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Association of BCR-ABL Alternative Splice Variants with Disease Progression, Treatment Response and Survival in Chronic Myeloid Leukemia Patients Treated with Firstline Imatinib Monotherapy

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Abstract

Background: Alternative RNA splicing has diverse biological effects in heath as well as disease. It also contributes to cancer onset and progression. Chronic Myeloid Leukemia (CML) results due to BCR-ABL fusion oncogene that is created due to chromosomal translocation t [9; 22] [q34; q11]). BCR-ABL is target of tyrosine kinase inhibitors (TKIs). BCR-ABL through alternative splicing can generate b2a2, b3a2 and some other rare splicing variants. BCR-ABL variants may vary in their response to TKI treatment and disease progression potential, which is a major factor contributing to dismal treatment outcome in CML. Objective: The objective of this study is to investigate correlation of BCR-ABL splice variants with TKI treatment outcome and survival in three phases of CML that has rarely been studied previously.

Methods: BCR-ABL splice variants were studied using reverse transcriptase PCR (RT-PCR). in 70 CML patients from three phases of CML who were receiving imatinib (TKI) treatment.

Results: Frequencies of different BCR/ABL splice variants like b3a2, b2a2 and b3a2+b2a2 were 49 (70%), 15 (21.4%) and 6 (8.6%), respectively. Splice variant b2a2 were more common (53.3%) in chronic phase CML (CP-CML) while b3a2 had higher frequency in advanced phases of CML (44.9%). CML patients with b2a2 transcript had better complete cytogenetic response and major molecular response to TKI treatment overall (100% vs. 24.5%) as well as in CP-CML (100% vs. 85.7%) and superior survival when compared to patients with b3a2 splice variant. All patients who died had male gender, less than 33 years age, b3a2 transcript, advanced phases of CML and imatinib resistance.

Conclusions: Splice variant b3a2 was associated with CML progression, poorer survival and inferior treatment outcome as compared to b2a2. Further investigations on BCR-ABL splice variants and their roles in CML pathogenesis can provide deeper insights into CML biology and new targets for BCR-ABL positive leukemia treatment.

Introduction

Alternative RNA splicing is responsible for diversity of molecular structure and function in higher animals and even plants [1]. It gives rise to different biological molecules from single gene which produces diverse biological effects in heathy individuals as well as different disease conditions [2]. It contributes to cancer progression [3] and recent reports show implication of RNA splice variants in prognosis [4, 5], drug resistance [6] and metastasis [7], which indicates the scope of targeting splice variants or associated mechanisms as promising new approaches for treating cancers in this era of resistance to even very effective anti-cancer drugs. Blood cancers need more innovative approaches to new drug design because of limited role of surgical treatment and recent studies have also shown role of alternative splicing in leukemias [8]. Chronic Myeloid Leukemia (CML) results due to translocation between chromosomes 9 and 22 translocation t[9; 22] [q34; q11]), that creates fusion oncogenes BCR-ABL responsible for leukemogenesis [9]. BCR-ABL fusion oncogene is a classic example where two splice variants of BCR-ABL, fusion transcripts b2a2 and b3a2, induce carcinogenesis in majority of CML patients [9, 10]. There are some published studies reporting correlation of BCR-ABL splice variants with clinical parameters in chronic phase CML [10-13] but there are limited reports about correlation of BCR-ABL fusion transcripts with treatment outcome and survival in CML patients from different disease phases [2]. Therefore, we studied different splice variants of BCR-ABL and their significant correlation with CML progression, treatment outcome and survivals in all three stages of CML.

Methods

Peripheral blood samples along with clinical data were collected from 70 clinically diagnosed CML patients from different hospitals of Lahore, Pakistan during 2011-2019. The written informed consent about proposed study was obtained from all study subjects and it was approved by scientific as well as ethical review boards of all institutions that participated in the study . Clinical characteristics of the patients were obtained from patients' medical records. RNA was extracted, cDNA prepared and RT-PCR was performed according to protocols established by our group earlier and reported in our previous article [13]. All response criteria were adopted per European LeukemiaNet guidelines 2013 [9]. SPSS version 26 (IBM Corp., Armonk, N.Y., USA) was employed for statistical analyses.

Results

Demographic features of the patients

A total of 70 clinically diagnosed CML patients were studied (N=70). The patients had male to female ratio of 1.33 to 1. Our patient population has median age of 34 years with range of 13-63 years. It included 10 pediatric patients (age of 18 years old or less) and 60 adult patients. Twenty-six patients were in chronic phase (CP-CML), 26 in accelerated phase (AP-CML) and 18 in blast crisis phase (BP-CML).

