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Original Article

Monitoring of Disease Activity in Chronic Myeloid Leukemia-chronic Phase Patients Treated with Indian Generic Veenat (NATCO) Imatinib Mesylate: A Tertiary Care Experience

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Aim: Real-time quantitative polymerase chain reaction (RT-PCR) is a sensitive technique to monitor treatment response in chronic myeloid leukemia (CML). Standardization of RT-PCR protocols and interpretation of results are important to maintain the reliability of this molecular test.

Methods: We present the analysis of treatment monitoring after administration of Indian generic imatinib mesylate (IM) (Veenat) in 73 patients with CML-chronic phase using RT-PCR.

Results: A consistent decrease in the median breakpoint cluster region-abelson1 (BCR-ABL1) percentage denoting a continued time-bound response to IM is observed. At 6 months, 87.5% patients showing ≥ 1-log reduction in BCR-ABL1 levels subsequently developed ≥ 3-log reduction as compared to 42.8% patients showing < 1-log reduction. All patients with ≥ 3-log reduction in BCR-ABL1 at 12 months maintained treatment response at 18 months.

Conclusion: The efficacy of Veenat (NATCO) is comparable to Gleevac (Novartis). Patients who attain ≥ 1-log reduction at 6 months and ≥ 2-log reduction at 12 months are more likely to subsequently attain a major molecular response.

Key words: BCR-ABL1, chronic myeloid leukemia, imatinib mesylate, log reduction, real-time quantitative polymerase chain reaction

INTRODUCTION

Chronic myeloid leukemia (CML) is characterized by t (9;22) (q34.1; q11.2) that was first demonstrated in 1960.[1,2] Philadelphia chromosome quantification using cytogenetics was used for monitoring of CML treatment for almost three decades. It has been estimated that 10^7 leukemic cells may still present in a CML patient in complete cytogenetic remission.[1] Identification and measurement of breakpoint cluster region-abelson1 (BCR-ABL1) transcripts by real-time quantitative polymerase chain reaction (RT-PCR) have enabled a better assessment of treatment response. The past two decades have witnessed an increased effort in the standardization of protocols, primers, plasmids, and interpretation of results. This is important in maintaining the reliability of molecular tests used in CML. Imatinib mesylate (IM) was the first clinically available tyrosine kinase inhibitor and has revolutionized the treatment of CML. To achieve better treatment outcomes, serial monitoring of individual patients to predict disease course has become mandatory. Recent recommendations to define response to treatment in CML patients have been made by the European LeukemiaNet (ELN) in 2013 and the National Comprehensive Cancer Network (NCCN) in 2014.[3,4] This study was aimed to standardize the RT-PCR technique for BCR-ABL1 detection in our laboratory and to monitor the response to IM in CML-chronic phase (CP) patients. To the best of our knowledge, this is the first comprehensive report from India on this technique.

METHODS

The study was conducted at the Army Hospital Research and Referral, a Tertiary Care Centre in Delhi, India. Seventy-three cases of CML-CP diagnosed on clinical and hematological grounds (including bone marrow aspirate and biopsy) between October 2010 and October 2011 were included in the study. All CML patients diagnosed as accelerated phase/blast crisis or CML-CP patients already on treatment were excluded from the study. Pretreatment BCR-ABL1 transcript levels were measured by RT-PCR, and all these patients were started on Indian generic IM Veenat (Natco Pharma Ltd, Oddway International) 400 mg once daily. BCR-ABL1 transcript levels were measured at 3, 6, 9, 12 and 18 months after initiation of IM therapy. To measure BCR-ABL1 transcript levels at each time point, 3 mL of peripheral blood was collected in ethylenediaminetetraacetic acid, and RNA

