Review Paper

Multi-Drug Resistant Chronic Myeloid Leukemia: Report of Two Cases and Review of Literature

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Abstract

Introduction: In the last one decade first generation tyrosine kinase inhibitor (TKI), imatinib mesylate was the choice of treatment in Chronic Myeloid Leukemia (CML) but recently, a considerable number of patients have developed resistance to first as well as second generation TKIs.

Objective: To present two cases which were first and second generation TKIs resistant and to review multidrug resistant CML.

Materials and Methods: Relevant articles and literatures were retrieved from different journals and web pages to find out the mechanism of development of drug resistance in chronic myeloid leukaemia.

Discussion: Two cases of drug resistant CML were found in Combined Military Hospital (CMH), Dhaka cantonment. Extensive review of the literature showed that two mechanisms, namely Breakpoint Cluster Region-Abelson (BCR-ABL) dependent and independent mechanisms, are mainly responsible for multi drug resistant (MDR) CML. In Bangladesh, it is difficult to identify the exact mechanism of development of drug resistance in CML. In a few cases, sample from CML patients are sent overseas to detect any new mutation in TKI binding domain of BCR – ABL fusion gene but it is not possible to find out other mechanisms responsible for drug resistance in CML.

Conclusion: To overcome the drug resistance in CML, it is important to use the conventional drugs used in the treatment of CML meticulously and regularly as irregular use and discontinuation of treatment will permit the development of drug resistance.

Key-words: BCR-ABL fusion gene, Tyrosine kinase inhibitors, Chronic myeloid leukemia.

Introduction

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by the accumulation of Philadelphia chromosome positive (Ph+) myeloid cells. CML has an estimated incidence of 1 – 1.5 per 100000 populations and represents approximately 15% of all leukemias diagnosed in adults with an onset at 40 – 50 years of age[1]. CML, a type of myeloproliferative disease, is characterized by the overproduction of both mature and immature myeloid cells causing high levels of white blood cell counts, splenomegaly, weight loss, fatigue and anemia[2].

Philadelphia chromosome positive (Ph+) myeloid cells are present in more than 90% of CML patients. As a result of balanced reciprocal translocation between the Abelson gene (ABL1) on the long arm of chromosome 9 and the breakpoint cluster region gene (BCR) on the long arm of chromosome 22, t(9;22) (q34;q11), Philadelphia chromosome is formed. BCR-ABL1fusion gene is stirred as a result of such balanced translocation[3]. In different types of leukemia, various sizes of BCR-ABL fusion protein are synthesized. In more than 90% of CML and 30% - 35% of acute lymphoblastic leukemia (ALL) patients, a BCR-ABL protein of 210 kDa is noticed. BCR-ABL proteins of 190 and 230 kDa are tracked out in ALL and chronic neutrophilic leukemia respectively[4]. Malignant cell transformation results from tyrosine kinase activity of BCR– ABL oncoprotein. This oncoprotein phosphorylates target proteins through the activation of several signal transduction pathways leading to uncontrolled cell proliferation, decreased cell apoptosis, adhesion and differentiation of hematopoietic stem and progenitor cells. All these alterations form the phenotypic physiognomy of CML[5]. CML has an evolutional course comprising three clinical phases, known as chronic, accelerated and blast crisis phases, based on clinical and pathological

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features. The transition from chronic to accelerated phase and blast crisis phase results from secondary chromosomal aberrations such as trisomy 8, trisomy 19, an extra Ph chromosome and isochromosome 17q (p53 gene on 17p is lost)[3].

Tyrosine Kinase inhibitors (TKI) are the treatment modality of chronic phase CML, acting against the kinase activity of BCR – ABL fusion protein for the effective treatment of CML[6]. The complete cytogenetic response for early-phase patients treated with imatinib was observed in more than 80% of patients; for accelerated phase this was 40% and when the patients turned out to blast crisis phase, the response rate have shown to fall 20%[7].

Though imatinib has emerged as a very potent agent for treating CML but its remedial ability is narrowed due to amplification or emergence of point mutations in BCR – ABL[8]. At present, 100 different BCR-ABL kinase domain mutations have been identified in imatinib resistant CML patients (Fig 1). But the frequently encountered mutations in BCR – ABL domain are usually located in regions such as imatinib-binding site, at the Adenosine Triphosphate (ATP) binding site and in the activation loop[9].

