Original Article

Simple multiplex RT-PCR for identifying common fusion BCR-ABL transcript types and evaluation of molecular response of the a2b2 and a2b3 transcripts to Imatinib resistance in North Indian chronic myeloid leukemia patients

Rashid Mir, Ahmad I[1], Javid J[1], Zuberi M[1], Yadav P[1], Shazia R[1], Masroor M[1], Guru S[1], Ray PC[1], Gupta N[2], Saxena A[1]

Prince Fahd Bin Sultan Research Chair, Faculty of Applied Medical Sciences, University of Tabuk, Saudi Arabia – 71491, [1]Cancer Genetics Lab, Department of Biochemistry and Associated Hospitals, [2]Department of Medicine, Maulana Azad Medical College and Associated Hospitals, New Delhi, India

Correspondence to: Dr. Alpana Saxena, E-mail: drrashidmamc@gmail.com

Abstract

INTRODUCTION: Chronic myeloid leukemia (CML) is characterized by the Philadelphia chromosome, an abnormally shortened chromosome 22. It is the result of a reciprocal translocation of chromosomes 9 and 22, creating BCR-ABL fusion transcripts, b3a2, b2a2, and e1a2. The aim of our study was to determine the type of BCR-ABL fusion transcripts for molecular diagnosis and investigate the frequency of BCR-ABL fusion transcripts in CML patients by multiplex RT-PCR in CML. **MATERIALS AND METHODS:** A single reaction with multiple primers multiplex PCR was used to detect and investigate the type and frequency in 200 CML patients among which 116, 33, and 51 were in CP, AP, and BC phase, respectively. **RESULTS:** The study included 200 CML patients, among whom breakpoints in b3a2, b2a2 transcripts were detected in 68% and 24%, respectively, while 8% of the patients showed both b3a2/b2a2. A statistically significant difference was seen between frequency of BCR-ABL fusion transcripts and gender ($P = 0.03$), molecular response ($P = 0.04$), and hematological response ($P = 0.05$). However, there was no correlation found between frequencies of BCR-/ABL fusion transcripts and other clinicopathological parameters like age, type of therapy, thrombocytopenia, and white blood cell count. **CONCLUSION:** Multiplex reverse transcriptase-polymerase chain reaction is useful and saves time in the detection of BCR-ABL variants; the occurrence of these transcripts associated with CML can assist in prognosis and treatment of disease.

Key Words: BCR-ABL gene, CML, a2b2 and a2b3 transcripts

Introduction

Chronic Myeloid leukemia (CML) is a pluripotent hematopoietic stem cell disorder.[1] Diagnosis of CML is based on the detection of BCR-ABL gene and is a product derived from translocation of chromosome 22 to chromosome 9. This reciprocal translocation gives rise to BCR-ABL fusion oncogene, which translates a chimeric protein, p210 BCR-ABL that is characterized by constitutive activation of its tyrosine kinase activity. It is found in 95% of CML, 20-40% of adult acute lymphoblastic leukemia (ALL), 5% of childhood ALL, and 2% of myeloblastic leukemia (AML). The BCR-ABL gene encodes different fusion proteins that vary in size depending on the breakpoint in the BCR gene. In general, three breakpoint cluster regions in the BCR gene have been described: M-BCR, m-BCR, and u-BCR [Figure 1].[2] Break points occurring in M-bcr joins exon 13 (also known as b2) or 14 (also known as b3) with exon 2 of abl (a2) resulting in the fusion transcripts b2a2 and b3a2, respectively.[3,4]

As a result of alternate splicing these transcripts lead to the production of a 8.5 kb transcript coding for a 210-kDa (p210) chimeric protein.[5,6] The breakpoint in the m-BCR region results in an e1a2 junction, which is translated into a smaller p190 (e1a2) BCR-ABL protein.[7] In some cases, the breakpoint between BCR exon 19 and 20 in the u-BCR region induces a larger p230 BCR-ABL protein.[8-11] The e1a2 fusion transcript is seen primarily in t (9; 22)-positive acute lymphoblastic leukemia and lymphoid blast phase of the CML, but rarely in chronic phase of CML.

