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ORIGINAL ARTICLE

Frequency of the minor BCR-ABL (*e1;a2*) transcript oncogene in a Mexican population with adult acute lymphoblastic leukaemia

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KEYWORDS
Acute lymphoblastic leukaemia;
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Abstract
Background: The minor BCR-ABL (*e1;a2*) transcript oncogene is the most common genetic alteration in adults with acute lymphoblastic leukaemia (ALL). It is associated with a poor prognosis.
Aim: To determine the frequency of minor BCR-ABL (*e1;a2*) transcript oncogene expression in ALL patients in Mexico.
Material and methods: A cohort of 411 patients with *de novo* ALL were tested for the oncogene using reverse transcription polymerase chain reaction (RT-PCR).
Results: The oncogene was found in 14% ($n = 57$) of the study population. Mean age was 29 years, and 53% were male. Median leucocyte count was 53×10^3 μl.
Conclusion: Prevalence of BCR-ABL expression by RT-PCR has not previously been reported in Mexico. Our laboratory found a higher prevalence than that reported in Latin-American series, but lower than that reported for the European population.

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PALABRAS CLAVE
Leucemia aguda linfóblastica;
Cromosoma Philadelphia;
Oncogén BCR-ABL

Frecuencia del oncogén BCR-ABL (*e1;a2*) rompimiento menor en población mexicana con leucemia linfóblastica aguda del adulto

Resumen
Introducción: El oncogén BCR-ABL (*e1;a2*) rompimiento menor constituye la alteración de mayor frecuencia en la leucemia aguda linfóblastica (LAL) del adulto. Su presencia se asocia con pronóstico adverso.
Objetivo: Determinar la frecuencia de la expresión del oncogén BCR-ABL (*e1;a2*) en portadores de LAL en México.
Material y métodos: Se estudiaron 411 pacientes con diagnóstico de LAL *de novo* para la búsqueda del oncogén mediante Reacción de cadena de polimerasa por Punto final (RT-PCR).
Resultados: El 14% ($n = 57$) de la población estudiada presentó expresión positiva. La edad promedio fue 29 años, el 53% correspondió al sexo masculino, la mediana de leucocitos fue 53×10^3 μl.
Conclusión: En México no hay reportes de la frecuencia de expresión de BCR-ABL por RT-PCR, nuestro laboratorio encontró una frecuencia mayor que lo reportado en las series Latino-Americanas y menor a lo reportado para población europea.

Introduction

Acute lymphoblastic leukaemia (ALL) is one of the most common types of cancer found in Mexico, with an average incidence of 5 cases per 100,000 inhabitants.[1] On average, 70 new cases of ALL are admitted to the Haematology Department of the General Hospital of Mexico each year. Several cytogenetic abnormalities are involved in the development of this type of cancer. The t(9;22) (q34;q11) translocation, known as the Philadelphia chromosome or *Ph* gives rise to the BCR-ABL fusion transcript. This transcript, together with abnormalities such as t(4:11), is associated with an adverse prognosis.[2] Incidence of this gene varies; reports suggest it to be 5% in the paediatric population,[3–5] and 25–50% in adults.[6–9] The minor BCR-ABL transcript codes for a chimeric protein (190 kDa) with tyrosine kinase activity, which is implicated in both the activation of various cell signalling pathways (RAS-GTP) and cell apoptosis (PI3K).[10–13] The BCR-ABL transcript has been associated with an adverse prognosis in most international studies.[14] The introduction of therapies that act on specific molecular targets, such as BCR-ABL tyrosine kinase (TK) inhibitors (*Glivec*©, Novartis) has improved overall survival rates when compared to traditional chemotherapy. There are various methods for isolating the BCR-ABL transcript, the most common being conventional karyotyping, fluorescent *in situ* hybridization (FISH), and polymerase chain reaction.[15–17] In Mexico, the Philadelphia chromosome is found in around 3.8% of the paediatric population[18] and 16.7% of adults,[19] isolated by reverse transcription polymerase chain reaction (RT-PCR) and conventional cytogenetics, respectively. In our laboratory, we amplify the BCR-ABL fusion transcript by means of RT-PCR, and perform around 60 tests on ALL patients each year. In this study, we describe the frequency of minor BCR-ABL expression in ALL patients compared with the international literature.

