Int.j.pathol.2019;17(2)66-71

Original Article

Hematological Response to Imatinib Mesylate in Chronic Myeloid Leukemia in a Tertiary Care Centre

Nazish Farooq[1], Muhammad Ihtesham Khan[1], Saleem Ullah[2] and Abid Jameel[3]
[1]Pathology Department Khyber Medical College, [2]Khyber Medical University,
[3]Oncology Department, Hayatabad Medical Complex

ABSTRACT

Objective: To determine the hematological response to imatinib therapy in cases of chronic myeloid leukemia, hence determining the effectiveness of imatinib in our population.

Methods: This cross-sectional study was conducted in Pathology Department, Hayat Abad Medical Complex (HMC) hospital, Peshawar, from October 2013 to March 2014 (6 months duration). About 99 cases of Chronic Myeloid Leukemia (CML) in chronic phase, of all ages and both sexes, diagnosed through peripheral blood smear and bone marrow examination, and showing BCR-ABL translocation by Fluorescent in situ hybridization technique (FISH) were included in the study. The sampling technique used was Non-Probability purposive sampling. Patients who were nonconsenting, BCR-ABL negative, those in accelerated phase and blast transformation were excluded from the study. All the cases were given 400 milligrams of imatinib mesylate. Hematological response was assessed by determining hemoglobin level, red cell count, total leukocyte count and platelet count on monthly basis for six months of therapy. The findings were recorded, and results were made. Quantitative variables were calculated via Mean and standard deviation. Frequency and percentages were used for qualitative variables.

Results: About 99 patients were included in the study for determining hematological response to imatinib mesylate for period of six months. About 57 (57.6%) cases were male while 42 (42.4%) cases were female. Male to female ratio was1.4:1. Age range of the study population was 21 – 60 years (mean: 39 ±8.1SD). The mean hemoglobin levels improved from 9.96±2.14 g/dL at the start of therapy to 12.45±0.97 g/dL at sixth month of therapy in males and a gradual improvement from 9.15±1.86 g/dL to 11.86± 0.98 g/dL in case of female patients. The mean red cell count improved from 3.24±0.60 millions/mm^3 to 4.19±0.30 millions/mm^3 in male patients and from 3.06±0.54 millions/$mm^3$to 4.02±0.32 millions/mm^3 in female patients. The mean Total Leukocyte Count at the start of therapy was 123421.7±117547.9 thousand/mm^3 and 134782.5±110407.2 thousand/mm^3 for male and female patients, respectively. After six months treatment these levels improved to 7264.3±1882.2 thousand/mm^3 and 8014.6±3810.8 thousand/mm^3 for male and female patients respectively. The mean Platelet count lowered from 508017.5±426502 cells/$mm^3$to 232482.5±108276.5 cells/mm^3 in case of male patients and from 419857.1±256539.5 cells/$mm^3$to 213278.6±89839.02 cells/mm^3 in female patients at start and conclusion of imatinib mesylate treatment, respectively. Thus, the blood counts greatly improved in response to imatinib mesylate therapy at the end of 6 months.

Conclusion: Imatinib is highly effective in our setup as it successfully improved the blood counts and no resistance was reported in any of the cases. It can be used as first line treatment option.

Key words: Chronic myeloid leukemia, blast crises, chronic phase, accelerated phase, Hematological response, Imatinib mesylate

Introduction

Chronic myelogenous leukemia (CML) is a hematological malignancy in which there is excessive abnormal proliferation of cells of myeloid lineage in the bone marrow.[1-3]

AUTHOR CORRESPONDENCE:
Dr. Nazish Farooq
Assistant Professor, Pathology Department
Khyber Medical College.

