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Clinico Hematological Profile and Phase Distribution of Chronic Myeloid Leukemia

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Summary

Objectives: To evaluate the Clinico hematological profile based on the age, sex and Clinico hematological presentations and frequencies of three phases of chronic myeloid leukemia (CML), This study highlight the Ph positively by real time polymerase chain reaction (RT-PCR) technique contribute towards understanding the disease biology, and have important implications for diagnosis and management of CML patients.

Study design: This is an experimental and observational study.

Place and duration: This study was conducted in medical ward and pathology department of Peoples University of Medical and Health Sciences for women (PUMHS-W) Nawabshah from June 2013 to June 2014.

Materials and methods: Total 83 patients including 52 male, 31 female at their age ranges between 23 and 57 years admitted in medical ward of PUMHS hospital were selected for study. The clinical history and physical examination of these patients were noted. All the blood samples and bone marrow biopsy sent to the pathology department of PUMHS for the analysis of complete blood count, peripheral blood and bone marrow examination for the diagnosis of three phases of chronic myeloid leukemia.

Results: Out of 83 patients, 52 were male and 31 were female with male to female ratio of 1.6:1, the mean age of these subjects was 39.5 ± 16.5 years. The mean total leukocyte counts, platelet counts, hemoglobin levels and marrow blast frequencies were 121,000 ± 35,000/cmm, 285,000 ± 122,000/cmm, 7.5 ± 4.9 and 15 ± 9 respectively. The majority of patients 62 (74.6%) were classified in the chronic phase (CP), 17 (20.4%) in the accelerated phase (AP) and 3 (5.0%) in blast crisis (BC). The most frequent patient age ranges were 21-30 years for CP, 41-50 years for AP and 41-50 years for BC.

Conclusion: This study concluded that most CML patients are from a younger age group (33-47 years). Males were more commonly affected than the females. The detection of ph chromosome positively by resent and advanced RT-PCR technique is mandatory for the diagnosis and treatment of CML patients.

Keywords: Chronic myeloid leukemia; Phase distribution; Response to therapy; RT-PCR; Philadelphia chromosome

Introduction

Chronic myeloid leukemia (CML) is a clonal malignant neoplasms of pleuripotent hematopoietic stem cell characterized by the excessive proliferation of mature granulocytes and their precursors in the bone marrow and peripheral blood caused by in 90% of cases due to the presence of Philadelphia chromosome and rarely by Hyperdiploidy of >50 chromosomes [1]. The translocation between chromosome 9 and 22 t (9,22) (q34;q11) leads to the formation of break point cluster region and Abelson's (BCR-ABL) a new hybrid fusion genes that encodes for an oncoprotein (P210) located in the cytoplasm that has a strong, capacity to activate tyrosine kinas resulting in the activation of several downstream signals that transform hematopoietic stem cells in to the leukemic cells, thus increased tyrosine kinas activity is currently thought to play a central role in the pathogenesis of CML [2]. In spite of leukemia induced factors, there are risk factors that enhance the CML and these factors include lower socio-economic status, occupational exposure to benzene, formaldehyde, high doses of ionizing radiation among the atomic bomb survivors, other risk factor such as alcohol abuse, obesity, weight gain during adulthood and effects of preservatives or pesticides used in the food industry causes CML [3,4].

Clinically in 50% of cases patients with CML are asymptomatic and remaining were present with anemia, spleenomegally, fever, bleeding tendency, hepatomegally, lymphadenopathy and complications such as renal failure, hearing loss and priapism, and laboratory findings include complete blood count, peripheral blood and bone marrow examinations showing low hemoglobin, total WBC count between 287×10^9/L and 535.7×10^9/L, thrombocytopenia or normal platelet count or thrombosytosis and peripheral blood smear showing increase number of mature and immature granulocytes including predominantly [5,6]. The Bone marrow pictures in CML without treatment showing hypercellularity due to excessive proliferation of the granulocytes with myelocytes predominantly and presence of blast cells from <10% to >20% in the bone marrow and peripheral blood according to the world health organization criteria that divide the CML in to chronic, accelerated phases and blast crisis, there is decreased or normal or increased megekayrioposis as well as moderate to marked reticulin fibrosis with presence of small megakaryocyte containing hypolobulated nuclei, sea-blue histiocytes and gaucher cell and

