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Prevalence of the *BCR/ABL1* transcripts in Mexican patients with chronic myelogenous leukemia

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ABSTRACT

RT-PCR studies in 93 patients with chronic myelogenous leukemia from the Mexican West were done in order to know the proportion of b2a2 and b3a2 *BCR/ABL1* transcripts. Forty-five patients showed the b3a2 transcript (48%), 37 (40%) displayed the b2a2 and in 11 cases (12%) both transcripts were detected. Statistical analyses showed that these figures are in accordance with two of three similar studies realized in Mexican population. Moreover, significant differences were found among Mexican people and patients from other countries, namely Ecuador, England, Italy, Poland, Japan, and Thailand. Ecuadorian patients showed differences with all the populations analyzed. These variations could be due to a different genetic background.

Key words. b2a2 and b3a2 transcripts. Chronic myelogenous leukemia. Mexican patients.

Prevalencia de los transcritos de BCR/ABL1 en pacientes mexicanos con leucemia mieloide crónica

RESUMEN

Con el objetivo de conocer la proporción de los transcritos b2a2 y b3a2 de BCR/ABL1, nosotros realizamos estudios de RT-PCR en 93 pacientes mexicanos con Leucemia Mieloide Crónica. Cuarenta y cinco pacientes (48%) mostraron el transcrito b3a2, 37 individuos (40%) el transcrito b2a2 y en 11 casos (12%) ambos transcritos fueron detectados. Análisis estadísticos muestran que estas cifras concuerdan con dos de tres estudios similares realizados en población mexicana. Por otra parte, diferencias significativas en las frecuencias de los transcritos fueron encontradas entre la población mexicana y pacientes de otros países (Ecuador, Inglaterra, Italia, Polonia, Japón y Tailandia). Los pacientes ecuatorianos mostraron diferencias con todas las poblaciones analizadas. Estas variaciones pudieran ser debidas a un componente genético diferente.

Palabras clave. *Leucemia mieloide crónica. Fusión BCR/ABL1. Transcritos b2a2 y b3a2. Pacientes mexicanos.*

INTRODUCTION

Chronic myelogenous leukemia (CML) is a hematological disorder characterized by a triphasic clinical course: a chronic phase, an accelerated phase, and a blast crisis. CML is characterized by a reciprocal translocation between chromosomes 9 and 22 -t(9;22)(q34;q11)- in at least 95% of patients, resulting in a 22q- or Philadelphia (Ph) chromosome.[1] This translocation produces a hybrid *BCR/ABL1* gene.[1,2] In most CML patients the breakpoints within the *ABL1* gene occur anywhere in an area larger than 300 kb upstream of the exon Ib, between exons Ib and Ia, or downstream from exon Ia; whatever it is, splicing yields a chimeric mRNA in which *BCR* sequences are fused to the *ABL1* exon a2.[1,2] The breakpoint within the *BCR* gene occurs in a 5.8 kb region known as major breakpoint cluster region (M-bcr).[3] The majority of breakpoints occur between exons 13 and 14 or bet-

ween exons 14 and 15, yielding the chimeric transcripts b2a2 and b3a2, respectively.[1,2] The b3a2 transcript is 75 base pair (bp) larger than the b2a2 transcript, but both encode a 210 kDa protein with increased tyrosine kinase activity.[4] Usually, CML patients display either the b2a2 or the b3a2 transcript; however, in 5% of the cases both transcripts are found.[1] The prevalence of b2a2 and b3a2 transcripts in CML patients has been assessed in many studies around the world, including Mexico.[5-15] Here, we determine the prevalence of b2a2 and b3a2 transcripts in CML patients from the Mexican West and compare it with other populations.

MATERIALS AND METHODS

Patients

Bone marrow samples from 93 CML patients from the Mexican West (the states of Jalisco, Colima, Michoacan, and Nayarit) were collected between January 2001 and December 2003 and analyzed by reverse transcription polymerase chain reaction (RT-PCR). Subjects at diagnosis and/or under treatment were included; they were 59 males and 34 females with a mean age of 35 years (range 1 to 75 years).

RT-PCR analysis

RNA was extracted from bone marrow or peripheral blood leukocytes by the acid guanidium thiocyanate-phenol-chloroform method.[16] cDNA was synthesized from 3 μg of RNA by oligo-dT priming (GIBCO). PCR for b2a2 and b3a2 *BCR/ABL1* transcripts was performed according to the following conditions: 35 cycles at 95 °C/1 min, 62 °C/1 min, and 72 °C/1 min; which had been preceded by denaturation at 96 °C for 3 min and followed by extension at 72 °C for 10 min; 2.5 U of Platinum Taq DNA polymerase, 2.5 mM $MnSO_4$ and the buffers supplied. The reaction was performed in 25 μL. The following primers were used; sense 5'-CGGGAGCAGCAGAAGAAGTGT-3' and anti-sense 5'-AAAGGTTGGGGTCATTTTCAC-3'.[17] Moreover, amplification of the Actin-B gene was used as an internal control with the primers sense 5'-CCAAGGCCAACCGCGAGAAGATGAC-3' and anti-sense 5'-GTCTGGCGGCACCACCATGTACCCT-3'. Ten microliters of every reaction were analyzed on a 6% poliacrylamide gel stained with $AgNO_3$. Positive cases showed bands of 238 bp (b2a2) and/or 313 bp (b3a2); actin-B amplification produced a band of 587 bp (data not shown).

