

Detection of BCR-ABL fusion gene and its transcript variants in chronic myeloid leukaemia patients — a multi-comparison study

Ali Javed,¹ Hamid Mukhtar,² Kadija tul Kubra,³ Sidrah Lodhi,⁴ Sajid Abaidullah⁵

Abstract

Objective: To compare blood cells and plasma for BCR-ABL quantification and to find out the frequency of b2a2, b3a2 and e1a2 transcripts in chronic myeloid leukaemia patients, and to assess the correlation of BCR-ABL transcripts with haematological counts, age and gender.

Methods: The study was conducted in April 2018 at King Edward Medical University, Lahore, Pakistan, and comprised chronic myeloid leukaemia patients from Mayo Hospital, Lahore. Ribonucleic acid was extracted using commercial extraction kits and detection of BCR-ABL messenger ribonucleic acid and its transcript variants was done by real time polymerase chain reaction. Data was analysed using SPSS 11.5.

Results: Of the 48 patients, fusion of b3a2 was detected in 32(66.66%) and b2a2 in 10(32.10%), while fusion e1a2 was not detected at all. No co-expression of transcripts was seen in any patient. No significant correlation was found between transcript type and any of haematological parameters ($p>0.05$). No significant correlation of transcript type with gender and age was found ($p>0.05$). BCR-ABL/G6PD ratios in peripheral blood cells were higher than that of plasma ($p<0.05$).

Conclusion: Plasma can be used as an alternative to blood cells for BCR-ABL quantification, and transcript types cannot be easily explained by clinical factors.

Keywords: BCR-ABL, RT-PCR, Chronic myeloid leukaemia. (JPMA 70: 1748; 2020)

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Introduction

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative neoplasm having annual incidence worldwide of 1-2 cases per 100000 population.¹ CML is characterised by the existence of Philadelphia chromosome formed by the translocation between chromosome 9 and chromosome 22. Fusion of breakpoint cluster region (BCR), a housekeeping gene belonging to chromosome 22 with Abelson (ABL) gene of chromosome 9 results in the formation of translocation t (9:22) and the fused BCR-ABL 1 gene.¹ BCR gene has adjacent breakpoints in it, and it encodes BCR protein, while ABL encodes Abelson murine leukaemia viral oncogene homolog 1.¹ BCR-ABL1 messenger ribonucleic acid (mRNA) is transcribed from this BCR-ABL1 gene. This mRNA translates to form BCR-Abl1 protein. This resultant protein starts the unwanted auto-phosphorylation and uncontrolled signalling to the downstream proteins which initiates the activation of undesirable cellular pathways.²

Due to the presence of different types of breakpoints in the BCR gene, BCR-ABL gene encodes fusion proteins of different sizes. All these differently encoded proteins

share high tyrosine kinase activity.³ Three main breakpoints in the BCR gene have been reported so far: major (M-BCR), minor (m-BCR) and micro (μ -BCR).⁴ Further, 90% of Ph+ve CML patients possess M-BCR. Two types of breakpoints are involved in M-BCR which form two types of fusion transcripts: b3a2 and b2a2. Transcript b3a2 (e14a2) involves the breakpoint after 14th exon of BCR (b3) and the transcript b2a2 (e13a2) involves the breakpoint after 13th exon of BCR (b2). In both transcripts, the breakpoint fuses with exon 2 (a2) of ABL gene. Protein p210 BCR-ABL is encoded by both of these fusion mRNA transcripts.⁵ Protein p210 BCR-ABL may also be encoded from other rare junctions. Other types of leukaemia may also contain some forms of this protein.^{6,7}

Fusion e1a2 involves the breakpoint in the m-BCR region. Protein p190 BCR-ABL is encoded by this transcript. This protein induces some types of acute lymphoblastic leukaemia (ALL).⁸ Another type of BCR-ABL protein, p230 BCR-ABL can also be seen in CML patients. Transcript e19a2 (having breakpoint in μ -BCR region) encodes this protein. Protein p230 BCR-ABL is often associated with neutrophil maturation.⁹ Rare variants of BCR-ABL have also been studied in some CML cases.¹⁰ BCR-ABL fusion transcripts have been reported to show significance in prognosis and treatment in CML patients.¹¹ Some studies show no prognostic significance of BCR-ABL transcript variants.¹²

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¹⁻³Government College University, Lahore, ^{4,5}King Edward Medical University, Lahore, Pakistan.

