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CASE REPORT

TRANSLOCATION t(3;12)(q26;q21) IN JAK2^{V617F} POINT MUTATION NEGATIVE CHRONIC IDIOPATHIC MYELOFIBROSIS: A CASE REPORT

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ABSTRACT

The myeloproliferative diseases (MPDs) or myelo-proliferative neoplasms (MPNs) are a group of diseases of the bone marrow in which excess cells are produced. Chronic idiopathic myelofibrosis (CIMF) is a stem cell defect characterized by splenomegaly with multiorgan extramedullary hematopoiesis, immature peripheral blood granulocytes and erythrocytes and progressive bone marrow fibrosis. The most common chromosomal abnormalities seen in CIMF patients include numerical changes of chromosomes 7, 8 and 9, and structural changes of 1q, 5q, 13q and 20q. At least 75.0% of patients with bone marrow abnormalities have one or more of these chromosomal anomalies. Detection of the Janus kinase 2 (JAK2) mutation may be a potential major breakthrough for understanding the pathobiology of MPNs, and is an essential part of the diagnostic algorithm. In this study, we describe a JAK2^{V617F} mutation negative CIMF patient who has the chromosomal translocation t(3;12)(q26;q21) in her karyotype.

Keywords: Chronic idiopathic myelofibrosis (CIMF), Chromosome aberration, JAK2^{V617F}, Myeloproliferative diseases (MPDs).

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INTRODUCTION

The myeloproliferative diseases (MPDs) or myeloproliferative neoplasms (MPNs) are a group of diseases of the bone marrow in which excess cells are produced. Essential thrombocythemia (ET), chronic myelogenous leukemia (CML), polycythemia vera (PV) and chronic idiopathic myelofibrosis (CIMF), have almost similar laboratory and clinical features and the most accurate way to differentiate them is a study of cytogenetic or molecular abnormalities in such patients. Chronic idiopathic myelofibrosis is a stem cell defect characterized by splenomegaly with multiorgan extramedullary hematopoiesis, immature peripheral blood granulocytes and erythrocytes and progressive bone marrow fibrosis [1]. Chronic idiopathic myelofibrosis is also known as myelofibrosis with myeloid metaplasia, agnogenic myeloid metaplasia, myelosclerosis, osteosclerosis and a leukemic myelosis. It is characterized by varying degrees of bone marrow fibrosis and extramedullary hemopoiesis, with concomitant anemia, poikilocytosis with characteristic teardrop forms in peripheral blood, and circulating immature granulocytes and erythroblasts. Men are slightly more often affected than women. The majority of patients are between 50 and 70 years of age [2]. The limited number of cytogenetics studies reported in CIMF, at least partly reflects the technical difficulties researchers face when trying to obtain good cytogenetic prepa-

rations from fibrotic bone marrow. The most common chromosomal abnormalities include numerical changes of chromosomes 7, 8 and 9, and structural changes of 1q, 5q, 13q and 20q. At least 75.0% of patients with bone marrow abnormalities have one or more of these chromosomal anomalies [3,4]. The short arm of chromosome 9 contains Janus kinase 2 (*JAK2*), a gene recently identified to have a critical gain of function mutation. The JAK2 mutation is a tyrosine kinase that has an important role in the cell signalling pathways [5-7]. Detection of the JAK2 mutation may be a potential major breakthrough for understanding the pathobiology of MPNs, and is an essential part of the diagnostic algorithm [8]. The $JAK2^{V617F}$ point mutation frequency in patients with polycythemia vera (PV) is more than 90.0%, and approximately 37.0-57.0% in patients with CIMF and ET [5,8]. In this study, we describe a $JAK2^{V617F}$ mutation negative CIMF case with chromosomal translocation t(3;12)(q26;q21).To our knowledge, this is the second described case with translocation t(3;12)(q26;q21) [9], and the first case with such chromosomal breakpoints in a patient with CIMF.

