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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.175.181

Research Article
Prevalence of BCR-ABL T315I Mutation in Different Chronic Myeloid Leukemia patients Categories

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Abstract

Background and Objective: Chronic Myelogenous Leukaemia (CML) is a clonal myeloproliferative tumor distinguished by the existence of the Philadelphia chromosome (Ph) resulting from the t (9, 22) (q34, q11) translocation. The BCR-ABL gene and the fusion protein, which has constitutive tyrosine kinase activity, are the outcome of this translocation. The purpose of this study is to determine the prevalence of the BCR-ABL T315I mutation in CML patients. **Materials and Methods:** Descriptive cross-sectional studies were conducted on 100 CML patients who visited RICK hospital between May, 2018-2019. T315I mutation analysis was done on all patients utilizing (RT/PCR) followed by RLFP to quantify the prevalence of Kinase Domain Mutation analysis (KDM) in CML. **Results:** The link between haematological parameters and ABL mutations in CML patients was shown to be a substantial positive correlation between T315I and haematological parameters (HB and WBC) but no correlation with PLT. The data revealed that 43 out of 99 CML had T315I, with highly prevalent gene express (43.4%) detected in all CML 56.6%. The correlation of T315I mutations with clinical status was positive significant (p-000). **Conclusion:** It can be concluded that T315I mutation became significantly higher in CML patients than in other groups of mutations. The detection of ABL kinase domain mutations may be a proper and valuable strategy for optimizing therapeutic methods and preventing treatment delays.

Key words: Chronic myeloid leukaemia, BCR-ABL T315I gene, mutation, haematological parameters, chromosome, tyrosine kinase

Citation: Khair, H.E., B.A. Mohamed, B.Y. Nour and H.A. Waggiallah, 2022. Prevalence of BCR-ABL T315I mutation in different chronic myeloid leukemia patients categories. Pak. J. Biol. Sci., 25: 175-181.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

CML is a clonal bone marrow disorder and cancer of white blood cells characterized by proliferation and accumulation of myeloid cells and precursor's cells. It is known that radiation exposure increased the risk of CML. CML is one of the genetic abnormalities that result from chromosomal translocation. the translocation whereby the part of the BCR ("Breakpoint Cluster Region") gene from chromosome 22 is fused with the ABL (Abelson) gene on chromosome 9 formed new fusion gene called Philadelphia chromosome that produces fusion protein known tyrosine kinase (P210) is continuously activated without regulated from other messenger protein in contrast fused protein play important role in the cell cycle (cell survival and proliferation), inhibit DNA repair and making the cell more susceptible to developing further genetic abnormalities[1,2].

The BCR-ABL tyrosine kinase transfers a phosphate from ATP to tyrosine residues on a unique substrate, causing the formation of a large number of malignant myeloid cells in chronic myeloid leukemia patients[3]. Tyrosine kinase inhibitors are a treatment of CML in 2001 after food and drug administration approved imatinib mesylate (Gleevec or Glivec) as the 1st drug to be developed targeted against the protein kinase. The function of imatinib was an inhibitor of tyrosine kinase enzymes activity and block ATP binding to tyrosine kinase active site to prevent phosphorylated protein[2,3]. The imatinib was found to sufficiently inhibits the progression of CML in most patients (65-75%) with normal white blood cell and stabilized in bone marrow[4,5].

BCR-ABL kinase domain mutations are the most common molecular mechanism of loss in response to BCR-ABL kinase inhibitors. More frequently, point mutation is a genetic abnormality that usually takes place during DNA replication or translation of mRNA caused by a change in a single nucleotide base in nucleic acid. In contrast, a different codon is formed so that a mutated protein is created[6]. Approximately 20-30% of patients with CML fail to respond to the 1st line of treatment (imatinib) after an initial response[7]. The ABL mutation can be classified into four types according to the site of mutation catalytic loop (C-loop), the activation-loop (A-loop), the phosphate binding-loop (P-loop) and the direct binding site[8].

T315I is a missense mutation caused by a change in a single nucleotide in contrast new amino acid is mutated from a threonine residue to an isoleucine residue at position 315 (T315I). T315I concede one of the important BCR-ABL kinase domain mutations that make resistant to drugs by the affected binding site of imatinib leads to a hydrogen bond being formed with imatinib that prevents imatinib from binding with the ATP site. T315I comprises approximately 14% of detected mutations[9] affected binding sites of imatinib lead to a hydrogen bond being formed with imatinib that prevents imatinib from binding with ATP site[10,11]. Thus the study aimed to identify the prevalence of T315I mutation in different groups of CML patients.

