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BCR-ABL kinase domain mutations in CML patients, experience from a tertiary care center in North India

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ABSTRACT

Background: Chronic Myeloid Leukemia is characterized by the presence of the Philadelphia Chromosome (Ph) which contains the *BCR::ABL1* fusion gene that occurs due to a reciprocal translocation between chromosomes 9 and 22. This accounts for up to 15 % of all adult leukemias [1]. Most patients treated with first line tyrosine kinase inhibitor (TKI) imatinib achieve durable response but may undergo relapse at some stage [2]. The most important mechanism that may confer imatinib resistance is point mutation within *BCR::ABL* kinase domain. Other generation *ABL* tyrosine kinase inhibitors such as dasatinib, nilotinib, bosutinib and ponatinib help to overcome imatinib resistance [3]. Sensitivity of the patient to each of the above TKIs depends upon the individual candidate mutation present. Thus, it is important to perform mutation analysis for effective therapeutic management of CML patients once they show imatinib resistance. We used direct sequencing to identify the different types of mutations responsible for resistance of imatinib treatment from north India.
Methods: In this study, the patient resistance for the imatinib were analyzed for *BCR::ABL* kinase domain mutation by direct sequencing and the detected mutations along with their percentage prevalence were reported.
Results: 329 patients with CML-CP were analyzed for *BCR::ABL* kinase domain mutation. Total 66 (20.06 %) patients out of 329 had mutation in at least one of the domains of *BCR::ABL* conferring resistance to different generations of TKI. Mutations in *BCR::ABL* kinase domain was observed in different domain of *BCR::ABL*. ATP binding P-Loop (42.42 %), Direct binding site (36.36 %), C-Loop (10.60 %), A-Loop (6.06 %), SH2 contact (3.03 %), SH3 contact (1.51 %).
Conclusion: Total 20.06 % patients (66/329) show mutation in at least one of the structural motifs of BCR-ABL kinase domain, which further confer the resistance to a particular generation of TKI.

1. Introduction

Chronic myelogenous leukemia (CML) constitutes about 15 % of adult leukemias and annually affects 1–2 people per 100,000 and is characterized by the presence of the Philadelphia chromosome (Ph+), which contains the oncogenic *BCR::ABL1* fusion gene [1] .The chimeric protein carries a constitutive tyrosine kinase activity with associated activation of downstream mitogenic, proliferative, and anti-apoptotic pathways [1]Imatinib mesylate, used as frontline therapy for chronic myeloid leukemia (CML), is a selective inhibitor of tyrosine kinase (TKI) that binds competitively to the adenosine triphosphate (ATP) docking site of tyrosine kinase proteins, including ABL itself and the hybrid *BCR::ABL* protein [4]However, TKI resistance occurs in 20–30 % of CML patients [1]Imatinib resistance can be divided into primary resistance and secondary resistance based on therapy response. Primary resistance is defined as the inability to achieve a landmark response by a defined time point, while secondary (or acquired) resistance requires the loss of an established response.

Approximately 60–80 % of the resistance in patients is generated due to point mutations in the *BCR::ABL* gene [5]. The main regions where mutations occur and lead to drug resistance are P loop, A Loop, C Loop and the direct binding site [6]. The P Loop refers to the phosphate-binding loop. When imatinib binds, this loop undergoes a change in its conformation to allow a better fit for the drug so that it may

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easily associate with tyrosine 253 [Y353] with a hydrogen bond. Point mutation at Y253 will interfere with this binding [7]. The A Loop refers to the Activation loop. It closes the kinase active site making *ABL* inactive and thereby allowing the drug to bind. Thus, mutations in this loop will hamper with *ABL* specificity and promote drug resistance. C Loop refers to the catalytic loop. M351T mutation occurs in this loop and causes change in the conformation of the *ABL*. The direct binding site refers to T315 which is responsible for formation of Hydrogen bond between the drug and *ABL*. A point mutation of threonine to isoleucine results in allosteric effects which does not allow the drug to bind with *ABL* region and thus produces the strongest resistance to the TKIs [6]. Mutations are also known to occur in SH2 and SH3 contact domains which denote the contact area for SH2 and SH3 domain containing proteins [8].

Imatinib is a potent, highly selective *BCR::ABL* kinase inhibitor approved for adults with newly diagnosed CML in chronic phase (CML-CP). *BCR::ABL* mutations can be a major cause of imatinib resistance once patient starts showing resistance to this drug. *BCR::ABL* kinase domain mutations detected after Imatinib failure have varied from 19 % to 90 %, depending on the methodology applied [9]. No north Indian data till date is available on analysis of the prevalence of *BCR::ABL* kinase domain mutations influencing the drug resistance in north India.