Tyrosine kinase inhibitor, imatinib showed promising success in the treatment of patients with CML but evolution of resistance in CML patients have emerged as a major problem in the recent years. To surmount the resistance noticed with imatinib treatment, other selective TKIs have been created. Second generation TKIs such as dasatinib, nilotinib and busatinib exhibited better performance for the treatment of CML as compared to imatinib[10]. In the face of the development of second generation TKIs, a small percentage of CML patients and a significant number of patients in advanced phase are either initially refractory to TKIs or ultimately develop resistance[10]. Therefore, a significant number of CML patients are now becoming multi-drug resistant and new challenge to manage such patients.

The aim of this review was to present two cases which recently we have envisaged in our setting as well as to evaluate the mechanisms of developing multi-drug resistance in CML patients.

Case 1

A 45-year-old female patient, wife of a corporal of Bangladesh Army, got admitted into a peripheral military hospital, Chittagong, Bangladesh with the complaints of generalized weakness and a small lump in the left side of the abdomen for a period of two months in May 2010. There she was diagnosed as CML, chronic phase on the basis of complete blood count (CBC), blood film findings and bone marrow examination. At that time capsule Hydroxyurea was started and became under control within one month. For better management she was then transferred to a tertiary level military hospital, Dhaka, Bangladesh. In this hospital she underwent all routine laboratory investigations including repeat bone marrow aspiration and examination. Also her bone marrow sample was sent overseas for BCR-ABL fusion gene study which was detected confirming the diagnosis of CML. Then she was given capsule imatinib mesylate (Cap Enlevin) in standard dose (4 capsules daily as a single dose) and advised for follow up with complete blood count every week. After one month, bone marrow examination and analysis of BCR-ABL fusion gene study was performed and found negative. She was advised to continue the capsule enlevin in the same dose and follow up with CBC fortnightly in her nearest peripheral hospital, Chittagong. She was staying well with such medication until November 2014 when her CBC showed moderate anaemia (8.7 g/dl), increasing white blood cell (WBC) [$34X10^9$/L] with significant number of blasts (13%), basophils (4%) as well as myelocytes and meta-myelocytes (19%) suggesting the transition to accelerated phase. Again she was transferred to the military hospital, Dhaka where all relevant investigations including BCR-ABL fusion gene study was done and BCR-ABL fusion gene was again detected. After evaluating all investigations, she was confirmed as the case of CML, accelerated phase and was given tablet dasatinib in standard dose and transferred back to her nearest military hospital where she was under close observation of a hematologist. But within two months, her condition deteriorated and transferred to Dhaka again. Now all laboratory investigations were performed and her bone marrow sample was sent overseas to the same laboratory for detection of BCR-ABL mutation study which revealed the presence of mutation in the P-loop of BCR-ABL domain. Other investigations revealed that the patient landed to blast crisis phase. After critical evaluation, she was prescribed with tablet nilotinib in the usual standard dose. Unfortunately she did not respond to this second generation TKIs and within one month she succumbed to death.

Case 2

A 28-year-old young soldier got admitted into military hospital with the complaints of tiredness, fatigue, increased

sweating and feverish feeling for the last two weeks. After performing all relevant investigations including bone marrow examination and BCR-ABL fusion gene study, he was diagnosed as CML, chronic phase and started treatment with capsule enliven in usual standard dose. His disease became under control after two months and discharged with the advice of continuing the medicine and performs CBC fortnightly. With this medication, the soldier was continuing well but after six months he again got admitted with marked pallor, bleeding manifestations and fever. His CBC revealed that the patient transited to blast crisis and started with tablet dasatinib immediately as well as bone marrow sample was sent overseas for BCR-ABL gene mutation study. Unfortunately, before getting the results of all relevant investigations including mutation analysis of the BCR-ABL domain, the patient passed away.

Discussion

Tyrosine Kinase inhibitors showed outstanding success in the treatment of CML patients, but the major problem recently encountered by the clinicians is the development of resistance. Two mechanisms, namely BCR-ABL dependent and independent mechanisms, are mainly responsible for MDR CML. These mechanisms include mutations in the TKI binding domain of BCR-ABL (Fig 1), over expression of BCR-ABL, ATP-binding cassette (ABC) transporters, aberrant ceramide metabolism, inhibition of apoptosis and changes in the expression levels of microRNAs[6].

Four regions (P-loop, SH-3, SH-2 and A-loop) of the BCR-ABL domain are crucial for effective binding of imatinib. Point mutations in any of these regions either reduce and/ hinder the binding of TKI and the oncogenic BCR-ABL protein leading to the emergence of drug resistance. Among the four sites, P-loop mutation was more commonly found and responsible for 43% of drug resistance in the accelerated and blast crisis phases. Another mechanism of development of imatinib resistance may also be observed when BCR-ABL oncogene is over expressed. These two mechanisms are BCR-ABL dependent (Fig-3)[11].

