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Molecular Epidemiology of BCR-ABL Rearrangement Variants in Chronic Myeloid Leukemia and Acute Lymphocytic Leukemia from Major Institute of Pakistan

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Abstract

In Pakistan the disease burden of Chronic Myeloid Leukemia (CML) and Acute Lymphoblastic Leukemia (ALL) is quite high, yet there is a lack of scientific evidence regarding the spectrum of BCR-ABL rearrangement variants in CML and ALL. Knowing about BCR-ABL rearrangement is important in determining the prognosis and treatment strategy of disease at the time of diagnosis. This study included a total of 685 patients, out of which there were 644 CML patients and 41 ALL patients, from October 2016-July 2019. From the CML group, 270 patients were reported to have the BCR-ABL1 transcript, from which 50% were males. Whereas in the ALL group, 35 patients were reported to have the BCR-ABL1 transcript out of which 65.7% were males. Proportions of BCR-ABL transcript type differed between the two groups, with b3a2 (63.3%) and b2a2 (34.8%) transcript types having the highest frequency in CML patients, whereas e1a2 (77.1%) and b3a2 (11.3%) transcript types were found to have the highest frequency in ALL patients. Our data shows transcript genotypes in CML and ALL patients in an Asian population, which may be useful to guide the clinical management and assess prognosis. Since the majority of our CML population had the b3a2 transcript, they have a better prognosis and treatment response.

Keywords:

Chronic myelogenous leukemia • Chromosomal translocation • Molecular pathology • Imatinib treatment

Introduction

Leukemia is identified to be the cause of some of the highest mortality rates worldwide [1-4]. From among these leukemias, Chronic Myeloid Leukemia (CML) is accountable for approximately 15% of all adult leukemias [5], 9% of adolescent leukemias and a mere 2% of childhood leukemias [6]. Also known as Chronic Myelogenous Leukemia, the disease is a neoplasm that occurs in about 1 to 2 per 100,000 adults and is a myeloproliferative disorder with a characteristic hallmark of increased proliferation of the granulocytic cell line, without the loss of the cells' ability to differentiate 5. The diagnosis involves identifying the Philadelphia chromosome (Ph) harboring BCR-ABL1 fusion transcript by using cytogenetic and/or molecular techniques [7]. The disease itself is very rarely seen in children, with the average age of diagnosis typically being 60-70 years old in Caucasian populations [8,9]. In contrast, the age range typically seen in Pakistani populations is 21-50 years, which is much younger than other countries. Acute Lymphoblastic Leukemia (ALL), in contrast, is the most common pediatric cancer in the world [10]. The pathology involves the lymphoid cell line which results in observable immature progenitors in the blood progressing rapidly into a fatal disease within a matter of weeks to months, especially if medical attention is not received [11]. In 2015 alone, 876,000 global cases were reported, of which 111,000 cases did not survive - a devastating mortality rate of 12.6% [12[. A subtype of ALL, known as Philadelphia Positive Acute Lymphoblastic Leukemia (Ph + ALL), is seen much more rarely in children, yet it is comparatively more common in adults 10. Interestingly, this particular subtype shares a key diagnostic feature with CML; both pathologies are associated with BCR/ABL fusion gene variants [13].

A trademark diagnostic feature for patients suspected of CML or Ph+ ALL is a reciprocal chromosomal translocation that results in what is known as the Philadelphia chromosome t(9;22) (q34;q11), giving rise to the BCR/ABL fusion gene 2. Translocation of the proto-oncogene tyrosine-protein kinase (ABL1) on chromosome 9 to the Breakpoint Cluster Region (BCR) located on chromosome 22, results in a BCR-ABL1 fusion gene 4. There are many leukemogenic proteins formed by translation of BCR-ABL1 fusion genes in CML and ALL, all of which differ based on the transcription variant of the BCR-ABL1 gene present 4. Although prior studies have thoroughly investigated these proteins, research of the frequency of various fusion genes among CML and ALL patients is still ongoing 3. Transcription variants are indispensable pieces of information, that may aid in the prognostic criteria of a patient's response to treatment, as well as treatment outcomes 3.Variations in breakpoint location sites within the BCR lead to transcription variants, termed b2a2, b2a3, b3a2, b3,b3, c3a2, e1a2 and many more 3. Majority of CML patients exhibit transcripts within the major BCR (M-BCR) forming mRNA molecules, with b2a2 or b3a2 junctions that encode for the 210kDA fusion protein, p210BCR-ABL 1. Comparatively, ALL patients have been found to exhibit a majority of transcripts within the minor BCR (m-BCR) forming mRNA molecules, with e1a2 junctions that encode for the 190kDA fusion protein, p190BCR-ABL [14]. These protein isoforms, p210BCR-ABL, p190BCR-ABL and p230BCR-ABL, are encoded by BCR-ABL1 fusion gene hybrids and enhance tyrosine kinase activity which lead to disrupted signaling pathways, ultimately causing resistance to cell death, enhanced proliferation and differentiation arrest 4. Certain variants of the fusion gene have been known to have worse clinical outcomes in comparison to other variants, particularly with specific tyrosine kinase inhibitors (TKIs) such as imatinib [15]. A study by Sharma et al. showed that upon administration of imatinib to patients, 59% of the patients with the b2a2 variant showed major cytogenetic response, compared to only 28% in patients with the b3a2 variant of BCR-ABL 15. Different variants of BCR-ABL found through mutational analysis are treated using different TKIs [16]. Looking at Pakistan in particular, the disease burden of CML and ALL is relatively high, with CML contributing to about 20% of all diagnosed leukemia cases in Pakistan annually 1. A previous study conducted in 2014 found BCR-ABL1 gene variants in CML and reported b3a2 as the most

