1 INTRODUCTION

Chronic myeloid leukaemia (CML) is one of the entities in myeloproliferative neoplasm, typically characterized by the presence of Philadelphia (Ph) chromosome. The Ph chromosome is produced as a result of reciprocal translocation between long arm of chromosome 9 and 22 or t(9;22)(q34;q11) leading to the formation of BCR-ABL1 fusion gene. Translocation involving long arm of chromosome 9 and short arm of chromosome 12 or t(9;12)(q34;p13), resulting in ETV6-ABL1 gene has been uncommonly reported in CML.

Philadelphia-Negative Chronic Myeloid Leukaemia with ETV6-ABL1 Fusion Gene: A Case Report

Abstract—Chronic myeloid leukaemia (CML) is typically characterized by the presence of Philadelphia chromosome, the product of reciprocal translocation between 9q34 and 22q11, resulting in generation of BCR-ABL1 fusion protein. In rare cases, another translocation involving 9q34 and 12p13, encoding ETV6-ABL1 fusion protein has been reported. Here we described a man with Philadelphia-negative CML but positive for the ETV6-ABL1 fusion gene. A 44-year-old gentleman was referred for hyperleucocytosis. Physical examination revealed lymphadenopathies and hepatosplenomegaly. The bone marrow examination was consistent with CML in chronic phase. Bone marrow cytogenetic displayed normal male chromosome. Molecular method using multiplex Reverse Transcriptase-Polymerase Chain Reaction analysis capable of detecting 28 mutations, had only identified ETV6-ABL1 fusion gene, or translocation (9;12)(q34;p13). Cytoreductive and imatinib therapy were initiated. Unfortunately, he had poor response to this treatment; hence he was advised for allogeneic haematopoietic stem cell transplantation. The present case highlights the importance of molecular study in identifying the cryptic chromosomal translocation and the therapy resistance of ETV6-ABL1-positive CML to imatinib.

Keywords—Chronic Myeloid leukemia, Philadelphia-negative CML, ETV6-ABL1 fusion gene, case report.
1.1 Case Presentation

A 44-year-old man was referred from a district hospital for anaemia and leucocytosis. He complained of vomiting, diarrhoea and loss of appetite for one week duration, and there was no significant loss of weight. Physical examination revealed lymphadenopathies and hepatosplenomegaly. Blood count from an automated blood counter, Sysmex XE-2100D (Sysmex, Japan) showed hyperleukocytosis with total white cell count (TWBC) of 450.0 x 10^9/l; that constituting 88.7% neutrophils, 1.8% lymphocytes, 5.5% monocytes, 3.7% eosinophils and 0.3% basophils. He had moderate anaemia (haemoglobin, Hb, 8.5g/dl) and thrombocytopaenia (platelet, 75 x 10^9/l). Peripheral blood film examination reported neutrophilia, myelocyte peak, eosinophilia, basophilia and 3% blast cells. Bone marrow examination revealed hypercellular marrow fragment and cell trails, composed of marked number of neutrophilic series, which predominantly neutrophils (64%) and myelocytes (19%). The eosinophilic precursors were mildly increased, accounting for 4%, and there was no excess of blast cells (Fig. 1).

![Figure 1: Bone marrow smear examination. The bone marrow examination revealed hypercellular cell trails, composed of marked number of neutrophilic series, which predominantly neutrophils (64%) and myelocytes (19%). The eosinophilic precursors were mildly increased, accounting for 4%, and there was no excess of blast cells.](image)

Nevertheless, chromosomal analysis of bone marrow aspirate displayed no Ph chromosome in four male karyotypes. Multiplex Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis that consisted of two steps; master-PCR and split out-PCR were performed using HemaVision®-28N kits (DNA Diagnostic A/S, Denmark) and the PCR products from these steps were subsequently analysed using Bioanalyzer 2100 (Agilent Technologies, USA). The kit is able to identify 28 types of gene mutation. We had detected a transcript with molecular size of 1235 base pair as revealed by the presence of PCR amplification in M6 tube in master-PCR analysis (Figure 2). The specific mutation was then identified when split out-PCR analysis of five M6 tubes (M6A-M6E) showed 1216 base pair amplicon in M6C tube. According to the protocol, the presence of amplification in the respective tubes would be interpreted as follows: M6A - inversion 16, M6B - BCR-ABL1, M6C - ETV6-ABL1, M6D - ETV6-PDGFRB and M6E - ETV6-MN1. Our sample was positive for ETV6/ABL1 or t(9;12)(q34;p13) with specific breakpoint at exon 5 ETV6 gene and exon 2 ABL1 gene (Figure 3). The result also confirmed the absence of BCR-ABL1 fusion gene since there was no PCR amplification in M6B tube (Figure 3). The results were then validated by direct sequencing analysis performed by DNA Diagnostic A/S (Denmark) (Figure 4). Amplification Refractory Mutation System-PCR analysis for JAK2 V617F gene mutation was performed as an in house method showed negative result; which excluded other diagnosis of myeloproliferative neoplasms.

The patient was immediately treated with hydroxyurea 1.5g twice daily for a week and the dose was adjusted according to his blood counts. However after about two weeks, he complained of dizziness and blurring of vision for one week. He was found to have leukaemic infiltration of the retina of both eyes. At this time, his TWBC count was markedly raised again (444 x 10^9/l) and he had moderate anaemia (Hb, 10.5g/dl) and thrombocytopaenia (platelet, 95 x 10^9/l). Examination of the peripheral blood film, bone marrow aspirate and trephine biopsy discovered similar findings as in the previous slide. CT-scan of the brain demonstrated a small left temporal intraparenchimal haemorrhage, measuring 1.4 x 1.4 cm.

