Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-251
Volume 106 Issue 6

The Prevalence of G250E and Y253F among ABL Kinase Domain Mutations and its Relation with Resistance to Tyrosine Kinase Inhibitors in Patients with CML in Middle Euphrates of Iraq

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Abstract

Background: Chronic Myeloid Leukemia (CML) is a hematopoietic stem cell disease, associated with a reciprocal translocation between chromosomes 9 and chromosome 22, lead to the formation of the BCR-ABL fusion gene (Philadelphia chromosome). This fusion gene is believed to play a golden role in the initial development of CML with constitutive tyrosine kinase activation. Successful use of tyrosine kinase inhibitors (TKIs) play a role in improve survival and increase the prevalence of CML, but unfortunately mutations in the BCR-ABL kinase domain may cause, or contribute to increasing, resistance to TKIs in CML patients.

Objective: This study was designed to assess the association of two common BCR-ABL kinase domain mutations (G250E and Y253F) with resistance state of CML patients on TKIs in the Iraqi Middle Euphrates region.

Patients and Methods: A retrospective case-control study in which 85 patients with chronic myeloid leukemia in chronic phase (45 patients as cases group and 40 patient as a control group) were selected from three hematooncology centers in the middle Euphrates in Iraq during the period from January 2016 till October 2016 out of a total of 240 CML patients (108 male and 132 female) who were registered during this period in these three centers and all patients on TKI (Imatinib and Nilotinib).

Results: One patient from the cases group (1/45) was carriers of one of two selected ABL kinase domain mutations and no one of the control groups. G250E was detected in 1/45 (2.2 %) and also had significant risk association to develop resistance to TKIs odd ratio, C.I., 2.73, 0.1081-68.9424. This mutation had no significant correlation with demographic or hematological features.Y253F was not detected in any one of our study groups CML patients.

Conclusions: G250E among selected ABL kinase domain mutations were detected in our CML patients with resistance to TKIs. This mutation may play a role in the development variable degree of resistance to first and second-generation TKIs whether primary or secondary.

Keywords: CML; TKIs; ABL Domain kinase mutations

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Citation: Al-Rekabi SHA, Al-Musawi MSM, et al. (2020) The Prevalence of G250E and Y253F among ABL Kinase Domain Mutations and its Relation with Resistance to Tyrosine Kinase Inhibitors in Patients with CML in Middle Euphrates of Iraq. Prensa Med Argent, Volume 106:6. 251. DOI: https://doi.org/10.47275/0032-745X-251.

Received: April 08, 2020; **Accepted:** April 30, 2020; **Published:** May 05, 2020

Introduction

Chronic Myeloid Leukemia (CML) is a hematopoietic stem cell disease, characterized by a reciprocal translocation between chromosomes 9 and 22, resulting in the formation of the Philadelphia chromosome (Ph) [1]. This translocation t (9;22) results in the head-to-tail fusion of the breakpoint cluster region (BCR) gene on chromosome 22 at band q11 and the Abelson murine leukemia (ABL) gene located on chromosome 9 at band q34. The product of the fusion gene (BCR-ABL) is believed to play a central role in the initial development of CML. This genetic abnormality was first named in 1960 so it was one of the first malignancies to be linked to a clear genetic abnormality [2]. In less than the last 10 years, the prognosis of CML has changed from that of fatal disease to a disorder amenable simply to lifelong oral medication and compatible with a normal lifespan [3]. This change has been made possible by a deep understanding of the molecular pathogenesis and a determination to develop targeted and selective drugs [4]. Introduction of Imatinib (Gleevec, Novartis) into clinical practice nearly one decade ago, has dramatically changed treatment and follow-up of CML [5]. Imatinib specifically targets tyrosine kinase activity of the oncogenic protein encoded by the BCR/ABL gene. Then, new other tyrosine kinase inhibitors (TKIs) were developed [6,7]. ABL

kinase domain mutations are identified in about 30–50% of CML cases and it is variable as a consequence of different methods of detection, nature of resistance, and disease phase examined and is the most frequently identified mechanism of treatment resistance [8]. More than 100 kinase domain mutations are known till now to cause varying degrees of resistance to the TKIs. These point mutations usually result in amino acid changes, which decrease the binding affinity of TKIs, but not the usual substrates [9].

Materials and Methods

A retrospective case-control studying which 85 patients with chronic myeloid leukemia in chronic phase (45 patients as case group and 40 patient as a control group) were selected from three hematooncology centers in the middle Euphrates in Iraq (Karbala, Babylon and Al Najaf centers) during the period from January 2016 till October 2016 out of a total of 240 CML patients (108 male and 132 female) who were registered during this period in these three centers.

Patients

Case group includes 45 CML patients resistant to TKI treatment out of 67 resistant patients eligible for the study, who complete >6 months of treatment with TKI, 43 patients were in primary resistant state and 2 patient with secondary resistance. The other 22 patients refuse to participate in this study.

