EARLY MOLECULAR RESPONSE WITH IMATINIB THERAPY IN CHRONIC MYELOID LEUKEMIA AND ITS ASSOCIATION WITH BASELINE WHITE BLOOD CELL COUNT AND SPLEEN SIZE

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ABSTRACT

Objectives: To determine the frequency of early molecular response in patients of chronic myeloid leukemia treated with Imatinib and its association with baseline white blood cell (WBC) count and spleen size.
Study Design: Cross sectional study.
Place and Duration of Study: Combined Military Hospital, Rawalpindi Pakistan, from May to Nov 2017.
Material and Methods: Seventy eight patients of Chronic Myeloid Leukemia (CML) in chronic phase (CP) were included in the study. Inclusion criteria were: 18 years or older, diagnosed with CML in CP with positive BCR ABL1. Patients who were in accelerated/blast phase, or already taking any Tyrosine Kinase Inhibitors or chemotherapy were excluded from the study. Base line WBC count, spleen size and BCR-ABL1 IS values were recorded. All the enrolled patients were placed on Imatinib therapy (400 mg/day) and RT-PCR for BCR ABL1 transcript was repeated after three months.
Results: In our study, 60.15% of patients achieved EMR at 3 months after Imatanib therapy (*p*-value <0.001). In univariate analysis, there was significant association of spleen size, baseline WBC count and percentages of blasts in bone marrow with BCR ABL1 (IS) at 3 months (*p*-value <0.001), while on multivariate regression model, significant association was found only in spleen size (*p*-value <0.001) with EMR.
Conclusion: A significant number of patients achieved EMR with Imatinib therapy. Spleen size at diagnosis was the only significant factor associated with achieving EMR. It is imperative to identify patients at an early stage who are unlikely to achieve EMR and therefore have poor over-all survival.

Keywords: Chronic Myeloid Leukemia, Imatinib, Spleen.

INTRODUCTION

Chronic myeloid leukemia (CML) is characterized by presence of genetic translocation between chromosome 22 and 9, label ledas the Philadelphia (Ph) chromosome[1]. It has been reported that CML accounts for approximately 15% of adult leukemias and approximately 85% of patients with CML are diagnosed in the chronic phase[2]. Untreated chronic phase usually progresses to accelerated or blast phase in three to five years. Philadelphia chromosome is present in 90-95% cases of CML[3]. BCR-ABL protein produced as a result of Philadelphia chromosome, has dys-regulated tyrosine kinase activity. It activates different pathways promoting survival and growth in leukemic cells[4]. Imatinib and other targeted tyrosine kinase inhibitors are the mainstay of treatment in CML, which has resulted in significant improvement in survival[5]. Imatinib a first generation tyrosine kinase inhibitor is phenyl amino pyrimidine derivative. It acts as an analog to tyrosine kinase protein, occupying the tyrosine kinase site and thereby decreasing its activity. Imatinib also inhibits movement of tyrosine kinase protein across nuclear membrane thus inhibiting its anti-apoptotic effects. Patients on TKI therapy are categorized on hematological, molecular and cytogenetic responses[6]. National comprehensive cancer network (NCCN) and the European leukemia net (ELN) for CML recommend to continue TKI treatment indefinitely in all responding patients[6,7]. However, side-effects,

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impairment of quality of life for many patients and the economic burden due to the high prices of drugs are the limiting factors for this continuing therapy. For many years the main goal of the treatment of CML with TKIs has been the achievement of the "major molecular response" (MMR) which is present if BCR-ABL1 transcripts levels decline to ≤0.1% on international scale (IS) at any time. Recently the goal of treatment is being shifted to the early molecular response (EMR) i.e. BCR-ABL1 transcript ≤10% at 3 months on IS as a predictor of a deeper response and of a better outcome[7]. In a recent study, authors reported EMR after 3 months of Imatinib therapy as 77.6% (n=410/528)[8]. In previous years multiple studies have been done to identify factors associated with poor response to therapy. On the basis of these studies, several scores have been derived to prognostify patients with CML in chronic phase[9]. Most of these studies were conducted on western population and in pre TKI era. We wanted to study the response of TKI therapy in our population (i.e south Asian, Pakistani) and association of risk factors including spleen size and WBC count at diagnosis with EMR.

