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The Frequency of *BCR-ABL1* Fusion Transcripts in Iranian Patients with Three Different Types of Leukemia

Mohammad Hamid,[1,*] and Hanieh Bokharaei[1]

[1]Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

[*]*Corresponding author*: Mohammad Hamid, Molecular Medicine Dept., Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran. Tel/Fax: +98-2166480780, E-mail: hamidi43@yahoo.com

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Abstract

Background: Different *BCR-ABL* fusion transcripts occur more or less frequently, in three different types of leukemia
Objectives: This study was done to determine the frequencies of *BCR-ABL* fusion transcripts in leukemia patients from Iran.
Methods: This experimental study was carried out from 2001 to 2015 in Pasteur Institute of Iran. The leukemia patients containing 348 chronic myeloid leukemia (CML), 72 acute lymphoblastic leukemia (ALL), and 34 acute myeloid leukemia (AML) were studied. Total RNA was extracted from peripheral blood samples and analyzed by multiplex RT-PCR in 486 leukemia patients to detect different types of BCR-ABL transcripts. Fisher's exact test was used in comparing qualitative variables in the case control study. Associations with a P value < 0.05 were considered significant.
Results: The BCR-ABL transcript frequencies for CML, ALL and AML patients were 92.0%, 12.5% and 14.7% respectively for all transcripts. The majority of CML patients with positive BCR-ABL expressed one of the $p210^{BCR-ABL}$ transcripts (86.6%) while the remaining showed other transcripts ($p190^{BCR-ABL}$ 25 (7.8%)) and $p230^{BCR-ABL}$ 2 (0.6%)). The rate of co-expression of the p190/p210 transcripts were 16 (5%). In other types of leukemia patients the rates of expression of those transcripts were different.
Conclusions: For the first time, we reported co-expression of p210/p190 which may be caused by alternative splicing in Iranian patients. This study we showed no significant correlation between *BCR-ABL1* variants, age, sex type, and WBC count of studied leukemia patients.

Keywords: Leukemia, Chronic Myeloid Leukemia, Iran, *BCR-ABL* Fusion Protein

1. Background

The Philadelphia chromosome is basically caused by reciprocal translocation between chromosomes 9 and 22: t (9; 22)(q34;q11). This translocation causes the formation of BCR-ABL fusion gene, and is associated often with chronic myeloid leukemia (CML) [1]. More than 95% of patients with CML are positive for a BCR-ABL gene in their leukemia cells [2].

However, the BCR-ABL gene is just not limited to CML type. It is also reported in 10% to 20% of adults and in 2% to 5% of children with acute lymphoblastic leukemia (ALL), and in very rarely cases of acute myeloid leukemia (AML) [1, 3].

In the majority of CML patients (95%) the breakpoint in the BCR gene falls within the major breakpoint cluster region (Mbcr), and the resultant BCR-ABL mRNA molecules with a b2a2 (40%) or b3a2 (55%) junction encode a 210 kDa fusion protein ($p210^{BCR-ABL}$) [1]. In 5% of CML patients, both b3a2 and b2a2 transcripts can be made as a result of alternative splicing [1, 3]. In approximately 60% of Philadelphia chromosome positive ALL patients and in some cases of CML and AML the BCR breakpoint falls within the minor breakpoint cluster region (m*bcr*) that causes hybrid transcript containing an e1a2 junction, which is translated into a 190 kDa fusion protein ($p190^{BCR-ABL}$) [4, 5]. In the remaining Ph positive ALL patients (40%), the breakpoint occurs in the M*bcr* region [1, 6]. The micro breakpoint cluster region (μ-bcr) between BCR exon 19 and 20, result in the joining of exon 19 (e19) of bcr with a2, e19a2, coding for a 230-kDa (p230) protein has been report in rare cases of CML [7].

2. Objectives

This study was done to define the frequency BCR-ABL transcript variants among Iranian patients with different type of leukemia. This is the first report of the frequency of BCR-ABL transcript variants among ALL and AML patients from Iran.

3. Methods

This study was carried out in Pasteur Institute of Iran. The type of BCR-ABL rearrangement was studied in 348 chronic myloid leukemia (CML), 72 acute lymphoblastic leukemia (ALL) and 34 acute myeloid leukemia (AML) from

2001 to 2015. Written informed consent was obtained from all the patients or their family members. Initial diagnosis was carried out based on clinical presentation and morphological criteria of blood and bone marrow.

Peripheral blood (10 mL) in EDTA tubes was collected. Buffy coat was isolated by density gradient centrifugation on Ficol-Hypaque. Total RNA was extracted by Trizol (Invitrogen). One microgram of total RNA was reverse transcribed with random hexamer primers and Super-Script III Reverse Transcriptase (Invitrogen, Carlsbad, CA). The concentration of cDNA was determined by a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Rockland, DE) at 260 /280 nm.

The BCR/ABL transcript was amplified using multiplex RT-PCR. The primers were designed based on a previous study [8, 9], which are adapted to detect the following fusion transcripts: p190 (e1a2), p210 (b2a2, b3a2), p230 (e19a2) and p203 (b3a3, b2a3).