MATERIALS AND METHODS

Study area: The study was carried out at National Health Laboratory, Khartoum, Sudan I duration between May, 2018-2019.

Data collection: Descriptive cross-sectional studies were done on 100 patients with CML who attended RadioIsotopes Center of Khartoum after verbal consent from every patient participating. Most of participating were female and above 40 years (55.8 and 62.8%), respectively. These patients were in different groups of the disease 25 in the new case, 25 relapse patients and 49 on treatment according to clinical status.

Mutation analysis: Mutation analysis for T315I was determined by using RT/PCR (Bioron, Römerberg, Germany) followed by RLFP (Bioron, Römerberg, Germany) were performed for all patients to calculate the prevalence of KDM in CML. Five millilitres of blood were obtained and divided into 2 EDTA tubes, 2.5 mL for haematological testing and 2.5 mL for molecular techniques. The automated blood counter analyzer was used to determine the Complete Blood Count (CBC) (Sysmex KX 21N, Tokyo, Japan).

Experimentation: Total RNA was isolated from peripheral blood samples using a high pure RNA Isolation kit (Roche Applied Science Germany), the extraction procedure is based on 1 µg of RNA was used for reverse transcription and the reaction medium was made p to 20 µL with free DW Water, The cDNA synthesis was executed at 42°C for 10 min and incubating the reaction at 95°C for 3 min. The c DNA was then stored at 20°C.

PCR was performed to amplify the BCR-ABL kinase domain by using 2 primers BCR exon13, ABL exon 7. After rehydration with 250 free DW overnight and diluted the stock primer with 80 DW (1:10). 5 µL from patient's cDNA has been mixed with 10 µL of universal master mix, 8 µL H_2O, mixed with 1 µL primers (exon 7, 13) then amplify the gene per thermocycler finally common product sample was used to amplify ABL-BCR kinase domain mutation.

PCR thermocycler, involving initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60.5°C for 30 sec and extension at 72°C for 45 sec with a final extension at 72°C for 5 min. Amplification products corresponding to 183 bp were then visualized after electrophoresis in an ethidium-bromide-stained 2% agarose gel and then nest PCR was performed to amplify T315I BCR-ABL fusion transcript by using, specifically designed primers, reverse primers GTT GCA CTC CAT CAA GTA GTCCA and forward primers AAGACCTTGAAGGAGGACA CCATG. To precisely nest PCR reactions were prepared in a volume of 24 µL master mix with 1 µL of product sample and vortex the sample then loaded into the in PCR machine for 2 hrs.

We used a nested PCR product followed by a restriction enzyme to detect T315I mutation by using thermo scientific HpyF3I (DdeI) restriction enzyme. The restriction enzyme digestion combination contains the following ingredients: Buffer 2 L, restriction enzyme DdeI 2 µL, PCR product 10 µL and nuclease-free water 18 µL. DdeI mixes were incubated at 37°C for 16 hrs for full digestion before being tested on a 2.0% agarose gel. Five micro liter of digesting product was applied to the gel's wells. The 1st well of the gel received 5 µL of DNA ladder (50-1000 bp). For 30 min, the gel was run till the bromophenol blue reached the bottom of the gel, which was then observed under UV light. The size of the band of positive patients was (183 pb) in contrast non-mutant was (134+49 pb).

Statistical analysis: SPSS (version 20, SPSS Inc., Chicago, IL) software was used for statistical analysis. The data was expressed as a percentage. Chi-square ×2 was used to give descriptive analysis of percentages of categorical variables. Comparisons of continuous variables using the Student's t-test and one-way ANOVA for parametric data. To estimate odds ratios and 95% confidence intervals, we built a logistic regression model. In all statistical comparisons, an $\alpha<0.05$ represented a statistically significant difference.

Ethical approval: This study was ethically approved by the Committee of Ethical Clearance in the Faculty of Medical Laboratory Sciences at the University of Gezira and subjects provided written consent.

RESULTS

Demographic data of CML patient: The demographic information for all data for the 99 study subjects who attended RICK Hospital from May, 2018-2019 is given in Table 1, the age above 40 (76.8%) and <40 (23.2%) years old. The gender distribution of the patients was (44.4%) males and (55.6%) females. Patients from Western and Central Sudan were the most common (30.3 and 23.2%, respectively).