CASE PRESENTATION

In March 2013, the 68-year-old female patient with leucopenia, thrombocytosis and anemia was hospitalized at the Department of Hematology, Oncology and Radiotherapy, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina. A complete blood cell count revealed: white blood cells (WBC) 2.0×10^9/L, hemoglobin (Hb) 8.8 g/dL, erythrocytes 3.2×10^{12}/L and platelets 880.0×10^9/L. The clinical symptom of the patient was diagnosed as suspected chronic MPN. Physical examination was abnormal with a very increased spleen size. A bone marrow biopsy showed myelofibrosis with reticulin grade 3 and a marked increase of small to medium-sized dysplastic CD61 (+) megakaryocytes. The cytogenetic analysis and $JAK2^{V617F}$ mutation testing were undertaken at the Polyclinic for Laboratory Diagnostic, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

Cytogenetic Karyotype Analysis. A bone marrow aspirate was introduced into complete RPMI 1640 medium (Euroclone, Milano, Italy) with 10.0% fetal bovine serum (FBS) (Euroclone) and two different culture methods were used: one culture was harvested on the same day, while the second culture was harvested 24 hours after the first one. After harvesting, chromosomes were prepared and GTG-banded for karyotyping according to standard laboratory protocol. These banded preparations were analyzed on an Olympus BX 51 microscope with digital camera Olympus DP12 (Olympus Corporation, Tokyo, Japan). The description of karyotype followed the recommendations of the International System for Human Cytogenetics Nomenclature, ISCN 2005 [10].

Balanced translocation between 3q and 12q was detected in 18 metaphases, while a normal female karyotype was found in two metaphases. Her karyotype was 46,XX, t(3;12)(q26;q21)[18]/46,XX[2] (Figure 1).

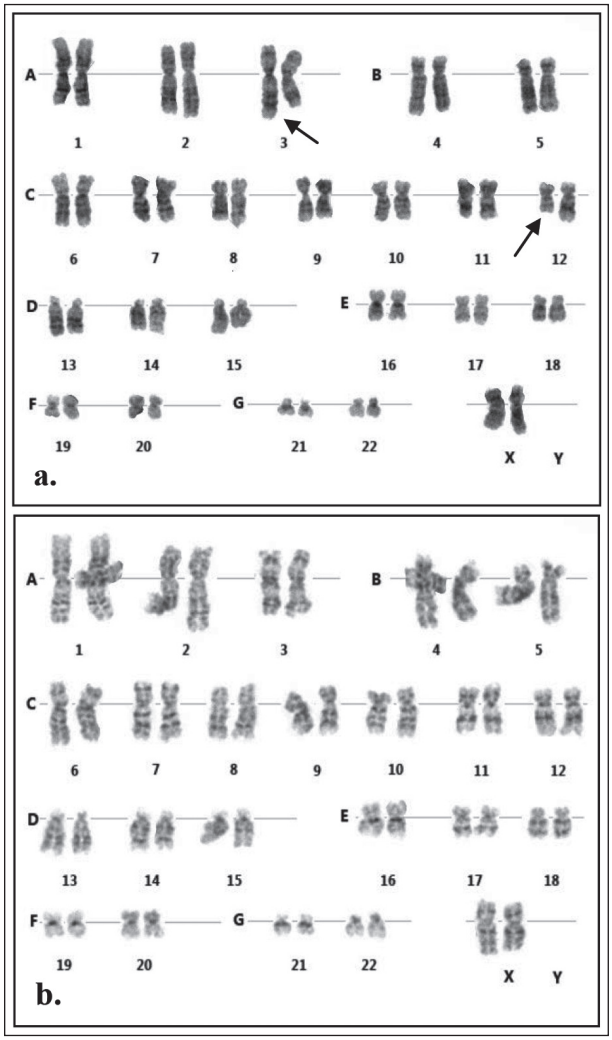

Figure 1. The representative karyotypes from a bone marow G - banded methaphase of the patient showing: a. 46,XX,t(3;12)(q26;q21) (Arrows indicate the abnormal chromosomes); b.46,XX

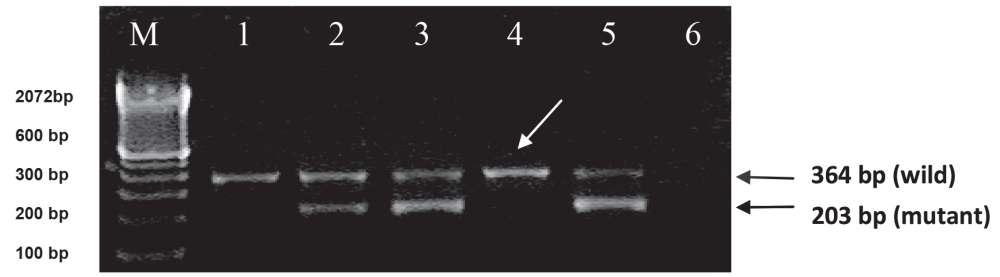

Figure 2. Allele-specific PCR. Lines 1-3: tested patients; line 4: patient with CIMF- absence of the mutant allele sequence; line 5: positive control - Presence of the lower band (203bp) indicates the mutation is carried by the patient; the top band (364bp) acts as an internal PCR control; line 6: negative control (H_2O). M- MWM 100-2072bp (Life Technologies, USA).