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these changes are return to the normal state after treatment and the immunohisto-chemistry is used for differentiating the myeloblastic and lymphoblastic crisis of CML [7,8]. The recent developments in the confirmation of diagnosis of CML by sensitive tests such as qualitative real time-polymerase chain reaction (RT-PCR) to identify transcript variants of BCR-ABL fusion genes and quantitative droplet digital PCR as well as RT-PCR tests are used for ratio of BCR- ABL transcripts levels with normal genes on the international scale (>1016 and monitoring the response to therapy of patients with chronic myeloid leukemia [7,8]. The Cytogenetic must be performed by chrosome banding analysis (CBA) of marrow cell metaphases for the detection of BCR-ABL+ nuclei and additional chromosome abnormalities among patient with CML, if marrow cell cannot be obtained, CBA can be substituted by inter-phase fluorescence in site hybridization (I-FISH) of blood cell using dual color dual fusion probes [9]. The marked improvements in the management of CML with first line gold slandered therapy of imatinib mesylate (IM), the first tyrosine kinase inhibitor (TKI) targeting the BCR-ABL1 oncoprotein that causes leukemia, the second line therapy of TKI such as nilotinib and desatinib and allogenic bone marrow transplantation are used in case of failure of three TKI in CP as a 3rd line therapy while treatment of AP and blast phase is required prolong use of TKI and allogenic bone marrow transplant [10] BCR- ABL – positive cells are genetically unstable and are prone to develop multiple and heterogeneous genomic abnormalities such as point mutations >90 in the kinas domain (KD) resulting in the transformation of the luekemic phenotype from chronic to acute hence leading to resistance to the tyrosine kinase inhibitors [11].

Material and Methods

Inclusion criteria

The study was conducted in the Pathology Department of the Peoples University of Medical and Health Science (PUMHS) at Shaheed Benazirabad between June 2013 and May 2014. Samples were collected from outpatient clinics and inpatients suspected to be suffering from blood cancer. In this study, all newly diagnosed CML patients (based on hematological profile) older than 17 years of age were included. A detailed history was obtained for each patient, and questionnaires and physical examinations were also administered Additional file 1. Investigations included complete blood counts with differential and bone marrow aspirations. After completion of the investigation, patients were categorized into various CML phases based on the World Health Organization (WHO) criteria. The chronic phase (CP) was defined as myeloid blasts less than 10% in the peripheral blood or bone marrow. The accelerated phase (AP) was defined as blasts 10-19% of white blood cells in peripheral and/or nucleated bone marrow cells; persistent thrombocytopenia (<100 × 109/L) unrelated to therapy or persistent thrombocytosis (>1000 × 109/L) unresponsive to therapy; increasing white blood cells and spleen size unresponsive to therapy and or cytogenetic evidence of clonal evolution. Blast crisis (BC) phase was defined as peripheral blood blasts ≥ 20% of peripheral blood white blood cells or nucleated bone marrow cells; extra medullary blast proliferation; and large foci or clusters of blasts on bone marrow biopsy.

Exclusion criteria

Ph chromosome - negative or BCR-ABL - negative CML, Pregnant or breastfeeding woman, and patients taking imatinib for treatment of CML were excluded.