Primary resistance patients: CML patients who complete 6 months of TKIs treatment and their molecular response after treatment is either under warning category (BCR-ABL level 1-10 % IS after 6 months or 0.1-1% IS after 12 months of treatment) or under failure category (BCR-ABL level >10% IS after 6 months or >1% IS after 12 months) according to the ELN guideline for the molecular monitoring of CML patients on TKI [10-12].

Secondary resistance patients: CML patients who complete > 6 months of TKIs treatment and their molecular response to treatment was optimal (BCR-ABL level <1% IS after 6 months or <0.1% IS after 12 months) according to the ELN guideline but lose their response at any time during the period of treatment, that does not fit for the optimal response criteria [11,12].

Exclusion Criteria

In this study, we excluded all patients with newly diagnosed, lost their data, and poor compliance with treatment. Follow up adherence of patients to treatment was approved as possible by oral or phone communications with each patient to exclude the possibility of poor compliance as a cause for response failure.

Control Group Definition

Control group includes 40 CML patients out of 195 CML patient of good response to TKI treatment randomly selected, according age and gander, who complete >6 months of treatment with TKI and their molecular response to treatment was at optimal category (BCR-ABL level <1% IS after 6 months or <0.1% IS after 12 months) according to the ELN guideline without any evidence of secondary resistance [11,12].

Ethical Issue

The study protocol was approved by the Ethics Committee of the College of Medicine, University of Kufa. In addition to an oral permission for blood sampling and do laboratory analysis was attained from all patients included in this study.

Assessment of Exposure

After categorization of CML patients eligible for the study into study and control groups, the presence or absence of most common 7 ABL kinase domain mutations and their correlation with the differences in the response criteria of patients to TKIs according to ELN guideline were assessed. These mutations identified from important and published articles in CML field.

Potential Confounders

Other causes of resistance to TKIs treatment (like other less common Kinase Domain mutations and ineffective GIT absorption of drug) were not assessed in this study due to limitation in the cost and time.

ASO-PCR

Blood samples were collected from all participated patients. First of all DNA was extracted from blood samples using commercial available DNA extraction Kit (Promega, USA) following manufacture instructions. ASO-PCR was performed in 30µl reaction. Briefly, two master mixes were prepared, for G250E and Y253Fmutations detection. PCR master mixes were prepared according to the stander procedure as the manufacture company advice. We design 5 different ASO-PCR primer sets according to the frequency of known ABL gene mutations (2 mutations) for normal allele and mutant allele (primers synthesis by Macrogen Korea). The sequences of mutant primers were adapted from a previously published article and sequences of normal primers (wild type primers) were designed using BLAST search and Primer 3. The sequences of forward and reverse primers used for ASO-PCR are shown in below [13].

- **Normal forward Primers for normal alleles:** N/G250E-F: gaagcacaagctgggcgg and N/Y253F-F: ctgggcgggggccagta, N/M244V-F: for G250E and Y253F respectively.
- **Mutant forward Primers for mutant alleles:** m/G250E-F: gaagcacaagctgggcga and m/Y253F-F: ctgggcgggggccagtt, m/M244V-F: for G250E and Y253F respectively.
- **Reverse primers:** 244-R: gccaatgaagccctcggac (This primer was used for mutations: G250E and Y253F)

Statistical Analysis

Statistical analysis was performed using SPSS 22 (statistical package for social sciences) and Excel 2010 programs. Data analysis was done using t- test, analysis of variance (ANOVA) & chi –square test for tables with frequencies, percentages, range mean &standard deviation.

Results

Two ABL domain kinase mutations had been evaluated by using ASO-PCR which were G250E and Y253F in both control and study group .No mutations within control group and only 1 patient (nearly 2.2%) of study group had mutations tables 1, 2 and 3 with figures 1.

Association of positive mutation with general patients finding like age, gender, type and duration of TKI management and type of resistant if present table 3.

Association of Positive Mutation with Hematological Findings

G250E: In positive case the mean hemoglobin concentration was 9.90g/l, while in negative cases mean hemoglobin concentration was

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Table 1: General characters of cases and control groups.

General characters		Control group	Cases group	P Value
Gender	Female	23 (57.5 %)	26 (57.8 %)	0.979
	Male	17 (42.5 %)	19 (43.2%)	
Age (Mean in years+SD)		46.25+10.37	43.9+12.95	0.506
Type of treatment	Imatinib	36 (90%)	33 (73%)	0.050
	Nilotinib	4 (4%)	12 (27%)	
Duration of treatment (Mean in months+SD)		21.4+9.3	18.9+12.6	0.319
Geographical distribution	Babylon	13 (32%)	17 (38%)	0.326
	Al Najaf	11 (28%)	11 (24%)	
	Karbala	10 (25%)	12 (27%)	
	Al Qadisiyah	4 (10%)	3 (7%)	
	Al Muthana	2 (5%)	2 (4%)	

Table 2: Type of ABL domain kinase mutations in study and control group.

