ART & TECHNOLOGY PUBLISHING PTE. LTD.

Cell Biology Research
2024 Volume 5, Issue 1
ISSN: 2529-7627(Online)
ISSN: 2529-7643(Print)

Frequency of BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 Fusion Genes in B-Cell Acute Lymphoblastic Leukemia

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Abstract

Objective: To describe the frequency of BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 fusion genes in patients with B-cell acute lymphoblastic leukemia at the Instituto Nacional de Salud del Niño of San Borja, Lima, Peru. *Methods:* We studied 375 samples from pediatric patients diagnosed with B-cell acute lymphoblastic leukemia, which were received at the Genetics Service. BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 fusion genes were detected in bone marrow or peripheral blood using reverse transcriptase polymerase chain reaction (RT-PCR). *Results:* Of the total samples, 58 were positive for BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 fusion genes, with the following frequencies: 2.13%, 4.53%, 0.53%, and 8.27%, respectively. *Conclusions:* The TEL-AML1 fusion gene is the most frequent in our pediatric population and is associated with a favorable prognosis.

Keywords

Lymphoblastic leukemia
Pediatrics
Molecular biology
Cytogenetics
Immunophenotyping

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of cancer in pediatric patients, which has seen improvements in survival with a high rate of cure through clinical trials [1]. The process by which ALL develops involves the uncontrolled abnormal proliferation of white blood cells or leukocytes in the bone marrow (BM), and then it spreads through other tissues via the peripheral blood (PB) [2]. The most common clinical features of this disease include hemorrhages, petechiae and ecchymosis, fever, bruising, and bone pain [3], leading to prompt emergency attention

and timely treatment based on the type of leukemia presented by the pediatric patient.

B-cell ALL is the most common type of ALL in children. Numerous advances in pathogenesis have provided a better understanding of the genes involved in the disease's biology with respect to diagnostic and prognostic implications. The most common genetic abnormalities in this pathology are the following fusion genes: BCR/ABL1 (variants p210 and p190), E2A/PBX1 (TCF3/PBX1), MLL/AF4 (KMT2A-AFF1) and TEL/AML1 (ETV6-RUNX1), corresponding to the following cytogenetic translocations: t(9;22)(q34;q11), t(1;19)(q23;p13), t(4;11)(q21;q23) and t(12;21)(p13;q22), respectively [4,5].

Well-established prognostic variables include patient factors such as age, initial leukocyte count 10^3/mm^3, genetic and immunophenotypic characteristics of leukemic blasts, and individual response to therapy [6].

In the context of the development of molecular biology at the national level, the frequency of fusion genes in the pediatric population with B-cell ALL is of utmost importance. Thus, the molecular characterization of fusion transcripts and their association with complementary cytogenetic and immunophenotyping studies will allow us to identify the proper management in the diagnosis, treatment, and prognosis of ALL at the Instituto Nacional de Salud del Niño de San Borja (INSNSB).

2. Methods

2.1. Study type

An observational, descriptive, and retrospective study was conducted at the Instituto Nacional de Salud del Niño de San Borja, from 1 January 2016 to 31 December 2020.

2.2. Population and sample

A sample population of 375 cases of patients under 18 years of age who met the criteria for B-cell ALL was collected. They were admitted to the molecular genetics department of the Genetics Service at INSNSB for a diagnostic study involving the molecular panel of fusion genes BCR/ABL1 (p210 and p190), E2A/PBX1, MLL/AF4, and TEL/AML1.

2.3. Data collection instruments

Patients diagnosed with B-cell ALL were identified through the review of molecular genetics results, cytogenetics, flow cytometry, and leukocyte count, ensuring that they met the inclusion and exclusion criteria. Patients were assigned a numerical code to maintain information confidentiality. Data on demographic and laboratory information (molecular, cytogenetic, flow cytometry, leukocyte count, and sample type) were retrieved from the INSNSB computer system, Sis Galen Plus. The data were recorded on a data collection form as an instrument, which was validated through a feasibility and viability agreement by the INSNSB Subunit of Research and Technological Innovation (SUIIT).