extraction was performed on the same day using QIAamp RNA Blood Mini-kit (QIAGEN) following stringent measures to avoid contamination. cDNA was prepared from extracted RNA by high-capacity cDNA reverse transcription kits. Quantitative detection of BCR-ABL1 M-bcr p210 b2a2 or b3a2 fusion gene transcripts was performed using BCR-ABL M-bcr Fusion Quant kit (Ipsogen cancer profiler) and Rotor Gene 3000. This assay exploits the RT-PCR double dye (FAM-TAMRA) oligonucleotide hydrolysis principle. Ready-to-use primer and probe mixes containing a mixture of specific reverse and forward primers for the ABL control gene and Mbcr fusion gene plus a specific FAM-TAMRA probe, supplied within the BCR-ABL M-bcr Fusion Quant kit were used. ABL was used as the control gene in three standard dilutions (10^3, 10^4, 10^5 copies/5 μL) and water was used as negative control. All test and control sample measurements were performed in duplicate. Standard curves were established using standard dilutions of ABL and BCR-ABL1 MBcr (10^1, 10^2, 10^3, 10^5, 10^6 copies/5 μL). The results were expressed as a percentage calculated from the ratio of BCR-ABL copy numbers to ABL copy numbers. The results were reported only if the ABL copy numbers were ≥ 10,000, and for ABL copy numbers < 10,000, a repeat test was advised.

The baseline BCR-ABL: ABL percentage of our laboratory was established by calculating the median of first 30 cases of newly diagnosed CML-CP patients. The BCR-ABL: ABL percentage of our laboratory was calculated to be 60. Therefore, a value of 0.06 represented a 3-log reduction from the standardized baseline value. A ≥ 3-log reduction in BCR-ABL1 transcript levels was defined as a major molecular response (MMR).

We divided our patients into two groups depending on whether their response to treatment was optimal or suboptimal at each time point (3, 6, and 12 months) according to both ELN and NCCN criteria. At 3 months, a cut-off of BCR-ABL1 ≤ 10% and > 10% was used to define optimal and suboptimal response, respectively (as per both ELN and NCCN criteria), and the two groups were compared with respect to subsequent development of ≥ 3-log reduction. At 6 months, criteria of BCR-ABL1 < 1% or ≥ 1% (as per ELN criteria) and BCR-ABL1 ≤ 10% or > 10% (as per NCCN and ELN warning criteria) were used to monitor the response to therapy. At 12 months, a criteria of BCR-ABL1 ≤ 0.1% or > 0.1% (as per ELN criteria) and BCR-ABL1 ≤ 1% or > 1% were used.

Statistical analysis was done using SPSS software (Version 20, SPSS Inc., Chicago, IL, USA) and the Chi-square test with a Yates correction was applied to obtain *P* values.

RESULTS

In the 73 patients included in this study, the percentage of BCR-ABL1 at baseline varied from 1.38% to 121%. The median and range of percentages of BCR-ABL1 at 3, 6, 9, 12, and 18 months were 15.74 (0–99.34), 2.19 (0–64.28), 0.52 (0–93.21), 0.24 (0–67.67), and 0.02 (0–2.5) [Figure 1]. The BCR-ABL1 percentage values were available for 33, 42, 8, 51, and 43 patients at a follow-up period of 3, 6, 9, 12, and 18 months, respectively. The rates of MMR at 3, 12, and 18 months were 3%, 20%, and 30%, respectively. Undetectable BCR-ABL1 levels were achieved in 3%, 4.76%, 25%, 24%, and