Clinical Characteristics of patients at the time of diagnosis

Overall, median treatment duration in our CML patients 4.5 years with a range between 1.1-11.1 years. Median follow-up duration was 5.1 years, ranging from 3.3 to 9.6 years. Chronic myeloid leukemia (CML) patients showed different presenting at the time of diagnosis. Overall, 56 out of 70 (80%) patients showed splenomegaly as main symptom. Fever was the second most common symptom (n=36, 51.4) followed by hepatomegaly (n=16, 22.9%), weakness (n=14, 20%), and gum bleeding (n=10, 14.1%) (Table 1).

Characteristic	Number (%)
Age (years)	
Median	34
Range	13-63
Gender	
Female	40 (57)
Male	30 (43)
Clinical features at the time of diagnosis	
Splenomegaly	56 (80)
Fever	36 (51.4)
Hepatomegaly	16 (23)
Weakness	14 (20)
Bleeding	10 (14.3)
CML phase at presentation	
Chronic phase	26 (37.15)
Accelerated phase	26 (37.15)
Blast phase	18 (25.70)
BCR-ABL splice variant	
b3a2	49 (70)
b2a2	15 (21.4)
b3a2+b2a2	06 (8.6)

Table 1: Patients' characteristics

Phase of CML (Number)	b2a2 Number (%)
CP	8 (53.3)
AP	2 (13.3)
BC	5 (33.4)

Table 2: Frequency of b2a2 BCR-ABL splice variant in three CML phases

Frequency of BCR-ABL splice variants and their clinical outcome

BCR/ABL splice variants b3a2 and b2a2 were detected in 70% (49/70) and 21.4% (15/70) CML patients, respectively, with 6 (8.6%) patients having both fusion transcripts (b3a2+b2a2). None of the rare BCR-ABL transcripts was identified in our study subjects (Table 1). With respect to different CML phases, splice variant b2a2 were more common (53.3%) in CP-CML while b3a2 was more frequent in AP-CML (44.9%) (p=0.05). Considering all three disease phases, both transcripts (b3a2+b2a2) co-existed in 4 (66.7%) patients with CP- and 2 (33%%) in AP-CML but none in BC-CML (Tables 2-

4). The co-existence of b2a2 and b3a2 transcripts was observed only in male patients with 40 years of age or younger ($p<0.05$). Individual b3a2 or b2a2 splice variants types had no significant association with age or gender.

Phase of CML (Number)	b3a2 Number (%)
CP	14 (28.6)
AP	22 (44.9)
BC	13 (26.5%)

Table 3: Frequency of b3a2 BCR-ABL splice variant in three CML phases

Phase of CML (Number)	b2a2+ b3a2 Number (%)
CP (N=26)	4 (66.7)
AP (N=26)	2 (33.3)
BC (N=18)	0

Table 4: Frequencies of combined expression of BCR-ABL splice variant b2a2 and b3a2 in three CML phases

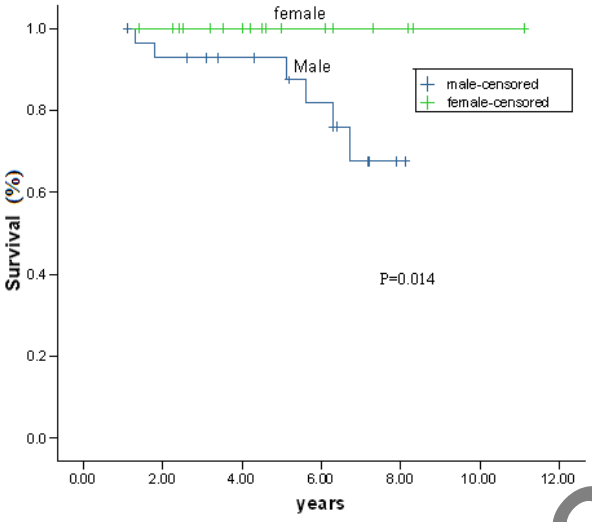

Figure 1: Survival probability of Male and Female CML Patients

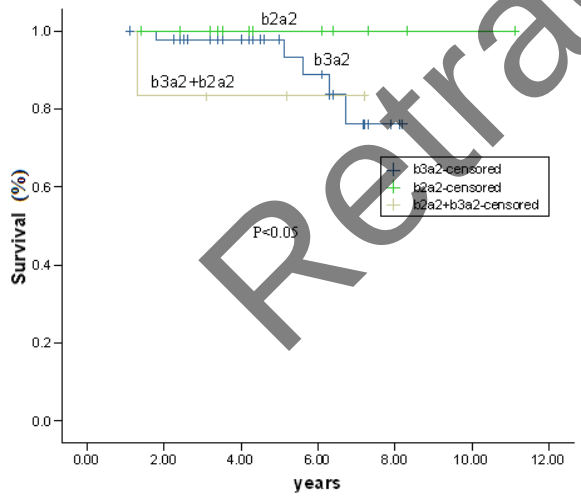

Figure 2. Association of overall survival of CML Patients with different BCR-ABL splices variants