Materials and methods

An experimental, prospective, longitudinal study conducted from February 2000 to January 2010 in the molecular biology laboratory of the Haematology Department. The study was approved by the institution's independent ethics committees. Male and female patients with *de novo* diagnosis of ALL that agreed to give peripheral blood samples after having signed the informed consent form were included in the study. ALL was diagnosed in accordance with the French–American–British (FAB) classification systems, with the help of immunophenotyping and cytochemistry assays. Clinical data were sourced from the patient's medical records (Table 1).

Methodology

Leukaemia cells

Bone marrow samples were collected from ALL patients that had signed the informed consent form. Samples were collected in heparinized tubes containing Lymphoprep (Nycomed Pharma AS, Oslo, Norway) and centrifuged to obtain mononuclear cells.

Reverse transcription polymerase chain reaction (RT-PCR)

Total-cell RNA was isolated with Trizol (Life Technologies, Paisley, UK), and 1 μg of RNA was used for cDNA synthesis by means of MMLV (Life Technologies, Paisley, UK). The CMLB primers 5′ATCTCCACTGGCCACAAAATCATACA3′.

ALLA 5 AGATCTGGCCCAACGATGGCGAGGGC3 were used for PCR amplification. Results were validated by sequencing two positive samples (ABI PRISM 3100, Applied Biosystem, San Francisco, USA). Each cDNA was tested by PCR using primers specific for the constituent β_2 *microglobulin* gene.

Table 1 General clinical characteristics of patients with acute lymphoblastic leukaemia.

Characteristics	BCR-ABL	
	Negative *N* (%)	Positive *N* (%)
Total patients = 411	354 (86.13)	57 (13.86)
Sex		
Men	194 (54.8)	30 (53)
Women	160 (45.2)	27 (47)
	Median (range)	Median (range)
Median age (years)	29 (16–62)	25 (18–56)
Laboratory tests		
Baseline leucocyte count ($\times 10^3/\mu l$)	55.9 (0.7–789)	54 (1.2–207)
Haemoglobin (g/dl)	7.18 (4–10.9)	7.05 (5.4–11.5)
Platelets ($\times 10^3/\mu l$)	53 (0.88–388)	45 (2–432)
	N (%)	*N* (%)
FAB classification		
L1	7 (2)	0 (0)
L2	347 (98)	57 (100)
Immunophenotype		
B-cell	111 (81.6)	12 (100)
T-cell	25 (18.3)	0 (0)
Nervous system infiltration	7 (2)	0 (0)

PCR cycles of 1 min 94 °C, 1 min 55 °C, 1 min 72 °C were repeated 35 times. The PCR products were stained with ethidium bromide and visualized in a 1.5% agarose gel.

Results

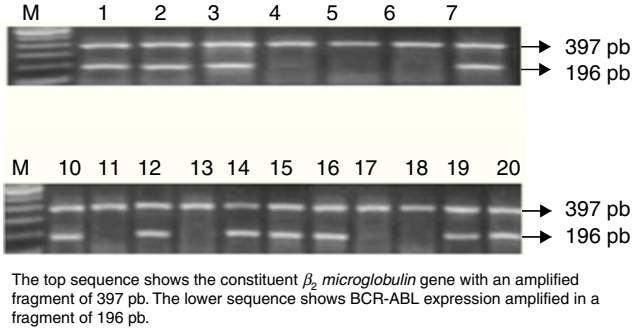
Patient characteristics

A total of 411 patients with a mean age of 29 years (range 16–62) were studied. By morphology, most ($n = 98\%$) presented acute lymphoblastic leukaemia (ALL-L2), with 81.6% corresponding to the B-cell immunophenotype. Only 2% showed central nervous system infiltration at diagnosis.

Expression of the minor BCR-ABL oncogene

All 411 *de novo* ALL cases were studied for BCR-ABL oncogene expression. RNA quality was evaluated by amplification of the constituent β_2 *microglobulin* gene, which amplifies a fragment of 397 bp by RT-PCR. BCR-ABL was isolated in 57 patients, amplifying a fragment of 196 bp. This represents 13.8% of the study population.