Results

In our study total 83 patients, including 52 male and 31 female with male to female ratio of 1.6:1 and their mean ages was 39.5 ± 16.5 years were selected. The major clinical features in these subjects were anemia, massive splenomegally, Hepatomegally, history of fever with cough and bleeding. The Mean hemoglobin levels, 9.5 ± 2.9 g/dl, total leukocyte counts /cumm, 121000 ± 35000 /cumm differential leukocyte count % including mature leucocytes 43% such as neotrophils 21 ± 7, lymphocyte 8 ± 2, eosinophils 9 ± 2, monocytes 5 ± 3 and immature cells 57% composed of Blast 18 ± 12, promylocytes 4 ± 1, Myelocytes 25 ± 5, Metamyelocytes 9 ± 2, band cells 13 ± 3 platelet counts / cumm, 285000 ± 220000 / cumm respectively were noted in the present study (Table 1). The examination of peripheral blood smear in these patients was showing the nomocytic normochronic red blood cells with variability in size and shapes [12,13]. Plenty nucleated red blood cells and many mature and immature leucocytes including predominantly myelocytes with number of the blast cells form 6% to 30% were present in the chronic, accelerated phases and blast crises were seen while bone marrow smear were showing hypercellularity due to excessive proliferation of myeloid cell line predominantly of myelocytes hypolobated megakaryocyts with few blue histocytes and pseudogaucher cell. Total 83 patients with CML were divided in to chronic phase (CP) 62 (74.6%), 17 (20.4%) in the accelerated phase (AP) and 3 (5.0%) in blast crisis (BC). The parameters included in this study are present Table 2 show frequency of three phases of CML based on age, sex, spleenic size and number of blast cells in peripheral blood and bone marrow smears (Table 2).

Discussion

The Chronic myeloid leukemia(CML) being a commonest leukemia in Asia, needed Clinico hematological profile and frequency of three phases of CML with early diagnosis and treatment among the Asian populations to improve survival rate in CML reported by Altekruse et al. [7].

Hence Ahmed et al. [14] Reported that frequency of chronic phase (CP), accelerated phases (AP) and blast crisis (BC) were 77.8%, 15.5% and 6.7% respectively were observed in among the 45 patients suffering from CML with their mean age 37.9 yrs, and male: female ratio of 2.2:1 while Clinico hematological features were Anemia and massive splenomegally, hemoglobin 9.94 g/dl. The mean total leukocyte count 214.3x109/L, platelet count 551.4×109/L, and marrow blasts were 9.3% respectively. Buchner-Daley L, Brady-West D were reported the presenting features of 70 patients diagnosed with chronic myeloid leukemia, with male to female ratio of 2.4:1, had age incidence of 37 years while Weight loss and splenomegally were the most frequently seen and frequencies of three phases of CML were similar with above study. Bhatti et al. [13] studied the 335 patients with CML had mean age of 35.5 yrs, with male to female ratio of 2:1 while similar Clinico hematological features and frequency of three phases of CML were recorded. Mutaleb et al. [8] were detected Philadelphia (PH) chromosome positive CML cases by Real Time-Polymerase Chain Reaction RT-PCR in 58 (90.1%) out of 63 patients with male to female ratio of 2.0:1.0 and the mean values of age, hemoglobin levels, total leukocyte counts, platelet counts, and marrow blast frequencies were 37.4 years, 12.2 g/dl, 101×10^9/L, 409×10^9/L, and 2.8% in (CP),while 45.4 years, 8.7 g/dl, 121×10^9/L, 418×10^9/L, and 15% in (AP) and 45.5, 9.2 g/dl years, 311×10^9/L, 396×10^9/L, and 26% in (BC) respectively, They recorded the frequency of CP (81.2), AP (14.5), BC (4.1) respectively and the risk factors contributing to the early onset of CML were