2.4. Data collection procedures

A description of demographic and laboratory variables was performed, using percentages and absolute frequencies for qualitative variables (sex, sample type, fusion gene, maturation stage, and cytogenetics), and measures of central tendency, such as the mean and standard deviation or median, for quantitative variables (age and leukocyte count), depending on whether they followed a normal distribution.

2.5. Data analysis

Descriptive statistics were employed for measures of central tendency using SPSS 26 statistical analysis software.

2.6. Ethical considerations

This research did not require active enrollment of any patient, so approval from the Ethics Committee of INSNSB was obtained for data collection. The information obtained was stored in the electronic data capture system (REDCap) for digital information

storage under specific codes created for this study.

2.7. RNA extraction and quantification

To extract ribonucleic acid (RNA), total blood was used, which was subjected to a monophasic solution of phenol and guanidine thiocyanate (phenol/chloroform method). Subsequently, the RNA was resuspended in molecular-grade water [7]. For RNA quantification, the Qubit® RNA Broad Range (BR) Assay Kit from Life Technologies (Q33230) was used with a Qubit Fluorometer (v2, Invitrogen by Life Technologies).

Next, the RNA underwent complementary DNA (cDNA) synthesis, which is necessary for its amplification using polymerase chain reaction (PCR). The synthesis of cDNA and PCR together is referred to as reverse transcriptase polymerase chain reaction (RT-PCR).

2.8. cDNA synthesis

For cDNA synthesis, the final RNA quantification in our laboratory standardization was 600 ng/µL for each sample. In this process, ProtoScript® II ReverseTranscriptase from New England Biolabs was used in a two-step procedure. 20 µL of the mixture was prepared for each sample in a 0.2 mL PCR tube. In the first step, 600 ng of template RNA (3 µL in the final mix), 2 µL of random primer mix, and 3 µL of molecular-grade water were used. The PCR tube with the complete mixture was then placed in the thermocycler at 65°C for 5 minutes. In the second step, 10 µL of 5X Buffer and 2 µL of reverse transcriptase were added. The tube was then placed in the thermocycler, where the 20 µL mixture was incubated at 25°C for 5 minutes, followed by 42°C for one hour. Subsequently, for enzyme inactivation, the temperature was raised to 65°C for 20 minutes. Finally, the produced cDNA was used for PCR or stored at -20°C for future use.

2.9. Polymerase chain reaction

The ABL1 gene was used as an internal control or housekeeping gene for PCR amplification in leukemias. The primer pairs used for the amplification of fusion genes in this study are shown in **Table 1**.

Table 1. Primers used in PCR

Genes	Sequence (5' → 3')
ABL1 primers (internal control)	
Forward	ATC TGC CTG AAG CTG GTG GGC T
Reverse	TGT GAT TAT ATA GCC TAA GAC CCG GGG AG
BCR-ABL1 primers (p210)	
Forward	GAA GTG TTT CAG AAG CTT CTC TCC
Reverse	GTT TGG GCT TCA CAC CAC CAC CAT TCC
BCR-ABL1 primers (p190)	
Forward	GACTGCAGCAGCTCCAATGAGAAC
Reverse	GTTTTGGGCTTCACACCACCACCATTCC
E2A-PBX1 primers	
Forward	CACCAGCCTCATGCACAAC
Reverse	TCGCAGGAGAGATTCATCACACG
MLL-AF4 primers	
Forward	CCGCCTCAGCCACCTAC
Reverse	TGTCACTGAGCTGAAGGTCG
TEL-AML1 primers	
Forward	TGCACCCTCTCTGATCCTGAAC
Reverse	AACGCCTCGCTCATCTCTTGC

For PCR reactions in a 0.2 mL PCR tube with a final volume of 25 µL, 3 µL of cDNA and final concentrations of 400 hM (forward and reverse primers), 2.5 mM dNTP, 1X buffer, 2.5 mM $MgCl_2$ and 1 U of Taq polymerase were used. The PCR tube was placed in the thermocycler with the following programming: initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 65°C for 1 min, and extension at 72°C for 1 min, with final extension at 16°C for 1 min.

