Mutation Profiling of BCR-ABL Kinase Domain in Chronic Myeloid Leukaemia Patients with Imatinib Resistance

Yuslina Mat Yusoff (✉ yuslina@imr.gov.my)

Sharifah Zahra Anoar

INSTITUTE FOR MEDICAL RESEARCH (IMR) <https://orcid.org/0000-0003-4925-6798>

Zahidah Abu Seman

Institute for Medical Research

Siti Shahrum Mohamed Said

Hospital Kuala Lumpur

Nor Rizan Kamaluddin

Institute for Medical Research

Julia Abdullah

Institute for Medical Research

Aliza Mohd Yaacob

Institute for Medical Research

Ezalia Esa

Institute for Medical Research

Zubaidah Zakaria

Institute for Medical Research

Research note

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Abstract

Objective: Emergence of mutation in the BCR-ABL kinase domain (KD) impairs imatinib mesylate (IM) binding capacity, thus contribute to IM resistance. Identification of these mutations is important for treatment decision and precision medicine in CML patients. Our study aims to determine the genomic landscape of BCR ABL KD mutations in CML patients with IM resistance.

Result: BCR-ABL KD mutations were observed in 23 patients (26.7%). Fifteen different types of mutations have been identified; Y253H, E255K, T267A, K285I, A287T, M290R, F311I, T315I, F317L, F359V, F359I, F359C, K357T, A399T and E459K. We also discovered three novel mutations; M290R, K285I and K357T and two silent mutations at codon 389 and 401. Mutational analysis is recommended to identify patients at risk of disease progression. Therefore, early detection of such mutations may allow timely treatment intervention to prevent or overcome resistance.

Keywords: Chronic Myeloid Leukaemia; BCR-ABL kinase domain; imatinib resistance mutation.

Introduction

Chronic Myeloid Leukaemia (CML) is a type of blood cancer which is characterized by a myeloproliferative neoplasm that originated in an abnormal pluripotent bone marrow stem cell [1]. CML affects 20% adults and its frequency is similar worldwide with a slight predominance in males [2]. According to Malaysian National Cancer Registry Report 2007–2011, leukaemia is the sixth most common cancer in Malaysia with 4.4% incidence, in which CML accounts for 12.2% of all leukaemia cases [3].

The hallmark of CML pathogenesis is associated with Philadelphia chromosome (t(9;22) (q34;q11)) that results in creation of breakpoint cluster region-Abelson (BCR-ABL) fusion gene, which encodes constitutively activated tyrosine kinase [4–7]. CML progresses through 3 phases which classified as chronic phase, accelerated phase and blast phase [6]. Imatinib Mesylate (IM) is the first established TKIs has become a prominent therapy for first line CML treatment. IM acts by binding to adenosine triphosphate (ATP) binding site of tyrosine kinase domain. This action result in inhibiting tyrosine autophosphorylation and at the same time cause phosphorylation of its substrates in order to prevent tyrosine kinase enzyme activity [8, 9].

Resistance to IM occurs when patients do not respond to IM commonly due to mutation on BCR-ABL KD that cause failure to destroy clonal expansion of leukaemic cells [10]. This situation leads to affecting patients monitoring treatment and survival outcome. Our study aims to determine the genomic landscape of BCR ABL KD mutations in CML patients with IM resistance, to determine the prevalence of these mutations in our population and to identify novel, pathological mutations. Hence, it would be beneficial for us to understand the mutation status in our CML patients which will help us to provide better personalised treatment.

Methods

Subjects

A total of 86 CML patients with IM failure were included in the study. They were recruited from Ministry of Health hospitals in 2016 to 2018. These patients were in chronic, accelerated or blast phase, treated for at least 12 months, with IM dose of 400 mg as frontline treatment. Their demographic data including age, race, gender and disease phase were recorded.

RNA Extraction

RNA extraction conducted using QIAamp RNA Blood Mini Kit according to QIAamp RNA Blood Mini protocol (Qiagen, Hilden, Germany) with slight adjustment. The samples measured for RNA purify and concentration using NanoDrop Spectrophotometer (Thermo Fisher, Massachusetts, USA) and stored at -80 °C refrigerator until proceed with cDNA synthesis.

cDNA synthesis

The RNA samples were reverse transcribed using Quantitect Reverse Transcription kit (Qiagen, Hilden, Germany) according to protocol provided by manufacturer. The cDNA samples were stored at -20 °C refrigerator until proceed with reverse transcriptase Polymerase Chain Reaction (PCR) process.

