Clinical significance of the ABCB1 and ABCG2 gene expression levels in acute lymphoblastic leukemia

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ABSTRACT

Objectives: Acute lymphoblastic leukemia (ALL) is a clonal disease that accounts for 20% of acute leukemias in adults. A high percentage of adult patients (ranging from 70 to 80%) reach complete remission; however, the 5-year survival rate is only 20–40%. One of the main obstacles to treatment success is the drug resistance of leukemic cells. Therefore, our research group analyzed the ABCB1 and ABCG2 gene expression levels in 61 patients diagnosed with ALL and assessed whether the levels affected the clinical parameters and 40-month survival rate.

Methods: The ABCB1 and ABCG2 gene expression levels were analyzed using real-time polymerase chain reaction in 61 patients diagnosed with ALL and 99 healthy donors as controls. The association between ABCB1 and ABCG2 gene expression levels and clinical variables was determined using the Chi-square test and Fisher’s exact test. Overall survival (OS) was determined using the Kaplan–Meier method.

Results: The results showed high ABCB1 and ABCG2 gene levels, which were 4.5 and 2.3 times the levels of healthy donors, respectively. A total of 52% of the study patients expressed high ABCB1 levels and were significantly associated with the high-risk patient group and a decreased 40-month survival rate of 78%. Only 49% of the patients expressed high ABCG2 gene levels. No association was found between the clinical parameters and the ABCG2 gene expression levels.

Conclusions: Early detection of ABCB1 gene expression levels could be important for the diagnosis and monitoring of ALL patients.

KEYWORDS

ABCB1; ABCG2; acute lymphoblastic leukemia

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by deregulation of the normal mechanisms of cellular proliferation, differentiation, and apoptosis inhibition. Its incidence in Mexico is five cases per 100,000 inhabitants [1,2]. The rates of the ALL cure range from 60% to 70% in children and 30% in adults, although they may vary from one country to another [1,2]. Cellular resistance to chemotherapeutic drugs is the main cause of treatment failure in most onco-hematological diseases [3], which translates into high rates of mortality from these conditions. One of the best characterized and perhaps most important mechanisms involved in chemotherapeutic drug resistance is the expression of drug resistance genes of the ATP-binding cassette (ABC) transporter family [4,5]. The ABCB1 gene [also known as multidrug resistance gene 1 (MDR-1)] is located on chromosome 7q21 and consists of 28 exons, encoding a 170-kDa membrane transport protein termed P-glycoprotein (P-gp). P-gp functions as an ATP-dependent efflux pump that transports exogenous and endogenous substrates from inside the cells to the extracellular space. Its normal expression has been identified in various human tissues, including intestinal epithelium, adrenal gland, placenta, kidney, liver, endothelial cells and testicular tissue, and P-gp naturally protects these organs against xenobiotics [8]. P-gp substrates include Adriamycin, daunorubicin, paclitaxel, vincristine, vinblastine, and imatinib. In cancer, P-gp was identified as a protein responsible for resistance to many drugs [6,7]. The ABCG2 gene is located on chromosome 4q22.1 and encodes a 72-kDa membrane transport protein termed breast cancer resistance protein (BCRP) whose function is to expel a wide variety of chemical compounds from tissues, including the brain, kidney, breast, lung, liver, blood–brain barrier, and placenta. Mitoxantrone, methotrexate, cladribine, topotecan, and imatinib are among the chemotherapeutic substrates of this protein.
These drug transporters are reportedly overexpressed in various types of cancer, including breast, lung, liver, brain and prostate cancer, leukemia, and lymphomas, and are a leading cause of drug resistance and treatment failure that contribute to decreased survival rates [8,9]. The clinical impact of the overexpression of these genes in adult ALL patients is controversial [10,11]. The present study shows that the ABCB1 and ABCG2 genes are overexpressed in ALL patients and that ABCB1 overexpression is associated with a worse prognosis and a decreased survival rate.