2.10. Electrophoresis

The PCR products underwent electrophoresis using a 2% agarose gel. Safe-Green staining (abm), which contains 6X loading dye, was added to the samples. A transilluminator was used for visualizing the amplicons. A molecular weight (MW) marker with a size range of 100 bp to 1 kb was used. A comparison was also made with cytogenetics using Giemsa-trypsin-Giemsa (GTG) banding.

Digitized photographs of the gels were properly archived and encoded in computer folders. Data from cytogenetic studies, flow cytometry, and leukocyte count were obtained from the same institution.

3. Results

Out of the 375 samples processed in the molecular genetics area for molecular rearrangements corresponding to the BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 fusion genes, 58 samples showed positivity for one of the fusion genes in B-cell ALL panel for the period 2016–2020, while 317 samples tested negative for all four molecular rearrangements.

For the 58 positive samples, we analyzed general characteristics such as age, gender, and sample type. We also added clinical characteristics, including leukocyte count and immunophenotype for each sample. The average age of the BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 fusion genes positive was 13.9, 10.6, 5, and 8 years, respectively. The majority of individuals were male, with 40 male patients compared to 18 female patients. The sample type for all positives was equally represented by bone marrow (BM) and peripheral blood (PB), with 29 cases each. The average leukocyte count for the BCR-ABL1, E2A-PBX1, MLLAF4, and TEL-AML1 fusion genes was $108.2\times10^3/mm^3$, $55.3\times10^3/mm^3$, $155.6\times10^3/mm^3$, and $19.1\times10^3/mm^3$, respectively, with a total average of $84.6\times10^3/mm^3$. The distribution of immunophenotypes analyzed by flow cytometry was as follows: 26 common, 19 Pre-B, 3 Pro-B, and 10 mature stage (see **Table 2**).

Regarding the 375 processed samples, the BCR-ABL1 fusion gene was detected in 8 out of 375 (2.13%) children. In 6 of these 8 patients, the t(9;22) (q34;q11) cytogenetic translocation was observed. The E2A-PBX1 fusion gene was found in 17 out of

Table 2. General characteristics and immunophenotypes of ALL-positive patients classified according to BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 fusion genes

Parameters		BCR-ABL1	E2A-PBX1	MLL-AF4	TEL-AML1	Total
n		8	17	2	31	58
Average age (years)		13.9	10.6	5	8	9.4
Gender (F/M)		2/6	6/11	0/2	10/21	18/40
Sample (BM/PB)		3/5	7/10	2/0	17/14	29/29
Average leukocyte count ($\times10^3/mm^3$)		108.2	55.3	155.6	19.1	84.6
Immunophenotype	Common	5	1	0	20	26
	Pre-B	0	15	0	4	19
	Pro-B	1	0	2	0	3
	Mature	2	1	0	7	10

Table 3. Distribution of BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML1 positive fusion genes from INSNSB patients (2016–2020) associated with chromosomal translocations detected by conventional cytogenetics

Laboratory test	Molecular biology and cytogenetics	*n*	%
Molecular biology	BCR-ABL1	8	2.13
	E2A-PBX1 (TCF-PBX1)	17	4.53
	MLL-AF4 (KMT2A-AFF1)	2	0.53
	TEL-AML1 (ETV6-RUNX1)	31	8.27
	Negative	317	84.54
	Total	375	100
Cytogenetics	t(9;22)(q34;q11)	6	1.67
	t(1;19)(q23;p13)	9	2.51
	t(4;11)(q21;q23)	2	0.56
	t(12;21)(p13;q22)	0	0.00
	Other alterations (numerical and/or structural)	275	76.66
	No cell growth	67	18.66
	Total	359	100

375 (4.53%) children. Among the positive cases, 9 of them had the t(1;19)(q23;p13) in their karyotype. The MLL-AF4 fusion gene was detected in 2 out of 375 (0.53%) children. Similarly, the t(4;11)(q21;q23) was observed in their karyotype. For the TEL-AML1 fusion gene, it was detected in 31 out of 375 (8.27%) children, with the t(12;21)(p13;q22) not being detected in cytogenetics (see **Table 3**).