`Mean age in years	Sex	Socioeconomic status
40 ± 17 years	Male 52 (63.8%) Female 31(37.4%) Male to female ratio 1.6:1.0	Poor 63 (75.9%) Lower middle class 15 (18.0%) Upper middle class 4 (4.8%)
Symptoms / clinical history		Physical examination
Asymptomatic; 10(12.03%) Symptoms due to Anemia 79 (95.1%) Pallor Fatigue, lethargy, Body aches, dizziness, nausea & vomiting Difficulty in breathing, Symptoms due to splenomegally 70 (84.3%) Abdominal distension Abdominal discomfort Pain left side of abdomen History of bleeding 12 (14.4) Symptoms due to infection; fever with cough 18(21.6%) Hypermetabolic state; loss of weight night sweat 10(12.0%)		Anemia Mild 19 (22.8) Moderate 39 (46.9%) Severe 25 (30.1%) Splenomegally Massive (≥ 10cm) 60 (70.2%) Moderate(4-9 cm) 55(66.2%) Mild (1-3 cm) 14 (16.8%) Hepatomegally 15(18.0%) Lymphadenopathy 8 (9.6%)
Hematological parameters		Result & Value
Hemoglobin g/dl, Total leucocyte count /cumm Differential leucocyte count; The % of mature and immature cells calculated out of 100 leucocytes / HPF Platelet count /cumm		9.5 ± 2.9 g/dl 121000 ± 35000 /cumm Mature cells 43% (neotrophils 21±7, lymphocyte 8± 2, eosinophils 9± 2, monocytes 5 ±3) Immature cells57% (Blast 18±12, promylocytes 4± 1, Myelocytes 25± 5, Metamyelocytes 9±2, band cells 13±3) 285000 ± 220000 / cumm
Examination of PBS		Examination of bone marrow
The red blood cells are nomocytic normochronic with variable in size and shapes. Plenty nucleated red blood cells are seen and many mature and immature leucocytes are seen, the majority of cells are myelocytes.		The bone marrow is hypercelurar due to excessive proliferation of myeloid cell line predominantly of myelocytes with few blue histocytes and pseudogaucher cell. The megakaryocytes or hypolobated.

N= Number of Patient PBS=peripheral blood smear
HPF= High Power Field %= percentage

Table 1: The evaluation of chronic myeloid leukemia based on age in year, sex, socioeconomic status and Clinico laboratory findings. (N=83)

Phases of CML	Chronic Phase	Accelerated Phases	Myeloid Blast Crisis
Frequency	62 (74.6%)	17 (20.4%)	3 (5.0%)
Age	30 ± 7	45 ± 6	45.5 ± 9.5
Sex	Male 42	Male 11	Male 2
	Female 20	Female 6	Female 1
	Ratio 2.1.1	Ratio 1.8.1	Ratio 2.0;1.0
Spleenic Size	< 10 / cm	>10 / cm	> 15 / cm
Number of blast cells in peripheral blood and bone marrow smears	6 ± 2	16 ± 4	26 ± 5

Table 2: The frequency of three phases of CML based on age, sex, spleenic size and number of blast cells in peripheral blood and bone marrow smears N=83

Formalin applied on fish for preservation, calcium carbide on fruits to ripen, brick dust in chili powder, urea to whiten rice and puffed rice, sawdust in loose tea, soap in Ghee, artificial sweetener, coal tar, textile dyes in sweetmeats and occupationally exposure of benzene, ionizing radiation in x-ray department, any form of formaldehyde used in industries including formalin. Studies detected Abelsons and break point cluster region (ABL-BCR) positive cases of CML in 40 patients, out of 48 patients by RT-PCR test with the mean age of 37.6 ± 14.1 years, male to female ratio 1.8:1 spleenic size 9.8 ± 5.8 cm, TLC 284.5 $\times 10^9 \pm 267.5 \times 10^9$. That indicated 92% specificity sensitivity and reliability of this test. Yaghmaie et al. [14] detected expression of one of the P120BCR-ABL transcripts including b3a2 (62%) and b2a2 (21%) among the 83% out of 75 Iranian patients, while the remaining showed one of the transcript of b3a3 and b2a2 while the similar two types of transcripts and additional cytogenetic abnormalities such as double PH chromosome, +8, +19 among the Indian 208 patients with CML ph chromosome positive had male to female ratio of 1.8:1 and mean age of 38 years of all the three phases of CML were observed by Anand et al. [15].

Conclusion

From the above discussions, following conclusion and recommendation were made. In our study, total 83 patients including 52 male and 31 female with male to female ratio of 1.6:1 and their mean ages was 39.5 ± 16.5 years and frequency of three phase of CML was 62.(76.4%) were in the chronic phase (CP), 17 (14.58%) respectively. The male are affected more than the female and chronic phase of CML was common in younger age group. The Philadelphia chromosome detection by RT-PCR in CML patients due to the limited sources, we can't perform this advanced test for the molecular analysis of CML.

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