Original Article

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Benefits of the early detection of M351T mutation, by allele-specific oligonucleotide polymerase chain reaction, in imatinib-resistant chronic myelogenous leukemia (CML) - A retrospective analysis

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ABSTRACT

Introduction: BCR-ABL kinase domain mutations represent the most important disease-related factor in chronic myelogenous leukemia (CML) resistance. Highly resistant clones may pre-exist and emerge rapidly. Patients with CML can acquire more than one BCR-ABL1 mutation, which may result in increased oncogenicity. **Materials and Methods:** Retrospective analysis of 50 patients of imatinib resistance was done in GCRI, from January 2014 to May 2014. Response to imatinib was defined according to the European LeukemiaNet 2009 criteria. Allele-specific oligonucleotide-polymerase chain reaction (ASO-PCR) was performed on genomic DNA, extracted from peripheral blood mononuclear cells. **Results**: Average age was 40.75 years, 33 were males and 17 females. 47 (94%) were in chronic phase, 2 (4%) in accelerated phase, and 1 (2%) in blastic crisis. 29/50 were having low EUTOS score, whereas SOKAL score was low in 20, intermediate in 21 while only 9 had high SOKAL at presentation. Median duration of imatinib was 48 months. 43/50 had one or more than 1 mutation, T315I mutation in 5 (10%) patients, and M351T in 32% (16/50). **Conclusion**: The presence of M351T mutation in mutant clone leads to the development of T315I mutations development, and the detection of M351T mutation in the initial months of the therapy has a prognostic significance. ASO-PCR is more sensitive method of the detection of such mutations as compared to direct sequencing. We report low cytogenetic response (25%) and durability of response to 600 mg of imatinib, even in M351T mutation, after 400 mg of imatinib for median period of 4 years.

Key words: BCR-ABL kinase domain mutation, Imatinib resistance, M351T mutation

Introduction

Chronic myeloid leukemia (CML) exemplifies importance of molecular pathways and their exact role in oncology. Imatinib mesylate first tyrosine kinase inhibitor (TKI) introduced into clinical practice has an ability to bind ATP-binding pockets of the ABL kinase domain (KD). This subsequently prevents the change in conformation of the protein to the active form of the molecule, leading to subsequent death of target cells.[1]

These domains are amenable to mutations, and such mutations can lead to imatinib resistance. Multiple clinical trials such resistances have been frequently encountered (approximately 50–60%)[2] of patients. Clinical resistance is the hallmark for checking any underlying mutations. This fact is enough to speculate that such mutant clones might be present even before the start of therapy and might be pre-existing.[3] Imatinib selects sensitive clones, and thus, resistant clones are spared, leading to the early clinical resistance to standard therapy. Patients with CML can acquire more than one BCR-ABL-1 mutation, which may result in increased oncogenicity compared to each individual mutation.[4] The presence of M351T mutation in mutant clone leads to the development of T315I mutations development. The detection of M351T mutation in the initial months of the therapy has a prognostic significance.[5] Allele-specific oligonucleotide-polymerase chain reaction (ASO-PCR) is more sensitive method of the detection of such mutations as compared to direct sequencing.

Materials and Methods

We performed this retrospective analysis of 50 CML patients with imatinib-resistant clones documented by mutational analysis. Hospital records of these patients were analyzed for clinical correlates such as median age, gender, phase of CML, and prognostic score, i.e., EUTOS and Sokal score, median time duration for resistance to treatment, response to imatinib (hematological, cytological, and molecular), and cumulative dose of imatinib given.

Genomic DNA was extracted from peripheral blood mononuclear cells using QIAamp DNA Mini Kit (Qiagen, according to the manufacturer's recommendations). Quantity was estimated using Qubit fluorometer version 2.1. Mutated or wild-type sequences were specifically amplified in a separate PCR reaction performed on DNA in 25 µL reaction mixture using PCR master mix (Fermentas, Thermo Scientific, according to manufacture instruction). Healthy volunteer was used as negative control.

Following PCR cycle was designed using allele-specific and reverse primers: For the Thr315Ile mutation, F315C (wild): 5 'GCC CCC GTT CTA TAT CAT CAC '3 or F315T (mutated): 5' CCC GTT CTA TAT CAT CAT '3 and Reverse: 5 'GGATGAAGT TTT TCT TCT CCAG '3, annealing at 64°C; 158-bp PCR product. For the Phe311Leu mutation, F311T (wild): 5' CAC CCG GGA GCC CCC GT '3 or F311C (Mutated): 5' CAC CCG GGA GCC CCC GC 3 and Reverse: 5'CCCCTACCTGGTGGATGAAGT'3 with annealing at 64.4°C; 174-bp PCR fragment. For the Met351Thr mutation, F351T (wild): 5'CCA CTC AGA TCT CGT CAG CCA T '3 or F351C (mutated): 5'CCACTC AGA TCT CGT CAG CCA C '3 and Reverse: 5'GCC CTG AGA CCT CCTAGG CT '3, annealing at 71.3°C; 112-bp PCR fragment.