For BCR/ABL1, the p190 variant was considered in electrophoresis, which was more prevalent in our study and is primarily associated with ALL in pediatric patients (**Figure 1A**) [8]. Thus, for this fusion gene, its amplification size of 521 bp with its internal control (ABL1) of a size of 277 bp can be observed, along with the negative controls. Cytogenetics (**Figure 1B** and **1C**) could correlate this positivity with the t(9;22)(q34;q11.2) translocation, which was found in a complex karyotype in two of the three cell lines (**Figure 1B** and **1C**). The third cell line, which was normal (46,XY), was not considered in the figure for visualization.

In the case of E2A-PBX1(TCF3-PBX1), an amplification size of 373 bp can be observed with its internal control (ABL1) of a size of 277 bp, along with the negative controls (**Figure 2A**). Cytogenetics (**Figure 2B**) was associated with t(1;19)(q23;p13), which was found in the first of the two reported cell lines in the diagnosis. The second cell line, which was normal (46,XY), was not included in the figure for visualization.

For the MLL-AF4 fusion gene (KMT2A-AFF1), an amplification size of 673 bp was observed with its internal control (ABL1) of a size of 277 bp, along with the negative controls (**Figure 3A**). Cytogenetics (**Figure 3B**) was associated with t(4;11)(q21;q23), which was found in the first of the two cell lines reported at diagnosis. The second cell line, which was normal (46,XX), was not included in the figure for visualization.

For TEL-AML1 (ETV6-RUNX1), a size of 298 bp can be observed with its internal control (ABL1) of a size of 277 bp, along with the negative controls (**Figure 4A**). Cytogenetics (**Figure 4B**), did not visually correlate with the t(12;21)(p13;q22) due to the translocation occurring at breakpoint regions where the banding pattern is similar, but other alterations were found in the first cell line, such as t(1;16)(q32;q24), t(4;21)(p10;q10), and t(8;12)(p10;p10). The second cell line, which was normal (46,XY), was not included in the figure for visualization.