Email: nazishfarooqheam@gmail.com

The underlying genetic defect in CML is a reciprocal translocation between chromosome 9 and 22, which is referred to as Philadelphia (Ph) chromosome which is a cytogenetic hallmark of CML and carries the BCR-ABL gene. This encodes BCR-ABL oncoprotein that has tyrosine kinase activity, which autophosphorylates downstream protein kinases leading to uncontrolled

abnormal proliferation of myeloid cells in the bone marrow and thus, transformation to CML.[1-5]

The worldwide incidence of CML is about 1-2 cases per 100000 population and in adult population it is about 14% of the new cases of leukemia.[1-2]In Pakistan, CML is the commonest myeloproliferative disorder in adults.[6] The peak incidence of CML in Pakistan ranges from 21–50 years after which there is a continuous declining trend to the age of 80 years.[6] In the West, the peak age of CML is in 6th decade. But in Pakistan, CML occurs in young age.[6] The mean age of patients of CML in Pakistan is 42 years, with a male predominance, and present with advanced stages of CML due to inadequate health care facilities and non-affordability on the part of patients.[6]

CML progresses through three phases i.e. chronic, accelerated and blast phase.[1,2,5]Most of the patients present in the chronic phase which may last for 2-7 years.[2] The disease in this stage is usually not severe, and in some cases even without any symptoms.[2] Accelerated phase is recognized by the falling platelet counts and shows less satisfactory results to treatment as compared to chronic phase.[2] The blast phase is recognized by increase in immature cells in blood and bone marrow and is fatal; patients survive only for a few months.[2] Progression from chronic phase to blast phase is due to further genetic defects additional to the Philadelphia chromosome.[2] Fatigue, and pain or fullness in abdomen are the main clinical features of the chronic phase of CML.[1] Accelerated phase presents as bleeding and thrombosis while blast phase manifests as anemia, fever and cachexia.[1]

The routine diagnostic investigations for CML includes a leukocyte count which shows leukocytosis with neutrophil and metamyelocytes peak in blood film and bone marrow aspiration. The presence of the Philadelphia (Ph) chromosome is detected by cytogenetic analysis, fluorescence in situ hybridization or by molecular studies. Reverse transcriptase-polymerase chain reaction (RT-PCR) has high sensitivity in detecting minimal residual disease in patients on therapy. Bone marrow examination is necessary in patients of CML. It confirms the diagnosis and also helps determine the stage in which the patient is.[1]

Treatment for CML in chronic phase includes the use of imatinib which is the first-generation tyrosine kinase inhibitor.[1,2,3,5] It was first approved in 2001 for the treatment of CML due to its high level of activity in phase 2 studies.[3,5] Nilotinib, bosutinib and dasatinib are second generation tyrosine kinase inhibitors.[1,2,3,5] Second generation tyrosine kinase inhibitors are used when patients fail to show response to imatinib.

The response of patients for imatinib is assessed by determination of hematological response, cytogenetic response and molecular response at 0,3,6 and 12 months of starting therpy.[5-7] Hematological response is assessed by determining the blood counts. Cytogenetic and molecular response is detected by PCR and cytogenetic analysis; a "Complete Hematological Response" is said to have been achieved if the blood counts normalize and there are no sign symptoms of the disease. A "Complete Cytogenetic Response" means that there are no Ph-positive metaphases.[7]

Failure to achieve complete hematological response by 3 months indicate treatment failure and that second-generation Tyrosine kinase inhibitors should be used.[5,8] Patients who get the "T315I gatekeeper" mutations show resistance to all Tyrosine Kinase Inhibitors [1,2]Patients with advanced stage disease, and those fail to respond to at least two tyrosine kinase inhibitors are candidates for allogeneic stem cell transplantation.[1,2,5]

There is scanty data regarding response to imatinib in cases CML in our setup. The present study was done to determine the hematological response to imatinib in our setup, thus highlighting the efficacy of imatinib.

Methods

It was a cross-sectional descriptive Study, done in the Department of Pathology, Hayatabad Medical Complex (HMC) hospital, Peshawar from October 2013 to March 2014(6-month duration). A total of 100 cases of CML (Chronic Phase), of all ages and both sexes, diagnosed with peripheral blood smear and bone marrow examination, showing "BCR/ABL translocation" by Fluorescent in situ hybridization technique (FISH) were included in the study. The sampling technique used was Non-Probability purposive sampling. Patients who were non-consenting, negative for BCR-ABL translocation, those in accelerated Phase and blast transformation were excluded from the study. Data regarding age, sex and basic hematological parameters at diagnosis were noted in the proforma. All the cases were given 400 mg imatinib mesylate daily. Hematological response was assessed by determining Hemoglobin level, red cell count, total leukocyte count and platelet count on monthly basis for six months after therapy. Quantitative variables were determined by mean and standard deviation. Qualitative data was measured by frequency and percentages.

