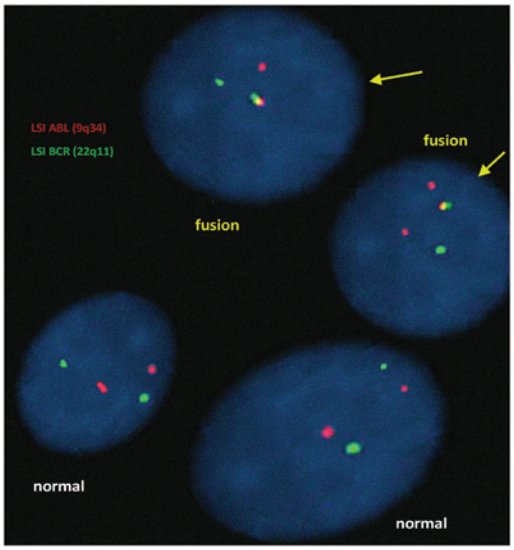

Figure 2. Fluorescence *in situ* hybridization was used for the detection of (9;22)(q34;q11)[10]. Breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1 translocation was detected in 67% of the 500 nuclei counted.

The cytogenetic analysis revealed that 10 of the cells that were examined expressed the Ph chromosome and demonstrated the 46XY,t(9;11;22)(p15;q34;q11.2)[10] translocation (Fig. 1). BCR/ABL translocations were detected in 67% of the 500 nuclei counted using the FISH test (Fig. 2). The hematological analyses revealed that the WBC count was 186.5x10^3 cells/μl, the PLT count was 235x10^3 PLTs/μl and the hemoglobin level was 11.1 mg/dl, which indicated that the patient had anemia.

Discussion

CML is a cancer of the blood that occurs when two proteins on chromosomes 9 and 22 translocate to create the novel Ph chromosome. CML is responsible for the production of the BCR/ABL gene. BCR/ABL interferes with the function of white blood cells, making it challenging for the body to fight off infections. The Ph chromosome (9;22)(q34;q11) is found in 90-95% of CML patients; however, only 5-8% of CML patients demonstrate a variant that involves a third chromosome other than (9;22) in a three-way Ph chromosome complex (1,8,17).

To the best of our knowledge, the present study is the second case of a three-way complex variant involving chromosome 46XY,t(9;11;22)(q34;p15;q11.2) to be reported, with one case reported previously, which was identified by searching the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman) using the terms 'Chronic myeloid leukemia' and 'complex variant translocation (9;11;22)(q34;p15;q11)' (18).

The formation of the variant Ph translocations is a controversial matter. The studies by Morel *et al* (2003) and Emberger *et al* (2001) on complex variant Ph translocations concluded that the variant may occur as a result of a single event, which involves the simultaneous breaking of the chromosomal regions involved, followed by a mismatched rejoining of the broken ends, termed concerted genomic rearrangement (18,19). The studies by Sessarego *et al* (1995) and Reddy and Sulcova (2001) stated that the mechanism behind variant translocation is a two-step process, which includes the initial formation of a standard t(9;22)(q34;q11) translocation, followed by a translocation that usually involves one derivative chromosome from the Ph translocation and a third chromosome (20,21).

In the present study the patient was successfully treated with 400 mg/day imatinib mesylate. Imatinib mesylate is an orally designed tyrosine kinase protein inhibitor that inhibits BCR/ABL gene translocation and the constitutive abnormal tyrosine kinase produced by the Ph chromosome. The drug also inhibits the platelet-derived growth factor and stem cell factor receptor tyrosine kinases. The treatment of CML with imatinib has demonstrated notable success, by prolonging the survival time and improving the quality of life for the patient (2,11,17).

In conclusion, the present study reports a rare case of a CML patient in the chronic phase of disease, who demonstrates the BCR/ABL translocation that involves the three-way translocation variant 46XY,t(9;11;22)(q34;p15;q11.2)[10].

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