According to the geographical distributions, the Sudanese tribes with the highest proportion of patients were from the Western and Northern parts (44.4 and 33.3%, respectively). According to the clinical status of CML patients, 49.5% were receiving treatment, while 25.3% were experiencing relapses or new cases.

Haematological analysis

Haematological examination results in CML: Our study included 99 CML patients who visited RICK hospital, 49 of whom were on treatment, 25 of whom were new cases and 25 of whom were resistant to treatment. The distribution of haematological parameters according to the status of CML patients was shown in the data record in Table 2. The analysis of complete blood count revealed that the mean of HB was lower in new cases and replacement (HB 10.58 and 10.78 g dL^{-1}) than in treatment CML (HB11.88 g dL^{-1}). On the other hand, there was a significant decrease in WBC count (4.87) in treated patients when compared to a new case and relapse patients (137.22±79.93, 50.49), respectively and platelet was increased in the new case and relapse patients when compared to treatment. The relationship between haematological parameters and CML patient status appeared as a significant positive relationship between clinical status and (HB, WBC) and a significant negative relationship between PLT and clinical status.

Haematological examination result of T315I KD mutation: Table 3 shows the relationship between haematological parameters and ABL mutations in CML

Table 1: Baseline characteristics among patients with chronic myelogenous leukemia

Characteristics	Patient (N = 99) (%)
Age group	
<40	23 (23.2)
≥40	76 (76.8)
Gender N (%)	
Male	44 (44.4)
Female	55 (55.6)
Geographical distribution N (%)	
Khartoum	17 (17.2)
Central	23 (23.2)
Northern	16 (16.2)
Eastern	11 (11.1)
Western	30 (30.3)
Southern	2 (2.0)

N: Number of patients in a study group and SD: Standard deviation

Table 2: Association between haematological parameter and clinical status

Status	HB (g dL^{-1}) (Mean±SD)	WBC ($\times 10^9 L^{-1}$) (Mean±SD)	PLTS ($\times 10^9 L^{-1}$) (Mean±SD)
Relapse	10.78±1.40	50.49±41.66	225.97±98.21
New case	10.58±1.16	137.22±79.93	245.68±138.73
Under treatment	11.88±1.84	4.87±1.68	209.86±81.78
p-value	0.001*	0.000*	0.365

Table 3: Correlation between haematological parameters and T315I mutations of CML patients

Mutations	HB (g dL^{-1}) (Mean±SD)	WBC ($\times 10^9 L^{-1}$) (Mean±SD)	PLTS ($\times 10^9 L^{-1}$) (Mean±SD)
T315I			
Normal T	11.52±1.79	25.60±41.99	211.13±78.38
Mutant I	10.95±1.51	81.35±86.26	238.40±127.24
p-value	0.095	0.000*	0.220

Table 4: Distribution of BCR-ABL kinase domain mutations according to the status of CML patients

Clinical status	Relapse N (%)	New case N (%)	Under treatment N (%)	p-value
T315I				
Normal T	20 (80.0)	2 (8.0)	34 (69.4)	0.000*
Mutant I	5 (20.0)	23 (92.0)	15 (30.6)	

Table 5: Distribution of BCR-ABL kinase domain (T315I) mutations according to the status of CML patients

Codon	Relapse N(%)	Under treatment N (%)	p-value	OR (95% CI)
T315I				
Normal T	22 (80.0)	34 (69.4)	-	1 (reference)
Mutant I	5 (20.0)	15 (30.6)	0.334	1.76 (0.56- 5.59)

*p<0.05 significantly association

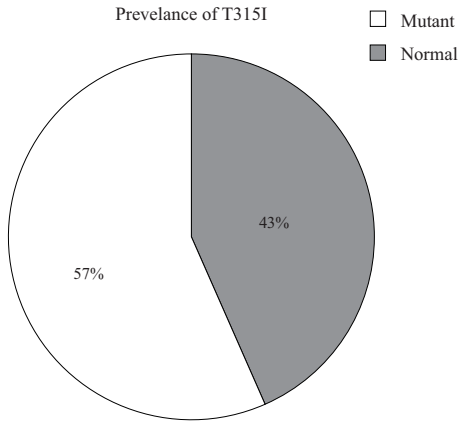

Fig. 1: Prevalence of BCR-ABL kinase domain mutations among CML patients

patients appeared as a significant positive correlation between T315I and haematological parameters (HB and WBC) but no correlation with PLT.