Results

About 100 cases were diagnosed as CML during the study period. One case was in blast crises, so it was excluded from the study. The remaining 99 cases were included in the study for determining hematological response to imatinib mesylate for period of six months.

Age of the study population ranged from 21 – 60 years with mean of 39 ±8.1SD. About 57(57.6%) cases were male while 42 (42.4%) cases were female, with male to female ratio being 1.4 :1.
Demographic data of the study sample is shown in Figure 1.

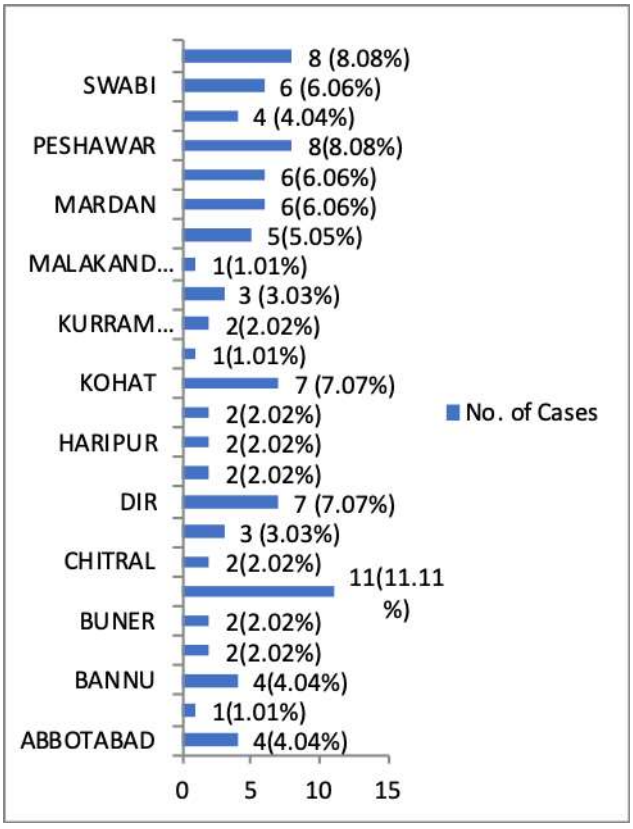

Figure 1. Demographic distribution of CML patients

The mean hemoglobin levels improved over the course of therapy, i.e it improved from 9.96±2.14 g/dL at the start of therapy to 12.45±0.97 g/dL at sixth month of therapy in males, and a gradual improvement from 9.15±1.86 g/dL to 11.86± 0.98 g/dL in case of female patients (Table: 1 & Figure 2.a).

The mean red cell count rose from 3.24±0.60 millions/mm^3 to 4.19±0.30 millions/mm^3 in case of male patients and from 3.06±0.54 millions/mm^{3}to 4.02±0.32 millions/mm^3 in female patients. This showed a gradual improvement in RBC count over the course of imatinib mesylate therapy (Table 1 & Figure 2.b).
The mean Total Leukocyte Count at the start of therapy was 123421.7±117547.9 thousand/mm^3 and 134782.5±110407.2 thousand/mm^3 for male and female patients, respectively. After six months treatment these levels came to 7264.3±1882.2 thousand/mm^3 and 8014.6±3810.8 thousand/mm^3 for male and female patients respectively (Table 1 & Figure 2.c).
The mean Platelet count lowered from 508017.5±426502 cells/mm^{3}to 232482.5±108276.5 cells/mm^3 in case of male patients and from 419857.1±256539.5 cells/mm^{3}to 213278.6±89839.02 cells/mm^3 in female patients at start and conclusion of imatinib mesylate treatment, respectively (Table 1 & Figure 2.d).