Allele - Specific Oligonucleotide - Polymerase Chain Reaction. Genomic DNA was extracted from the whole peripheral blood of the patient using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Screening for the $JAK2^{V617F}$ point mutation was performed by allele-specific olignonucleotide-polymerase chain reaction (ASO-PCR) on 80 ng DNA prepared from the peripheral blood specimen according to the method of Baxter *et al.* [5]. The method uses one 1 μM common reverse primer and two 0-5 μM forward primers (Life Technologies, Grand Island, NY, USA) (Table 1). The first forward primer was specific for the mutant allele sequence (203 bp), while the second amplified a 364 bp product that served as an internal PCR control (Figure 2). The patient's DNA was amplified in a 36-cycle PCR reaction as follows: an initial denaturation step at 94 °C for 11 min., followed by 36 cycles of 30 seconds at 94 °C, 30 seconds at 56 °C, 30 seconds at 72 °C, and a final extension step at 72 °C for 7 min. The PCR products were electrophoresed on 2.0% agarose gel, and the fragments were visualized by ethidium bromide under UV transilluminator. The result of the PCR experiment was the absence of the mutant allele sequence (Figure 2, lane 4). Based on all performed clinical and laboratory tests, especially the bone marrow biopsy, a diagnosis of CIMF was made at that time.

DISCUSSION

The incidence of chromosomal abnormalities in CIMF varies from 30.0 to 75.0%. In nearly two-thirds of these patients, three cytogenetic aberrations were seen: del(13q), del(20q) and partian trisomy 1q [3]. Balanced chromosomal translocations in CIMF are very rare events, however, several case reports have been published [3,11-13]. Moreover, the 12q rearrangements seem to be the most common translocation karyotypic abnormalities in CIMF. Cho and Hyun [14] cited a few articles which suggest that two clustering breakpoints on chromosome 12q (12q21 and 12q24) may be related to the etiology of myelofibrosis. It is still unknown which genes were involved, but this knowledge should help in the identification of the genetic basis of this disease.

To the best of our knowledge, Huret [9] reported the first balanced translocation t(3;12)(q26;q21) in only one case to date, a 47-year-old male patient with a treatment related acute myeloid leukemia (t-AML). A balanced translocation involving the band 3q26, contains the *EVI1* gene. This gene sequence has a role in cell cycle progression and in hematopoietic differentiation [15]. The connection of an abnormal karyotype with the prognosis of disease is questionable, while some studies have supported, and other studies have not favored it as a prognostic factor [16].

Table 1. Allele-specific oligonucleotide-polymerase chain reaction primers.

Reverse	5'-CTG AAT AGT CCT ACA GTG TTT TCA GTT TCA-3'
Forward specific (mutant)	5'-AGC ATT TGG TTT TAA ATT ATG GAG TAT ATT-3'
Forward internal control	5'-ATC TAT AGT CAT GCT GAA AGT AGG AGA AAG-3'

Taken together, these findings suggest that a gene on bands 3q26 (*EVI1* gene) and 12q21 are probably involved in CIMF tumorigenesis. Performing the JAK2 mutation as a molecular test with the highest positive predictive value for the diagnosis of chronic MPDs, is not a diagnostic gold standard for CIMF, because the mutation may not be present in as many as 40.0 to 60.0% of patients with CIMF [5,7,8,17]. However, this mutation screening test may be useful to diagnose prefibrotic IMF and to differentiate IMF from myelofibrosis caused by secondary causes [18].

CONCLUSIONS

Many studies showed that karyotypic aberrations occur in 32.0 to 48.0% of CIMF patients at diagnosis. The most frequently detectable cytogenetic abnormalities are structural and numerical changes of chromosomes 1, 5, 7, 8, 9, 13 and 20. In this study, we report the first case of CIMF with translocation t(3;12)(q26;q21). Further studies are required to determine the genes involved in this chromosome breakpoint. This knowledge should help in the identification of the genetic basis of the CIMF pathogenesis.

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