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BCR-ABL/ABL1 Ratio is Significantly Reduced in CML Patients Treated with Imatinib: A Follow-up Study

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ABSTRACT

Introduction: Previous studies have emphasized the importance of monitoring BCR-ABL/ABL1 ratio by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for follow-up during therapy.
Aims: This study aimed to study the effect of imatinib therapy on BCR-ABL/ABL1 ratio in newly diagnosed CML patients in north Indian population.
Methodology: After baseline investigations, patients were started on imatinib therapy and follow up was done for upto one year. Hematological response was assessed at regular intervals. To assess molecular response, Quantitative Real-Time PCR was used to quantitate BCR-ABL transcripts for the 30 CML cases, using ABL1 as control gene. Both pretreatment as well as post treatment BCR-ABL/ABL1 ratios were calculated for the 30 CML cases.
Results: The BCR-ABL/ABL1 ratio was measured at baseline, 6 months and at 12 months as depicted in Table 2. It was seen that BCR-ABL1/ABL1 ratio was significantly reduced at 6 months from baseline (p=0.027) Similar findings were seen at 12 months of therapy whereby BCR-ABL1/ABL1 ratio was significantly reduced as compared to baseline. (p=<0.001)
Conclusion: It can be concluded that Imatinib is effective in reducing BCR-ABL/ABL1 ratio in Indian population after six and twelve months of therapy.

Keywords: CML, Imatinib, BCR-ABL/ABL1 ratio

INTRODUCTION

Imatinib mesylate (IM), the first *BCR-ABL1* tyrosine kinase inhibitor (TKI), is a first-line therapy for chronic phase (CP) chronic myeloid leukemia (CML), used commonly in routine practice[1] However, some patients fail to respond durably to TKIs. Previous studies have emphasized the importance of monitoring BCR-ABL/ABL1 ratio by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for follow-up during therapy.[2] This study aimed to study the effect of imatinib therapy on BCR-ABL/ABL1 ratio in newly diagnosed CML patients in north Indian population.

AIM:

To study the effect of Imatinib therapy on BCR-ABL/ABL1 ratio in newly diagnosed CML patients.

METHODOLOGY

Thirty Chronic myeloid leukemia patients who attended Medicine OPD or were admitted in Medicine ward, Lok Nayak Hospital, New Delhi were included in the study. All the thirty cases were in chronic phase (CP-CML). After baseline investigations, patients were started on imatinib therapy and follow up was done for upto one year. Hematological response was assessed at regular intervals. To assess molecular response, Quantitative Real-Time PCR was used to quantitate BCR-ABL transcripts for the 30 CML cases, using

ABL1 as control gene. Both pretreatment as well as post treatment BCR-ABL/ABL1 ratios were calculated for the 30 CML cases. *Ipsogen* BCR-ABL1 Mbcr kit (from QIAGEN, Netherlands,) was used for this purpose and protocol followed was as per the manufacturer's instructions. Statistical analysis was conducted using Microsoft Excel 2016 program. Data was presented as, number and median . Mann-U Whitney test was used to test any significant difference between the groups. $p< 0.05$ was considered to be significant.

Quantitative REAL-TIME PCR for BCR-ABL1 fusion gene

A peripheral blood sample was collected at follow-up either at 6 months or at 12 months after initiation of imatinib therapy to assess the molecular response. Molecular response was assessed by calculating BCR-ABL1/ABL1 ratio. This was done by quantification of BCR-ABL1 p210b2a2 or b3a2 transcripts. *Ipsogen* BCR-ABL1 Mbcr kit (from QIAGEN, Netherlands,) was used for this purpose and protocol followed was as per the manufacturer's instructions as described below.