BCR-ABL independent mechanisms are ATP binding cassette (ABC) transporters, human organic cation transporters (hOCT1), aberrant ceramide metabolism, inhibition of apoptosis and changes in the expression level of miroRNAs (Fig-3). ABC transporters are an extremely preserved transmembrane protein family and in the export/import of various substances into or out of the cells in addition to efflux or influx of drugs across organelles and the cell membrane[12]. ABCB1, also known as multi-drug resistance (MDR1) transporter, is one of the well studied ABC transporters that played an important function in imatinib resistance in CML patients by elevating the expression of MDR1[13]. Genetic changes in MDR1 gene causes increased expression of P-glycoprotein efflux pump leading to imatinib resistant[14].

Human organic cation transporter (hOCT1) is another transmembrane protein that influences the uptake of substances through the cell membrane and imatinib is one the substrate of hOCT1. Lower level of hOCT1 cause reduced intracellular concentration of imatinib, thereby diminishing the drug activity in the cell[15].

Aberrant ceramide metabolism is also responsible for drug resistance in CML patients. For programmed cell death, ceramide, synthesized in the sphingomyelin pathway, plays an important role. Ceramide synthesis is also stimulated by Fas/CD95 triggered cell death. Sphingosine is synthesized from ceramide by ceramidase and production of sphingosine 1 phosphate (S1P), which has antiapoptotic effect, is mediated by sphingosine kinase (SK) from sphingosine. Therefore, ceramide/ SIP dimer is a tightly regulated process in respect to its antagonistic effects and vital for drug resistance. Accumulation of S1P in the cell promotes imatinib resistance in CML patients by preventing the degradation of BCR-ABL protein[16]. On the other hand, application of ceramide in combination with imatinib, nilotinib or dasatinib caused synergistic apoptotic effects of sensitive and drug-resistant CML cells and increasing level of ceramide decreases cell survival by stimulating proapoptotic molecules[17,18].

Inhibition of apoptosis is another mechanism mediated by different genes or proteins having diverse functions in the apoptotic signaling pathway[19]. P53 protein is a product of Tp53 tumour suppressor gene and P53 protein in the cell is crucial in Deoxyribonucleic Acid (DNA) repair, apoptosis and genomic stability[20]. Progression of CML through accelerated phase and blast crisis is related to the mutation in exon 8 region of Tp53 gene as well as such mutation lower the molecular response during treatment with imatinib, thus causing the development of drug resistance CML[21]. Bcl – 2 is another protein family that plays important role in mitochondria-dependent apoptosis. Transformation of CML from chronic phase to the aggressive form is associated with over expression of Bcl – 2 and thereby causing imatinib resistant CML[22].