2. Material and methods

Sample Collection: Peripheral blood (3 mL) was collected after taking written informed consent from CML patients. The study was approved by the Institutional Ethics Committee Dean/2022/EC/3415 clinical information of the patients was collected from the hospital information system (HIS) of the Institute.

Inclusion criteria: All CML patients with *BCR::ABL* fusion irrespective of age or gender were included.

Exclusion criteria: CML patients without *BCR::ABL* fusion were excluded.

In this study, samples of the patients found resistance to imatinib were analyzed for *BCR::ABL* kinase domain mutation analysis by direct sequencing of *BCR::ABL* kinase domain by Sanger method at MRU Laboratory, Dept. of Anatomy, Institute of Medical Sciences, BHU, Varanasi, India. Total 329 samples were collected between March 2019 to December 2020 from different areas of North India. The results were analyzed using both variant reporter software and NCBI Blast software using *ABL (*NM.14752) as reference sequence.

Statistical analysis: statistical analysis procedures were performed using SPSS version 24.0. We followed established best practices to summarize the data and employed appropriate statistical tests to assess the significance of observed differences. The significance level was set at $p < 0.05$ for all tests, and survival outcomes were evaluated using the Kaplan-Meier method and the stratified log-rank test.

3. Result

The study included a total of 329 newly diagnosed CML patients who presented to our clinic between March 2019 and December 2020. Among them, 232 (70.51 %) patients were initiated on Nilotinib. 31 patients were excluded from further analysis due to non-compliance or loss to follow-up, leaving a total of 66 CML CP patients who were started on Nilotinib as a second-line therapy and remained available for regular follow-up. Baseline characteristics for this subset are presented in Table 1. The median duration of prior Imatinib therapy was 42.6 months, with the majority (20.06 %) exhibiting secondary Imatinib resistance. The minimum follow-up duration was 6 months, and 60 out of 66 patients (90.9 %) completed one year of follow-up.

A total of 329 patients were analyzed for the *BCR::ABL* kinase domain mutation, of which 212 were males and 117 were female. The age was in the range of 18–72 years. In the cohort of 329 patients, 263 were found negative for the any mutation in *BCR::ABL* kinase domain.

Table 1
Characteristics of patient with Imatinib resistance ($n = 329$).

Parameters	Value
Median age, y (range)	41.1 (18–76)
Men/women	212 (64.4 %)/117 (35.5 %)
Median duration of Imatinib therapy, months (range)	42.63 months (3–144)
Primary/Secondary resistance	16(24.24 %) / 50(75.75 %)
BCR-ABL1 mutations at baseline, n (%)	14/66 (21.21 %)
Median duration of Nilotinib therapy, months (range)	23.9 (6- 48)

Only 66 patients out of 329 (20.06 %) were found positive for the mutation in the *BCR::ABL* kinase domain. In these 66 patients positive for mutations showed a higher frequency of mutation at T315I, Y253H, G250E and F317L.

We found 16 (24.24 %) patients with of T315I amino acid substitution (Table 2) which corresponds to $c > T$ at 1308 nucleotide position (Fig. 1). 10 (15.15 %) patients with of Y253H amino acid substitution (Table 2) which corresponds to t > C at 1121 nucleotide position. 8 (12.12 %) patients with of F317L amino acid substitution (Table 1) which corresponds to $c > A$ at 1315 nucleotide position. 7 (10.60 %) patients with of G250E amino acid substitution (Table 2) which corresponds to $g > A$ at 1113 nucleotide position. While other BCR::ABL kinase domain mutations had a relatively lower prevalence (Fig. 2).

To further identify the prevalence of mutations in the respective motifs of kinase domain. We did the blast analysis using NCBI BLAST tool and identified 28 mutations (42.42 % prevalence) in ATP binding P-Loop (amino acid 248–256), 24 mutations (36.36 % prevalence) in direct binding site (amino acid 315–317), 7 mutations (10.60 % prevalence) in C-loop (amino acid 350–363) 4 mutations (6.06 % prevalence) in A-loop (381–402). While 2 mutations (3.03 % prevalence) and 1 mutation (1.51 % prevalence) respectively were found in flanking regions of direct binding site SH2 and SH3 contact regions (Fig. 3 and Table 3).

4. Discussion

Imatinib has been proven as a potential tyrosine kinase inhibitor before evolution of *BCR::ABL* kinase domain mutation in structural motifs of *ABL* kinase domain. The goal of the present study was to investigate the presence of *ABL* kinase domain mutations in Philadelphia positive (PH+) cases of chronic myeloid leukemia patients in population of North India. Only 66 patients out of 329 (20.06 %) were

Table 2
. Distribution frequency of different kinase domain mutations in patients screened.