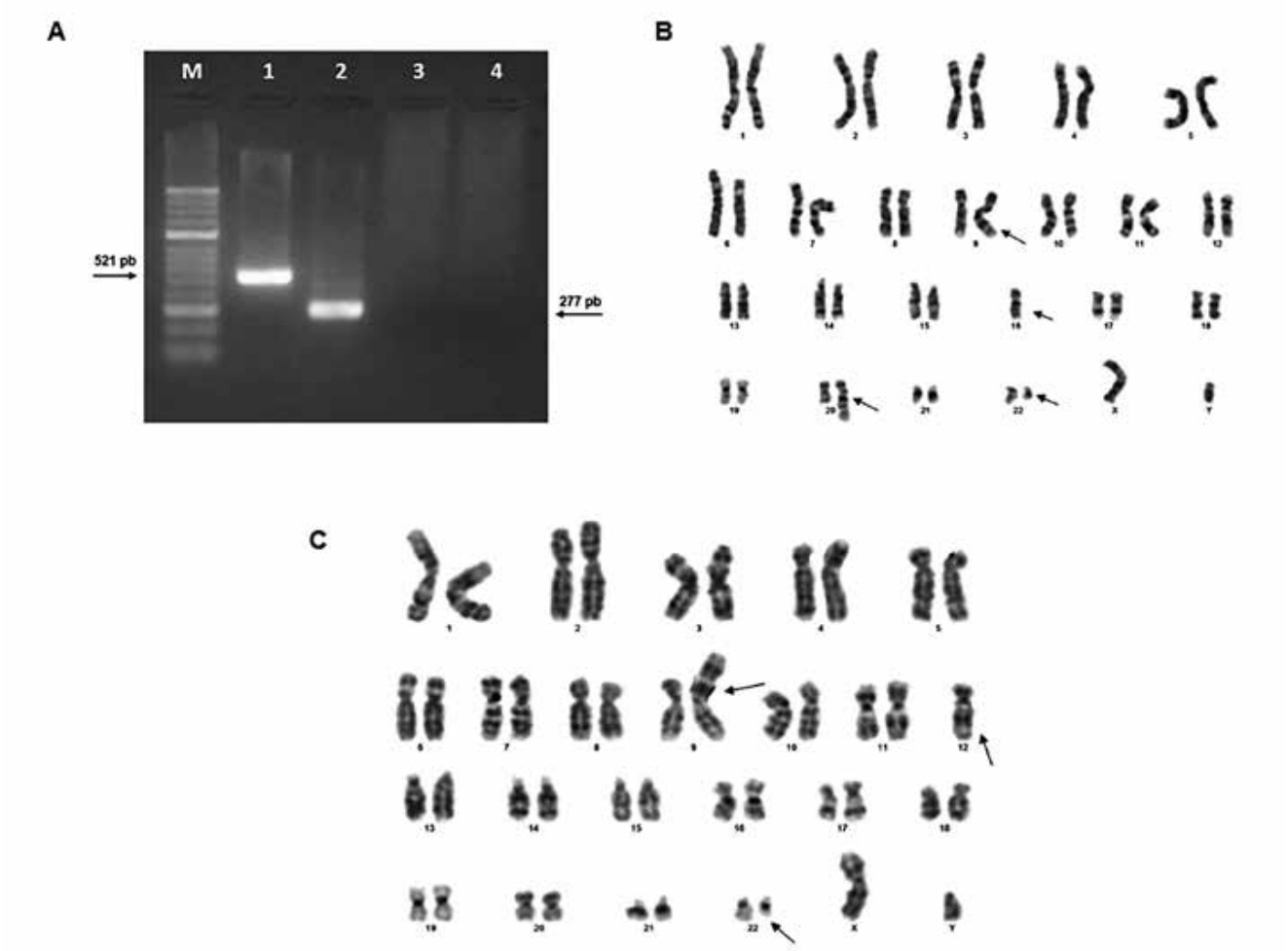

Figure 1. (A) PCR product for BCR/ABL1 fusion gene (p190 variant); M: molecular weight marker (100 bp), lane 1: 521 bp product of BCR-ABL1 p190, lane 2: 277 bp product of ABL1, lane 3: negative control of BCR-ABL1 p190, and lane 4: negative control of ABL1. **(B)** Karyogram of the first cell line with karyotype 45,XY,t(9;22)(q34;q11.2),-16,add(20)(q11.2). **(C)** Karyogram of the second cell line with karyotype 45,XY,der(9)t(9;12)(p22;q13)t(9;22)(q34;q11.2). All altered chromosomes are marked with arrows.