Results

As per Table 1, of the 50 patients of imatinib-resistant cases, 33 were males and 17 were females. Average age of the study group was 40.75 years. Of the 50 selected, 47(94%) were in chronic phase, whereas 2 (4%) were in accelerated phase, 1 (2%) was in blastic crisis. 29 of 50 were having low EUTOS score, whereas SOKAL score was low in 20, intermediate in 21, while only 9 were having high SOKAL at presentation.

We treated every patient with imatinib 400 mg per day, and dose was escalated to 600 mg at the time of clinical evidence of imatinib resistance, 28% (14/50) of the patients did not achieve complete hematological response at the end of 3 months of standard dose of imatinib (i.e., 400 mg per day). 86% (43/50) of the study group patients failed to achieve complete cytogenetic response (CCyR) at the end of 1 year of imatinib-standard dose. Only 8% (4/50) of the patients in this study group achieved major molecular response (MMR) at the end of 18 months of standard-dose imatinib.

Cumulative imatinib dose was <400 mg per day in 11 (22%) patients, >600 mg per day in 1 patient, whereas it was 400–600 mg in 38 (76%) of the cases. 88% (n = 44) were having drug to drug compliance >85%.

Of the study population, 96% (43/50) showed either one or more than 1 mutation. T315I mutation was detected in 5 (10%) patients. 1 was having isolated T3151. M351T mutation was seen in 32 (64%) patients. Of which 32% (16/50) were having isolated M351T mutation.

Median duration of imatinib resistance and mutation detection was 36 months. Median duration of imatinib treatment in these patients before the detection of mutation was 48 months. Three patients stopped imatinib, because of pregnancy, and 1 patient was intolerant to imatinib.

Table 1: Clinical characteristics of patients with mutations

Patient characteristics	Total *n*=50 (%)	Mutations *n*=43 (%)
Average age	40.75 years	41.12 years
Male	33 (66)	28 (65.11)
Phase of CML: Chronic phase	47 (94)	41 (95.34)
Accelerated phase	2 (4)	1 (2.32)
Blastic phase	1 (2)	1 (2.32)
EUTOS score: High	21 (42)	19 (44.18)
Low	29 (58)	24 (55.81)
SOKAL score: High	8 (16)	7 (16.27)
Intermediate	21 (42)	17 (39.53)
Low	21 (42)	19 (44.18)
Response evaluation: 3-month CHR	36 (72)	29 (67.4)
12-month CCyR	7 (14)	5 (11.6)
18-month MMR	4 (8)	3 (6.97)
Major cytogenetic response	1 (2)	1 (2.32)
Minor cytogenetic response	1 (2)	-
Cumulative imatinib dose<400 mg/day	11 (22)	9 (20.93)
400–600 mg/day	38 (76)	33 (76.74)
>600 mg/day	1 (2)	1 (2.32)
Compliance: >or 85	44 (88)	38 (88.37)
=or<85	6 (12)	5 (11.6)
Median imatinib 400 mg (range)	48 months (6–90)	48 months (6–90)
Treatment before imatinib: Hydroxyurea	6 months	Average 6 months

CHR: Complete hematological response, CCyR: Complete cytogenetic response, MMR: Major molecular response

As per Table 2, response to 600 mg of imatinib in T315I mutation, none achieved CCyR (n = 5) and 2 lost the hematological response also. In isolated M315T mutation (n = 16), 4 (25%) of the patient achieved CCyR and 1 achieved CCyR after 1 year. About the molecular response, only 3 (18%) achieved it and 2 lost the MMR within 12 months. 1 lost MMR after 12 months of imatinib 600 mg.

Discussion

High incidence (32%) of M351T was detected in our study, as compared to data by another Indian study by Shrivastava and Dutt, they reported an incidence of M351T of 10% in their study.[6]

They studied imatinib resistance in chronic phase only and not in accelerated phase and blastic Crisis. Second, the method they used was bidirectional sequencing analysis of the amplicon, acquired from RNA of the sample containing adequate BCR-ABL was compared to human genome sequencing [Table 3]. However, sensitivity and reliability of mutation detection are critically dependent on the method employed, awe used ASO-PCR while they used bidirectional sequencing as the method of detection of mutation. Direct sequencing is not specifically designed to detect the mutation concerned.[5]

ASO-PCR is proved to be a very economical, sensitive, and rapid technique for the detection of KD mutations M351T, F317L, and F311C ABL mutation and is more sensitive than mutation detection by sequencing.[5] In a study done at AIIMS by Mir *et al*., 100 CML patients were screened for M351T mutation after 3 years of imatinib initiation. (40%) 40/100 were positive for M351T,[5] which is comparable to ours.