Access this article online

Quick Response Code:

Website: www.indianjcancer.com

DOI: 10.4103/0019-509X.176741

PCR studies to monitor both response and minimal residual disease have adopted several modifications including a qualitative RT-PCR,[12,13] and quantitative RT-PCR measurements such as the commercial real time RT-PCR, using for internal controls fluorescent double-stranded DNA dyes or fluorescein probes, or the non-commercial real-time quantitative PCR (RQ-PCR) using for internal controls ABL, BCR, Beta-2-microglobulin, or others.[14,15]

RQ-PCR techniques have become a standard for the molecular monitoring of minimal residual disease in hematologic malignancies. RQ-PCR-based molecular monitoring of BCR-ABL transcripts is the most sensitive tool for assessing disease burden in patients with CML and is typically able to detect one leukemia cell in a background of approximately 100,000 normal cells. Molecular monitoring of residual disease by quantitation of BCR-ABL mRNA is required to stratify treatment response and to detect early response The treatment of patients with Philadelphia chromosome (Ph)-positive CML with Imatinib mesylate has resulted in complete cytogenetic response rates of 65% to 85%, major molecular response (MMR) rates (i.e. 3-log reduction in transcript levels from a standardized baseline) of 40% to 70%, and complete molecular response rates (i.e., undetectable *BCR-ABL* transcripts) of 10% to 40%.[15-18] The frequency of BCR-ABL gene splice variants associated with CML was calculated in our study. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was chosen as the method of choice to detect the BCR-ABL gene as this technique is one of the most sensitive methods for this purpose.

Materials and Methods

Patients and samples

The institutional ethics committees of Maulana Azad Medical College, New Delhi, India, approved the study and all patients provided written informed consent. Peripheral

blood samples i.e. 5 ml venous blood samples were collected in EDTA vials from CML patients. Buffy coat was isolated and washed in red cells lysis buffer. All samples were stored at −80°C until the RNA was extracted.

The study included diagnosed CML patients of all the three stages from chronic phase, accelerated phase and blast crisis. All patients were treated with Imatinib with a dose of 400 mg/day. All patients gave written informed consent to participate in the study before entry, and the study was reviewed and approved by a recognized ethics review committee. The exclusion criteria included chronic myelomonocytic leukemia (CMML) patients, other myeloproliferative disorder patients.

Patient evaluation

Patients were evaluated for hematologic and molecular responses and relapse at specified intervals. The patient follow up was maintained regularly and peripheral blood samples were obtained and analyzed, after every 3 months of treatment until achieved and confirmed. The classic criteria used for Imatinib mesylate responses in CML for hematologic and molecular responses are depicted in [Table 1a and b], respectively.

RNA isolation

Total RNA was isolated from mononuclear cells with guanidinium isothiocyanate (Trizol LS™ - Invitrogen), according to the protocol provided by the manufacturer. The presence of RNA was confirmed by running the product on 2% agarose gel.

cDNA synthesis

The concentration of RNA was measured spectrophotometrically. cDNA was then synthesized using M-MuLV Reverse Transcriptase and other reaction components (Fermentas CAT# K1622), according to the protocol provided by the manufacturer.

B2-microglobulin PCR

cDNA quality was checked by B2 microglobulin PCR.[19] The thermo cycling conditions were 40 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s and extension at 72°C for 60 s, then a final extension at 72°C for 10 minutes. The PCR products were then analyzed on a 2.5% agarose gel. The expected band size was 443 bp [Figure 2a].