BCR ABL mutation analysis

The reverse transcriptase-PCR assay was performed for BCR-ABL gene amplification. The first PCR amplified from synthesized cDNA using a set of designated primers of tyrosine kinase domain of the bcr-abl p210 transcript. The following programme was used on Mastercycler→Nexus Gradient (Eppendorf, Hamburg, Germany) that included denaturation for 5 minutes at 95 °C, 30 cycles of 30 seconds at 95 °C, 60 seconds at 60 °C, and 150 seconds at 72 °C. The last elongation step was extended to 7 minutes. The total PCR reaction volume was 25 µL consist of 2.5 µL of 10X PCR buffer, 1.5 µL of 25 mM MgCl₂, 1 µL of 10 mM dNTP, 2.5 µL of 50 ng cDNA, 1.25 U Hot Start taq DNA Polymerase, and 1.25 µL of 10 µM each primer. The second PCR was amplified using 3 pairs of ABL kinase domain designated primers. The following programme was used on Mastercycler→Nexus Gradient (Eppendorf, Hamburg, Germany) that included denaturation for 5 minutes at 95 °C, 30 cycles of 30 seconds at 95 °C, 30 seconds at 60 °C, and 40 seconds at 72 °C. The last elongation step was extended to 7 minutes. Total reaction volume was 75 µL for each set consist of 7.5 µL of 10X PCR buffer, 5 µL of 25 mM MgCl₂, 1 µL of 10 mM dNTP, 0.5 µL of 1st step PCR product, 3.75 U Hot Start taq DNA Polymerase and 3.75 µL of 10 µM each primer. All mutations were confirmed by Sanger sequencing. The detected mutations were verified according to standard amino acid substitution nomenclature using CLC Main Workbench 7 software (Qiagen, Hilden, Germany) for alignment with reference sequence Genbank accession number X16416.1 (Fig. 1).

Results

Baseline Characteristics

Demographic data for 86 CML patients with IM-resistance were shown in Table 1. Gender distributions for male patients are 48 (55.8%) and female patients are 38 (44.2%) respectively. Their age ranges from 13 to 81 years old with the median age of 46.5. Among all patients, 46.5% were Malays, 34.9% Chinese, 10.5% Indians and 8.1% others which includes indigenous population i.e.; Bajau, Bisaya and Dusun.

Frequency of BCR-ABL KD mutations

Mutations in the BCR-ABL gene were found in 23/86 (26.7%) patients, 10 (43.5%) were in chronic phase, 8 (34.8%) in accelerated phase and 5 (21.7%) in blast phase. Seventeen different point mutations in BCR-ABL gene were detected in these 23 patients. Of these 17 mutations, 15 were missense mutations namely Y253H, E255K, T267A, K285I, A287T, M290R, F311I, T315I, F317L, K357T, F359V, F359I, F359C, A399T and E459K mutations, whereas 2 were silent mutations, T389T and F401F.

Types of mutations

Spectrum of mutation varies between patients. Eighteen out of 23 patients (78.3%); among them, six patients with Y253H mutation, four patients with F311I mutation and two patients with T315I mutation. The remaining patients had single mutation of F359V, F317L, T267A, F359I, E255K and F401F mutation respectively. Meanwhile, 5 patients showed multiple mutations; K285I/E459K mutation, Y253H/M290R mutation, K357T/F359C mutation, Y253H/T389T and Y253H/A287T/A399T (Table 2).

We would like to highlight that from all the mutations identified, 3 mutations namely K357T, K285I, and M290R appeared to be novel mutations which have not been reported so far, in cancer database namely Catalogue of Somatic Mutations in Cancer (COSMIC) (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>) [11].

The highest frequency of mutation is in the phosphate binding loop (P loop) region of ABL kinase domains (Y253H and E255K) with a frequency of 43.5% (10/23). Mutations were found in the IM Binding site of KD (F311I, T315I, and F317L) in 30.4% patients (7/23). Three patients were discovered with mutation on the catalytic domain (C loop) of ABL KD (K357T, F359V, F359I and F359C). There were three patients whom developed T389T, F401F and A399T mutation at activation loop (A loop) ABL KD. Additionally, the following mutations were observed in one patient each: K285I, A287T and M290R (located on α C-helix). T267A and E459K mutation were in other mutation category which means outside of the mentioned region.

