Proceeding S.Z.P.G.M.I. Vol: 30(1): pp. 15-18, 2016.

Chronic Myelogenous Leukemia Diagnosis: Peripheral Blood, Bone Marrow, Cytogenetics, and PCR

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ABSTRACT

Introduction: CML is an acquired clonal disorder resulting from a chromosomal abnormality; t (9:22) (q33:q11) forming a fusion gene BCR-ABL presenting as hyper-leukocytosis with immature granulocytes in blood, basophilia, anemia, bone marrow hyper-cellularity owing to a greatly expanded myeloid mass, high M:E and bone marrow fibrosis. The conventional diagnostic techniques rely mainly on morphology and clinical presentation. However, cytogenetics and molecular techniques provide us a more reliable diagnosis and provide the quantification of disease, which is of special importance in monitoring of treatment response of different therapeutic regimens. **Objectives:** To determine the presence of Philadelphia chromosome and BCR-ABL fusion gene in morphologically diagnosed CML. **Materials and methods:** CBC, bone marrow biopsy, reticulin stain, cell culture for karyotype, and RQ-RT-PCR were carried out. **Results:** Out of 50 cases of CML, hemoglobin range was 5.2-14.3 g/dL with a mean of 9.6 g/dL. Total WBCs ranged 65-353 $x10^9$/L with a mean of 210.8 $x10^9$/L. Blast cells ranged between 2-7% on peripheral blood with a mean of 7%. Out of 50 cases of CML, platelets were ranging between 123-1548 with a mean of 430.3. Trephine Biopsy showed moderate fibrosis in 35 (70%) cases of CML and 14 (28%) cases showed marked fibrosis in CML patients while only one (02%) cases showed mild fibrosis. **Conclusion:** All cases, which were morphologically diagnosed as CML, were also positive for Philadelphia chromosome and BCR-ABL gene by cytogenetic and RQ-RT-PCR, respectively. However, tumor burden was different when we use cytogenetic and molecular methods.

Key words: CML, Karyotype, RQ-RT-PCR

INTRODUCTION

Chronic Myeloid Leukemia (CML) is a chronic myeloproliferative disorder developed by an acquired chromosomal translocation t (9:22) (q34:q11), resulting in Philadelphia chromosome.[1]. Among the total patients with leukemia, nearly 15% are of CML or nearly 4600 new patients each year with a man to women ratio 1.5:1. High intensity ionizing radiation and DNA topoisomerase II inhibitors have been found to have association with a potential to induce t (9; 22)-positive leukemia.[2] Clinical features may include lethargy, shortness of breath on exertion, weight loss, bruising, bleeding gums, visual disturbances, pain in the splenic area, and gout. Priapism may occur in male.[3] Blood shows mildly anisocytic anemia, a few nucleated red cells, normal to increased reticulocytes, Thrombocytopenia or marked degree of thrombocytosis, hyper-leukocytosis with immature granulocytes, basophilia (less than 15%), and less than 10% blast cells.[4] The bone marrow is markedly hyper-cellular, and hematopoiesis constitutes more than 90% of the total nucleated element. An increased granulopoiesis, decreased erythropoiesis, and normal to increased megakaryopoiesis with hypo-lobate megakaryocytes is observed. Increased myeloid to erythroid ratio (M: E) is noted. Eosinophilia and basophilia may be present in bone marrow and in blood simultaneously.[5] Bone marrow fibrosis in proportion to number of megakaryocytes is present in nearly 50% of the patients with CML.[6] Cytogenetic analysis is used to diagnose the patients

with morphological diagnosis of CML. Philadelphia chromosome was shown in almost 95% of patients in cytogenetic analysis. Approximately 5% have complex translocations involving chromosomes other than rearrangements of BCR-ABL or chromosomes 9 and 22. Reverse transcriptase PCR method is used for the monitoring and diagnosis of BCR-ABL fusion gene in CML.[7] Allogenic SCT (stem cell transplantation) offers the ultimate solution for patients with Chronic Myeloid Leukemia (CML). Before Imatinib, interferon (IFN)-based therapy were main options for treatment, and easy cytoreductive treatment with Hydroxyurea if SCT was not possible.[8] Imatinib can induce hematologic, cytogenetic, and molecular remission in all stages of CML, with minimal toxicity.[9]

Objectives

To determine the presence of Philadelphia chromosome and BCR-ABL fusion gene in morphologically diagnosed CML.