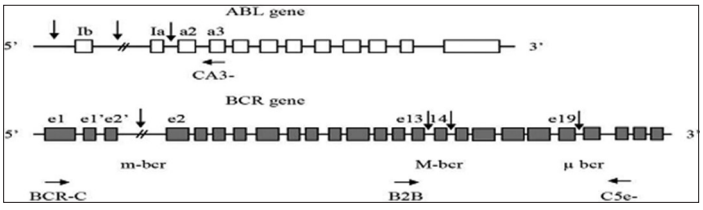

Figure 1: Schematic representation of BCR-ABL splicing variant transcripts

Multiplex RT–PCR for BCR-ABL

BCR-ABL transcripts were detected using multiplex RT-PCR primers for p210 and p190 primer sequences, as already described,[20] listed in Table 2. PCR was carried out in a total volume of 25 μl reaction mixture containing 1 U/μL Taq polymerase, 240 μM dNTP, 1.8M $MgCl_2$, and 0.6 μM of primers. A program was employed, under the following conditions: an initial denaturation step at 95°C for 10 min then followed by 40 cycles of denaturing at 94°C for 40 s, primer annealing at 55°C, extension at 72°C for 45 s, and a final extension step at 72°C for 5 minutes. The expected bands were as follows: 808 bp, normal BCR; 481 bp, e1a2; 385 bp, b3a2; 310 bp, b2a2.

Statistical analysis

The Chi-square method was applied for comparing obtained BCR-ABL1 variants of the CML patients by gender. Statistical analysis was performed using the SPSS 16.0 software package. Chi-square analysis and Fisher exact test were carried out to compare the difference of frequencies between groups of patients. A P value of < 0.05 was considered statistically significant.

Results

Clinical-pathological profiles of CML patients

Peripheral blood samples from 200 CML patients were collected with the informed consent of all patients and approval of the ethics committee, Maulana Azad Medical College and associated Hospitals, New Delhi. 116 patients were in chronic phase, 33 in accelerated phase, and 51 were in blast phase. Clinical-pathological features including the age and gender of patients, BCR-ABL type, thrombocytopenia, molecular response, hematological response (HR), and age group are shown in Table 3.

Frequency of BCR-ABL transcripts

In the present study, multiplex RT-PCR was used to detect all the BCR-ABL rearrangements [Figure 2b]. Of the 200 patients studied, we could reliably detect b2a2, b3a2 and b3a2/b2a2 transcripts in 48 (24%), 136 (68%), and 16 (8%) patients, respectively. Among 200 CML patients, 128 were males, 85 of which patients were expressing b3a2 transcript, 33 b2a2, 10 both b3a2/b2a2 transcripts and 72 females of which 51, 15, 6 showing b3a2, b2a2, and b3a2/b2a2, respectively, with a statistical

Table 1a: Hematologic responses criteria in CML patients

Complete or major hematological response	Partial or minor hematological response	No or minimal hematological response
Platelet count: >150×109/L	Platelet count: <450×109/L	Platelet count: <450×109/L
WBC count: <10×109/L	WBC count: >10×109/L	WBC count: >20×109/L
Basophils: <5%	Basophils: >10%	Basophils: >15%
Absence of blasts and promyelocytes in peripheral blood	Presence of blasts and promyelocytes in peripheral blood	Presence of blasts and promyelocytes in peripheral blood
Spleen: Nonpalpable spleen	Spleen: Palpable spleen	Spleen: Palpable spleen

significance (P = 0.03) between gender and BCR-ABL type. Table 4 shows clinical data of patients according to their BCR-ABL transcripts.

BCR-ABL transcripts with Molecular response

A significant correlation was found between BCR-ABL transcripts frequency and molecular response (P = 0.04). 72.1% b3a2 transcripts were detected by Major MR group, whereas 64% b3a2 frequency was detected in No MR group of patients, Major molecular responders showed 24.7% b2a2 frequency, 23.3% b2a2 transcripts was shown by No MR patients. Only 3% MMR patients had both transcripts whereas 12.6% No MR patients showed both b3a2/b2a2 transcripts.

BCR-ABL transcripts with hematological response

Patients were divided into three groups Major HR, Minor HR, and Loss HR. 22.2% in MHR, 31.0% Minor HR, 23.4% in Loss HR showed b2a2 transcripts, 74.4% in Major HR, 55.1% in Minor HR and 65.4% Loss HR patients were showing b3a2 transcripts and 3.3% in Major HR, 13.7% in Minor HR and 11.1% in Loss HR were showing both transcripts. A significant correlation was found between BCR-ABL transcripts frequency and HR (P = 0.05).

Discussion

CML is a stem cell pluripotent myeloproliferative disorder and consistently associated with the BCR-ABL fusion gene. In this study simple multiplex RT-PCR was used for the diagnosis of different BCR-ABL transcripts in CML patients of north India. All the transcripts can be known by multiplex RT-PCR by using more than one pair of primers.