In our studies, gender was significantly associated with patient survival as manifested by higher survival of female patients in comparison to their male counterparts (P=0.014) (figure 1). Moreover, patients possessing b2a2 fusion transcript had a superior response to imatinib monotherapy and subsequently superior overall survival as compared to patients positive for splice variant b3a2 ($P<0.05$). The patients in which b3a2 and b2a2 co-existed similarly also showed superior survival relative to their counterparts with only b3a2 fusion transcript (figure 2). 12.3% (6/49) of CML patients harboring b3a2 died out of which 4 (8.2%) were in AP and 2 (4.1%) in BP while none of the patients with b2a2 died during course of the study (OS 87.7% vs. 100%, p=0.00001). 3-year survival of patients with b3a2 was 95.9% (47/49) as compared to 100% (15/15) 3-year survival for patients with b2a2. All these patients who died were male, younger than 33 years (median age 27 years, age 24-32 years), resistant to imatinib and receiving escalated doses of imatinib (600-800mg) and had b3a2 splice variant. Overall, majority of patients with b2a2 (n=8, 53%) showed stable complete cytogenetic response (CCyR) and major molecular response (MMR) lasting for 3 years or more while only 12 out of 49 (24.5%) patients with b3a2 splice variant showed sustained CCyR and MMR ($P<0.001$) (Tables 3-4). When comparing CP-CML, all 8 (100%) CP-CML with b2a2 showed sustained CCyR and MMR as compared to only 85.7% (12/14) CP-CML patients with b3a2 splice variant (P=0.05).

Therefore, our results show that BCR-ABL splice variants are associated with disease progression, treatment outcome and survival in CML patients.

Discussion

In present study, we attempted to find out association splice variants of cancer-causing genes with cancer progression, clinical parameters, anti-cancer treatment outcome and patient survival by taking example of CML (chronic myeloid leukemia) as example. As BCR-ABL oncogene is a hallmark of not only CML but cancer genetics and anti-cancer molecular targeted therapies [9, 10], we investigated BCR-ABL splice variants in CML and correlated it CML disease phase, disease progression, survival and treatment response. Our results revealed significantly different occurrence of BCR-ABL splice variants in our CML study population manifested by the frequencies of 70% and 21.4% for b3a2 and b2a2, respectively and while both transcripts co-existed in 8.6% of the patients studied. Nevertheless, no rare transcript of BCR-ABL oncogene was detected in our study. Our results showing higher b3a2 frequency are consistent with the other reports from Pakistan [13], Iraq [14], Togo/West Africa [15], Kashmir [16], Iran [17], India [18], Syria [19], Korea [20], Germany [21], Austria [22] and US [23] while few researchers have reported higher frequencies of b2a2 than b3a2 in Sudan and Ecuador [24,25]. It demonstrates a solid biological basis

of higher b3a2 splice variant frequency than b2a2 in CML patients.

Our investigations into different BCR-ABL splice variants in CML showed their significant correlation with clinical phases of the CML disease, i.e., b2a2 splice variant was observed mostly in chronic phase, while b3a2 was present more commonly in patients with accelerated phase CML. It shows association of b3a2 splice variant with CML disease progression. Our findings are supported by studies carried out by Reiter *et al* who reported that b3a2 preferentially acquires additional cytogenetic aberrations during imatinib treatment as compared to b2a2 which results in quicker progression to due to increased activity of a cysteine endopeptidase called ESPL1/Separase [22]. It demonstrates the role of alternative splicing in CML progression.