Materials and methods

Study population

Sixty-one ALL patients were included in this study, and 99 healthy donors as controls were recruited to this study after signing the informed consent form. The healthy donors had a mean age of 43 years (range from 18 to 52 years); 58 patients were men, and 41 were women. The ALL diagnosis was based on morphological findings and corroborated by immunophenotyping. The mean age of the patients was 36 ± 15 years, with a range from 18 to 78 years; 27 patients were men, and 34 were women. Of the 61 patients with ALL, 14 (23%) had lymphadenopathy, 6 (9.8%) had splenomegaly, and 7 (11.5%) had hepatomegaly. The white blood cell (WBC) and platelet counts were 45 x109/L (range 0–245) and 50.18 x109/L (range 7–252), respectively. The predominant immunophenotype was B cell in 58 (95.1%) patients, and only 3 (4.9%) patients had a predominant T cell immunophenotype. Cytogenetic abnormalities were observed in 16 (26.2%) patients, and the presence of the minor BCR–ABL oncogene transcript was identified in 2 out of 58 patients (3.4%). The initial treatment was based on the HGMLAL07 institutional protocols. The induction regimen consisted in administering vinca alkaloids, steroids, and anthracyclines. The use of L-asparaginase was not considered. Central nervous system prophylaxis was performed with the weekly administration of intrathecal chemotherapy during induction, and intermediate methotrexate doses were administered during the consolidation phase. The clinical characteristics of the patients are outlined in Table 1.

RNA extraction and cDNA preparation

Total cellular RNA was extracted from peripheral blood from the patients and controls using the TRizol® Reagent (Life Technologies, Paisley, UK). A total of 2 µg of RNA was used for cDNA synthesis using the Moloney murine leukemia virus (M-MLV) reverse transcriptase (Life Technologies, Paisley, UK).

Real-time polymerase chain reaction (qRT-PCR) analysis

The mRNA expression levels of the ABCB1 (Hs01069047), ABCG2 (Hs0105379), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs00985689) genes were measured using the TaqMan® gene expression assay (Applied Biosystems, Foster City, CA, USA). The GAPDH gene was used as an endogenous control, and each sample was analyzed in triplicate. The relative expression levels were calculated using the 2−ΔΔCt method with bone marrow as a calibrator. The high and low expression cut-off points were determined by the mean values observed in healthy donors.

Statistical analysis

The association between ABCB1 and ABCG2 gene expression and clinical variables was performed using the Chi-square test and Fisher’s exact test. Overall survival (OS) was determined using the Kaplan–Meier method, and the significance was established using the log-rank test, considering a value of p ≤ 0.05 significant. The statistical software Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

The relative expression levels of the ABCB1 and ABCG2 genes were analyzed in 61 patients and 99 healthy donors. The results showed high expression, with a 4.5-fold difference in ABCB1 and 2.3-fold difference in ABCG2 (p = 0.001) from the group of healthy donors (Figure 1). The frequencies of patients with ALL with

| Table 1. Clinical-pathologic characteristics of 61 adults with ALL. |
|-----------------------------|-----------------------------|
| **Clinical features**        | **Values**                  |
| Age                         | Mean ± SD (range) 36 ± 15 (18–78) |
| Median                      | 32                          |
| Sex                         | M 27 (44.3), F 34 (55.7)    |
| Lymphadenopathy (%)         | 14 (23)                     |
| Splenomegaly, 2 cm or larger (%) | 6 (9.8)                |
| Hepatomegaly, 2 cm or larger (%) | 7 (11.5)             |
| CNS (%)                     | 5 (8.1)                     |
| Laboratory data             |                             |
| PB Blast count              | Mean ± SD (range) 92 ± 10 (60–100) |
| Median                      | 94                          |
| WBC count, 10⁹/L (range)    | 45 (0–245)                  |
| Hemoglobin level, g/L (range) | 8.24 (2.80–14.20)          |
| Platelet count, 10⁹/L (range) | 50.18 (7–252)             |
| DHL (range)                 | 706 (90–4602)              |
| Immunophenotype (%)         | 58 (95.1)                   |
| B-lineage                   | 3 (4.9)                     |
| Cytogenetics (%)            |                             |
| Unsuccessful karyotype      | 25/61 (40.98)               |
| Normal karyotype            | 20/61 (32.78)               |
| Abnormal karyotype          | 16/61 (26.22)               |
| Molecular biology (%)       | 2/58 (3.44)                 |
| BCR-ABL                     |                             |
high expression levels of the ABCB1 and ABCG2 genes were 52% (32/61) and 49% (30/61), respectively.