PRINCIPLE:

Total RNA is reverse-transcribed and the generated cDNA is amplified by PCR using a pair of specific primers and a specific internal double-dye probe (FAM-TAMRA).the probe binds to the amplicon during each annealing step of the PCR. When the Taq DNA polymerase extends the DNA segment from the primer bound to the amplicon, it displaces the 5' end of the probe, which is then degraded by the 5'→3' exonuclease activity of the Taq DNA polymerase. Cleavage continues until the remaining probe melts off the amplicon. This process releases the fluorophore and quencher into solution, spatially separating them and leading to an increase in fluorescence from the FAM and a decrease in fluorescence from the TAMRA.

TABLE 1: PCR Cocktail preparation(1X) for quantitative RT-PCR for BCR-ABL1 fusion gene

COMPONENT	VOLUME (µL)
TaqMan Universal PCR master mix	12.5µL
Primers and probe mix	1µL
Nuclease-free PCR grade water	6.5µL
Sample (cDNA ,100ng RNA equivalent)	5µL
Total volume	25µL

TABLE 2 : PCR conditions for quantitative RT-PCR for BCR-ABL1 fusion gene

Mode of analysis	Quantitation
Hold	Temperature:50 °C Time: 2 mins
Hold 2	Temperature : 95 °C Time : 10 mins
Cycling	50 times 95 °C for 15 secs 60 °C for 1 min with acquisition of FAM fluorescence in channel Green: single
Extension	Temperature :72 °C Time: 10 mins

Interpretation of results:

Data analysis principle:

Using TaqMan technology, the number of PCR cycles necessary to detect a signal above the threshold is called the threshold cycle (Ct) and is inversely proportional to the amount of target present at the beginning of the reaction.

Using standards with a known number of molecules, a standard curve is established to determine the precise amount of target present in the test sample. The *ipsogen* standard curves used 3 plasmid standard dilutions for the ABL1Control Gene and 5 standard dilutions for the BCR-ABL1Fusion Gene, in order to ensure accurate standard curves.

Standard curve and quality criteria:

For each gene (ABL1 and BCR-ABL1), raw Ct values obtained from plasmid standard dilutions were plotted according to the log copy number (3,4 and 5 for C1,C2 C3 ; and 1,2,3,4,5 for F1, F2, F3, F4, F5).

Normalized copy number (NCN)

The ABL1 standard curve equation was used to transform raw Ct values for the

unknown samples into ABL1 copy numbers ($ABL1_{CN}$)

The BCR-ABL1 standard curve equation was used to transform raw Ct values for the unknown samples into BCR-ABL1 copy numbers ($BCR\text{-}ABL1Mbcr_{CN}$)

Ratio of these CN values gives the normalized copy number (NCN):

$NCN = [\ BCR\text{-}ABL1Mbcr_{CN} / ABL1_{CN}\ ] * 100$

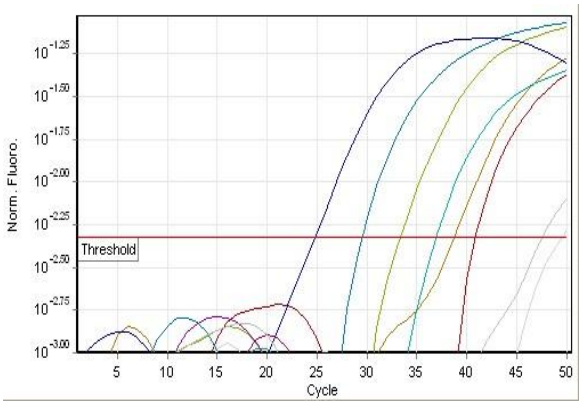

Figure 1: Standard amplification curve for BCR/ABL.