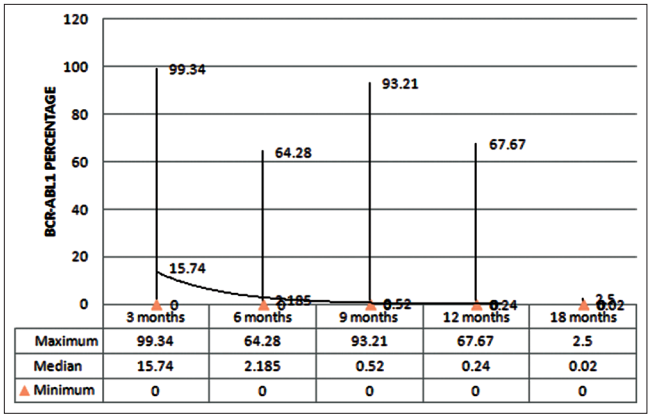

Figure 1. Maximum, minimum, and median BCR-ABL1 values at baseline

44.1% at 3, 6, 9, 12, and 18 months, respectively [Table 1]. These data were comparable with previous studies that used Gleevac (Novartis Pharmaceuticals Corporation, East Hanover, New Jersey), implying comparable efficacy of Veenat (NATCO) and Gleevac. Since follow-up data for all patients at all time points was not available, the proportion of patients who achieved MMR/CMR at any time point and subsequently maintained it was calculated. Of the 73 patients included in the study, 15 patients (20.5%) and 20 patients (27.4%) eventually achieved MMR and undetectable BCR-ABL1 levels, respectively. Overall, 18 patients (24.6%) showed increasing levels of BCR-ABL1 transcripts. A single patient achieved MMR at 3 months but showed relapse at 6 months. This patient tested positive for a BCR-ABL1 kinase domain mutation by direct sequencing.

No statistically significant relationship between the attainment of ≥ 1-log reduction response at 3 months and MMR was found (χ^2 = 2.09, P > 0.05) [Table 2]. A total of 87.5% (21/24) patients showed ≥ 1-log reduction in BCR-ABL1 percentage at 6 months and MMR as compared to 42.8% (3/7) patients showed < 1-log reduction at 6 months (χ^2 = 3.88, P < 0.05) [Table 3]. No statistically significant relationship between the attainment of ≥ 2-log reduction at 6 months and MMR was found (χ^2 = 2.6, P > 0.05) [Table 4]. None of the patients showing < 2-log reduction treatment response at 12 months subsequently developed an MMR as compared to 96.8% (31/32) patients with ≥ 2-log reduction (χ^2 = 32.14, P < 0.001) [Table 5]. All patients (18/18) with ≥ 3-log reduction in BCR-ABL1 percentage at 12 months maintained treatment response at 18 months. This is in comparison to 54.1% (13/24) patients with < 3-log reduction at 12 months who developed MMR at 18 months (χ^2 = 8.9, P < 0.01) [Table 6].

DISCUSSION

IM has revolutionized the treatment of CML since it was approved for use as first-line therapy in 2001. Its efficacy in the management of CML especially in patients in the CP of the disease and its superiority over other therapeutic options has been demonstrated in various trials.[5-9] All these studies used the IM (Gleevac) marketed by Novartis, the cost of which becomes a limiting factor in developing countries. We used the Indian generic IM (Veenat) marketed by NATCO in the present study and demonstrated that its efficacy is comparable to Gleevac.

Table 1: Treatment response (BCR-ABL1 transcript levels) at 3, 6, 9, 12, and 18 months after initiation of imatinib mesylate

BCR-ABL1 levels	3 months (%)	6 months (%)	9 months (%)	12 months (%)	18 months (%)
Undetectable	1 (3.03)	2 (4.76)	2 (25)	12 (23.5)	19 (44.1)
≥ 3-log reduction	1 (3.03)	0	0	10 19.60)	13 (30.23)
≥ 2- < 3-log reduction	6 (18.18)	8 (19.04)	3 (37.5)	14 (27.45)	9 (20.93)
≥ 1- < 2-log reduction	4 (12.12)	16 (38.09)	2 (25)	12 (23.5)	2 (4.65)
< 1-log reduction	11 (33.33)	8 (19.04)	0	1 (1.96)	0
Stable/increasing	10 (30.30)	8 (19.04)	1 (12.5)	2 (3.92)	0
Total	33	42	8	51	43

Table 2: Response to imatinib mesylate 400 mg at 3 months ($\chi^2 = 2.09$; $P > 0.05$), and follow-up data for 10 patients who showed < 1-log reduction at 3 months were not available

Subsequent response	≥ 1-log reduction in BCR-ABL1 level at 3 months	< 1-log reduction in BCR-ABL1 level at 3 months	Total
≥ MMR	9	5	14
< MMR	3	6	9
Total	12	11	23

MMR: Major molecular response

Table 3: Response to imatinib mesylate 400 mg at 6 months ($\chi^2 = 3.88$; $P < 0.05$), and follow-up data for two patients who showed ≥ 1-log reduction and nine patients with < 1-log reduction at 6 months were not available

Subsequent response	≥ 1-log reduction in BCR-ABL1 level at 6 months	< 1-log reduction in BCR-ABL1 level at 6 months	Total
≥ MMR	21	3	24
< MMR	3	4	7
Total	24	7	31