Multiplex RT-PCR is the simultaneous detection of more than one target sequence in a single reaction tube using more than one primer pair. The benefit of multiplex approach is to simplify and shortens the procedure of RT-PCR. This co-amplification of two or more targets in a single reaction is dependent on the compatibility of the PCR primers used in the reaction. Therefore, the detection of p210 and p190 in single patient require multiple procedures and it is time consuming and expensive. Multiplex PCR assay is clinically useful, efficient, and fast procedure for the detection of genetic changes especially in CML. RT-PCR is by far the most sensitive method to detect residual disease in CML and can enable a single leukemia cell to be detected in a background of 10^5 to 10^6 normal cells.

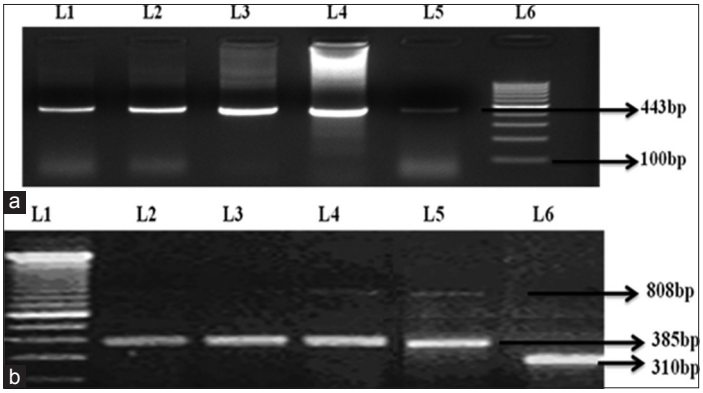

Figure 2: (a) EtBr stained gel electrophoresis image of B2-microglobulin cDNA quality check PCR. (b) EtBr stained gel electrophoresis image of BCR-ABL transcripts. L1.100bp ladder, L2, L3, L4, L5. b3a2 transcript (385 bp). L6. b2a2 transcript (310bp)

Primers used could detect p210 transcripts, such b2a2 or b3a2, and p190 BCR-ABL transcripts, such as e1a2 in 200 north Indian patients. The study showed that the frequency of b2a2, b3a2, b2a2/b3a2 transcripts were 24%, 68%, and 8%, respectively.

The incidence of b2a2, b3a2 in CML was found to be 40.9% and 59.0% respectively by Jose Alexander *et al*.[21] Marjan *et al*. found that the frequency of b2a2, b3a2 transcripts was 20% and 63%, respectively, in Iranian population.[22] A study on Sudanese patients by Emadaldin

Table 1b: Molecular responses criteria in CML patients

Major Molecular Response	Minimal or No Molecular response
It indicates nonquantifiable and nondetectable BCR-ABL gene transcript (BCR-ABL/ABL) <0.10.*	It indicates quantifiable and detectable BCR-ABL gene transcript (BCR-ABL/ABL) >0.10.*
Check every three months	Check every three months

*BCR-ABL to control gene ratio according to international scale (IS)

Table 2: Sequence of oligonucleotides used in multiplex RT-PCR for detection of BCR-ABL transcript as the target gene and BCR transcripts as the internal control

	BCR-ABL primers
C5e	5'-ATAGGATCCTTTGCAACCGGGTCTGAA-3'
B2B	5'-ACAGAATTCCGCTGACCATCAATAAG-3'
BCR-C	5'- ACCGCATGTTCCGGGACAAAAG-3'
CA3	5'-TGTTGACTGGCGTGATGTAGTTGCTTGG-3'

Table 3: Clinical pathological features of CML patients

Clinical pathological features	No. of cases
Patients	200
Gender	
Males	128
Female	72
Stage	
CP-CML	116
AP-CML	33
BC-CML	51
Therapy	
Imatinib	200
Interferon	36
Age group	
Age >45	66
Age <45	134
Molecular response	
MMR	97
No MR	103
Hematological response	
MHR	90
Minor HR	29
Loss HR	81
Thrombocytopenia	100