Correspondence: Ali Javed. Email: ali.javed102@yahoo.com

The diagnosis of typical CML can be done by assessing presence of consistent unexplained leucocytosis, presence of Ph chromosome abnormality by routine cytogenetics or molecular BCR-ABL anomalies by fluorescence in situ hybridisation (FISH) and by molecular methods.¹³ Reverse transcriptase polymerase chain reaction (RT-PCR) detects BCR-ABL by amplifying the region around fusion point between BCR and ABL. It is extremely sensitive for minimal residual disease detection. Quantitative RT-PCR is now used as standard method for molecular monitoring of CML.¹⁴

Generally, in tumour diagnosis or evaluation, the tumour-associated mRNA is extracted from the tissue which contains tumour cells. It is difficult to evaluate the residual leukaemia cells in the body by taking bone marrow or peripheral blood as the source of leukaemia cells because leukaemia cells are not evenly distributed in peripheral blood and bone marrow cells. Extraction of the tissue containing the tumour is also a difficult task. In some studies, plasma has been shown to possess tumour-specific deoxyribonucleic acid (DNA), RNA and proteins.¹⁵ Tumour-associated free mRNA has been reported to exist in whole blood and separated plasma.^{16,17} So, for the evaluation of treatment efficiency or residual tumour burden, quantification of tumour-associated free mRNA in whole blood or plasma / serum can be an efficient method.

The current study was planned to determine the frequency of b3a2, b2a2 and e1a2 transcripts in CML patients, and to determine their relationship with clinical factors like blood count, age and gender. A comparison was also planned of blood cells and plasma samples for BCR-ABL mRNA extraction and detection.

Patients and Methods

The study was conducted in April 2018 at King Edward Medical University, Lahore, Pakistan, and comprised CML patients from Mayo Hospital, Lahore. After approval from the ethics review board of Government College University Lahore, CML patients were included who were BCR-ABL positive either on the basis of FISH analysis or on the basis of routine cytogenetic tests. After taking informed consent from the patients, blood cell counts, including total leukocyte count (TLC), haemoglobin (Hb) and platelet count (PC) were recorded.

Venous blood 3-5ml was drawn from the antecubital vein by aseptic technique. Plasma from whole blood was separated by centrifuging the sample-containing Ethylenediaminetetraacetic acid (EDTA) vials at 2000-2500 rpm for 5-10 min. Supernatant (plasma) was pipetted out and saved in aliquots at -20°C. RNA

extraction from plasma samples was done using QIA symphony SP (QIAGEN) with QIA symphony® DSP Virus/Pathogen mini-kit. It is a silica-based nucleic acid purification method. Peripheral blood mononuclear cells of some patients were also used as a source for RNA extraction for the sake of comparison, BCR-ABL detection and quantification in paired plasma and blood cells. Isolation of peripheral blood mononuclear cells (PBMCs) was done by using Histopaque®-1077 (Sigma-Aldrich, Germany). RNA from PBMCs was extracted using TRIzol reagent. Extracted RNA was quantified using Nanodrop Spectrophotometer (Thermo Scientific™).

For RT-PCR, the complementary DNA (cDNA) of BCR-ABL1 transcript (GenBank: AJ131467.1) was synthesised from the extracted RNA using Superscript III 1st Strand Synthesis Kit (Invitrogen™). Quantitative RT-PCR amplification was done using CFX Connect Real-Time PCR Detection System (BIORAD). The amplification of target BCR-ABL1 transcript and internal control gene glucose 6-phosphate dehydrogenase (G6PD) (GenBank: MF796526.1) was carried out by using SsoAdvanced™ Universal SYBR® Green Super mix (BIORAD). G6PD standards were used for calculating quantity of BCR-ABL in samples. Primer sequences used in real time RT-PCR were designed by Primer-Blast (NCBI) (Table).

PCR was performed in total volume of 20µl by adding SsoAdvanced™ Universal SYBR® Green Supermix (BIORAD) (2x), Forward primer (10 µM), Reverse primer (10 µM), Nuclease free H₂O, cDNA (100ng). PCR was started with 1min at 94°C for the first step and then 40 cycles were run as follows: 1min at 94°C, 1min at 64°C, 1min at 72°C and final extension was 10min at 72°C. Normalised expression of BCR-ABL was obtained from BCR-ABL/G6PD ratios. Separate amplification reactions for b2a2, b3a2 and e1a2 transcripts were then carried out in line with literature¹⁸. Specific primers with same PCR components were used in these reactions.