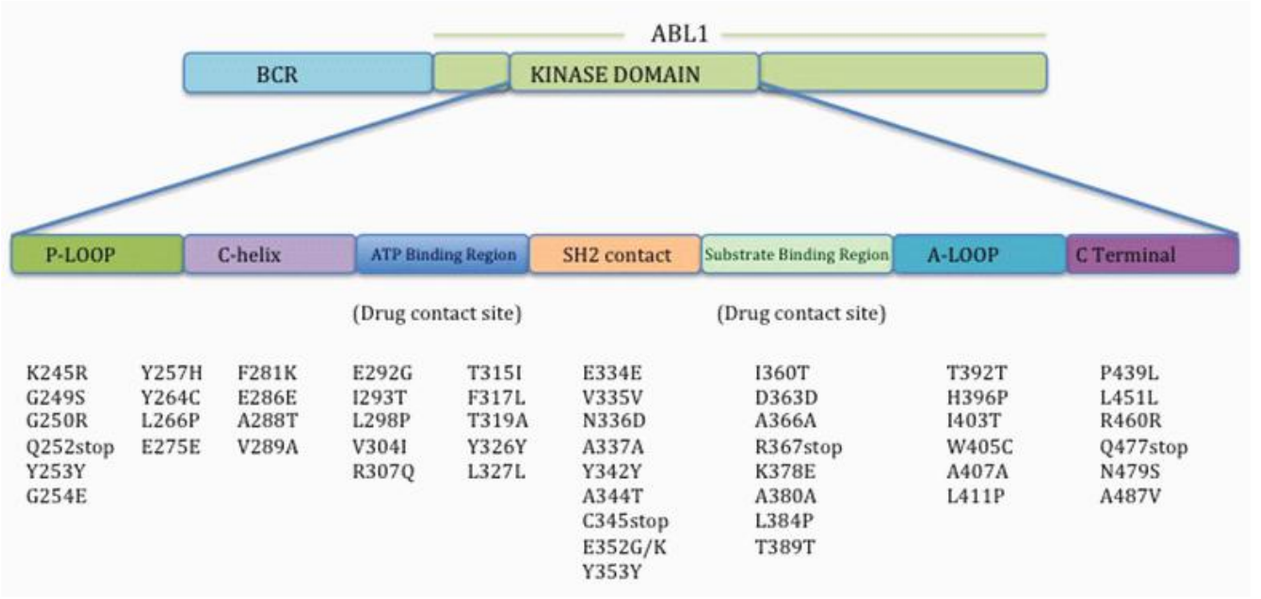

Fig-1: Distribution of the mutations in the different regions of BCR- ABL1kinase domain[7]

Fig-2: Photograph of case 1

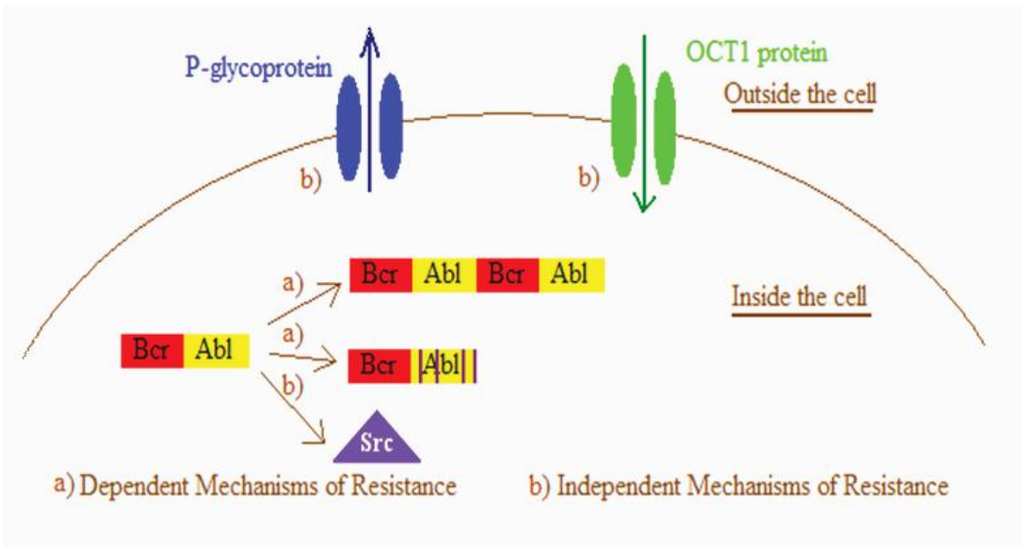

Fig-3: Different types of drug resistance mechanisms in CML patients

Scenario in Bangladesh

In Bangladesh, though exact data is not available about the incidence of CML or how many new cases are identified every year but it is assumed that considerable number of CML cases are reported and diagnosed in different medical installations. Before 2000, all patients were treated with either hydroxyurea or first generation TKIs and no resistant cases were identified. After 2000, a few cases were reported as resistant to first generation TKIs and at that time, possibly before 2008, second generation TKIs were not available. At present second generation TKIs are available and first generation TKIs resistant CML cases are treated with second generation TKIs. In such cases, sample were sent overseas to find out the cause of drug resistant and in few cases new mutation were identified in the TKI domain of BCR-ABL fusion gene which was responsible for drug resistance. In Bangladesh it is assumed that irregular use, discontinuation of treatment, use of drugs in under dose because of financial constraint, use of hydroxyurea and TKIs alternatively, use of forged drugs as well as environmental exposure to different mutagenic substances may be responsible for the development of drug resistance in CML cases. These factors may cause new mutations in TKI domain of BCR-ABL fusion gene and allow other mechanisms of drug resistance to play important role in causing multi drug resistance in CML.

Conclusion

Chronic myeloid leukemia is a highly investigated type of cancer for the creation of new therapeutic protocol to manage the disease. Various methods such as targeting the signaling pathway, direct protein targeting, nanotechnology and knockout/ knockdown techniques are now coming out as new research areas which will in future focus not only on the development of new drugs but also on drug resistance and its mechanisms culminating a better cure and increase quality of life.

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