Table 3 shows that, the mean of Hb (10.95±1.51) was lower in T315I than in CML patients (11.52±1.79), whereas, WBC and PLT were higher in T315I (81.35±86.26, 238.40±127.24) than in CML patients (25.60±41.99, 211.13±78.38), respectively. PLT yielded no significant results, whereas, haematological parameter WBC yielded significant results (p = 0.00).

Genetic analysis

Kinase domain mutation analysis: The demographic profile, disease characteristics and molecular analysis of KD mutation.

ABLBCR T315I oncogene: The data revealed that 43 out of 99 CML had T315I, with highly prevalent gene express (43.4%) detected in all CML 56.6%, as shown in (Fig. 1).

The correlation of T315I mutations with clinical status was positive significant (p-000) with new cases, on treatment and resistance as shown in Table 4. T315I distribution was highest in (new case, on treatment and relapse), as shown in (Fig. 2).

Table 5 shows T315I has a slightly increased odds ratio but insignificant relationship with treatment when compared to relapse (OR1.76 (0.56-5.59)).

Demographic data of T315I KD patients: Table 6 shows that, the percentage of T315 is over 40 was 62.8%, while those under 40 were 37.2%. Females are more affected than males (55.8%) (44.2%) T315I has a stronger affinity for the tribes of the West, Northern, Central, Eastern and South (37.2, 37.2, 16.3 and 2.3%, respectively). When compared to gender and geographical distribution, age was found to be a risk factor for T315I.

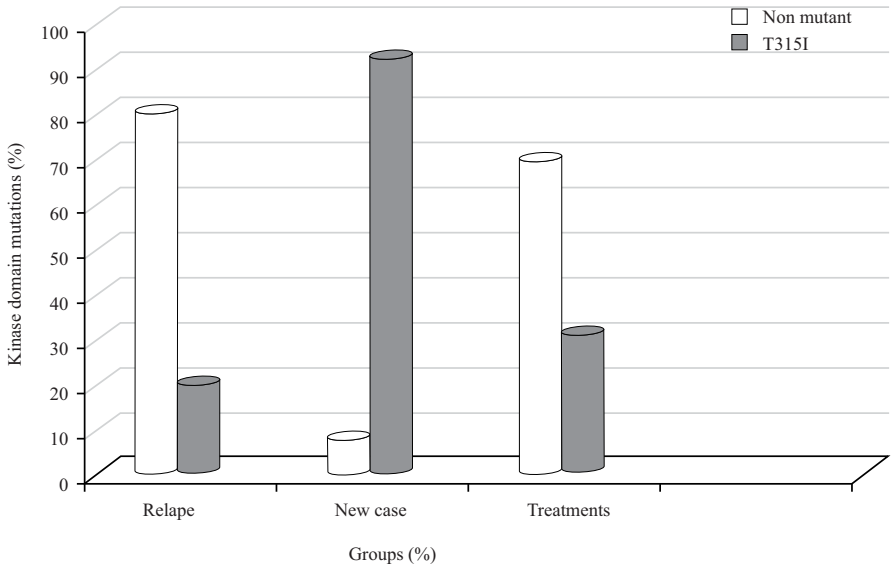

Fig. 2: Distribution of BCR-ABL kinase domain mutations percentage according to clinical status

Table 6: Risk factors associated with BCR-ABL (T315I) mutation among CML patients

	T315I		
Risk factors	Normal T	Mutant I	p-value
Age group			
<40	7 (12.5)	16 (37.2)	0.004*
≥40	49 (87.5)	27 (62.8)	
Gender N (%)			
Male	25 (44.6)	19 (44.2)	0.964
Female	31 (55.4)	24 (55.8)	
Geographical distribution N (%)			
Northern	17 (30.4)	16 (37.2)	0.804
Eastern	3 (5.4)	3 (7.0)	
Western	28 (50.0)	16 (37.2)	
Central	7 (12.5)	7 (16.3)	
Southern	1 (1.8)	1 (2.3)	

*p<0.05 significantly association

DISCUSSION

According to the 2013 ELN recommendations for CML management, all patients with a mutation in the ABL kinase domain had therapy failure. Mutations in the BCR-ABL kinase domain were the most common source of TKI resistance, with seven altered codons (G250E, Y253H, E255K, T315I, M351T, F359 and H396) accounting for 60-70% of cases[12,13].