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common variant, however it is difficult to generalize their results due to the small sample size population (n=25) 1. There are limited studies regarding BCR-ABL transcript types in CML and ALL patients, especially in various ethnic Asian populations such as those from Pakistan, subsequently leading to minimal insight into their response to treatment modalities. Furthermore, scientific evidence regarding Philadelphia chromosome variants has been carried out in other countries, with international data being more readily available in comparison to lower-middle income Asian countries such as Pakistan. Hence, our study aimed to assess the prevalence of transcript variant frequency of the BCR-ABL1 fusion genes, among CML and ALL patients in Pakistan.

Materials and Methods

This retrospective study was conducted by the Section of Molecular Pathology, at The Aga Khan University Hospital, Karachi, Pakistan. Medical records were retrieved, with the help of the institutional data management system, for all patients newly diagnosed with CML and ALL between October 2016 to July 2019. The diagnosis of both leukemias was made in accordance with the WHO criteria being used at the time of first presentation (WHO criteria 2008 and 2016). For both CML and ALL, the patients were divided according to gender and 3 age groups (<15 years old, between 15 to 60 years old and > 60 years old) to determine the frequency and proportion of BCR-ABL1 transcript types in each category.

Molecular analysis

Molecular analysis was performed in order to determine the BCR-ABL fusion gene p210 presence. From among the BCR-ABL1 positive patients, we aimed to identify the frequency of the various transcript types. Five milliliters of whole blood/bone marrow was collected in an EDTA containing tubes from each participant. The samples were transferred immediately to the laboratory for processing and preservation of nucleic acid (RNA) at -8°C. For BCRABL1 analysis, complementary DNA (cDNA) was synthesized from RNA extracted from the patient samples. Multiplex reverse transcriptase–polymerase chain reaction (RT-PCR) assay was performed with a Seeplex leukemia BCR/ABL kit (Seegene, Seoul, Korea), which is designed to detect eight common BCR/ABL transcripts including major, minor, and micro breakpoint cluster regions c3a2 (1012bp), b1a1 (745bp), b3a2 (476bp), b2a2 (401bp), e1a2 (348bp), b3a3 (299bp), b2a3 (224bp) and e1a3 (178bp), as per the manufacturer's instructions. Polymerase chain reaction (PCR) products were then separated and visualized on a 2% ethidium bromide stained agarose gel.

Statistical analysis

Statistical analysis was performed using the Statistical package for social sciences (SPSS-23) for data analysis (SPSS Inc., Chicago, IL, USA). Frequencies and percentages were calculated for categorical variables and mean, and standard deviation were computed for quantitative variables. The chi square method was also applied to compare the frequencies obtained for the CML and ALL patients.A total of 685 patients met our inclusion criteria, among which 644 (94.0%) patients were found to have Chronic Myeloid Leukemia (CML), while 41 (6%) patients had Acute Lymphoblastic Leukemia (ALL) between October 2016 to July 2019. Individual patient characteristics of CML and AML patients are summarized in Table 1. Further breakdown of gender across each age group for CML and ALL patients can be seen in Table 2. Gender and age distributions according to BCR-ABL positive and negative in CML and ALL patients are presented in Table 3. Transcript types found for both CML and ALL patients are depicted in Table 4.

Table 1. Patient characteristics of cml and all patients.