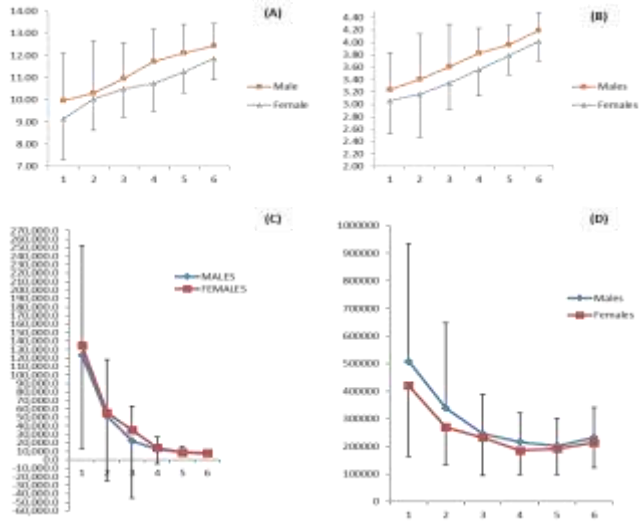

Figure 2: Effect of imatinib on red cell count, TLC, Hb and platelet count in CML cases. (A) Effect on Haemoglobin (g/dl) levels, **(B)** Effect on RBC count (million/mm^3), **(C)** Effect on total leukocyte count (thousand/mm^3) and **(D)** Effect on platelet count

Discussion

The survival of CML patients used to be very short before the introduction of imatinib.[9]Before development of imatinib, busulfan and hydroxyurea were used to control cell count in CML. Hydroxyurea only lowers the cell counts but does not change the course of disease.[5]Later , Interferon alpha was used as the main treatment of CML, until introduction of

Table 1: Effect of Imatinib therapy on Red cell count, Hemoglobin, Total Leukocyte Count and Platelet count in 57 male and 42 female cases of chronic myeloid leukemia.

Month	RBC (million/mm3)		Hemoglobin (g/dl)		Total Leukocyte Count (thousand/mm^3)		Platelet Count (cells/mm^3)	
	Males	Females	Males	Females	Males	Females	Males	Females
	Mean ±SD	Mean ±SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1	3.24±0.60	3.06±0.54	9.96±2.14	9.15±1.86	123421.7±117547.9	134782.5±110407.2	508017.5±426502.1	419857.1±256539.5
2	3.40±0.74	3.16±0.69	10.31±2.36	10.03±1.40	51545.24±63049.7	55311.4±76328	338708.8±308245.3	269097.6±135912
3	3.61±0.69	3.35±0.43	10.96±1.62	10.49±1.27	22404.76±28224.22	34907.54±67497.06	245578.9±142899.9	232238.1±138037.3
4	3.82±0.40	3.56±0.42	11.74±1.45	10.73±1.25	12215.48±12843.51	14071.93±17155.79	216526.3±105275.3	185738.1±88549.02
5	3.96±0.32	3.79±0.31	12.11±1.32	11.28±0.96	8850±7152.085	8771.053±4371.365	201763.2±99713.34	191743.9±94249.14
6	4.19±0.30	4.02±0.32	12.45±0.97	11.86±0.98	7264.286±1882.243	8014.561±3810.804	232482.5±108276.5	213278.6±89839.02

tyrosine kinase inhibitors in 2001.[5] Imatinib successfully changed the course of the disease by preventing the patients from going to accelerated phase and blast crises.[2,9,10]This led to increase in 10-year survival rate in patients of CML from20% to 80%-90% , and decrease in annual mortality rate from 15% to 1.5%.[1,11,12]Patients who respond to imatinib are predicted to have the same life expectancy as that of the normal population.[2,7,13,14,15]However, imatinib does not 'cure' CML.[2]Imatinib mesylate is a safe and effective anticancer agent ever developed so far.[2,10,16]It inhibits the BCR-ABL1 oncoprotein. This inhibits phosphorylation of proteins involved in transduction of cell signals.[1]Second and third-generation tyrosine kinase inhibitors were developed later in order to counteract the problem of drug resistance.[2]But they were found to cause more serious side effects and complications as compared to imatinib.[2,17,18,19]

Response to imatinib mesylate is elaborated in the European LeukemiaNet.[20] The hematologic response (HR) means returning of the peripheral blood counts to the normal.[8] After starting the imatinib, the peripheral blood counts normalize first; referred to as a complete hematological response (CHR), followed by cytogenetic and molecular response.[9] A Cytogenetic response defines the quantity of cells that are Philadelphia positive , through cytogenetic analysis or Fluorescent in situ hybridization technique, molecular response is detected by detecting the levels of BCR-ABL transcript.[8] Molecular response is measured by Reverse Transcriptase Polymerase chain reaction. PCR is costly and less available facility in our setup that patients cannot afford. So, we determined the response of imatinib by determining the hematological response in CML patients in the present study.