As different BCR-ABL splice variants differ in their potential to cause CML progression [22, 26], hence they also differ in their response to anti-leukemic treatment and survival. In our studies, CML patients harboring b2a2 fusion transcript showed a superior response to imatinib (drug targeting BCR-ABL oncoprotein) treatment and superior survival as compared to patients with b3a2 overall as well as in CP-CML. We found 12.2% mortality in b3a2 as compared to no deaths in patients with b2a2 and mortality was associated with male gender, younger age, advanced disease phases and higher imatinib doses. Overall survival of patients with b2a2 was significantly higher than patients harboring b3a2. All (100%) patients harboring b2a2 showed long-term CCyR and MMR in comparison to 24.5% CML patients with b3a2 showing sustained CCyR and MMR. Our results are supported by findings of de Lemos *et al.* who reported better molecular responses in CML patients with b2a2 BCR-ABL splice variant as compared to b3a2 [27]. Similarly, patients with b2a2 splice variants have been found to have superior CCyR than patients with b3a2 [28]. Azad *et al.* have reported higher imatinib resistance in CML patients with b3a2 splice variants as compared to b2a2 [16]. Recently, Sazawal et al. have reported higher frequency and better MMR in b3a2 splice variant as compared to b2a2 [29]. Moreover, co-existence of $p190^{BCR-ABL}$ $p210^{BCR-ABL}$ have been found be associated with and t-cell blast crisis in CML [30]. In addition to these common BCR-ABL splice variants, various rare BCR-ABL alternatively spliced BCR-ABL variants have been detected in CML patients and have been found be associated with failure to achieve molecular response, drug resistance and disease progression [31]. This clearly shows that different BCR-ABL splice variants, being different biological entities, are strongly associated with CML pathogenesis, treatment outcome, disease progression and survival. Therefore, CML patients should be tested for all alternatively spliced BCR-ABL variants at diagnosis, and specifically in case of drug resistance, failure to achieve molecular responses and disease progression. Per European LeukemiaNet (ELN) guidelines 2020, it is compulsory to detect type of BCR-ABL transcript type on first diagnosis and during assessment of treatment response by a qualitative reverse transcriptase PCR (RT-PCR) using peripheral blood cells from CML patients [32]. This highlights the need for further studies to understand the molecular mechanisms and differential roles of various BCR-ABL splice variants in CML progression and treatment outcome using state-of-the art molecular biological techniques [33].Such studies will open new windows to understand cancer pathogenesis in association with basic biological processes like alternative splicing, RNA editing etc. and to design novel patient-tailored anti-cancer drugs by targeting alternatively spliced variants and their associated mechanisms.

It is inferred from the our current study and other published scientific reports that BCR-ABL splice variants vary in their relative frequencies in CML patients. In our study population, different BCR-ABL splice variants showed significant correlation with the clinical phases of CML, responded differently to imatinib treatment and are associated with survival.

Although the number of patient samples in this study was small, there was degree of correlation of BCR-ABL splice variants with disease treatment outcome, progression, and survival. It is recommended to carry out similar studies with higher patient population and by employing cutting-edge cell biological, molecular, and immunological technologies to get deep insights into different biological, pathological, and clinical behaviors of different BCR-ABL splice variants in CML.

We report a significantly higher frequency of BCR-ABL splice variant b3a2 overall as well as in AP- & BC-CML as compared to CP-CML which shows association of type of splice variants with disease progression and hence severity and prognosis. CML patients possessing BCR-ABL fusion transcript b2a2 had a superior response to imatinib monotherapy and higher overall survival relative to BCR-ABL splice variant b3a2 , that indicates correlation of types of BCR-ABL fusion transcripts with TKI-based therapy and hence can further help in optimizing TKI treatment of CML in post-TKI era of BCR-ABL positive leukemia treatment. Co-existence of two transcripts b3a2 and b2a2 was observed mostly in CP-CML and not in BC-CML which indicates its possible role in early onset of disease. Larger studies are needed to further define the role of BCR-ABL splice variants in different phases of CML and their association with response to all FDA-approved TKIs, different types of survivals and prognosis. Studies of oncogene splice variants, their association with different disease

manifestations, along with other disease modifiers (mutants) and their correlation with personalized treatment of BCR-ABL positive leukemias undergoing multiple TKIs can provide novel insights into cancer biology and provide new targets for anti-cancer drug development in clinically progressed, severe and drug-resistant forms of different cancers and specifically BCR-ABL positive leukemias.

Competing Interest

Authors have no conflict of interest.

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Author Contributions

Zafar Iqbal, Tanveer Akhtar, Ahmad M. Khalid, Aamer Aleem, Mahmood Rasool, Nawaf Alanazi, Muhammad Farooq Sabar and Amer Mehmood conceptualized the study, supervised overall work and wrote / critically reviewed manuscript.

Ijaz H Shah, Muhammad Khalid, Mudassar Iqbal, Abid Jameel, Zeba Aziz analyzed clinical data.

Saba Shahzadi, Afia M Akram, , Maryam AlMajed, Buthinah AlShehab, Sarah AlMukhaylid, Nouf AlMutairi, Dhay Salah Almaghlouth, Alhanoof Rashid A Alsuwaidani, Khaled Aljarrah, Muhammad Farooq Sabar, Muhammad Arshad and Rashid Ayub conducted experiments, wrote article and and analyzed data.

Zafar Iqbal and Nawaf Alanazi contributed equally to the manuscript and thus share first authorship.

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