Possible correlations between ABCB1 and ABCG2 gene overexpression and several prognostic factors of ALL were examined (Tables 2 and 3). The Spearman correlation tests showed a significant positive correlation between the high levels of ABCB1 mRNA expression and the absence of ganglion growth \( (p = 0.016) \), the B cell immunophenotype \( (p = 0.014) \), the low-risk group \( (p = 0.032) \), and a decreased survival rate \( (p = 0.001) \). No association was found between the clinical parameters and the ABCG2 gene expression levels. The coexpression of high levels of both genes (ABCB1 and ABCG2) was significant \( (p = 0.001) \).

The 40-month OS was assessed based on expression levels. The results showed that the ABCB1 gene expression levels affected the OS of ALL patients \( (p \leq 0.05, \text{log-rank test}) \). Of the positive patients with high expression levels, 25/32 (78.1%) died. Conversely, no patient with low expression levels died, whereas 19% (4/21) of the patients who did not express the gene died (Figure 2).

The ABCG2 expression levels had no significant effect on the 40-month OS rate of the ALL patients.

**Discussion**

The present study analyzed the ABCB1 and ABCG2 gene expression levels in adults diagnosed with ALL. The results demonstrated high variability of the expression levels in each sample tested, which could result from different factors, including environmental agents, benzene exposure, ionizing radiation, heat shock proteins, and the presence of single nucleotide polymorphisms in the promoter region \( [12–16] \). The present study found ABCB1 and ABCG2 gene overexpression in 52% and 49% of patients compared with the control group, respectively. The results are similar to other patient series in which high expression

**Table 2. Significance of the expression gene ABCB1.**

|                          | Negative | High | Low | \( P \) |
|--------------------------|----------|------|-----|--------|
| Age (years)              |          |      |     |        |
| <35                      | 10       | 19   | 4   |        |
| \( \geq 35 \)            | 11       | 13   | 4   |        |
| Sex                      |          |      |     |        |
| Male                     | 9        | 15   | 3   |        |
| Female                   | 12       | 17   | 5   |        |
| Hepatomegaly             |          |      |     |        |
| Yes                      | 2        | 3    | 2   |        |
| No                       | 19       | 29   | 6   |        |
| Splenomegaly             |          |      |     |        |
| Yes                      | 2        | 2    | 2   |        |
| No                       | 19       | 30   | 6   |        |
| Lymphadenopathy          |          |      |     |        |
| Yes                      | 4        | 5    | 5   |        |
| No                       | 17       | 27   | 3   | 0.016* |
| Leukocytes               |          |      |     |        |
| \(<30 \)                 | 14       | 22   | 3   |        |
| \( \geq 30 \)            | 7        | 10   | 5   |        |
| Serum LDH level \(<500\) | 9        | 19   | 5   |        |
| \( \geq 500 \)           | 12       | 13   | 3   |        |
| Immunophenotype          |          |      |     |        |
| B Lineage                | 20       | 32   | 6   | 0.014* |
| T Lineage                | 1        | 2    | 2   |        |
| Cytogenetics             |          |      |     |        |
| Successful karyotype     | 6        | 14   | 5   |        |
| Normal karyotype         | 7        | 11   | 2   |        |
| Abnormal karyotype       | 8        | 7    | 1   |        |
| BCR                      |          |      |     |        |
| Positive                 | 1        | 3    | 0   |        |
| Negative                 | 20       | 29   | 8   |        |
| Risk                     |          |      |     |        |
| High                     | 17       | 15   | 6   |        |
| Low                      | 4        | 17   | 2   | 0.032* |
| Response                 |          |      |     |        |
| Good responder           | 15       | 18   | 5   |        |
| Poor responder           | 6        | 14   | 3   |        |
| Coexpression             |          |      |     |        |
| No coexpression          | 21       | 2    | 1   |        |
| ABCB1 and ABCG2          | 0        | 30   | 7   | 0.001* |
| Status                   |          |      |     |        |
| CCR                      | 17       | 7    | 8   |        |
| Death                    | 4        | 25   | 0   | 0.001* |

*Significance level \( p \leq 0.05 \).
levels of 36.7% for the ABCB1 gene and 32% for the ABCG2 gene were reported [15–16]. ABCG2 expression is reportedly 2.4-fold lower in the pediatric population than in adults, and ABCB1 gene expression is higher in the ALL population than in patients with acute myeloid leukemia [17–19].

