Immunophenotyping of Childhood Acute Lymphoblastic Leukemia in Qazvin City, Iran: A Cross-Sectional Study

Majid Vafaie¹, Mohammad Derhami², Hamid Sadeghi², Saeideh Gholamzadeh Khoei³*

¹. Clinical Research Development Unit, Qods Hospital, Qazvin University of Medical Sciences, Qazvin, Iran.
². Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.
³. Clinical Research Development Unit, Kowsar Hospital, Qazvin University of Medical Sciences, Qazvin, Iran.

* Corresponding Author:
Saeideh Gholamzadeh Khoei, PhD.
Address: Clinical Research Development Unit, Kowsar Hospital, Qazvin University of Medical Sciences, Qazvin, Iran.
Phone: +98 (28) 33328213
E-mail: s.gholamzade@yahoo.com

Background: Acute lymphoblastic leukemia (ALL) is the most prevalent childhood cancer. ALL is a heterogeneous type of malignancy and treatment protocols vary based on the immunological classification of ALL. The critical step for treating ALL is to identify immunological subgroups by flow cytometry findings.

Objective: In this study, immunophenotypic information was evaluated for the first time in children with ALL in Qazvin City, Iran.

Methods: This cross-sectional study reviewed the clinical and laboratory data of children with ALL during 2019-2020. Next, children with ALL were immunophenotyped by flow cytometry applying a panel of the specific monoclonal antibodies for some clusters of differentiation (CD) molecules, including CD20, CD21, CD10, CD34, CD38, and terminal deoxynucleotidyl transferase (TdT). The data were separately analyzed using SPSS software, version 24.

Findings: Of 52 children with ALL in the age range of 6 months to 15 years, 23 children (44.23%) had B-ALL-ProB (pro-B) cell immunotyping features, 26 (50%) had B-ALL-PreB (pre-B) cell immunotyping features, and 3 (5.7%) had T-cell immunotyping features. The ages of T-cell group children were higher than those of B-cell group children. The most common clinical and laboratory findings were fever (26 cases, 55.31%). In 55% of children, periodic acid-schiff (PAS) staining was positive. The presence of the terminal deoxynucleotidyl transferase (TdT) enzyme was higher in B-cell patients than in T-cell cases. Children with CD34+ were higher in the pro-B group than in the pre-B group.

Conclusion: our study shows that the immunophenotypic characteristics of children with ALL are more similar to previous reports and can be used for monitoring and prognosis of children with ALL in Qazvin City.

Keywords: Acute lymphoblastic leukemia, Immunophenotyping, Flow cytometry
1. Introduction

After unintentional injuries and violence, cancer is ranked as the second most frequent cause of mortality among children and teens [1]. Leukemia is the most common childhood cancer, accounting for about 33% of all pediatric cancers worldwide [2]. Evidence has shown that agents affecting fetus genetic mutations, stability, and cell development pathways may be responsible for a substantial part of childhood leukemia [3]. Acute lymphoblastic leukemia (ALL) is a major cancer in children with an incidence approximately five times higher than acute non-lymphoblastic leukemias [4].

Acute leukemia can be classified according to the following, morphology (FAB), cytochemistry, such as periodic acid-schiff (PAS) staining or identification of the terminal deoxynucleotidyl transferase (TdT) and myeloperoxidase enzymes, immunophenotype (WHO classification), cytogenetics, and combination of immunophenotype and cytogenetics (new WHO). The immunophenotypic classification identified 80% of ALL cases in B-lymphoblastic, 15% in T-lymphoblastic, and 1% in Burkitt lymphoma [5]. Chemotherapy protocols are associated with immunological subtypes and disease risk levels and vary based on good and bad prognostic factors [5]. Immunophenotyping of leukemic cells can detect different surface and cytoplasmic antigens related to varying stages of growth and lineage of malignant cells [6].

To distinguish and classify B-cells, wide surveys have been conducted based on superficial and cytoplasmic expressions of immunoglobulin M (IgM) and diverse CD markers, such as CD10, CD19, CD20, and so on [7, 8]. CD20 is considered one of the most sensitive and specific markers in B-ALL [9]. Primary screening with the CD20 molecule has empowered the classification of the leukemic cells into two categories B- and T-cells [10-12].

Flow cytometry is a rapid and reproducible method that allows fast identification of the leukemia lineage and hence proper initiation of the best treatment [13]. Immunophenotyping by flow cytometry has revolutionized our understanding of ALL diagnoses. These advances and fine-tuning of the diagnostic pathways used to assess ALL have led to sub-classification based on exact biological insights and in some cases, targeted therapy [14, 15].

Due to the importance of ALL in the Iranian population, the need to study ALL subgroups to determine the prognosis and select efficient treatment for patients, and the lack of statistics in this field in Qazvin Province, the present study was designed.

2. Materials and Methods

Study design, population, setting, and procedures

This cross-sectional study included children with ALL younger than 15 who were referred to the Qods Hospital in Qazvin City, Iran, from April 2019 to 2020. Diagnosis of ALL was mainly based on clinical demonstrations and immunophenotypic criteria. Accordingly, the information of patients with ALL, including age, sex, time of diagnosis, and clinical, and laboratory findings was recorded at the time of diagnosis.