MATERIALS AND METHODS

Fifty patients of CML patients were included in this study, with a clinical suspicion of CML.

Routine investigations: Complete Blood Examination and bone marrow aspirate and trephine biopsy. Cytogenetics: For karyotyping, cell culture was done on tissue culture media RPMI-1640 fortified with fetal calf serum, glutamine, and bicarbonate. Two ml of heparinized samples (PB/BM) was added under all possible aseptic conditions to 10 ml of tissue culture media pre-warmed to 37°C. It was incubated at 37°C for 24-72 hours. Phosphate buffer was used to wash cells by centrifuging multiple times. Colchicine was added to arrest growth of cells. Cells were separated from red cells by serial washing with fixative (methanol – acetic acid fixative in 3:1 ratio). Slides were prepared which were stained by Giemsa stain. G banding technique was carried out. Trypsinization procedure was followed. The slides were then stained with Giemsa stain. Short hand Chicago conference notation was followed and karyograms were prepared and these were then photographed.

Relative Quantitative Reverse Transcriptase Polymerase Chain Reaction for BCR-ABL Fusion Gene: Peripheral Blood/Bone marrow samples was obtained from the patients. RNA extraction was done by TRI reagent LS, using Chloroform, Isopropanol, and 75% ethanol. Complementary DNA (cDNA) was synthesized by Primers (ABL-R), dNTPs, MML-RT enzyme, RNAase inhibitor, and extracted-RNA. Amplification and detection was achieved through used of Taq polymerase, Primers (BCR mix), Reference dye, and cDNA.

RESULTS

Peripheral Blood

Out of 50 cases of CML, hemoglobin range was 5.2-14.3 g/dL with a mean of 9.6 g/dL. Total WBCs ranged 65-353 $x10^9$/L with a mean of 210.8 $x10^9$/L. Blast cells ranged between 2-7% on peripheral blood with a mean of 7%. Out of 50 cases of CML, platelets were ranging between 123-1548 with a mean of 430.3.

Fibrosis on Bone Marrow

Trephine Biopsy showed moderate fibrosis in 35 (70%) cases of CML and 14 (28%) cases showed marked fibrosis in CML patients while only one (02%) cases showed mild fibrosis (Fig 1).

Cytogenetic and Molecular Data:

Cytogenetics showed Philadelphia chromosome in all the 50 patients in Group A and a mean of 92% of positive cells in a range of 84-96%. Detection of BCR-ABL showed positive fusion gene in all the 50 patients of this group and it ratio to endogenous control gene showed a mean of 24.4% with a range of 5.5-96%.

DISCUSSION

Traditionally, CML diagnosis was based on morphology. CML is usually associated with greatly increased WBCs.[10] In 50 cases of CML, minimum total WBCs were 65 $x10^9$/L. The upper limit in our study touched to 353 $x10^9$/L. Therefore, threshold WBC count was 65 in this study. In our study, all patients had Chronic Phase CML (CML-CP). CML-CP is labeled when blast cells are <10% on peripheral blood and bone marrow separately.[11] In

