Abstract. Philadelphia (Ph)-positive chromosome or Ph translocation has been recognized in 90-95 chronic myeloid leukemia (CML) cases worldwide. However, only 5-8% CML patients show complex variant translocations. In the present study, hematological tests for a 47-year-old female CML patient were performed to determine the hemoglobin, platelets and total leukocyte values. A FISH test was carried out to recognize the BCR/ABL gene fusion, and a cytogenetic analysis was performed. The hematological results showed an increase in WBC (414000/mm$^3$) and a decrease in hemoglobin (8.9 mg/dl), indicating the anemic condition in the CML patient. Furthermore, cytogenetic karyotyping results showed 46,XX,t(6;9;22)(p21;q34;q11) and positive for Ph chromosome. In conclusion, in the present study, we report a rare three-way complex variant translocation in a CML patient.

Introduction

The clonal hematopoietic stem cell is associated with chronic myeloid leukemia (CML), which results due to the balance translocation among the long arms of chromosomes (9;22) (q34;q11) commonly known as Philadelphia (Ph) chromosome (1,2). CML consists of monocytic, megakaryocytic, myeloid, erythroid, B-lymphoid and T-lymphoid linkages (3). The nonfunctional crossbreed (PBCR-ABL) protein produced by the chimeric BCR-ABL gene with tyrosine kinase activity independently leads to myeloid proliferation and leukemic makeover (1,4). The translocation of chromosomes (9;22) (q34;p15) is perceived in almost 90-95% of patients with CML, and only 5-8% CML patients have established variant complex translocation, which is due to the participation of one or more chromosomes other than 9 and 22 chromosome (5-7).

BCR-ABL protein tyrosine kinase is inhibited by the imatinib mesylate (IM), a conventional oral therapy for patients suffering from CML, irrespective of phases, with a response rate of 65-90% in CML cases. IM acts by blocking the production and inducing apoptosis of BCR-ABL gene expression in CML cells. It plays a vital role regarding continued survival and better quality of life (8-11).

In the present study, we report a rare three-way Ph-positive complex variant translocation 46,XX,t(6;9;22)(p21;q34;q11) in a CML patient.

Patient and methods

Case report. We report here a 47-year-old female patient who had established CML in Sundayman Civil Hospital (Quetta) on the 8th August, 2014. She was referred to the hospital because of symptoms including fever, anemia, weight loss, sweating, depression, swelling on the body and high blood pressure. The laboratory characteristics of the patient were WBC (414000/mm$^3$), hemoglobin (8.9 g/dl), platelets (619000/mm$^3$), MCV (56.2 FL), MCH (22.5/pg), lymphocytes (7%), neutrophils (20%), HCT (30.2%), monocytes (0.3%), MCHC (39.9 g/dl), metamyelocytes (33%), blast (2%), myelocytes (10%) and normoblasts (04/100 WBCs). The result of ultrasound report of the CML patient showed mild hepatomegaly and massive splenomegaly. The patient was treated with Glivec (imatinib) 600 mg/day. Approval for the study was obtained from the Ethics Committee of the Institute/University (BUITEMS, Quetta, Pakistan).

Methods

CBC laboratory test. Hematological markers such as hemoglobin, platelets, red blood cells and white blood cells were determined within a day of sample collection by Nihon Codon Hematological analyzer (Tokyo, Japan).

Cytogenetic test. Bone marrow culture was used for cytogenetic analysis as reported in an earlier study (12). Twenty
GTG-banded unstimulated bone marrow specimens were examined. Karyotypes were performed as per the International System for Human Cytogenetic Nomenclature (13).

**FISH test.** Fluorescence *in situ* hybridisation (FISH) analysis was performed by directly labeled dual color LSI/CEP probe for the recognition of BCR/ABL gene. We counted 500 metaphase or interphase cells to obtain the BCR-ABL percentage.

**Results**

Hematological results showed an increase in WBC 414000/mm³ and a decrease in hemoglobin (8.9 mg/dl), indicating the anemic condition in the CML patient. Furthermore, cytogenetic karyotyping results showed 46,XX,t(6;9;22)(p21;q34;q11) in this CML patient. All 20 cells were positive for Ph chromosome (Fig. 1).

BCR-ABL fusion genes were detected in 91% of the 500 nuclei counted in the FISH analysis. The fluorescent red dots corresponded for the (9q34) ABL and green dots represents (22q11) the BCR gene. However, a cell showing two isolated green and red dots counted as a normal cell, indicating no translocation. Conversely, a cell displaying one red, one green with fused yellow signal was considered for irregular translocation.