Total patients screened for the mutation = 329 Total positive patients for mutation = 66 Total prevalence of mutation = 20.06 %. Mutation detected	No. of patients	Percentage prevalence
T315I	16	24.24 %
Y253H	10	15.15 %
F317L	8	12.12 %
G250E	7	10.60 %
F359V	4	6.06 %
E459K	4	6.06 %
E255K	4	6.06 %
E355G	2	3.03 %
F359I	2	3.03 %
V299L	1	1.51 %
Q252H	1	1.51 %
E275K	1	1.51 %
M244V	1	1.51 %
K247R	1	1.51 %
E255V	1	1.51 %
L248V	1	1.51 %
E255K	1	1.51 %
F359C	1	1.51 %

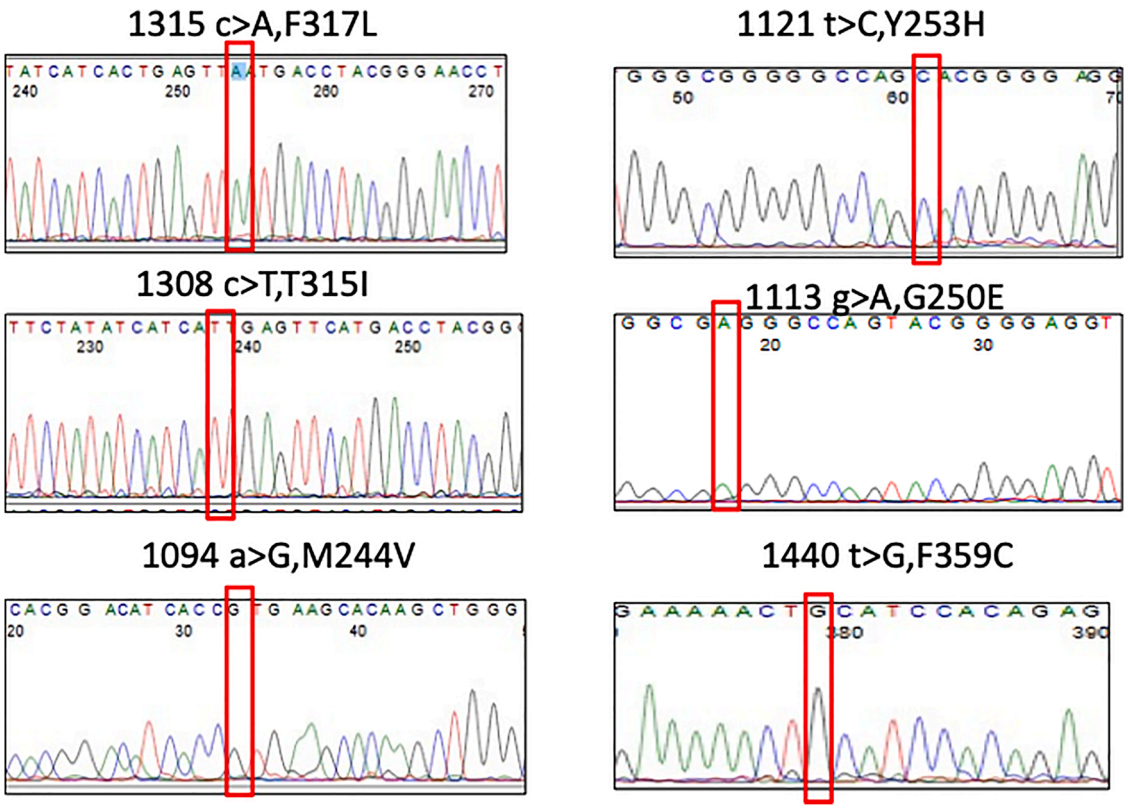

Fig. 1. Fluorescent peak trace of chromatograms obtained after Sanger sequencing showing the single base change in ABL kinase domain of BCR::ABL positive patients with imatinib resistance.