Patient characteristic	Total patients N=685 (%)	CML N=644 (%)	All N=41 (%)	P value
Gender				
male	396 (57.8)	368 (57.1)	28 (68.3)	0.161
female	289 (42.2)	276 (42.9)	13 (31.7)	
Age groups				
Age group <15 years	55 (8.0)	51 (7.9)	4 (9.8)	0.914
Age group 15-60 years	513 (74.9)	483 (75.0)	30 (73.2)	
Age group >60 years	117 (17.1)	110 (17.1)	7 (17.1)	
Bcr-abl Positive	305 (44.5)	270 (41.9)	35 (85.4)	0.000
Negative	380 (55.5)	374 (58.1)	6 (14.6)	

Table 2. Age distributions of CML and all patients according to gender.

Age groups	Total Patients N=685 (%)	CML patients N=644			All patients N=41		
		Female Patients N=276 (%)	Male Patients N=368 (%)	P value	Female Patients N= 13 (%)	Male Patients N=28 (%)	P value
Age group <15 years	55 (8.0)	20 (7.2)	31 (8.4)	0.541	2 (15.4)	2 (7.1)	0.723
Age group 15-60 years	513 (74.9)	204 (73.9)	279 (75.8)		9 (69.2)	21 (75.0)	
Age group >60 years	117 (17.1)	52 (18.8)	58 (15.8)		2 (15.4)	5 (17.9)	

Table 3. Patient characteristics of BCR-ABL negative and positive samples in CML and ALL patients.

Patient characteristic	Total Patients N=685 (%)	CML patients N=644			All patients N=41		
		BCR-ABL Negative N=374 (%)	BCR-ABL Positive N=270 (%)	P value	BCR-ABL Negative N=6 (%)	BCR-ABL Positive N=35 (%)	P value
Gender male female	396 (57.8) 289 (42.2)	233 (62.3) 141 (37.7)	135 (50.0) 135 (50.0)	0.002	5 (83.3) 1 (16.7)	23 (65.7) 12 (34.3)	0.368
Age groups							
Age group <15 years	55 (8.0)	34 (9.1)	17 (6.3)	0.017	1 (16.7)	3 (8.6)	0.845
Age group 15-60 years	513 (74.9)	265 (70.9)	218 (80.7)		4 (66.7)	26 (74.3)	
Age group >60 years	117 (17.1)	75 (20.1)	35 (13.0)		1 (16.7)	6 (17.1)	

Table 4. proportions of BCR-ABL transcript types in CML and all patients.

BCR-ABL Transcripts	Total patients N=305 (%)	CML patients N=270 (%)	All patients N=35 (%)	P value
Transcript type	96 (31.5)	94 (34.8)	2 (5.7)	0.000
B2a2	1 (0.3)	1 (0.4)	0 (0.0)	
B2a3	175 (57.4)	171 (63.3)	4 (11.3)	
B3a2	1 (0.3)	1 (0.4)	0 (0.0)	
B3a3	3 (1.0)	1 (0.4)	2 (5.7)	
C3a2	28 (9.2)	1 (0.4)	27 (77.1)	
E1a2	1 (0.3)	1 (0.4)	0 (0.0)	
E1a3				

The highest reported transcripts type in our population of CML patients positive for BCR-ABL (n=270) was found to be b3a2 (63.3%), with the typical and most common fusion seen in CML usually being b2a2 or b3a2 [17]. In an international study with over 45,000 CML patients from 45 different countries, the proportion of b2a2 and b3a2 transcripts was 37.9% and 62.1% respectively, with only 1.93% of patients expressing other rare transcripts 3. The percentages varied greatly across the continents for b2a2, however, with Africa having a 44.6% prevalence of b2a2 in their CML patients, while in Asia, the ratio was much lower at only 33.2% 3. Our results are similar to these international statistics, as well as the statistics from Asia, since from our total population of CML patients, 63.3% had the b3a2 transcript, 34.8% had the b2a2 transcript, and only 1.9% of the patients expressed other transcript types. These figures remain consistent in another study conducted in Asia, as well as a study conducted in India on 208 CML patients which showed that b3a2 and b2a2 were the most common transcript types (66.82% and 28.84% respectively) [18]. Similarly, another study conducted by Sazawal et al. concluded that b3a2 and b2a2 were the most common transcript types with prevalence of 72% and 26% respectively, from a total sample size of 400 patients [19]. Another study conducted in Iraq came to the same conclusion, which showed that b3a2 transcript was more common than b2a2 transcript in CML patients, with these 2 transcript types being the most prevalent in their CML patients [20]. This suggests that our study findings are corroborated by other similar investigations, providing more evidence to support our conclusions.