MATERIAL AND METHODS

This descriptive cross sectional study with prospective data collection was carried out in department of Oncology, Combined Military Hospital Rawalpindi from May 2017 to November 2017. Seventy eight patients of chronic myeloid leukemia in chronic phase were selected through non-probability purposive sampling technique. Inclusion criteria were: patients 18 years or older, diagnosed with chronic myeloid leukemia in chronic phase with positive BCR ABL1 transcript. Patients who were in accelerated/blast phase, or already taking any TKI or chemotherapy were excluded from the study. Informed written consent was taken from the patients. A detailed history and physical examination was carried out in all selected patients. Blood samples were drawn for baseline complete blood picture and WBC count. Baseline WBC count was measured through complete blood count from clinical laboratory before the initialization of Imatinib therapy and was recorded as 103/µl. In our study early molecular response was defined as BCR-ABL1 transcripts level ≤10% on IS at 3 months after initiation of imatinib therapy. Spleen size was measured by clinical method and recorded in centimeter below left costal margin. The baseline BCR-ABL1 value by RT-PCR on international scale was recorded. Bone Marrow aspiration was performed to ascertain the diagnosis. All the enrolled patients were placed on Imatinib therapy (400 mg/day) and RT-PCR for BCR ABL1 transcript was repeated after three months. Data were analyzed using SPSS version 23. Quantitative variables like age, baseline WBC count, spleen size, RT-PCR values for BCR-ABL1 at baseline and at 3 months of Imatinib therapy were measured as mean ± SD. Frequencies and percentages were calculated for qualitative variables like gender and EMR at 3 months of

Table-I: Baseline characteristics of patients.

Variables	Value
Age years; median (range)	48 (21–74)
Gender, male : female	2.12 : 1
Total leukocyte counts, 109/L; median (range)	124 (26–570)
Hemoglobin g/dl; median (range)	10.8 (6.5–15.1)
Platelets, 109/L; median (range)	297 (25–1458)
Basophils % in peripheral blood; median (range)	4 (1–16)
Blasts % (Peripheral blood); median (range)	2 (0-6)
Blasts % (Bone Marrow); median (range)	3 (1-9)
Spleen cm; median (range)	9 (5-22)

Imatinib therapy. Linear regression analysis was performed to calculate the best cut-off values for baseline spleen size and WBC in predicting EMR. Effect modifiers like age and gender were controlled by stratification. A *p*-value <0.05 was considered as significant.

RESULTS

Seventy eight patients of CML (chronic phase) were identified and enrolled after meeting eligibility criteria. Median age was 48 years (range 21-71) and 53 (67.8%) of the study population were male, while 25 (32.2%) were female. Male to female ratio was 2.12 to 1. Baseline characteristics of our study population are shown in table-I. Early molecular response (EMR) to Imatinib was assessed by measuring BCR-ABL1 transcript at baseline and 3 months through real time PCR on international scale (IS). WBC count at the time of dignosis is shown in fig-1. There was a significant percentage decline in PCR for BCR-ABL1 on IS as 47 (60.15%) patients achieving EMR at 3 months after imatanib therapy (*p*-value <0.001) as seen in fig-2. In univariate analysis, there was significant association of spleen size (*p*<0.001), baseline WBC count (*p*<0.001), percentages of blasts in bone marrow and peripheral blood (*p*<0.001), percentage of basophils in peripheral blood (*p*=0.007) with BCR ABL1 (IS) at 3 months as seen in table-II. A multivariate regression model was followed for investigating association of all these covariates with BCR ABL1 (IS) at 3 months in which significant association was found only with spleen size (*p*-value <0.001) as seen in table-III.

DISCUSSION

Treatment of CML changed significantly over the past years, especially after the introduction of tyrosine kinase inhibitors with first generation Imatinib, second generation Nilotinib, Bosutinib and third generation drugs such as ponatinib. Treatment with TKI have markedly improved survival in CML patients. With the introduction of second and third generation TKI, early identification of patients who are likely to respond poorly to first line TKI