The following sequence of primers were used in multiplex RT-PCR for BCR-ABL fusion transcripts: C5e 5'ATAGGATCCTTTGCAACCGGGTCTGAA 3', B2B 5'ACAGAATTCCGCTGACCATCAATAAG3', BCR-C 5' ACCGCATGTTCCGGGACAAAAG 3' and CA3 5'TGTTGACTGGCGTGATGTAGTTGCTTG G3'. The multiplex RT- PCR program was 4 minutes at 95°C for initial denaturation followed by three steps, including 94°C for 30 seconds, 64°C for 50 seconds and 72°C for 50 seconds with 32 cycles later than a final 10 minutes at 73°C in the thermocycler 9700 (Applied Biosystems, Foster City, CA, USA).

In statistical analysis quantitative variables were expressed as mean $\pm$ SD, while qualitative variables were shown as percentages. Fisher's exact test was used in comparing qualitative variables. P values lower than 0.05 were considered as statistically significant.

4. Results

The BCR-ABL transcript frequencies for CML, ALL and AML patients were 92.0%, 12.5% and 14.7% respectively for all different of transcript. The majority of CML patients with BCR-ABL positive expressed one of the $p210^{BCR-ABL}$ transcripts (86.6%) while the rest had other transcripts ($p190^{BCR-ABL}$ 25 (7.8%)) and $p230^{BCR-ABL}$ 2 (0.6%)) or co-expression of $p210^{BCR-ABL}$ and $p190^{BCR-ABL}$ (16 (5%)). The b3a2 and b2a2 transcripts were detected in 173 (62.5%) and 104 (37.5%) of the BCR-ABL transcript positive CML patients respectively. In 66.7% of all patients with Philadelphia chromosome positive ALL were produced the shorter isotype p190 *BCR-ABL1* from the e1a2 type mRNA. Table 1 shows BCR-ABL transcript types, age, gender and WBC count data for the patients according to leukemia types.

We didn't find any correlation between the obtained *BCR-ABL1* variants, sex type, age and WBC count of studied leukemia patients.

5. Discussion

The frequencies of BCR-ABL gene in CML patients obtained by this study is consistent with frequencies reported in related studies [1]. The transcript distribution in CML has been reported in European and some other populations with frequencies for b2a2 and b3a2 transcripts being nearly of the order of 40% and 55% [10-13], which is relatively close to the previous from Iran report [8] and our study. However, a study on an Ecuadorian population, showed very different frequencies: 5% for b3a2 and 95% for b2a2 [6]. This difference in frequencies may possibly be due to the genetic background of the populations. Moreover, it was found that CML patients at diagnosis expressed low e1a2 transcript frequency, besides the usual *BCR-ABL1* p210 [14, 15] or only 5% [16], or no co-expression whatsoever in previous Iranian study [8]. In this study, co-expression of p210 and p190 in 5%, which is closed to Mexican study, was detected [16].

There are rare reports about frequency of *BCR-ABL1* variants in ALL type. In 49 patients diagnosed with ALL in Ecuadorian patients, 42.8% presented BCR-ABL fusion transcript, from these; all of them presented the e1/a2 rearrangement [6]. Moreover, it is found in 10% to 20% of adults and in 2% to 5% of children which two thirds of ALLs had e1/a2 transcript in Caucasian patients [1]. In this study, the frequencies of BCR-ABL gene were 12.5% which $p190^{BCR-ABL}$ was predominantly associated with ALL (66.7%). This may indicate a preferential functional involvement of this form of hybrid product in metabolic pathways of the lymphoid lineage progenitors.

There were very rare reported of BCR-ABL rearrangement in AML, which is reported in this investigation for the first time [1] (Table 1).

We didn't find correlation between *BCR-ABL1* variants, sex type, age and WBC count of studied leukemia patients significantly. As a final point, the frequencies study of different BCR-ABL transcript variants involved in leukemia in ethnic groups valuable to approach for a better understanding of the causes that lead to different BCR-ABL transcript variants in different types of leukemia.

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Table 1. BCR-ABL Transcript Frequencies and Clinical Data in Iranian Leukemia Patients

Disease	BCR-ABL Positive[a]	Protein Variant, kDa	Case[a]	Age, y[b]	Gender, M/F	WBC Count (10^9/L) in BCR-ABL Positive[b]	WBC Count (10^9/L) in BCR-ABL Negative[b]
CML, (n = 348)	320 (92)	p210	277 (86.6)	45.9 ± 16.9	185/163	303 ± 250	247 ± 237
		p190	25 (7.8)			141 ± 183	
		p210/p190	16 (5)			43 ± 46	
		p230	2 (0.6)			560	
						Total: 277 ± 249	
ALL, (n = 72)	9 (12.5)	p210	3 (33.3)	31.8 ± 15.6	48/24	227 ± 224	193 ± 199
		p190	6 (66.7)				
AML, (n = 34)	5 (14.7)	p210	2 (40)	39.7 ± 13.3	15/19	349 ± 419	219 ± 190
		p190	3 (60)				

[a] Values are expressed as No. (%).
[b] Values are expressed as maen ± SD.

Footnotes

Authors' Contribution: Mohammad Hamid, designed the study and wrote the manuscript; Hanieh Bokharaei performed the experiments and analyzed the data.

Conflict of Interest: No conflict of interest

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