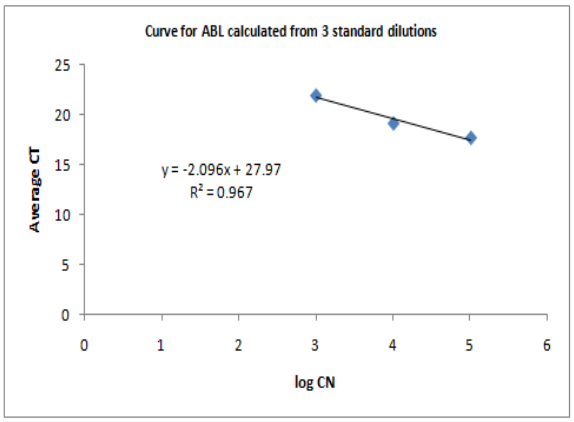

Figure 2: Standard curve for ABL calculated from 3 standard dilutions

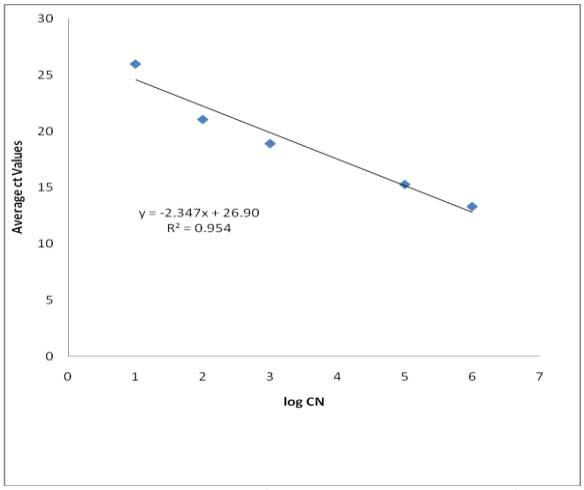

Figure 3: Standard curve for BCR-ABL calculated from 5 standard dilutions.

RESULTS

The age of the cases ranged from 18 to 80 years and the mean age for cases was 39.70 ± 18.04 years. The BCR-ABL/ABL1 ratio was measured at baseline, 6 months and at 12 months as depicted in Table 3. It was seen that BCR-ABL1/ABL1 ratio was significantly reduced at 6 months from baseline (p=0.027) Similar findings was seen at 12 months of therapy whereby BCR-ABL1/ABL1 ratio was significantly reduced as compared to baseline. (p=<0.001)

TABLE 3: BCR-ABL1/ABL1 ratios at baseline and follow up:

	At baseline(a)	After six months of imatinib therapy(b)	After 12 months of imatinib therapy(c)	p-value (P_1) (a& b compared)	p-value (P_2 (a& c compared)
No.of patients (n)	30	16*	9*	p=0.027	p=<0.001
Range of BCR-ABL/ABL1 ratio	27.238-5583.095	0.001-200.450	0.003-153.457		
Median BCR-ABL/ABL1 ratio	371.83	1.714	0.072		

DISCUSSION

The treatment of chronic myeloid leukaemia (CML) has been improved tremendously by Imatinib, an inhibitor of tyrosine kinase causal to CML.[4,5] Imatinib is considered for first-line treatment of CP-CML by the National Comprehensive Cancer Network and the European LeukemiaNet (ELN).[6] The therapeutic options for newly diagnosed CML patients continue to evolve with new drugs such as nilotinib and dasatinib being approved for

frontline therapy.[7,8] In the present study, it was attempted to study the molecular response to imatinib by monitoring BCR-ABL/ABL1 ratio at baseline and at follow up. It was seen that BCR-ABL/ABL1 ratio were significantly reduced, suggesting that imatinib is effective in eliciting a response. However, the sample is too less (only 30 cases) and thus results need to be authenticated in a larger sample size for a longer duration of follow-up. This is because patients who initially show a response might lose it and develop resistance.[9,10] However, it can be conclusive to say that imatinib is effective in short-term for majority of the patients.

CONCLUSION

It can be concluded that Imatinib is effective in reducing BCR-ABL/ABL1 ratio in Indian population after six and twelve months of therapy. However, results need to be confirmed on a larger sample of patients also taking account the Imatinib failure cases. It can also be proposed that QRT-PCR for measuring BCR-ABL levels in peripheral blood can be used as a method for monitoring patients on Imatinib therapy.

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