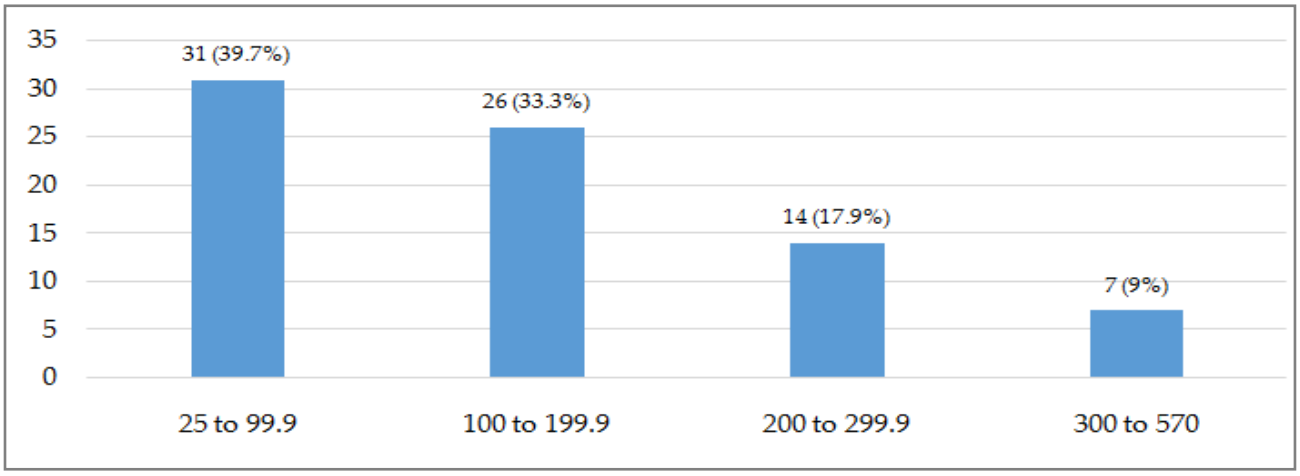

Figure-I: WBC count at diagnosis.

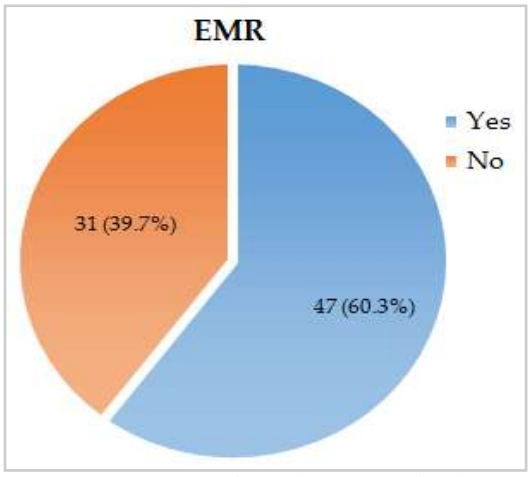

Figure-II: Number of patients achieving early molecular response.

therapy is important. These patients can be shifted to second generation TKI or alternative therapies like allogenic stem cell transplant. It is commonly observed that early molecular response (EMR) predicts overall survival and progression free survival[10]. A recent study by Marin *et al* concluded that assessment of BCR ABL1 transcripts at 3 months is the most important factor in identifying patients at high risk of progression[10]. Those patients who did not achieve early molecular response at three months were less likely to achieve molecular and cyto-genetic targets at 6 and 12 months and therefore have decreased overall survival and progression free survival[10]. Our study showed that, 60.15% of patients of CML in chronic phase being treated with Imatinib 400 mg/day achieved early molecular response (EMR). In multivariate analysis significant association was found only with spleen size (*p*-value <0.001). International studies show a variety of results ranging from 41-72%[10,11]. This difference could be due to several factors. Our study was done on south Asian population, which is known to have a different epidemiology in regards to CML, with a younger median age[12], racial differences and specific polymorphism that affects sensitivity to TKI therapy[13]. Secondly majority of our patient population has a low literacy rate and poor access to health care. This leads to poor compliance and adherence to treatment. Non-compliance and lack of adherence is a common problem in patients suffering from chronic diseases[14]. As reported by Marin *et al* various factors affect adherence in chronic myeloid leukemia patients, including lower age group, adverse effects of TKI therapy, psychological differences among patients and perception about disease[15]. Early molecular response may also predict the sensitivity of leukemic cells to Imatinib. It is known that BCR ABL protein may have anti apoptotic properties[16]. Treatment with TKI may induce apoptosis in philadelphia positive cells and in theory can remove all these cells and restore normal function[16]. As the disease evolves, genetic mutations in addition to BCR ABL are produced

Table-II: Regression analysis done to study association of independent variables with BCR-Abl at 3 months in response to Imatinib therapy

Independent Variables	Univariate Linear Regression (ULR)	Multivariate Linear Regression (MLR)
	p-Value	*p*-Value
TLC at diagnosis	<0.001	0.731
Spleen Size	<0.001	<0.001
Basophils in peripheral blood	<0.008	0.556
Blasts % in bone marrow	<0.001	0.450
Blasts % in peripheral blood	<0.001	0.127

Dependent variable (BCR Abl at 3 months) to see response to Imatinib therapy

Table-III: Multivariate Data Analysis to study association of independent variables with BCR-Abl at 3 months in response to Imatinib therapy