Statistical analysis was done using SPSS 11.5. FISH and RT-PCR results were compared by kappa test. Correlations between BCR-ABL/G6PDH ratios in paired plasma and peripheral blood cell samples were performed using Pearson's correlation coefficient test because the data was following normality. Chi-square test was used to compare transcript type with gender and age groups. Analysis of variance (ANOVA) (Post-Tukey test) was applied for comparison of transcript type with PC, Hb and TLC.

Results

Of the 48 patients, 40(83%) were diagnosed on the basis of FISH. Overall, 18(37.5%), 36(75%) and 17(35.4%) patients had TLC >100x10³/µl, Hb <12mg/dl and PC <150

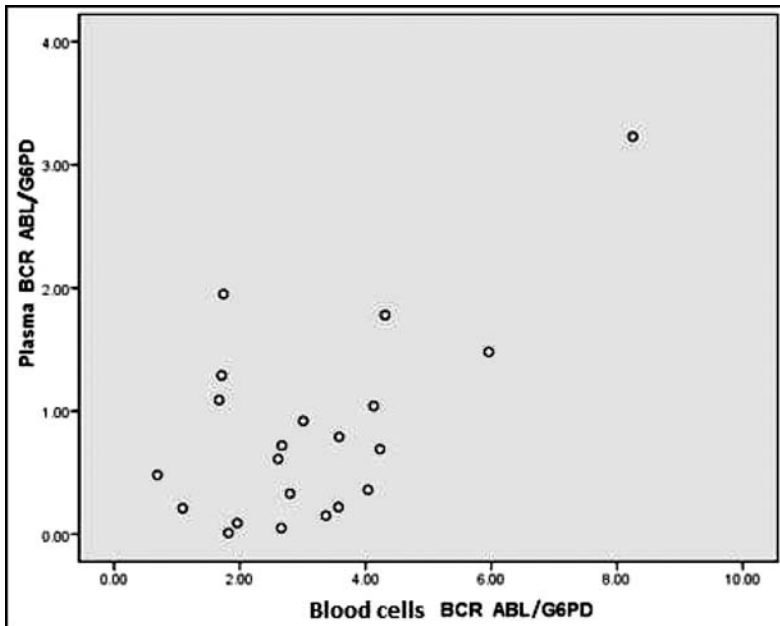


Figure-1: Relationship between BCR-ABL/G6PD ratio in plasma and blood cells.

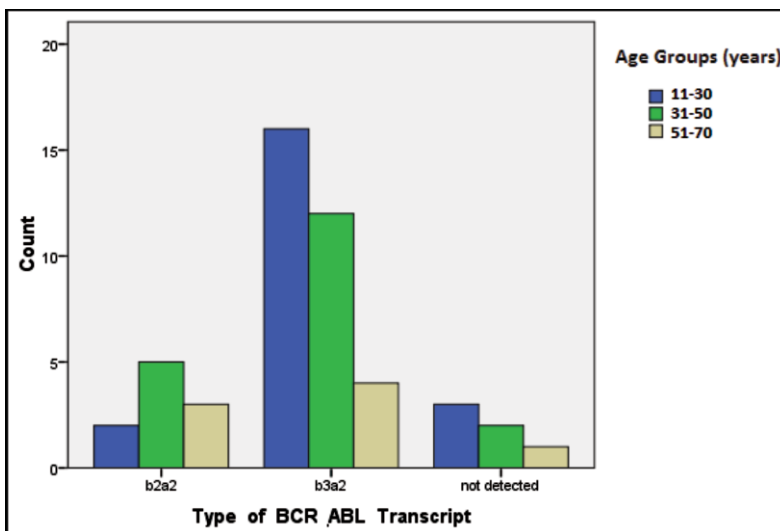


Figure-2: Distribution of the age group by the type of fusion transcript of BCR-ABL in patients.

Table: Sequences of primers used in polymerase chain reaction (PCR).

Gene	Primer Sequence
BCR-ABL1-Forward	5'-CTGAATGTCATCGTCCACTCA-3'
BCR-ABL1-Reverse	3'-CGCGTCTTTGCTTTATTACACA-5'
G6PD-Forward	5'-TGAGCCAGATAGGCTGGAA-3'
G6PD-Reverse	5'-TAACGCAGGCG-ATGTTGTC-3'
BCR e1-Forward	5'-CGAAGACCGGGCAGAT-3'
BCR b2-Forward	5'-GCATTCCGCTGACCATCAAT-3'
BCR b3-Forward	5'-TCCACTCAGCCACTGGATTAA-3'
ABL a2-Reverse	5'-TGGGTCCAGCGAGAAGGTT-3'

$\times 10^3/\mu\text{l}$ respectively.