We encountered a very high level of mutations compared to small sample size, and it may be because our patients progressed well within natural history of CML. This fact is reflected by median months on imatinib, i.e., 48 (4 years). BCR-ABL KD mutations account for 50–90% of the imatinib resistance observed in patients of CML-CP.[7,8] In the pre-imatinib era, CML-CP used to be around 4–6 years, accelerated phase for 18 months, and blastic phase for 6 months.

10% of mutations detected are M351T, in the western world. M351T has a low-level imatinib sensitivity. It is thought to be associated with a loss of function and may be selected on drug exposure.[1] M351T mutation reduces BCR-ABL kinase activity and transforming capacity.[9] Various studies showed that at higher concentrations of imatinib, different KD, and mutations retain sensitivities.[10] Thus, high doses of imatinib may render resistant clones as sensitive. However, what is the dose of imatinib required to achieve this? in the study done by Branford *et al*., they found that 600 mg imatinib was not sufficient to be clinically beneficial.[11] Same is what we studied in our data, 600 mg imatinib was not sufficient as far as M351T mutations are concerned.

As per Table 2, response to 600 mg imatinib, in isolated M315T mutation (n = 16), 4 (25%) of the patient achieved CCyR, 1 achieved CCyR after 1 year. About the molecular response only 3 (18%) achieved it, 2 lost the MMR within 12 months. 1 lost MMR after 12 months of imatinib 600 mg. As compared to the 74 patients data quoted by Breccia *et al*. regarding response to high-dose imatinib, CCyR was achieved in 37% patients.[12]

Table 2: Response to high-dose imatinib (600 mg)

Response to 600 mg imatinib	T315I (n=1)	M351T n=16 (%)
Average age	37	43.12
Male	1	10 (62.5)
Phase: Chronic phase	1	16 (100)
Accelerated phase	0	0
Blastic phase	0	0
EUTOS score: High	1	7 (43.75)
Low	0	9 (56.25)
SOKAL score: High	0	1 (6)
Intermediate	0	8 (50)
Low	1	7 (43.75)
Response evaluation CHR	No	12 (75)
CCyR	No	4 (25)
MMR	No	3 (18)
Major cytogenetic response	No	1 (6)
Minor cytogenetic response	No	-
Compliance: >or 85	1	14 (88)
=0<85	0	2 (12)
Median imatinib (math)	48	48

CHR: Complete hematological response, CCyR: Complete cytogenetic response, MMR: Major molecular response

Table 3: Various technologies available for identifying and quantifying BCR-ABL KD mutations[6]

Technology	Sensitivity, %	Specificity	Bias*	Availability
Direct sequencing	15–25	++	No	+++
Subcloning and sequencing	9	+++	No	++
D-HPLC	0.1–10	++	No	++
Pyrosequencing	5	++	No	+
Double-gradient denaturing electrophoresis	5	++	No	+
Fluorescence PCR and PNA clamping	0.2	++	Yes	+
Allele-specific oligonucleotide PCR (ASO-PCR)	0.01	++	Yes	+

PNA indicates peptide nucleic acid. *Bias indicates that the test is designed to detect specific mutations. ASO-PCR: Allele-specific oligonucleotide-polymerase chain reaction, D-HPLC: Denaturing high-performance liquid chromatography

The reason for the low response in our study was low dose of imatinib offered for a relatively prolonged period of time. 400 mg of imatinib was given for a median duration of 48 months. The other reason was possible emergence of clonal evolution which was not done in our study. Third, compliance was <85% in 12% of the cases, leading to low imatinib trough level might be the other reason for low response to high dose of imatinib in our patient. Thirdly selection bias also can be the other reason, as we retrospectively selected the resistant patient and retrospectively analyzed the response to both the standard dose as well as 600 mg dose of imatinib. Further analysis, in accordance to Marin *et al*.,[13] we found the response to 600 mg of imatinib was not durable. All of the 32 patients with M351T mutation treated with 600 mg Imatinib progressed within median of 21 months (range 8-30 months) of therapy, lading to escalation of imatinib.

M351I can be detected using more sensitive ASO-PCR method. M351T mutation precedes the emergence of T315I. If we detect M351I earlier, we may prevent the emergence of T315I.

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