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Figure 2: Bioanalyzer analyses of PCR products (tube M1-M8) from Master PCR step. (a) The result showed an abnormal band (arrow) at M6 tube and (b) the result from electropherogram showed an abnormal peak (arrow) at M6 tube with molecular size of 1235 bp. These findings (a and b) indicate the presence of chromosomal translocation.
Figure 3: Bioanalyzer analyses of PCR products (tube M6A-M6E) from Split Out PCR step.

(a) The result showed an abnormal band (arrow) at M6 tube and (b) the result from the electropherogram showed an abnormal peak (arrow) at M6C tube with molecular size of 1216 bp. These findings (a and b) confirmed the presence of translocation (9;12) and the specific breakpoint was ETV6 exon 5-ABL1 exon2.
Figure 4: Direct sequencing of the fragments amplified by RT-PCR. The electropherogram revealed the fusion of exon 5 of ETV6 to exon 2 of ABL1 (arrow).
Hence, he was treated with Ara-C 100mg daily for five days and hydroxyurea dosage was pushed up to 2g twice daily. Platelet transfusion was given intermittently as his platelet count was reducing and when the size of the bleed increased to 2.3 x 1.9 cm. He was started with imatinib 400mg daily after three months of diagnosis. Unfortunately, he had poor response to this treatment, even though it was given in escalated dose and together with hydroxyurea. He was advised for allogeneic haemopoietic stem cell transplantation since he had HLA-matched sibling. Currently, the patient is still thinking of the treatment option.

2 DISCUSSION

Philadelphia-negative CML with ETV6-ABL1 fusion gene is rare. Up to the present moment, 12 cases of CML including the current case reported to have this fusion gene [1]. It has been suggested that the ETV6-ABL1 fusion occurs as a result of at least two events, by which more than two breakpoint genes are involved. Firstly, a balanced translocation between chromosome 9 and chromosome 12 forms a fusion between ETV6 gene (exon 1-5) from 12p13.2 and NOTCH1 gene at 9q34.3 on der(9)t(9;12). Then the gene segment on der(9)t(9;12) breaks at the translocated ETV6 exon 5 gene and also at 9q34.12 region, splitting apart of ABL1 gene exon 2. This is finally followed by inversion of the gene segment and resulting in fusion of ETV6 exon 5 and the remaining ABL1 exon 2 [1, 2]. Therefore, it is not easy to detect t(9;12)(q34;p13)/ETV6-ABL1 at cytogenetic level. The cytogenetic analysis will be interpreted as normal or if the inserted segment is large, it will be described as insertion and/or deletion of chromosome 9 and/or 12 [1, 2]. Molecular characterisation either by fluorescence in situ hybridisation (FISH) or RT-PCR analysis is useful techniques in detecting this cryptic rearrangement as these methods are more sensitive [1-3]. In the present case, we used multiplex RT-PCR analysis that was able to confirm the presence of ETV6-ABL1 fusion gene and the absence of BCR/ABL1 fusion gene simultaneously. The result was also ascertained by direct sequencing analysis.

Even though there are extensive differences between ETV6 and BCR protein, previous studies have proved the indistinguishable biological activity of ETV6-ABL1 and BCR-ABL1 fusion protein in inducing leukaemia [4-9]. Both of ETV6 and BCR proteins are shown to have similar helix-loop-helix (HLH) domains which seem to activate the ABL1 protein. The HLH domains fuse to the kinase domain of ABL1 to facilitate protein oligomerisation process, a critical phenomenon for neoplasia-associated tyrosine kinases [4, 6-9]. The elevation of the tyrosine kinase activity transforms factor-dependent cell lines to factor independent and induces myeloproliferative disease [5, 7, 8]. In addition, BCR/ABL1 and ETV6/ABL1 seem to activate similar signal transduction pathways and transforming activity [8, 10]. However, it was found that the ETV6-ABL1 fusion protein is significantly more active compared to the p210 BCR-ABL1 fusion protein in a mouse model. This factor could be contributed by the difference of their ability to phosphorylate particular substrate [5].

There are variable reports on the clinical response of tyrosine kinase inhibitors (TKI) to patients with ETV6-ABL1 CML. Two studies have reported that chronic phase CML patients with normal karyotype and ETV6-ABL1 fusion gene do well with imatinib [11, 12]. However, the inhibitory effect of imatinib is transient and fails to induce complete remission in three other patients [1, 13-15]. We are unable to explain why our patient showed a poor response to imatinib. Indeed, a complete remission is successfully achieved when the second-generation TKI (nilotinib) is commenced [2, 14].

3 CONCLUSION

We highlight the importance of multiplex RT-PCR that can efficiently identifies a cryptic ETV6-ABL1 fusion and concurrently ruling out the presence of typical BCR-ABL1 fusions in patient with morphologically-diagnosed CML. We also confirm the therapy resistance of ETV6/ABL1-positive CML to imatinib. Therefore, the implementation of second generation TKIs as first line therapy in Ph negative-CML patients with ETV6-ABL1 is well supported.

APPENDIX

CML: chronic myeloid leukaemia
FISH: fluorescence in situ hybridisation
Hb: haemoglobin
HLH: helix-loop-helix
IMR: Institute for Medical Research
MOH: Ministry of Health
MREC: Medical Research and Ethics Committee
PCR: polymerase chain reaction
Ph: Philadelphia

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RT-PCR: multiplex reverse transcriptase-polymerase chain reaction
TKI: tyrosine kinase inhibitors
TWBC: total white blood cell

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CONFLICTS OF INTEREST
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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