STATISTICAL ANALYSIS

Chi-square test was applied to compare the prevalence of b2a2 and b3a2 transcripts in our study with those from other Mexican series.[5-7] Moreover, we did a Mexican group with our results and those previously reported.[5-7] in order to compare the prevalence of b2a2 and b3a2 transcripts of the Mexican population with those reported in other countries.[8-15]

RESULTS

In our sample the b3a2 transcript was the most prevalent one with a frequency of 48%, the b2a2 transcript was detected in 40%, and in 12% of the cases were found both transcripts. These results and those obtained in other studies realized in different Mexican regions[5-7] are shown in the table 1. Statistical analysis of these results displayed significant differences between some of them (Table 2).

Significant differences for the prevalence of b2a2 and b3a2 transcripts were found when Mexican patients were compared with other populations[8-15] (Table 3), namely Ecuador ($p < 0.001$), Japan ($p < 0.01$), England ($p < 0.01$), Thailand ($p < 0.01$), Italy ($p < 0.01$), and Poland ($p < 0.01$). Ecuador showed differences with all analyzed countries ($p < 0.001$, for every one). The remaining analyses did not show significant differences.

Table 1. Prevalence of the *BCR/ABL1* transcript types in CML Mexican patients from different regions.

Authors	n	b3a2 (%)	b2a2 (%)	b3a2/b2a2 (%)
Arana Trejo *et al.*[5]	226	39	53	8
Rosas Cabral *et* al.[6]	97	28	59	13
Ruiz-Argüelles *et al.*[7]	238	54	43	3
This study	93	48	40	12
All Mexican studies*	654	44	49	7

* Total sum of references 5, 6, 7 and our results.

Table 2. Statistical analysis (Chi-square test) among the different Mexican studies.

Comparison	b3a2 *vs.* b2a2	b3a2 or b2a2 *vs.* b3a2/b2a2
This study *vs.* Arana Trejo, *et al.*[5]	NS	NS
This study *vs.* Rosas Cabral, *et* al.[6]	$p < 0.01$	NS
This study *vs.* Ruiz-Argüelles, *et al.*[7]	NS	$p < 0.001$
Arana Trejo, *et al.*[5] *vs.* Rosas Cabral, *et* al.[6]	NS	NS
Arana Trejo, *et al.*[5] *vs.* Ruiz-Argüelles, *et al.*[7]	$p < 0.01$	$p < 0.01$
Rosas Cabral, *et* al.[6] *vs.* Ruiz-Argüelles, *et al.*[7]	$p < 0.001$	$p < 0.001$

NS: no significant.

Table 3. Prevalence of the *BCR/ABL1* transcript types in several countries.

Population[(Reference)]	n	b3a2 (%)	b2a2 (%)	b3a2/b2a2
England[8]	119	61	34	5
Japan[9]	57	60	30	10
Thailand[10]	91	66	34	ND
Poland[11, 12]	114	64	36	ND
Italy[13]	34	71	29	ND
Spain[14]	84	55	45	ND
Ecuador[15]	144	5	95	ND
Mexico*	654	44	49	7

* Source: Arana Trejo, *et al.*[5]; Rosas Cabral, *et al.*[6]; Ruiz-Argüelles, *et al.*[7] and our results. ND: not described.

DISCUSSION

Although the prevalence of *BCR/ABL1* transcripts in Mexican patients with CML varies in different studies,[5-7] the combined results show that a single transcript is found in 93% of the patients (b2a2, 49%; b3a2, 44%) whereas a minority of the cases (7%) displays both transcripts. Considering the presence of only one transcript, positive cases were 52% and 48% for b2a2 and b3a2, respectively. These numbers are similar to those that we calculate from studies done in other countries,[8-15] which were approximately 50% for every transcript (in most of the studies the presence of both transcripts was not described). The statistical test showed significant differences between Mexican subjects and individuals of six countries, but no with Spaniards. This fact probably reflects the Spanish genetic background of the Mexican mestizos' population. On the other hand, it is remarkable that the most frequent transcript in Latin American people (Mexicans and Ecuadorians) is b2a2 whereas in other countries is b3a2.[8-14]

Discrepancies within the Mexican population and between several populations could be fortuitous and related to variations in sample size or to methodological reasons; however, such differences may rather be related to different genetic background. Moreover, dual expression of b2a2 and b3a2 transcripts in CML patients is produced in individuals with linked polymorphisms within exon 13 and intron 13 which favor the elimination of exon 14 from both *BCR* and *BCR/ABL1* transcripts.[18] Therefore, certain DNA sequences with diverse population distributions could influence the occurrence of the breakpoint most frequently in one or another intron, or a biased alternative splicing that causes consequently, the preferential production of an isoform. It could explain partly the observed differences among the several populations like the Ecuadorian patients.

The significance of the type of transcript in the disease evolution (clinical parameters, platelet counts, duration of chronic phase, and survival) in CML patients has been already assessed in several studies.[6,8,9,11-12,19-24] Some researchers found association between the b3a2 transcript and elevated platelet counts[6,9,12,19,23] while others did not.[11,22,24] On the other hand, diverse studies did not find relation between any transcript and the duration of the chronic phase and/or survival,[8, 20-22,24] while Prejzner detected association between the b3a2 transcript and a longer survival, but, did not find differences in the duration of the chronic phase.[11] We did not look for any association between the type of transcript and clinical parameters because our group of study was

very heterogeneous respect to different disease's stages and therapies.

In conclusion, the discordance among the diverse studies may be accounted for multiple factors, such as late diagnosis, heterogeneous samples, therapeutic strategies, mutations and additional chromosomal changes, and different genetic backgrounds.

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