Explanatory Variables	Unstandardized Coefficients		Standardized Coefficients	*p*-value	95.0% Confidence Interval for B	
	B	Std Error	Beta		Lower Bound	Upper Bound
(Constant)	-24.878	3.602		<0.001	-32.058	-17.698
TLC count at diagnosis	-0.005	0.014	-0.026	0.731	-0.032	0.023
Spleen BCM	3.106	0.328	0.734	<0.001	2.451	3.761
Basophils in peripheral blood	0.288	0.487	0.041	0.556	-0.682	1.258
Blasts % in bone marrow	0.662	0.872	0.063	0.450	-1.076	2.400
Blasts % in peripheral blood	2.063	1.336	0.140	0.127	-0.600	4.725

Dependent Variable: BCR Abl at 3 months

in leukemic cells, thus diminishing the effect of Imatinib[17].Various mutations including hOCT1 (human organic cation transporter-1), MDR1 (multi drug resistance gene-1), ABCB1, ABCG2 alter the intracellular concentration of TKI. Various mutations in ABL1 kinase domain like T315I and others are implicated in resistance to TKI therapy and thereby poor response to treatment[17]. It is therefore implied that rapid fall of BCR ABL1 protein (i.e early molecular response) can identify patients who do not have additional genetic mutations and have a better survival in comparison to patients failing to achieve EMR. These patients may benefit from alternative therapies e.g. 2nd or 3rd generation TKIs or stem cell transplant. Median spleen size below left costal margin was 9 cm in our study. While international studies have varied results ranging from 3.4 cm to 10 cm. This could be due to the differences in characteristics of study population. Hasford *et al* (1998) study was conducted on German population had a median spleen size of 3.4cm, while Kuntegowdanahalli *et al* (2016) study was conducted on South Asian population had a median spleen size of 10cm[9]. Manual palpation and measurement of spleen size is not standardized as compared to other methods such as ultra sound or CT scan but is convenient and easy in the clinic setting. Splenomegaly may predict the extent of extra medullary hematopoiesis and so is enlarged in stem cell disorders with ineffective bone marrow function. In normal population hematopoiesis occurs predominantly in the bone marrow, where stem cells divide under environmental and cellular control[18]. When philadelphia (Ph) positive stem cells circulate in the blood and establish themselves in the spleen, they escape from the stricter environment and cellular control that was present in the bone marrow. This leads to genetic instability, defective differentiation and clonal evolution[19]. It was reported that splenic Ph positive stem cells were different from marrow Ph positive cells in regards to cytogenetic properties and cell kinetics[19]. While assessing the correlation of baseline WBC count with EMR, our results showed significance in univariate analysis (p<0.001) but on multivariate analysis it did not show any significance (p=0.731). These results are similar to a study conducted by J. Hasford *et al* (2011)[20]. In this prospective study, data from 2060 patients was used to evaluate various factors associated with response to imatinib therapy. End point was cytogenetic response at 18 months. They concluded that only spleen size and peripheral basophil count were significantly associated with response to Imatinib therapy. In our study peripheral basophil count was found to be significantly associated with achieving EMR in univariate analysis (p<0.008) while on multivariate analysis it did not show any significance (p=0.556). These differences could be because our study population was South Asian-Pakistani, which has different epidemiology as regards to CML. Secondly our study was limited by our small sample size. According to ENESTnd study, a phase 3, multicenter trial second generation TKI, Nilotinib has shown superior response in achieving EMR as compared to Imatinib[15]. In this study EMR was achieved in 91% of patients with Nilotinib, while 67% patients achieved EMR with Imatinib therapy. Patients who failed to achieve EMR, had larger median spleen size and higher WBC counts. Second generation TKI are now recommended as first line therapy, especially in patients with high risk features such as larger spleen sizes and higher WBC count. This study was unique as it was carried out in Pakistani-South Asian population, which is known to have a different epidemiology in regards to CML and literature review did not show a similar study carried out in this population. Our study was limited by the small sample size. We predominantly had male population in our study as majority of patients entitled in our institution are from military background. It is suggested that in future, larger multicenter studies should be carried out to evaluate impact of first and second generation TKI's including Imatinib, Nilotinib and Bosutinib on CML patients and their impact on remission and over all survival.

CONCLUSION

EMR was achieved in more than half of patients with CML in chronic phase, being treated with Imatinib at 3 months. Spleen size at diagnosis was the only significant factor associated with achieving EMR.

RECOMMENDATION

It is essential to identify patients at an early stage who are unlikely to achieve early molecular response and therefore have poor over-all survival. These patients can be considered for second or third generation TKI therapy or stem cell transplant.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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