Table 4: clinical data of patients according to their BCR-ABL transcripts

Clinical parameter	cases	a2b2	%	a2b3	%	a2b2/a2b3	%	*P*
Males	128	33	25	85	66.4	10	7.8	0.03
Females	72	15	20.8	51	70.8	6	8.3	
Cp CML	116	28	24.1	83	71.5	5	4.3	0.1
AP CML	33	9	27.2	21	63.6	3	9	
BC CML	51	11	21.1	32	62.7	8	15.6	
MMR	97	24	24.7	70	72.1	3	3.0	0.04
NO MR	103	24	23.3	66	64.0	13	12.6	
MAJOR HR	90	20	22.2	67	74.4	3	3.3	0.05
MINOR HR	29	9	31.0	16	55.1	4	13.7	
LOSS HR	81	19	23.4	53	65.4	9	11.1	
Imatinib mesylate	200	48	24.0	136	68.0	16	8.0	0.9
Interferon	36	10	27.7	22	61.1	4	11.1	
AGE >45	66	15	22.7	44	66.6	7	10.6	0.6
AGE <45	134	33	24.6	92	68.6	9	6.7	

et al. showed incidence of b2a2, b3a2 as 50% and 39.6%.[23] Dario *et al*. in Argentinean patients found frequency of b2a2, b3a2 to be 41.6% and 37.5%, respectively.[24] Hyan gyong *et al*. showed 31.75% and 66.4% b2a2, b3a2 frequency in Korean patients.[25] Rosiline *et al*., found frequency 15.3% and 84.6% for b2a2 and b3a2 in Malay patients.[20] The frequencies of both fusion transcripts were found to be 63.33% and 36.66% for b3a2 and b2a2 respectively in Pakistani population.[26] In Thailand, CML patients were positive for 61.00% b3a2 and 31.00% b2a2.[27] Verschraegen *et al*. found that the frequency of b2a2 and b3a2 transcripts was 30.2% and 67.9%, respectively.[28] In a study by Reiter *et al*., the incidence of b2a2 and b3a2 transcripts in CML patients with Philadelphia chromosome was 31.6% and 68.4%, respectively.[29] The frequency of b3a2 and b2a2 BCR-ABL transcripts is shown in Figure 3.

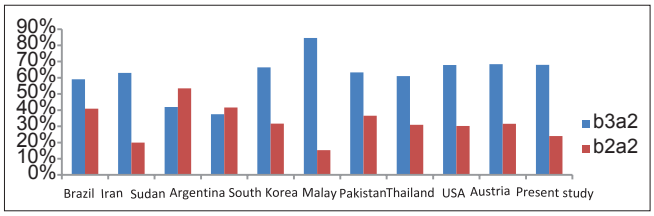

Figure 3: Frequency of b3a2 and b2a2 BCR-ABL transcripts

Most studies have shown that b3a2 expresses more in CML patients than b2a2; however, few studies have shown b2a2 transcript frequency more in CML patients than more common b3a2 transcript.[23,24] Several studies have reported that patients with b3a2 had bad prognosis than patients with b2a2 transcripts and response to Imatinib.[30,31] However, in our study we did not find any significant difference of BCR-ABL type and prognosis.

We found a significant association of BCR- ABL transcript type and gender. Similar results were detected by Emad-Aldin *et al*.[23] and Alder *et al*.[32] in Sudanese and German population, respectively.

We also found a statistically significant correlation of BCR-ABL transcript type with hematological and molecular responses. However no other correlations was found between CML BCR-ABL transcript type and other clincopathalogical parameters like age, stage, type of therapy, white blood count, platelet count, thrombocytopenia, etc.

We also detected 16 patients with both b3a2/b2a2 transcripts. Patients with both transcripts as a result alternate splicing mechanism were associated with poor prognosis with11/16 (69%) patients in progression phase, 13/16 (81%) showing no molecular response whereas 4/16 (25%) with minor HR and 9/16 (56%) with loss of HR. Hence, our study concludes that patents with both b3a2/b2a2 transcripts can be associated with bad prognosis of CML; however, there was no association of b2a2 or b3a2 transcripts found with bad prognosis of patients with CML.