Heparinized bone marrow and peripheral blood samples were prepared from children with ALL clinical and laboratory symptoms before treatment for the diagnosis.

Bone marrow samples were assessed by the flow cytometry department using CD markers, including CD20, CD21, CD10, CD34, CD38, PAS, TdT, and others at Mahak Hospital, Tehran City, Iran.

Accordingly, 52 samples in this study were divided into 49 children with B-ALL and 3 children with T-ALL subtypes by evaluating the expressions of CD20 and CD21. In addition, the expression of CD10, CD34, CD38, PAS, and TdT was used to classify the B-ALL group into pre-B and pro-B groups.

The results of all sample tests were evaluated by two hematopathologists and confirmed the malignancy.

Ethical approval

Patients’ consent forms were obtained and the Ethics Committee of Qazvin University of Medical Sciences authorized this study protocol.

Statistical analysis

Statistical analysis was performed using the SPSS software, version 24 and chi-square test. P<0.05 were considered significant.

3. Results

Among the 52 children enrolled in this study, 18 children (34.6%) were female and 34 (65.4%) were male, with a median age of 5.7 years (range 6 months-15 years). Also, 60% of children (31) were at the peak age of 2 to 5 years. Tables 1, 2, and 3 present the frequency
distribution of clinical and laboratory findings in the studied children. Based on the expression pattern of CD20, CD21, CD10, CD34, PAS, and TdT, we classified 52 children (containing recorded flow cytometric information) into three subtypes, including pro-B, pre-B, and T-ALL.

According to flow cytometry findings, 23 children (44.23%) had a pro-B immunophenotype with CD20-, 26 children (50%) had a pre-B with CD21-, and 3 children (5.7%) had T-ALL (Table 1).

Fever was observed in 55.31% of children with ALL, which was the most common clinical feature in patients.

The female-to-male ratio among children with ALL was 1:2; therefore the percentage of ALL in males was higher than in women (65.4% vs. 34.6%). However, the frequency analysis of ALL subtypes showed no significant difference between gender and ALL subtypes. While a significant difference was observed between age and ALL subtypes since the age of the children with T-ALL is less than B-ALL.

Frequency analysis of the clinical and laboratory findings, including splenomegaly, hepatomegaly, lymphadenopathy, central nervous system (CNS) involvement, median white blood cell (WBC), hemoglobin (Hb), and platelets Plt, fever, bleeding (petechiae or purpura), and bone pain in different ALL subtypes did not demonstrate a significant relationship.

PAS staining was positive in 55% of children. Also, the percentage of the TdT enzyme was higher in children with B-ALL than with T-ALL. The expression of CD34 in T-ALL was lower than in B-ALL and it was higher in pro-B patients than in pre-B (Table 3).

4. Discussion

In developed countries, one of the most crucial pediatric cancers is childhood acute leukemia with a floating occurrence rate of 10-45 cases per 106 children per year [2, 16]. In the last decades, prominent progress has been made in the therapy of childhood cancer, leading to the primary detection of the disease. Immunophenotyping is considered a vital component of the primary and valuable tool for the diagnosis and monitoring of minimal residual disease (MRD) [17]. Different goals were targeted in the immunophenotypic characterization of leukemic cells, including the evaluation of cell maturation, lineage assignment, and phenotypic abnormalities [17]. Hundreds of different monoclonal antibodies have been allocated to the CD antigens due to the diagnosis of various types of malignancies, leukemia, and lymphoma [7].

In this study, we used a panel of monoclonal antibodies against CD20, CD21, CD10, CD34, CD38, PAS, and TdT to characterize the phenotype of leukemic cells in 52 children involved in ALL.

According to the results, the frequency of children with T-ALL (5.7%) was much lower than B-ALL (94.23%). Our results were similar to those of a recent study conducted by Lanzkowsky et al. [5], in which 80% of patients were B-ALL and 20% were T-ALL patients. Other studies conducted in different countries, such as the USA, Italy, Germany, Saudi Arabia, Thailand, and others have also obtained similar results so that the frequency of T-ALL has always been substantially lower than B-ALL [18-22]. The T-cell immunophenotype is associated with a poorer outcome [23]. T-cell phenotype patients had lower age and this relationship was significant. As shown in Table 1, the median age of children with T-ALL (2 years) is less than children with B-ALL (5 years). Contradictory to our results in the studies conducted by Lanzkowsky et al. in 2016 [24] and Tong et al. in 2011 [9], the age of the T-ALL phenotype was significantly higher than the age of the B-ALL phenotype. The present disagreement is mainly due to the small population of children with T-ALL in our study.

The male gender seems to be also associated with an unfavourable prognosis in ALL. In this regard, Khawaja et al. in 2005 [25] and Chavoshi et al. in 2015 [26] reported that ALL was more prevalent among males than females. Consistent with the mentioned studies, 65.4% of children with ALL in our study were male.