Table 1
Demographic profile and disease status among
CML patients with IM-resistance

Demographic	Number of patients (%)
Gender	
Male	48 (55.8%)
Female	38 (44.2%)
Age	
Median (years, range)	46.5 (13–81)
Race	
Malay	40 (46.5%)
Chinese	30 (34.9%)
Indian	9 (10.5%)
Others	7 (8.1%).
BCR-ABL KD mutation	
Presence of mutation	23 (26.7%)
Absence of mutation	63 (73.3)
Disease status in patients with mutation	
Chronic phase	10 (43.5%)
Accelerated phase	8 (34.8%)
Blast phase	5 (21.7%)

Table 2
BCR-ABL KD mutation spectrum in CML patients with IM-resistance.

cDNA position with Nucleotide Change	Amino Acid Substitution (Gen Bank no. X16416 (ABL))	No. of Patients with Mutation	ABL Kinase domain
Single Mutation			
757 T > C	Y253H	6	P loop
763 G > A	E255K	1	P loop
799 A > G	T267A	1	Other mutation
931 T > A	F311I	4	IM binding site
944 C > T	T315I	2	IM binding site
951 C > A	F317L	1	IM binding site
1075 T > G	F359V	1	C loop
1075 T > A	F359I	1	C loop
1203 C > T	F401F	1	A loop
Multiple Mutation			
757 T > C & 869 T > G	Y253H / M290R*	1	P loop / αC-helix
757 T > C & 1167 A > G	Y253H / T389T	1	P loop / A loop
854 A > T & 1375 G > A	K285I* / E459K	1	αC-helix / Other mutation
1070 A > C & 1076 T > G	K357T*/ F359C	1	C loop
757T > C, 859 G > A & 1195 G > A	Y253H / A287T / A399T	1	P loop/ αC-helix / A loop
<p>*= Novel mutation.</p> <p>The most common sites include P-loop: phosphate binding loop (codons 244–256), IM binding site: imatinib binding region (codons 311, 315, 317), C loop: catalytic domain (codons 350–363) and A loop: activation loop (codons 381–407) [12, 13]. αC-helix: (codons 276–290) [14]. Genbank accession number X16416.1</p>			

Discussion

BCR-ABL KD mutations represented the major cause of resistance to TKI therapy. These mutations may alter the conformation of the BCR-ABL1 molecule and impair the imatinib-binding affinity [15, 16]. More than 90 various BCR-ABL KD mutations were reported that have been identified in patients with IM resistance [17]. Since IM has shown resistance that leads to treatment failure, the second line generation

(dasatinib, nilotinib, and bosutinib) and the third line generation (ponatinib) of TKI have been developed for patients' therapy option.

A variety of methods can be used as the primary screen for detection of mutations in BCR-ABL KD. In this study, we used conventional Sanger sequencing, which has been described as the gold technique for mutational screening of IM-resistant patients [17, 18]. We found 26.7% BCR-ABL1 KD mutation in CML patients with IM resistance in Malaysian population. Our finding is in concomitant with a study conducted by other researchers in similar population [19]. In their study, 22.4% of IM resistant CML patients showed the presence of mutation in their tyrosine KD of BCR-ABL1 gene. The present findings showed lower mutation frequency compared to the frequency reported in other study populations such as Korean (63%) [20], Chinese (58%) [21], Singaporean (45%) [22] and Caucasian (30–36%) [23, 24]. We discovered 15 different types of mutation at various location of ABL KD namely Y253H, E255K, T267A, K285I, A287T, M290R, F311I, T315I, F317L, F359V, F359I, F359C, K357T, A399T and E459K mutations. The common clinically relevant IM-resistant mutations reported are G250E, Y253F/H, E255K/V, T315I, M351T and F359V [25].

Remarkably, among all the mutations identified, 3 were novel mutations which have not been reported previously. These 3 new mutations or substitutions are M290R, K285I, and K357T. There is no record of mutation at codon 290 on COSMIC database. Hence, we proposed M290R which involved a substitution from Methionine (M) to Arginine (R) (ATG→AGG) as novel mutations of ABL KD. Meanwhile, K357R (mutation ID COSM6196747) was recorded on COSMIC database involving a substitution from Lysine (K) to Arginine (R). However, we identified K357T mutation which involved a substitution from Lysine (K) to Threonine (T) (AAA→ACA). According to Kamasani and colleagues (2017), they noted K285N involving a substitution from Lysine (K) to Asparagine (N) and K285R involving a substitution from Lysine (K) to Arginine (R) instead of K285I that we identified in this study involving a substitution from Lysine (K) to Isoleucine (I) (AAA→ATA) [14].