**Discussion**

CML is associated with clonal stem cell syndrome due to balanced translocation of long arms of (9;22) chromosomes in most cases (90-95%) of CML. However, 5-8% of CML patients present variant and complex translocation, referred to as Ph chromosome, later responsible for the production of a protein BCR-ABL gene fusion, which contains a protein kinase activity. The BCR-ABL genes interfere with WBC, making the immune system weak (25). This condition is observed in 90% of CML individuals (7).

Sessarego *et al* (5) and Aliano *et al* identified that complex variant translocation is present in almost 5-8% of CML patients, by the participation of one extra chromosome or more than one chromosome in addition to chromosome numbers 9 and 22 (5,26,27). In the present study, we also investigated a complex variant case of chronic myeloid leukemia patient with Ph chromosome 46,XX,t(6;9;22)(p21;q34;q11) in chronic phase. To the best of our knowledge, we are the thirteenth

<table>
<thead>
<tr>
<th>S.No</th>
<th>Translocations reported previous literature</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>46,XX,t(6;9;22)(q21;q34;q11)</td>
<td>(14)</td>
</tr>
<tr>
<td>2.</td>
<td>46,XY,t(6;9;22)(p21;q34;q11)</td>
<td>(15)</td>
</tr>
<tr>
<td>3.</td>
<td>46,XY,t(6;9;22)(p21;q34;q11)</td>
<td>(16)</td>
</tr>
<tr>
<td>4.</td>
<td>46,XY,t(6;9;22)(p21;q34;q11)</td>
<td>(17)</td>
</tr>
<tr>
<td>5.</td>
<td>46,XX,t(6;9;22)(p21;q34;q11)/47,idem,+8,i(17)(q10)</td>
<td>(18)</td>
</tr>
<tr>
<td>6.</td>
<td>46,XY,t(6;9;22)(p21;q34;q11)</td>
<td>(19)</td>
</tr>
<tr>
<td>7.</td>
<td>46,XX,t(6;9;22)(p21;q34;q11)</td>
<td>(20)</td>
</tr>
<tr>
<td>8.</td>
<td>46,XX,t(6;9;22)(p21;q34;q11)</td>
<td>(21)</td>
</tr>
<tr>
<td>9.</td>
<td>46,XX,t(6;9;22)(p21;q34;q11)</td>
<td>(22)</td>
</tr>
<tr>
<td>10.</td>
<td>46,XX,t(6;9;22)(p21;q34;q11),add(20)(p?)</td>
<td>(6,18)</td>
</tr>
<tr>
<td>11.</td>
<td>46,XX,t(6;9;22)(p21;q34;q11)/47,idem,+18</td>
<td>(23)</td>
</tr>
<tr>
<td>12.</td>
<td>46,XY,t(6;9;22)(p21;q34;q11)/46,idem,der(2)t(2;8)(q24;q1?)</td>
<td>(24)</td>
</tr>
</tbody>
</table>

Figure 1. Cytogenetic analysis shows a variant three-way translocation 46,XX,t(6;9;22)(p21;q34;q11). Arrowheads highlight all the derivative chromosomes.
study to report these cases; previously only 12 investigators reported such type of cases.

The mechanism of variant complex Ph translocation is an unknown phenomenon. Morel et al suggested a two-step mechanism. The first one involves the formation of (9;22)(34q11q) translocation and the second is the additional translocation involving one derivative chromosome from Ph translocation and a third chromosome (28). Reddy and Sulcova reported that the complex variant translocation formed by multiple simultaneous breakages of several chromosomes were followed by mismatch joining (29).

Imatinib (Glivec) is a potent inhibitor of the Bcr-Abl protein tyrosine kinase. The optimal doses of Glivec primarily attenuate the tyrosine kinase activity of the platelet-derived growth factor (PDGF) receptor β and c-Kit, without disturbing other associates of the type III receptor kinase family, such as Flt-3 and Fms (29,30).

In conclusion, we reported a rare case of the variant translocation involving Ph-positive chromosome 46,XX,t(6;9;22) (p21;q34;ql1) in a CML patient in the chronic phase.

Acknowledgements

The present study was partially supported by Office of Research Innovational and commercialization, BUTEMS, Quetta, Pakistan. (Registration ID no. 27934).

References

22. Morel
23. Vanelli T, Fogliatto LM and Zen PR: Cytogenetic response to