Mutations		Cases group	Control group	Total	P value	Odd ratio and 95% C.I
G250E	Positive	1/45	0/40	1/ 85	0.343	2.73
	Negative	44/45	40/40	84/85		0.108 -68.942
Y253F	Positive	0/45	0/40	0/85	-	
	Negative	45/45	40/40	85/85		

Table 3: Association of G250E mutation with general patients finding in cases group.

General Features for mutations (G250E)		Positive mutation	Negative mutation	P value
Mean of Age (years)		26	44.9	0.168
Gender	Male	0	18	0.387
	Female	1	26	
Type TKI treatment	First generation	1	32	0.542
	Second generation	0	12	
Mean treatment duration (months)		40	18.5	0.094
Primary resistance		0	42	0.827
Secondary resistance		1	2	

12.02 g/l+1.73. The mean total white blood cells count in positive case was 8.00x109\L, while in negative cases 8.39+3.54x109\L. The mean platelet count in positive case was 3.70x109/L and in negative cases group was 212+79.1x109/L.

Discussion

Mutations within the kinase domain of genetic abnormalities may contribute and lead to failure of management and loss of therapy effects. These mutations are the most commonly investigated mechanism of resistance to TKIs, but they are not the only one [14]. Really, the frequency by which mutations have been associated with TKIs resistance is variable top variation to the stage of CML often from twenty five percent to thirty percent of early chronic phase patients on specially first-line Imatinib to approximately 70% to 80% of plastic crisis patients [14,15]. The current study was conducted to investigate two frequent genetic abnormalities in published articles to provide the clinicians on how to best integrate the mutations analysis in the routine management of CML patients. A single amino acid substitution by point mutation at the abnormal gene development is the commonest way of acquired resistance to TKIs, which inhibit drug binding by main drug activities on certain pathway lead to loss drug targeting [16]. BCR-ABL kinase domain mutations have a considerable effect on TKIs primary and secondary resistance in some patients with CML and such resistance can appear at any time during TKIs treatment, so they have important roles in treatment failure [16], till now there are more than

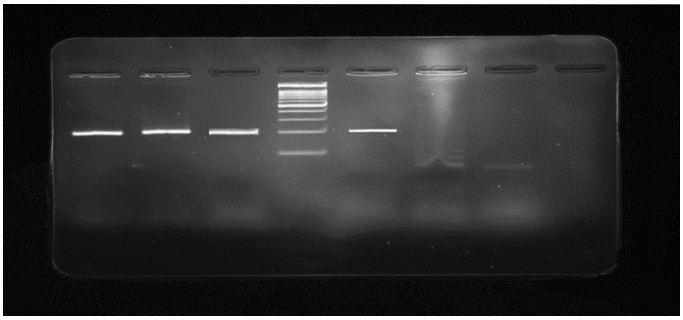

Figure 1: 41N-43N numbers of study group indicate amplification of normal allele, 42M-43M indicate that there is no amplification of mutant allele for 42 and 43 number of study group samples, while 41M of study group indicate amplification of mutant allele M; DNA ladder 100bp.

100 known BCR-ABL point mutation [13]. The European Leukemia Net proposal recommend analysis of these mutations in cases of CML at time of diagnosis in patient showing accelerated or plastic phase or in cases ranked under warning or failure response, as it play important role in treatment choice [10,11]. By ASO – PCR this study focused on two common mutations according to prevalence of mutations published by different studies [17-20]. One patient carried ABL domain kinase mutations, this mutated patient was from cases group. G250E record 2.2% of total study group. G250V mutation Glycine (G) is substituted to Glutamic acid (E) at 250 residue of BCR-ABL gene also lead clinical TKIs resistance, and it located on kinase domain exon 4 with 10.4% frequency among BCR-ABL1-mutated CML as recorded by COSMIC [13]. In this study it recorded 2.2% of mutated cases and was in agreement with other workers but much less than recorded by others workers like in India, Italy and Australia, whom founded the prevalence of G250E was 8%, 10% and 12% respectively among resistant cases to TKIs [21]. The main explanation for this difference is mainly sample size, which were hundreds of CML patients in these centers as compare with small sample size in this study. Mutated case was categorized as secondary resistance case, ranked under failure response and on Imatinib since diagnosis (40 months). Also there were no statistical differences between groups of sample size in this study and this mutation had good odds ratio (OR) 2.73 and it reflect the

association of presence of this mutation with TKIs resistance among resistance CML patients. There are no statistical differences between both sample size groups in related to demographic and hematological findings.

Conclusions

G250E among two ABL kinas domain mutations was detected in our CML patients with resistance to TKIs. This mutation may play a role in development variable degree of resistance to first and second generation TKIs weather primary or secondary.

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