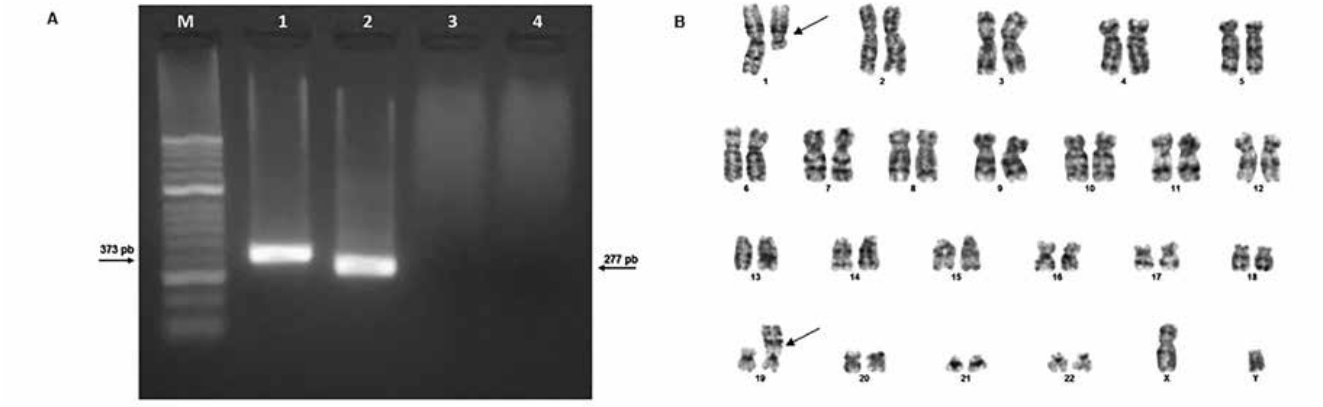

Figure 2. (A) PCR product for the E2A-PBX1(TCF3-PBX1) fusion gene; M: molecular weight marker (100 bp), lane 1: 373 bp product of E2A-PBX1, lane 2: 277 bp product of ABL1, lane 3: negative control of E2A-PBX1, and lane 4: negative control of ABL1. **(B)** Karyogram of the first cell line with karyotype 46,XY,t(1;19)(q23;p13.3). All altered chromosomes are marked with arrows.

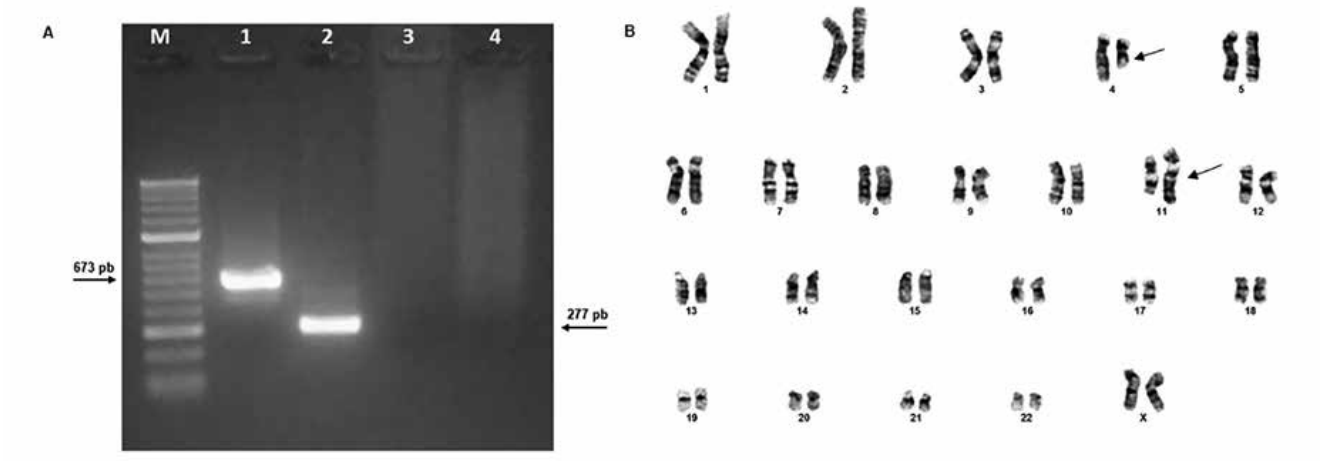

Figure 3. (A) PCR product for the MLL-AF4 fusion gene (KMT2A-AFF1); M: molecular weight marker (100 bp), lane 1: 673 bp product of MLL-AF4, lane 2: 277 bp product of ABL1, lane 3: negative control of MLL-AF4, and lane 4: negative control of ABL1. **(B)** Karyogram of the first cell line with karyotype 46,XX,t(4;11)(q21;q23). All altered chromosomes are marked with arrows.