Conclusion

In conclusion, multiplex-PCR is a simple, economical, time-saving technique that allows specific and simultaneous detection of the three most frequent BCR-ABL transcripts in patients with CML.

References

1. Chasseriau J, Rivet J, Bilan F, Chomel JC, Guilhot F, Bourmeyster N, *et al*. Characterization of the different BCR-ABL transcripts with a single multiplex RT-PCR. J Mol Diagn 2004;6:343-7.
2. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 1996;88:2375-84.
3. Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. Nature 1985;315:550-4.
4. Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON Baltimore D. The chronic myelogenous leukemiaspecific p210 protein is the product of the bcrabl hybrid gene. Science 1986;233:212-4.
5. Okamoto K, Karasawa M, Sakai H, Ogura H, Morita K, Naruse T. A novel acute lymphoid leukemia type BCRABL transcript in chronic myelogenous leukaemia. Br J Haematol 1997;96:611-3.
6. Lichty BD, Keating A, Callum J, Yee K, Croxford R, Corpus G, *et al*. Expression of p210 and p190 BCR-ABL due to alternative splicing in chronic myelogenous leukaemia. Br J Haematol 1998;103:711-5.
7. Hermans A, Heisterkamp N, von Linden M, van Baal S, Meijer D, van der Plas D, *et al*. Unique fusion of bcr and c-abl genes in Philadelphia chromosome- positive acute lymphoblastic leukemia. Cell 1987;51:33-40.
8. Pane F, Frigeri F, Sindona M, Luciano L, Ferrara F, Cimino R, *et al*. Neutrophilic chronic myeloid leukemia: A distinct disease with a specific molecular marker (BCR-ABL with C3/A2 junction). Blood 1996;88:2410-4.
9. Mittre H, Leymaire P, Macro M, Leporrier M. A new case of chronic myeloid leukemia with c3-a2 BCR-ABL junction. Is it really a distinct disease? Blood 1997;89:4239-41.
10. Haskovec C, Pozetto C, Polak J, Maritano D, Serra A, Klamova H, *et al*.