While looking at ALL patients, 85.4% of our population was diagnosed as BCR-ABL positive, which was higher than reports from a study in Germany that observed a prevalence of only 37% in their population of ALL patients [21]. A large-scale study analyzed group trials between 1990 and 2007 and reported an incidence of 36.2% for BCR-ABL for their ALL study population [22]. This again differs from our findings of the proportion of BCR-ABL presence, which are of a higher fraction. A large cohort study conducted across Austria, Switzerland, Italy and Germany over four years differed in these findings and found a prevalence of 1.3% for Ph+ among ALL patients, however, the study noted that their incidence was lower due to methodological reasons regarding cytogenetics and concluded the real incidence would be a higher percentage 23. The highest reported transcript type in our population of ALL patients was e1a2 transcript (77.1 %) followed by b3a2 transcript (11.3%) (p=0.000). One study had similar findings and reported 77% of the ALL patients were detected for e1a2, 20% for b3a2, and the remaining 3% for co-expression of both these transcript types 21. Our study did not report any findings for co-expression of transcript types in ALL or CML patients. A study conducted by Cimino, et al. in Italy also found that e1a2 transcript was prevalent in 60-75% of ALL patients who were BCR-ABL positive 24, near the range of our findings. From the cohort population of Ph+ ALL patients, there was a 49.2% incidence of e1a2 [23]. The large-scale study previously mentioned reported findings of 25% incidence of transcript type e1a2 and 11.3% had b3a2 [24]. Comparatively, the e1a2 transcript type was far less common and considered a rare finding in CML

patients (1%) [25]. Our study similarly observed a prevalence of 0.4% of the e1a2 transcript in CML patients. We did not report findings of any patient with transcript type b3a3 (0.0%), which is a rare transcript variant for ALL patients that was observed in a unique case study by Kim, et al. [26]. No published Pakistani paper regarding prevalence of transcript types could be found for comparison.

Discussion

Bringing gender distribution into the limelight, our study found that the proportion of CML patients according to gender remained consistent, with gender not causing any significant changes between numbers of CML patients. Rarer transcripts, such as e1a2, were found to be more common in women compared to men, as e1a2, c3a2 and b3a3 were all present in female patients but were not found in any male patients enrolled in the study. This is consistent with international data, with a study conducted by Baccarani et al. showing that 1.33% of females compared to 0.62% of males had the e1a2 transcript 3. However, in our study there was no statistical significance between the number of patients for both male and female categories (p-value=0.323). This differs from the previously mentioned study conducted by Baccarani et al. , as they found the transcript b2a2 to be more common in men compared to women (39.2% and 36.2% respectively) 3. This trend continues in Asia, as a study in Iraq demonstrated a gender skewed distribution and concluded that b3a2 is also found to be more prevalent in men compared to women 20. Looking at Pakistan in particular, a study conducted by Tashfeen et al. found that out of 528 newly diagnosed CML patients, 378 (71.6%) were male while only 150 (28.4%) were female [27]. Although our study also demonstrated a larger number of male cases compared to women, the difference was insignificant (e.g., 48 males to 46 females with b2a2 transcript type).

Focusing on gender distribution in ALL Patients, our study found that of 35 ALL patients positive for BCR-ABL, 23 were male (65.7%) and 12 were female (34.3%). Another ALL study conducted in Pakistan also observed a male predominance in the study population with a male to female ratio of 1.7:1 [28]. A study conducted in the European population also found a greater male (66%) to female (44%) ratio in their Ph+ ALL patients 23. One Brazilian study also observed a larger proportion of males (61%) to females (39%) [29]. Our studies reported that in males and females both the most prevalent transcript variant was e1a2 (73.9% and 83.3% respectively). There was one study that reported an ALL population of 100% female gender, of which 20% were found to have the BCR-ABL gene 13. Studies regarding the gender distribution for transcript types could not be identified from Pakistan for the purpose of comparison.

Looking at age distribution, our study found that CML is much rarer in cases under the age of 15. In CML, cases under the age of 15 were only 7.9% of the total population. An international study conducted by Baccarani et al. found that most continents, including Europe, Africa, Australia and South America, had far fewer cases in the 0-15 year age bracket, than among people from older age groups 3. It showed that across the world, higher ages correlate to greater chances of BCR-ABL positive CML 3. However, the number of patients with b3a2 decreased as adults approached the age of 60, with only 13% of patients being above the age of 60 3. About 80% of patients were found to be within the age of 15-60, and a major portion of those were found to be towards the lower end of that age group 3. In these cases, there seemed to be no contradiction between our data and data from our literature review. Looking at a study conducted in Pakistan on 145 patients, found CML patients were commonly found in the age groups between 20-30 and 30-40 years [30]. Similarly, another study in Pakistan by Khalid et al., found the mean age of CML patients was 34 years 28. All these results appear to agree with each other with no significant differences. It should be noted that much of the literature does not currently look into the prevalence of transcript types based on different age groups.