MMR: Major molecular response

Table 4: Response to imatinib mesylate 400 mg at 6 months ($\chi^2 = 2.6$; $P > 0.05$), and follow-up data for 11 patients with < 2-log reduction at 6 months were not available

Subsequent response	≥ 2-log reduction in BCR-ABL1 level at 6 months	< 2-log reduction in BCR-ABL1 level at 6 months	Total
≥ MMR	10	14	24
< MMR	0	7	7
Total	10	21	31

MMR: Major molecular response

The improved therapeutic outcome using IM has encouraged the routine use of more sensitive molecular monitoring techniques. RT-PCR is one of the most sensitive techniques, and it provides an accurate assessment of the total leukemia cell mass. This can be achieved effectively by the standardization of the laboratory procedures and interpretation of results. The present study has been done after the standardization of the RT-PCR technique using best practice guidelines drawn up by Foroni *et al.*[1] We currently do not have collaboration with a reference laboratory

Table 5: Response to imatinib mesylate 400 mg at 12 months ($\chi^2 = 32.14$; $P < 0.001$), and follow-up data for four patients who showed ≥ 2-log reduction and five patients with < 2-log reduction at 12 months were not available

Subsequent response	≥ 2-log reduction in BCR-ABL1 level at 12 months	< 2-log reduction in BCR-ABL1 level at 12 months	Total
≥ MMR	31	0	31
< MMR	1	10	11
Total	32	10	42

MMR: Major molecular response

Table 6: Response to imatinib mesylate 400 mg at 12 months ($\chi^2 = 8.9$; $P < 0.01$), and follow-up data for four patients who showed ≥ 3-log reduction and five patients with < 3-log reduction at 12 months were not available

Subsequent response	≥ 3-log reduction in BCR-ABL1 level at 12 months	< 3-log reduction in BCR-ABL1 level at 12 months	Total
≥ MMR	18	13	31
< MMR	0	11	11
Total	18	24	42

MMR: Major molecular response

which is essential for conversion of BCR-ABL1 percentage to the international scale.

In this study, a consistent decrease was observed in the median BCR-ABL1 percentage which denotes a continuing time-bound response of CML-CP patients toward IM. Patient compliance to the therapy, therefore, has an important role in the overall treatment response. An MMR of 40% at 12 months was reported by the IRIS trial that is comparable to 43% (22/51) patients with ≥ 3-log reduction (including undetectable BCR-ABL1 levels) in this study. An Indian study reported a MMR of 30% at 12 months.[6] This discrepancy may be attributable to the fact that the CML patients in accelerated phase and blast crisis were included in the study by Doval *et al.*,[6] whereas only patients in CP were included in our study.

In previous studies, it has been reported that the 10% BCR-ABL1 transcript levels at 3 months predicted the rate of subsequent treatment response.[10-12] Of the 33 patients whose BCR-ABL1 percentages at 3 months were available, 10 patients who had 1-log reduction at 3 months were lost to follow-up in our study. This could possibly explain why we did not find any

statistically significant relationship between ≥ 1-log reduction in BCR-ABL1 at 3 months and subsequent treatment response.

Both ELN and NCCN recommend that BCR-ABL1 > 10% requires a change of treatment.[3,4,10] Our findings corroborate with these recommendations. A statistically significant relationship between the attainment of ≥ 1-log reduction at 6 months and subsequent MMR has been found ($P < 0.05$). None of the patients who achieved ≥ 2-log reduction at 6 months failed to subsequently achieve an MMR. At 12 months, ELN has defined a BCR-ABL1 < 0.1% as optimal response and patients with 0.1–1% levels should be monitored more frequently. In our study, encouraging results of 97% patients with ≥ 2-log reduction at 12 months, with an MMR were obtained. One-hundred percent of patients who achieved MMR at 12 months maintained treatment response at 18 months.

In conclusion, the efficacy of Indian generic Veenat (NATCO) is comparable to Gleevac (Novartis). A ≥ 3-log reduction in 43% patients at 12 months was obtained in this study. Patients who attain ≥ 1-log reduction at 6 months and ≥ 2-log reduction at 12 months are more likely to subsequently attain MMR.

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