P230 BCR-ABL protein may be associated with an acute leukemia phenotype. Br J Haematol 1998;103:1104-8.
11. Lee JJ, Kim HJ, Kim YJ, Lee S, Hwang JY, Kim YL, *et al*. Imatinib induces a cytogenetic response in blast crisis or interferon failure chronic myeloid leukemia patients with e19a2 BCR-ABL transcripts. Leukemia 2004;18:1539-40.
12. Radich JP, Gooley T, Bryant E, Chauncey T, Clift R, Beppu L, *et al*. The significance of BCR-ABL molecular detection in chronic myeloid leukemia patients "late", 18 months or more after transplantation. Blood 2001;98:1701-7.
13. Radich JP, Gehly G, Gooley T, Bryant E, Clift RA, Collins S, *et al*. PCR detection of the BCR-ABL fusion transcript after allogeneic marrow transplantation for chronic myeloid leukemia: Results and implications in 346 patients. Blood 1995;85:2632-8.
14. Wang YL, Bagg A, Pear W, Nowell PC, Hess JL. Chronic myelogenous leukemia: Laboratory diagnosis and monitoring. Genes Chromosomes Cancer 2001;32:97-111.
15. Cross NC, Feng L, Chase A, Bungey J, Hughes TP, Goldman JM. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood 1993;82:1929-36.
16. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, *et al*. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006;355:2408-17.
17. Kantarjian HM, Talpaz M, O'Brien S, Jones D, Giles F, Garcia-Manero G, *et al*. Survival benefit with imatinib mesylate versus interferon alpha-based regimens in newly diagnosed chronic phase chronic myelogenous leukemia. Blood 2006;108:1835-40.
18. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, *et al*. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003;348:994-1004.
19. Diss JK, Fraser SP, Walker MM, Patel A, Latchman DS, Djamgoz MB. β-Subunits of voltage-gated sodium channels in human prostate cancer: Quantitative *in vitro* and *in vivo* analyses of mRNA expression. Prostate Cancer Prostatic Dis 2008;11:325-33.
20. Hassan R, Ramli M, Abdullah WZ, Mustaffa R, Ghazali S, Ravindran A, *et al*. One-step multiplex RT-PCR for detection of BCR/ABL gene in Malay patients with chronic myeloid leukaemia. Asia Pac J Mol Biol Biotechnol 2008;16:41-4.
21. de Lemos JA, de Oliveira CM, Scerni AC, Bentes AQ, Beltrão AC, Bentes IR, *et al*. Differential molecular response of the transcripts B2A2 and B3A2 to imatinib mesylate in chronic myeloid leukemia. Genet Mol Res 2005;4:803-11.
22. Yaghmaie M, Ghaffari SH, Ghavamzadeh A, Alimoghaddam K, Jahani M, Mousavi SA, *et al*. Frequency of BCR-ABL Fusion Transcripts in Iranian Patients with Chronic Myeloid Leukemia. Arch Iranian Med 2008;11:247-51.
23. Osman EA, Hamad K, Elmula IM, Ibrahim ME. Frequencies of BCR-ABL1 fusion transcripts among Sudanese chronic myeloid leukaemia patients. Genet Mol Biol 2010;33:229-31.
24. Darío A. Sastre, Carlos E. Argaraña, Viviana B. Heller, Mónica Gallo, Enrique N. Fernández and Cecilia M. RodríguezSastre DA, Argaraña CE, Hel An analysis of multiplex-PCR in the detection of BCR-ABL transcripts in hematological disorders. Genet Mol Biol 2007;30:520-3.
25. Goh HG, Hwang JY, Kim SH, Lee YH, Kim YL, Kim DW. Comprehensive analysis of BCR-ABL transcript types in Korean CML patients using a newly developed multiplex RT-PCR. Transl Res 2006;148:249-56.
26. Iqbal Z, Manzoor F, Iqbal M, Ali S, Sheikh N, Khan M, *et al*. Frequency of Bcr-Abl Fusion Oncogene Splice Variants Associated with Chronic Myeloid Leukemia (CML) J Cancer Ther 2011;2:176-80.
27. Udomsakdi-Auewarakul C, U-Pratya Y, Boonmoh S, Vatanavicharn S. Detection of molecular variants of BCR-ABL gene in bone marrow and blood of patients with chronic myeloid leukemia by reverse-transcriptase polymerase chain reaction (RT-PCR). J Med Assoc Thai 2000;83:928-35.
28. Verschraegen CF, Kantarjian HM, Hirsch-Ginsberg C, Lee MS, O'Brien S, Rios MB, *et al*. The breakpoint cluster region site in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. Clinical, laboratory, and prognostic correlations. Cancer 1995;76:992-7.
29. Reiter E, Greinix HT, Brugger S, Keil F, Rabitsch W, Mannhalter C, *et al*. Long-term follow-up after allogeneic stem cell transplantation for chronic myelogenous leukemia. Bone Marrow Transplant 1998;22:S86-8.
30. de Lemos JA, de Oliveira CM, Scerni AC, Bentes AQ, Beltrão AC, Bentes IR, *et al*. Differential Molecular Response of the Transcripts B2A2 and B3A2 to Imatinib Mesylate in Chronic Myeloid Leukemia. Genet Mol Res 2005;4:803-11.
31. Sharma P, Kumar L, Mohanty S, Kochupillai V. Response to Imatinib Mesylate in Chronic Myeloid Leukemia Patients with Variant BCR-ABL fusion Tran-scripts. Ann Hematol 2010;89:241-7.
32. Adler R, Viehmann S, Kuhlisch E, Martiniak Y, Röttgers S, Harbott J, *et al*. Correlation of BCR/ABL transcript variants with patients' characteristics in childhood chronic myeloid leukaemia. Eur J Haematol 2009;82:112-8.

How to cite this article: Mir R, Ahmad I, Javid J, Zuberi M, Yadav P, Shazia R, *et al*. Simple multiplex RT-PCR for identifying common fusion BCR-ABL transcript types and evaluation of molecular response of the a2b2 and a2b3 transcripts to Imatinib resistance in North Indian chronic myeloid leukemia patients. Indian J Cancer 2015;52:314-8.
Source of Support: Nil. **Conflict of Interest:** None declared.