The purpose of this research was to identify kinase domain mutations (T315I) in CML patients and assess their haematological parameters and medical status. The involved subjects in the current study are 99 CML patients were from a variety of tribes and geographic locations. The majority of them were female, 55.6%. Depending on their clinical situation, patients were separated into 3 groups (new case, under treatment, relapse). It has been discovered that people over the age of 40 made up 76.8% of the population, while those under the age of 40 made up 23.2% and most of T315I over 40 (62.8%), which was consistent with the literature review[14] T315I KD was a substantial risk factor with age when compared to gender, tribe. In our research, moderate anaemia was seen in CML patients. In new cases and relapse patients with CML, the mean of HB was lower. Overgrowth of blast cells inhibits the formation of other blood cells, resulting in a shortfall of red blood cells (anaemia). When compared to under treatment. This was in line with Liu, Z., Y. Shi, findings[14], which discovered moderate anaemia in CML-CP patients with high-risk scores.

A high significant count was discovered in the WBC analysis in both fresh cases and those who have relapsed on

treatment CML, WBC tests revealed a normal count, the increase in WBC count could be related to cell overgrowth or other mutations (BCR-ABL domain mutation. The relationship between haematological parameters and clinical status of CML patients was very significant with WBC and HB but not with platelets, according to our findings. The findings of previous studies[15,16] are similar to our research findings. Who started that there were significant results in HB and WBC of a new case and therapy CML patients.

There was a strong relationship between haematological parameters (WBC, HB) and T315I mutant patients. In T315I CML patients, my analysis found that HB reduced and WBC increased. Our research showed a correlation between mutations (T315I) and the status of CML patients (new, on treatment and relapse). In contrast, the worldwide described mutation prevalent most the be to discovered was T315I treatment line-first of causes common most the of one as well as literature, reviewed ABL to unique phenomena a considered be also should It patients. CML in resistance had acid amino polar a threonine replaces mutation T315I. The patient's domain kinase results as amino acid hydrophobic an isoleucine, with bonding, hydrogen in participates the between generated connection hydrogen key alteration, acid amino single this of disrupted be to demonstrated been has IM and domain kinase 1 ABL. This assigned binding protected BCR-ABL1 from IM inhibition and gave IM resistance[17].

The frequency of the T315I ABL kinase domain was 43.4% in our study, with the majority of them occurring in new cases. The results of this study correspond with those of Elias *et al.*[18], who found that the T315I mutation was 8 (61.5%), followed by E255K 2 (15.4%), M351T 1 (7.7%) and Y253H (7.7%). The results of this investigation differ from those of Dhahi *et al.*[19] and Quintas-Cardama *et al.* [20], who found 3% of T315I. T315I was estimated to be present in 7% of Resistance patients by Yap *et al.*[21]. CML patients who were resistant to IM had a detection rate of 12.5%[22]. Hughes *et al.*[23] found that 3% of people have T315I, 4% have E255K and 4% haveY253H.

Another study conducted by Elias *et al.*[24] reported that T315I mutation was T315I 9 (7.2%) E255K 4 (3.20%) M351T 2 (1.6%) Y253H 1 (0.8%). Tadesse *et al.*[25] detected non-P-loop mutations, T315I was detected in 4 patients (12.9%) and M351T was detected in one patient (3.2%). Among the P-loop mutationsY253H were detected in one patient (3.2%).

We recommend that genetic testing be performed to detect KD mutations using molecular techniques. This will aid in the selection of appropriate treatment strategies to prevent disease progression in CML patients. Because the number of samples used in the study was small, we will need to expand additional research into the ABL-BCR mutations that occur in CML patients using large samples including treated and non-treated patients.

CONCLUSION

The correlation of T315I mutations with clinical status was positive significant with new cases, on treatment and resistance, this study also revealed that a significant positive correlation between T315I and haematological parameters (HB and WBC) but no correlation with PLT. T315I mutation became much more common in CML patients than in other groups of mutations. The detection of ABL kinase domain mutations could be a useful and important tool for optimizing treatment approaches and avoiding therapy delays.

SIGNIFICANCE STATEMENT

This study discovers that T315I mutations had a favourable connection with the clinical state, with new cases, on therapy and resistance. This study will help the researcher to uncover the determination of ABL kinase domain mutations could be a helpful and crucial tool for optimizing treatment strategies and preventing therapy delays, may be arrived at.

ACKNOWLEDGMENT

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University.

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