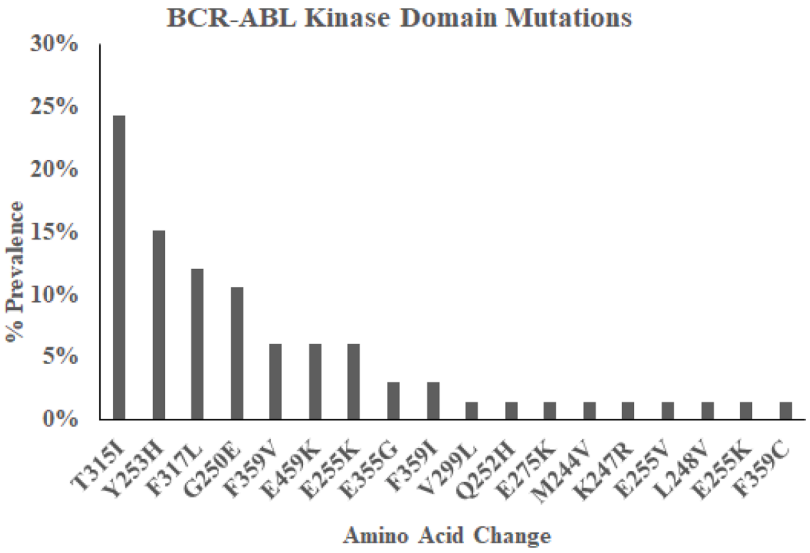

Fig. 2. Percentage prevalence of different kinase domain mutations in patients screened.

found positive for the mutation in the *BCR::ABL* kinase domain. These patients showed a higher frequency of mutation at T315I, Y253H, G250E and F317L [10]. Our study is in partial agreement with reported studies in India [11–13]. F317L mutation frequencies have been reported to be more prevalent in Caucasians when compared to Asians, with a particular emphasis on Indian populations. This observation could be attributed to a combination of genetic, environmental, and demographic factors. Genetic diversity, population genetics, and evolutionary history play crucial roles in determining the prevalence of specific mutations in different ethnic groups. Additionally, environmental exposures and lifestyle factors can influence mutation profiles. The observed disparities warrant further investigation, as they may have implications for disease management and treatment strategies, particularly in diverse patient populations. Furthermore, in our study, we found that the prevalence of T315I mutation is very high compared to other detected mutations in BCR-ABL kinase domain which was in concordance with other studies in India [12,13]. We also found Y253H and G250E had a high prevalence in our study, while GIMEMMA study by Specchia G, et al.,2021 reported high prevalence of Y253H, few contrasting reports of low prevalence of Y253H and moderately high

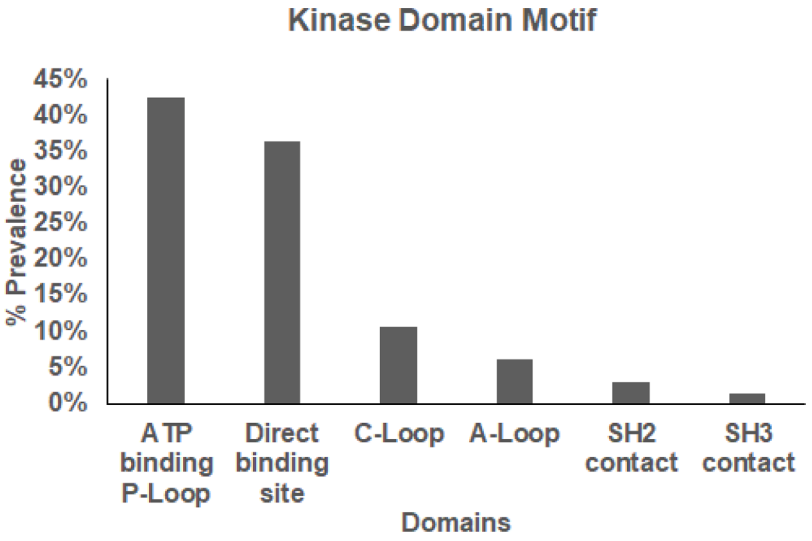

Fig. 3. Percentage prevalence of mutations in different kinase domain motifs in patients screened.

Table 3
Distribution of mutations in different kinase domain motif.

Kinase domain motif	Total no. of mutation	Percentage occurrence
ATP binding P-Loop	28	42.42 %
Direct binding site	24	36.36 %
C-Loop	7	10.60 %
A-Loop	4	6.06 %
SH2 contact	2	3.03 %
SH3 contact	1	1.51 %

prevalence of G250E were also reported in India [14]. Apart from the above mutations we also obtained F317L in high frequency which was not reported earlier. While other mutation were in low frequency as reported in GIMEMMA study [14]. The occurrence of low prevalence of *BCR::ABL* kinase domain mutations in our study could be due to break of follow-up of patients.

Declaration of Competing Interest

The authors whose names are listed immediately below certiry that they have NO affiliations with or Involvement In any organization or entity with any financial interest (such as honoraria; educational grants; participation In speakers' bureaus; membership, employment. consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discu>Sed in this manuscript.

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