Mean age of the 57 BCR-ABL-positive patients was 25 years (range 18–56); 53% ($n = 30$) were men, and 47% ($n = 27$) were women. Mean leucocyte count at diagnosis was $54 \times 10^3/\mu l$ (range $1.2–207 \times 10^3/\mu l$). All (100%) patients were of the B-cell immunophenotype, and none showed central nervous system involvement (Fig. 1).



The top sequence shows the constituent β_2 *microglobulin* gene with an amplified fragment of 397 pb. The lower sequence shows BCR-ABL expression amplified in a fragment of 196 pb.

Figure 1 BCR-ABL expression in patients with ALL.

Discussion

In this study, we evaluated the prevalence of the BCR-ABL transcript fusion (*e1;a2*) in a population of ALL patients in Mexico using reverse transcription polymerase chain reaction (RT-PCR). This technique has been used since the 1990s by various international groups in both the diagnosis and follow-up or ALL Ph+. The first studies in RT-PCR reported a prevalence of the minor BCR-ABL transcript of 50%, with no difference in either prognosis or clinical presentation.[20,21] Researchers in the GIMEMA 0496 trial reported a prevalence of minor *vs.* major breakpoint of 58.5% and 41.5%, respectively.[22] Prevalence continues to vary across Latin America, ranging from 5.7% in adults and between 2.3% and 2.7% in the paediatric population.[22–24] Prevalence in ALL patients in the US is estimated at 19%,[25] and from 25% to 39% in Asia (Table 2).[26–29] Very few studies in BCR-ABL prevalence in children have been conducted in Mexico, and none in adults. In our laboratory, we found prevalence to be greater than that reported for Latin America, and lower than that reported for the American and European population. These discrepancies could be due to the genetic diversity of the Latin American population.[30] Nowadays, it is particularly important to isolate the BCR-ABL transcript in ALL patients due to the potential benefits of tyrosine kinase (TK) inhibitors, such as Imatinib, nolotinib, or Dasatinib.[31–34]

Research suggests that the combination of BCR-ABL and TK inhibitor therapy reverses the disease by providing a specific molecular target. In contrast to previous interpretations, this marker is now thought to indicate a good prognosis. In conclusion, ALL is one of the most common malignancies seen in the Haematology Department. Isolation of BCR-ABL in ALL patients is of primordial importance, particularly in view of the potential action of tyrosine kinase inhibitors.[35–37] Advances in molecular biology, such as real time PCR, will allow clinicians to monitor BCR-ABL transcript levels more closely. An understanding of the prevalence of this fusion gene in the Mexican population will give greater insight into ALL, improve management and monitoring of the disease, and introduce more specific TK-based therapy.

Table 2 Prevalence of the BCR-ABL Ph+ oncogene worldwide, by cytogenetic and RT-PCR testing.

Region	Patients	*N*	Prevalence (%)	Testing technique	Reference
Asia					
China	Adults	389	28.3	RT-PCR	Li et al.[27]
China	Adults	137	37	RT-PCR	Bao et al.[28]
Malaysia	Children	299	7.8	RT-PCR	Ariffin et al.[29]
Japan	Adults	285	22	Cytogenetic	Takeuchi et al.[34]
India	Adults and children	33	24 children19 adults	RT-PCR	Gurbuxani et al.[30]
US					
Canada	Adults	53	24	RT-PCR	Brandwein et al.[35]
Mexico	Children	59	2.7	FISH	Pérez-Vera et al.[17]
Mexico	Children	2	3.8	RT-PCR	Jiménez-Morales et al.[18]
Chile	Adults	35	5.7	Cytogenetic	Arteaga-Ortíz et al.[19]
Chile	Children	44	2.3	Cytogenetic	Legües et al.[31]
Europe					
USA–UK	Adults	1521	19	RT-PCR	Rowe et al.[14]
France	Adolescents	100	6	Cytogenetic	Boissel et al.[36]
Italy	Adults	216	19	Cytogenetic	Aninno et al.[37]

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Conflict of interest

The authors declare that they have no conflict of interests.

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