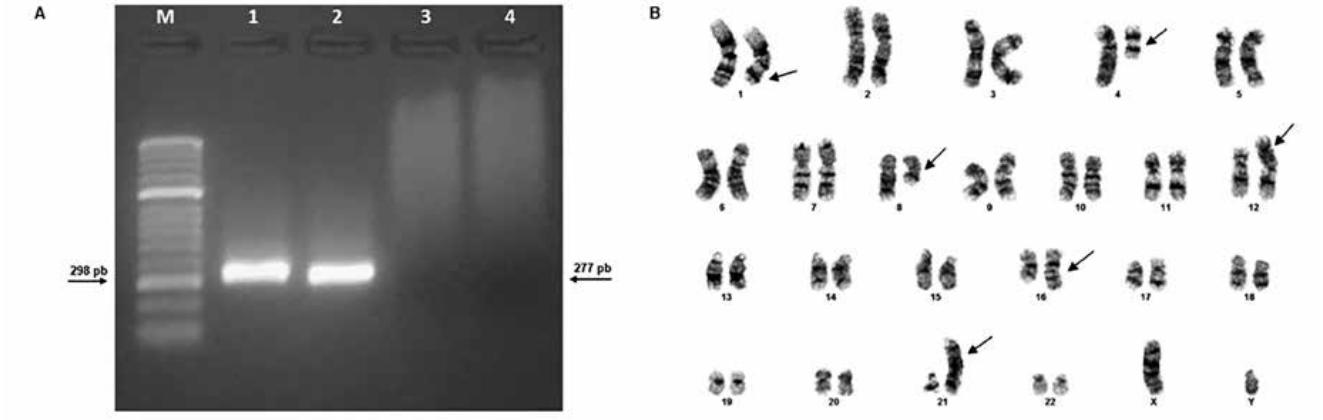

Figure 4. (A) PCR product for the TEL-AML1 fusion gene (ETV6-RUNX1); M: molecular weight marker (100 bp), lane 1: 298 bp product of TEL-AML1, lane 2: 277 bp product of ABL1, lane 3: negative control of TEL-AML1, and lane 4: negative control of ABL1. **(B)** Karyogram of the first cell line representing karyotype 46,XY,t(1;16)(q32;q24),t(4;21)(p10;q10),t(8;12)(p10;p10). All altered chromosomes are marked with arrows.

4. Discussion

The results presented in this study represent a significant advancement in molecular characterization within a public institution. Since INSNSB serves as a reference center for molecular diagnosis in pediatric ALL throughout Peru, it is essential to have all the tools necessary to facilitate successful diagnosis and treatment in all pediatric patients coming from various regions.

The frequency of fusion genes among our patients was as follows: BCR-ABL1 (2.13%), E2A-PBX1 (4.53%), MLL-AF4 (0.53%), and TEL-AML1 (8.27%). Similar values have been reported in Latin America. For instance, in 2016, a study in Mexico reported the TEL-AML1 had a frequency of 7.21%, followed by E2A-PBX1 at 5.15%, and the MLL-AF4 and BCR-ABL1 fusion genes were not found in the study [9]. Another study in Chile during the same year reported the presence of TEL-AML1 at 23% and BCR-ABL1 at 4% [10]. In Argentina, using 129 pediatric patients in 2005, the results were as follows: BCR-ABL1 (1.6%),

E2A-PBX1 (3.9%), MLL-AF4 (8.5%), and TEL-AML1 (11.6%) [11].

In the case of the BCR-ABL1 fusion gene, two peripheral blood samples tested positive for this gene but did not coincide with the chromosomal translocation t(9;22)(q34;q11). This discrepancy could be attributed to factors such as sample type. Molecular testing is more sensitive for detecting this fusion transcript [12]. Additionally, different breakpoint variants in the rearrangement of BCR-ABL1 can be easily analyzed using molecular techniques [13,14].