T315I mutation has been observed in 2 patients whom showed resistance to all second line generation of TKI therapy. Third line generation ponatinib have been developed to encounter T315I mutation which has high activity against ABL kinase point mutation including T315I by binding to the inactive conformation of the kinase domain in the murine ABL T315I [26, 27]. F317L has been recognised to be less sensitive to dasatinib and probably more responsive to nilotinib [11]. Y253H, E255K, F359V, F359I, F359C and F311I have been identified to be less sensitive to nilotinib and probably more responsive with dasatinib [17]. E459K has been shown to be less effective to nilotinib [10]. The other mutations that have been detected shown no studies of drug resistance so the most appropriate as alternative option is might be proceed with second line generation of TKI therapy [17].

The result of our study showed that 23 CML patients with BCR ABL KD mutations were in accelerated phase, (n = 8) and blast phase, (n = 5). These mutations were also detected in 10 patients with chronic phase, suggesting that point mutations in the ABL gene is not restricted to accelerated phase or blast phase patients only [28]. Therefore, mutation screening in chronic phase CML is recommended in

patients with inadequate initial response or those with evidence of loss of response. It is also recommended at the time of progression to accelerated or blast phase [12].

Evaluating CML patients with clinical signs of resistance for BCR-ABL KD mutation is an important component of disease monitoring, in determining how patients respond to treatment and to understand the BCR-ABL mutation as the cause of resistance. Not only the presence of mutations, but also the actual amino acid change should be investigated in CML patients displaying resistance to IM in order to optimize therapeutic response. Certain specific mutations in BCR-ABL have been linked with poor outcome and hence mutation screening is clinically relevant to identify CML patients who are likely to have poor outcome and to facilitate selection of subsequent therapy. Furthermore, presence of mutations in different regions of BCR-ABL TKD leads to different levels of resistance. The type of mutation potentially can indicate whether second or third generation BCR-ABL inhibitor or alternative therapeutic strategies should be given to such IM resistant patients and also can help to identify those who need IM dose escalation [17, 19].

Therefore, mutation analysis of BCR-ABL KD is recommended in CML patients with imatinib-resistance to identify patients at risk of disease progression. It should be offered as diagnostic service for these patients to guide therapy. Knowing the exact mutations responsible for IM resistance will help to select the most suitable TKIs for CML patients and improve their management. Furthermore, early detection of such mutations may allow timely treatment intervention to prevent or overcome resistance.

Limitations

Limitation of this study is the small size of the sample group. It is recommended that future studies be undertaken with larger samples to correlate the mutations with the clinical findings.

Abbreviations

CML
chronic myeloid leukaemia
BCR-ABL
breakpoint cluster region-Abelson
IM
imatinib mesylate
KD
kinase domain
TKI
tyrosine-kinase inhibitors
ATP
adenosine triphosphate
PCR

polymerase chain reaction
COSMIC
Catalogue of Somatic Mutations in Cancer
P loop
phosphate binding loop
C loop
catalytic domain
A loop
activation loop

Declarations

Ethics approval and consent to participate

Ethical approval for this study was waived by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia with registry number: NMRR-17-1368-36233. Samples were obtained from leftover diagnostic samples. Based on Malaysian guideline on the use of human biological specimen for research, no patient consent will be required for this study.

Consent for publication

The authors would like to thank the Director General of Health Malaysia for his permission to publish this article.

Availability of data and materials

Data is available on request from the corresponding author with permission from Ministry of Health, Malaysia (via email).

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

YMY designed the study and prepared the manuscript.

SZA collected samples, performed experiment, analysed data and prepared the manuscript.

ZAS collected samples, analysed data and prepared the manuscript.

SSMS designed the study.

NRK prepared and revised the manuscript.

JA collected samples and revised the manuscript.

ZZ, EE and AMY revised the manuscript.

All authors read and approved the final manuscript.

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Figures

Figure 1

Electropherogram of a patient with Y253H mutation. The figure shown substitution of T -> C at 757 cDNA position. This mutation leads to the alteration of amino acid tyrosine (Y) with amino acid histidine (H) at codon 253.