In our study, mean age of the study sample was 39 ±8.1SD. About 57(57.6%) cases were male while 42 (42.4%) cases were female, with male to female ratio being 1.4:1. Similar demographic data is reported in the study done by Doval DC, showing age range of the study population to be 18-66 years with mean of 41 years.[9]. In another study done by Qin YZ in 2016, mean age of the study sample was 39 years.[13] Male to female ratio was 1.6: 1 in his study.[13] In another study done by Bhatti S in 2012, mean age of the patients was 42 years, with male predominance. So, demographic data from these studies are similar to that reported in the present study.[6]

In the present study, it was observed that blood counts, including hemoglobin levels, RBC count, white cell count and platelet counts, returned to normal in all cases by the end of sixth month of treatment. Complete Hematological Response was achieved in 100% cases in the present study. None of the cases showed treatment failure. This response was far superior to that reported in different studies so far. In a study done by Tahlan in 2018, the complete hematological response was seen in 92% cases in patients onimatinib.[21] In another study done by Doval 97% cases achieved complete hematological response.[9] Similarly, complete hematological response rate of 97-98% was reported in other studies [22,23,24,25,26]. The International Randomized Study of Interferon and STI571 (IRIS) study showed that about 97% cases achieved CHR.[1,8,27,28] Patients whose total leukocyte count normalizes at 3 months of treatment is associated with better outcome and survival.[29]In the present study, hemoglobin value at the start of therapy was low in females in comparison to male patients. This fact may be attributed to low dietary

iron intake and periodic menstrual blood loss in females.

In the present study, there was no case showing failure to treatment or resistance. This was an important finding as failure to achieve complete hematological response is associated with poor outcome.[8] Literature suggests that about one third of patients with CML show inadequate response to imatinib.[8] Mutations in the BCR-ABL gene are present in about half of the cases of CML that are resistant to the imatinib.[8]

Recently, research has suggested that certain cases of CML can stay in remission after imatinib is stopped. But it is not clear that which cases will stay in remission and for how longer time. So, literature suggests that the imatinib treatment should be continued indefinitely.[29,30,31]

The response of CML patients to imatinib was good and comparable to that reported in various studies. None of the cases showed failure to treatment. Since test parameters showed good progress during the study period, with no resistance observed.

This is first large based local study in Khyber Pakhtunkhwa Province compiling response of imatinib in CML patients. As PCR and cytogenetic analysis were not routinely available nor the patients could afford them, the study focused on hematologic response solely. This was a major limitation of the present study, so there is a need to conduct larger studies that include the cytogenetic and molecular response in addition to hematologic response in order to generate further data on response to imatinib in our setup.

Conclusion

Imatinib is efficient and well tolerated treatment for CML in our setup. The rate of response in our series is equivalent to maximum of the researches. There was no issue of resistance to imatinib in the present study, therefore, Imatinib can be regarded as treatment of choice in our setup. Further studies should be done, including a large number of patients from various hospitals, and assessing the molecular and cytogenetic response to generate more detailed data.

Source of Funding: None

Conflict of Interest: None

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HISTORY	
Date Received	17-01-2019
Date sent for Reviewer	16-05-2019
Date Received Reviewer's Comments	22-05-2019
Date Received Revised Manuscript	25-06-2019
Date Accepted	08-07-2019

Authors' contributions:

- **Nazish Farooq:** Conception, Study Designing, Planning, Study conduction, Analysis, Critical review and Manuscript writing
- **Muhammad Ihtesham Khan:** Study conduction, Analysis, Manuscript writing and facilitation
- **Saleem Ullah:** Study conduction, Analysis, Manuscript writing and facilitation
- **Abid Jameel:** Conception, Study Designing, Planning, Study conduction and Critical Review