For the E2A-PBX1 fusion gene, eight samples out of all positive cases did not show the t(1;19)(q23;p13) translocation in cytogenetics. These "false negatives" in cytogenetics may be primarily due to the sensitivity of the assay. Molecular technology is more sensitive for the diagnosis of ALL [15]. Several laboratory-related factors can lead to the failure to identify genetic anomalies, including a low mitotic index of metaphases, which could be mistaken if only normal metaphases are analyzed. The type of sample, whether bone marrow or peripheral blood. can also play a crucial role, as the majority of abnormal white blood cells are found in the bone marrow, which is the primary source of all blood cell production [16]. Therefore, bone marrow samples may be more sensitive in certain cases than peripheral blood samples [17].

Cases have been reported where the karyotype detects the t(1;19)(q23;p13) translocation but it is not detectable with molecular tools. The explanation might be that positive cases for t(1;19) have an E2A-PBX1 fusion transcript that generally consists of exons 1–16 of E2A and exons 3–9 of PBX1. However, it does not exclude the presence of alternative fusions, as chimeric transcription consisting of exons 1–17 of E2A and exons 5–9 of PBX1 has been reported [18], which may not be detectable in RT-PCR using the standard primers used in our laboratory [4]. Likewise, cases of t(1;19)/der(19)t(1;19) have been observed within the context of hyperdiploid karyotype, only some of which express the E2A-PBX fusion gene and are associated with poor prognosis [19].

Regarding the MLL-AF4 fusion gene, it has the lowest prevalence. MLL rearrangements are primarily associated with infants and more aggressive leukemia, characterized by a rapid onset, hyperleukocytosis, and poor prognosis [20]. In our study, this rearrangement was associated with a Pro-B immunophenotype, which aligns with reports on t(4;11)(q21;q23), indicating a poor prognosis [21]. Pediatric patients positive for this rearrangement usually require more intensive treatment and are likely candidates for transplantation [22].

In the case of the TEL-AML1 fusion gene, which is associated with a favorable prognosis [23], it was the most prevalent (8.27%) in our population. This correlates with studies of ALL in other Latin American regions. For example, Argentina reported 11.6%, Chile 23.2%, and Mexico 25% [9-11]. TEL-AML1 could only be identified using RT-PCR because it is not visually detectable in cytogenetic studies due to its cryptic nature, i.e., it occurs at breakpoints of chromosomes 12 and 21 where the banding pattern is the same, so the alteration cannot be differentiated [24,25]. In some cases of TEL-AML1 positivity in molecular testing, other structural or numerical alterations were observed in the karyotype, which significantly impacts treatment decisions.

Therefore, the support of flow cytometry through immunophenotypic markers provided evidence that the common immunophenotypic was the most frequent, present in 26 of the total positive cases. This aligns with the most common variety reported in pediatric patients [26]. Including this complementary examination is crucial as a first-line tool in the classification of leukemia for determining the appropriate treatment strategy [27].

This study allowed for the detection of positive fusion gene transcripts in pediatric patients with ALL in the Peruvian population, using diagnostic support tools such as cytogenetics and immunophenotype. These tools combined provide the opportunity for timely personalized treatment at the time of diagnosis.

5. Conclusion

The TEL-AML1 fusion gene, considered to have a good prognosis, was the most common in the pediatric population with B-cell ALL at INSNSB. RT-PCR demonstrated higher sensitivity than cytogenetics for detecting the BCR-ABL1, E2A-PBX1, MLL-AF4, and TEL-AML fusion genes.

Acknowledgments

This work was supported by the Instituto Nacional de Salud del Niño de San Borja (INSNSB), through the Genetics Service, headed by Dr. Gioconda Manassero Morales; and also by the Research, Technology and Teaching Development Unit. The authors would also like to thank all colleagues of the Genetics Service in the area of cytogenetics and molecular genetics since these data were obtained through the invaluable work of all those who participated in these instances of the INSNSB.

Disclosure statement

The authors declare no conflict of interest.

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