BCR-ABL was detected in 42(87.5%) samples by RT-PCR. The mean BCR-ABL/G6PD ratio in 42 plasma samples was 0.88 ± 0.71 (range: 1.2-100.5). In 25(52%) paired plasma and blood samples, BCR-ABL/G6PD ratios in blood cells were much higher than in plasma. Mean BCR-ABL/G6PD ratio of blood cells was 3.13 ± 1.72 while that of plasma was 0.83 ± 0.78 . Though BCR-ABL/G6PDH ratios from plasma samples were low, ratios of BCR-ABL/G6PDH mRNA in plasma samples from CML patients correlated significantly with those from peripheral blood cell samples (Figure-1).

Transcript b3a2 was detected in 32(66.6%) patients, while 10(20.8%) patients had b2a2 transcript, and 6(12.5%) patients had neither transcript. No co-expression of transcripts was found in any patient.

Among 32(66.6%) patients in whom b3a2 transcript was detected, 19(59.3%) were males, while 14(43.75%) were females. Among the 10(20.8%) patients in whom b2a2 transcript was detected, 5(50%) each were males and females ($p > 0.05$). Mean ($\pm$ SD) age of patients with b3a2 transcript was 35 ± 13.4 years, and that of patients with b2a2 transcript was 42.4 ± 14.07 years ($p > 0.05$). Distribution of the age group by the type of fusion transcript was noted (Figure-2). There was no significant association of transcript type with PC, TLC and Hb count ($p > 0.05$).

Discussion

Since, tumour cells are not evenly distributed in the body, the evaluation of tumour load of body from tumour cells is not reliable. By quantifying BCR-ABL from plasma, we can calculate its copy number per unit of plasma which represents the true tumour load of the body. This shows that

free circulating mRNA in plasma can be effectively used to evaluate residual tumour load. Scatter plot (Figure-1) showed that though BCR-ABL/G6PD ratio in plasma was less than that in blood cell samples, a significant correlation was present between them ($p < 0.05$). Ting et al., in a similar study, found that the BCR-ABL mRNA copy numbers in peripheral blood cells samples were significantly higher than in plasma samples.¹⁹

In our study, patients with b3a2 transcript were the most,

followed by those having b2a2 (66.6% vs 20.8%). These results are in concordance with other studies.^{20,21} In contrast, some studies reported that the percentage of b2a2 transcript was more than b3a2 in CML patients.^{22,23} Alternative splicing can result in the co-expression of two transcripts. Our study didn't show co-expression of transcripts in any of the patient. So, results of our study add to the conflicting results of studies of similar nature found in literature.²⁰⁻²³

The current study revealed no significant correlation of transcript type with gender or age of CML patients. Transcript b3a2 was found more frequently in male patients, but there was no significant difference ($p>0.05$).

Transcript b3a2 was most frequent in age group 11-30 while most of the patients in age group 31-50 had b2a2 transcript, but the difference was not significant ($p>0.05$). Earlier studies have shown similar findings^{12,20} as well contrasting findings.^{21,24} Elevated TLC and decreased Hb levels can provide lead in diagnosing and monitoring of CML, but no evidence of blood counts used as a marker in diagnosing or monitoring BCR-ABL transcript variants has been reported yet. The current study also did not find any significant relation of type of transcript with blood counts, including Hb level, TLC and PC. Results of some of studies of similar nature revealed no significant correlation of BCR-ABL transcript type with blood cell counts.^{20,21} Some studies revealed statistically significant correlation of blood cell counts with BCR-ABL transcript type.^{12,25}

The findings of the current study suggest that plasma can be used as an alternative to peripheral blood cell samples for extraction and RT-PCR detection of BCR-ABL mRNA, but there is need to devise an efficient method of mRNA extraction and detection from plasma samples.

Conclusion

It was found that BCR-ABL mRNA could be detected and quantified in plasma by RT-PCR in CML patients. Also, haematological counts were not affected significantly by BCR-ABL breakpoints.

Disclaimer: The text is based on an M. Phil thesis submitted at Government College University, Lahore.

Conflict of Interest: A co-author was the designated authority who signed the ethical approval.

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