General data available on ALL transcript types has reported that the Philadelphia chromosome is typically only present in 3-5% of pediatric ALL patients 23. In reference to childhood ALL it was found that children were more likely to present with e1a2 than any other transcript, 24 which in accordance to our findings, holds true in our population. Looking at age distribution, our study found that ALL patients under 15 years of age were all found to be transcript type e1a2 (100.0%). A South American study differed from our results with a population of ALL patients with a 34.1% incidence of BCR-ABL of which e1a2 (28.6%) and b3a2 (50%) was detected, with the remaining 21.4% reported as e1a2/b3a2 co-expression 29. Our findings reported a higher incidence of e1a2 (77.1%), a lower incidence of b3a2 (11.4%), and no findings of co-expression (0%). Looking at age distribution, our study found above the age of 60 years were all found to be transcript type e1a2 (100.0%). Comparably, another study where 20% of the population was detected for e1a2, found that the patients were aged 27 and above 13. Our study observed 91.4% of BCR-ABL positive ALL patients were above the age of 15 (p=0.845). This finding aligns with our study and previous data available for ALL patients in our geographic region, as a study conducted in Pakistan in 2017 of 150 pediatric ALL patients between the ages of 1 and 15 were found not to be BCR-ABL positive [31]. Internationally, the prevalence of positive BCR-ABL for ages 15 to 20 (5%) was observed to be significantly smaller than those 20 to 50 (56%) and 50 to 65 (39%) (p=0.001) 21. These results mirror our findings with a small proportion of the youngest age group of the study population being BCR-ABL positive. One study analyzed the data of 2498 patients and found an increase in BCR-ABL frequency as age increased from 12.7% (15-24 years) to 30.6% (25-34 years), and 43.7% (34-44 years), suggesting an age dependency factor on diagnosis 22. No further increment was noted, however, for those older than 44 years (42%-44%) 22. Disregarding the division of patients based on BCR-ABL status, our total ALL population similarly presented with 92.9% of ALL patients above the age of 15. A large cohort study disagreed with these findings, as they reported that 70.5% of their ALL Ph+ patients were under the age of 9 23. Another study by Khalid et al. found ALL patients in their study were between the ages of 16 and 92, with a mean age of 33, however cytogenetic testing was not reported 28.

There is clinical significance for detection of BCR-ABL rearrangement in these patients, as it is pertinent in guiding treatment and accurate prognosis 3. Fusion oncogenes form as a result of environmental factors and manner of living that co-exist to create natural genetic variations, which differ according to geographic location 17. Frequency of transcript type incidence in ethnic groups may vary and subsequently affect management and prognosis 17. A previous study found specific splice variants such as b2a2 to have a better molecular response to treatment therapy such as imatinib in comparison to b3a2 [32]. One study found 59% of b2a2 patients reached a complete cytogenetic response (CCyR) to treatment; compared to only 28% of b3a2 patients (p=0.04) 15. Patients with transcript variant b3a2 who did not achieve CCyR were found to be higher in number than patients with b2a2 (75% and 25% respectively) 15. Similarly, patients with rarer transcript types such as e1a2 had a decreased molecular response to imatinib treatment [33]. ALL patients who were BCR-ABL positive were generally found to have a poorer prognosis, however presence of p210 was found to result in a better response to first line treatment than presence of p190 25. This information could potentially lead to further studies regarding the influence of ethnicity on the effects of management options and outcomes.

Conclusion

Our results show genotypes found in CML and ALL patients, which may be useful to guide clinical management and assess prognosis of such patients. Our data concludes that since the majority of our CML population is of b3a2 transcript, they have a better prognosis and treatment response 25. The findings of our study also highlight the need for more extensive studies in the Pakistani population and across Asia to determine the role of BCR-ABL variants in biology, clinical features, and response to treatment and prognosis. These observations may be utilized in understanding the rate of incidence of transcriptions in order to explore the pathophysiology and biology of BCR-ABL positive patients in comparison to patients negative for the fusion gene.

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Author ZA is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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