The association between the ABCB1 gene expression levels and clinical parameters is controversial. The discrepancies in the results of various studies may result from the methods used, including flow cytometry and semi-quantitative RT-PCR [20–26]. No association was found between the ABCG2 expression levels and the clinical parameters, which corroborated Sauerbrey et al., who analyzed 47 patients diagnosed with ALL and 20 patients with relapsed ALL by RT-PCR. The results showed that no correlation existed between the high expression levels and the clinical parameters [20].

The high ABCB1 gene expression levels were associated with a decreased 40-month survival rate compared with the low expression levels (21.8% versus 100%). However, patients with low or negative expression levels showed a higher survival rate in our study due to the response to treatment. These results also corroborated the results of Koorti et al. [17], who reported that high expression levels caused a decreased disease-free survival rate of 55.5% compared with patients with low expression levels (86.6% survival). In another case series, Brozek et al. [26] reported that the functional activity of the ABCB1 protein shortened the 5-year survival rate in 35% of positive patients versus 74% of patients without functional ABCB1 protein expression. This finding may be a result of cells expressing high ABCB1 levels expelling the administered chemotherapeutic agents as a drug resistance measure. No significant association was found between the 5-year survival rate and high ABCG2 expression levels. Cortez et al. [19] reported that low ABCG2 expression levels caused high toxicity in pediatric patients with ALL and were associated with a high risk for death and treatment toxicity.

Table 3. Significance of the expression ABCG2.

|                          | Negative | High  | Low   | P       |
|--------------------------|----------|-------|-------|---------|
| Age (years)              |          |       |       |         |
| <35                      | 4        | 18    | 11    |         |
| ≥35                      | 6        | 12    | 10    |         |
| Sex                      |          |       |       |         |
| Male                     | 3        | 12    | 12    |         |
| Female                   | 7        | 18    | 9     |         |
| Hepatomegaly             |          |       |       |         |
| Yes                      | 1        | 4     | 2     |         |
| No                       | 9        | 26    | 19    |         |
| Splenomegaly             |          |       |       |         |
| Yes                      | 2        | 2     | 2     |         |
| No                       | 8        | 28    | 19    |         |
| Lymphadenopathy          |          |       |       |         |
| Yes                      | 2        | 7     | 5     |         |
| No                       | 8        | 23    | 16    |         |
| Leukocytes               |          |       |       |         |
| <30                      | 7        | 16    | 16    |         |
| ≥30                      | 3        | 14    | 5     |         |
| Serum LDH level          |          |       |       |         |
| <500                     | 5        | 15    | 13    |         |
| ≥500                     | 5        | 15    | 8     |         |
| Immunophenotype          |          |       |       |         |
| B Lineage                | 10       | 28    | 20    |         |
| T-ALL                    | 0        | 2     | 1     |         |
| Cytogenetics             |          |       |       |         |
| Unsuccessful karyotype   | 4        | 13    | 8     |         |
| Normal karyotype         | 3        | 12    | 5     |         |
| Abnormal karyotype       | 3        | 5     | 8     |         |
| BCR                      |          |       |       |         |
| Positive                 | 0        | 2     | 2     |         |
| Negative                 | 10       | 28    | 19    |         |
| Risk group               |          |       |       |         |
| High                     | 5        | 21    | 12    |         |
| Low                      | 5        | 9     | 9     |         |
| Response                 |          |       |       |         |
| Good responder           | 6        | 15    | 17    |         |
| Poor responder           | 4        | 15    | 4     |         |
| Coexpression             |          |       |       |         |
| No coexpression          | 10       | 6     | 8     |         |
| ABCB1 and ABCG2          | 0        | 24    | 13    | 0.000(*)|
| Status                   |          |       |       |         |
| CCR                      | 6        | 14    | 12    |         |
| Death                    | 4        | 16    | 9     |         |

*Significance level $p \leq 0.05$.

Figure 2. Overall survival in ALL patients based on the ABCB1 and ABCB2 expression levels. The 40-month OS rate is analyzed in patients with high (21%), low (100%), and negative (81%) ABCB1 and ABCB2 gene expression levels, which have no significant relationship with survival ($p = 0.291$).
The results showed that the drug resistance genes ABCB1 and ABCG2 were expressed in adult patients diagnosed with ALL. High ABCB1 expression levels were associated with the risk group and disease status. The ABCG2 expression levels were not associated with any clinical parameter. These ABCB1 gene expression levels led to a 40-month survival rate of 21.8%. Therefore, examining the role of these genes in disease progression is very useful for decision making in ALL treatment.

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