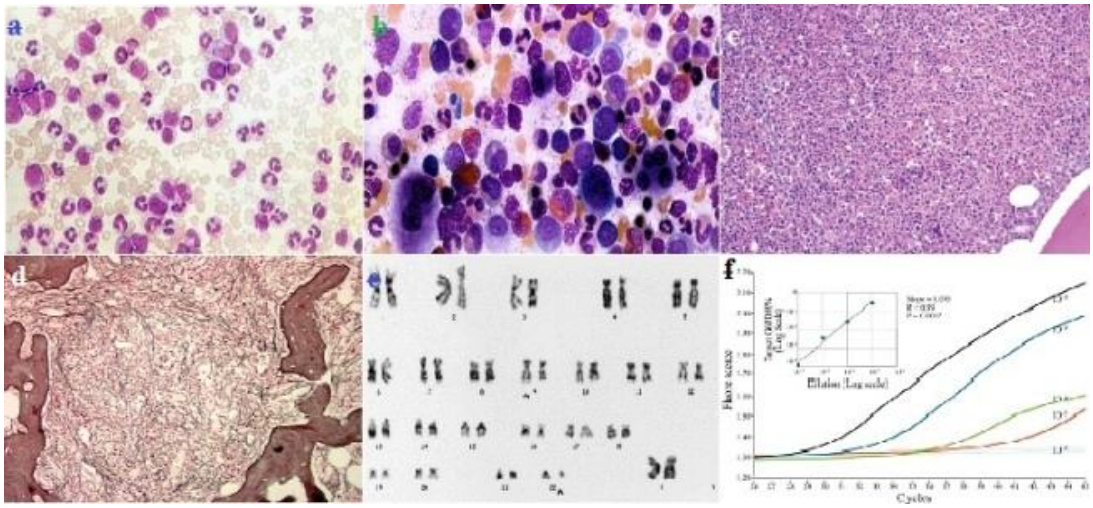

Fig. 1: Diagnostic workup for CML: a) Peripheral Blood, b) Bone Marrow, c) Core Biopsy, d) Reticulin Stain, e) CML Karyotype, f) RQ-RT-PCR for BCR-ABL gene.

our study, blast cells were 7% of mean value, ranging between 2-9%. Platelet count is usually normal, but may be reduced or elevated.[12] In our study, platelets ranges between 123-1548 $x10^9$/L but a vast majority (64%) showed platelets in normal ranges. Thrombocytosis was observed in 17 (34%) cases having platelets count >450 x 10^9/L. Only one patient showed thrombocytopenia. Mild to moderate degree of anemia is always present in CML patients. This phenomenon is due to replacement of bone marrow by greatly expanded granulocyte component.[13] In our study considerable anemia (Hemoglobin <10 g/dL) was in 32 (64%) cases having Hb <10.0 gm/dL. In addition, 18 (36%) cases had clinically insignificant anemia with Hb >10 gm/dL. Bone marrow shows increased cellularity and fibrosis.[14] These findings are supported by our study. Trephine Biopsy showed moderate in fibrosis in 35 (70%) cases of CML and 14 (28%) cases showed marked fibrosis in CML patients while only one (02%) cases showed mild fibrosis. Cytogenetics proved to be an important tool for detecting translocations or other chromosomal abnormalities. The most commonly, CML is associated with Philadelphia chromosome. In addition, Philadelphia chromosome negative CML is also an entity. This method is limited in sensitivity, if compared with FISH of PCR, in detecting leukemic cell. It is difficult to detect leukemic burden below 3-5% with conventional cytogenetics.[15] In our work, we found no cytogenetic abnormalities other than Philadelphia chromosome. Moreover, no patient was proved Philadelphia- chromosome negative. With the development of molecular techniques, it was made possible to detect BCR-ABL fusion gene. This is a highly sensitive technique to detect one in 10^{05} leukemic cells. There may be Philadelphia chromosome negative patients, which are BCR-ABL positive. These patients have either a masked Philadelphia translocation or a lower tumor burden than the detection limit of the cytogenetic techniques.[16] In our study, detection of BCR-ABL gene corresponds well to the detection of Philadelphia chromosome with conventional cytogenetic techniques. However, tumor burden was higher with molecular techniques as compared to the cytogenetic methods.

CONCLUSION

All cases, which were morphologically diagnosed as CML, were also positive for Philadelphia chromosome and BCR-ABL gene by cytogenetic and RQ-RT-PCR, respectively. However, tumor burden was different when we use cytogenetic and molecular methods. This is of implication while monitoring the disease on cytoreductive, interferon based therapy, or Imatinib mesylate follow up.

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