In the examination of the relationship between clinical and laboratory data, no difference was observed in lymphadenopathy, hepatomegaly, fever (as the most common findings), purpura, or bone pain with the Lanzkowsky investigation [24]. The only difference was related to splenomegaly so that in our study, 27.65% of children were included; however, in the Lanzkowsky study, 63% of children were included, and the observed difference was due to the lack of information related to pediatric ultrasound in our study.

Furthermore, according to the study by Lanzkowsky et al. [24], our results revealed no significant relationship between WBC, Hb and Plt counts with ALL subtypes. Although the amount of WBC count in children with T-ALL (363450 in mm3) was higher than B-ALL, it was not significant due to the small T-ALL population in this study.
CNS involvement which is an unfavourable prognostic factor in ALL was observed in 4 cases from 44 patients with ALL (9.09%), and it was more frequently observed in pro-B rather than pre-B children. However, this difference is not significant. Consistent with our results, in another study, the rate of CNS involvement was lower in ALL patients [27].

Of 47 children with PAS information, 26 cases (55%) were positive, while according to the study conducted by Lanzkowsky et al. [24], all cases were expected to be positive. In addition, the PAS-positive rate in pre-B (65.38) was higher than in pro-B (34.78). Besides, the total of 87.76% of TdT positivity children was the highest in B-ALL (85.7%) compared to T-ALL (33%), which was consistent with the previous report by Rezaei et al. [28].

In our study, 96% of B-ALL blasts and 33% of T-ALL blasts expressed the CD10 antigen and the difference between them was significant. Other studies have shown that CD10 appears specifically in B-ALL [28, 29].

Table 1. The frequency distribution of clinical Findings in children with acute lymphoblastic leukemia (ALL)

<table>
<thead>
<tr>
<th>Variables</th>
<th>B-ALL-ProB</th>
<th>B-ALL-PreB</th>
<th>T-ALL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometry</td>
<td>23(44.23)</td>
<td>26(50.0)</td>
<td>3(5.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender Male</td>
<td>16(69.56)</td>
<td>16(61.54)</td>
<td>2(66.66)</td>
<td>0.15</td>
</tr>
<tr>
<td>Female</td>
<td>7(30.44)</td>
<td>10(38.46)</td>
<td>1(33.33)</td>
<td></td>
</tr>
<tr>
<td>Median age</td>
<td>5.0 years (3-9)</td>
<td>5.0 years (3-9)</td>
<td>2 years (1-5)</td>
<td>0.003</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>7(30.43)</td>
<td>5(19.23)</td>
<td>1(33.33)</td>
<td>0.071</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>5(21.73)</td>
<td>3(11.53)</td>
<td>1(33.33)</td>
<td>0.068</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>6(26.08)</td>
<td>8(30.76)</td>
<td>1(33.33)</td>
<td>0.058</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>3(13.04)</td>
<td>1(3.84)</td>
<td>0(0)</td>
<td>0.078</td>
</tr>
<tr>
<td>Fever</td>
<td>9(39.13)</td>
<td>15(57.69)</td>
<td>2(66.66)</td>
<td>0.083</td>
</tr>
<tr>
<td>Bleeding (petechiae or purpura)</td>
<td>5(21.73)</td>
<td>6(23.07)</td>
<td>1(33.33)</td>
<td>0.057</td>
</tr>
<tr>
<td>Bone pain</td>
<td>4(17.39)</td>
<td>9(34.61)</td>
<td>1(33.33)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Abbreviations: ALL: Acute lymphoblastic leukemia; CNS: Central nervous system. P<0.05 were considered significant.

Table 2. The frequency distribution of laboratory findings in children with acute lymphoblastic leukemia (ALL)

<table>
<thead>
<tr>
<th>Variables</th>
<th>B-ALL-ProB</th>
<th>B-ALL-PreB</th>
<th>T-ALL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median WBC 10100/mm$^3$</td>
<td>13350/mm$^3$ (5625-24850)</td>
<td>7900/mm$^3$ (4100-23850)</td>
<td>3634/mm$^3$ (29900-697000)</td>
<td>0.086</td>
</tr>
<tr>
<td>Median Hb 7.50 g/dL</td>
<td>7.80 g/dL (5.80-8.80)</td>
<td>6.80 g/dL (5.35-10.20)</td>
<td>7.40 g/dL (5.6-9.5)</td>
<td>0.068</td>
</tr>
<tr>
<td>Median Plt 39000/mm$^3$</td>
<td>39000/mm$^3$ (20500-105000)</td>
<td>30000/mm$^3$ (14000-198000)</td>
<td>71500/mm$^3$ (54000-89000)</td>
<td>0.084</td>
</tr>
</tbody>
</table>

Abbreviations: ALL: Acute lymphoblastic leukemia; WBC: White blood cell; Hb: Hemoglobin; Plt: Platelet count. P<0.05 were considered significant.
Of 51 patients with CD information, 30 cases (58.82%) expressed CD34, the specific stem cell marker, which included only B-ALL (78.26% of pro-B and 46.15% of pre-B), corroborating the results of previous studies [9, 28]. The difference between subtypes of B-ALL and also between B and T-ALL is significant (P<0.05). This may be one of the reasons that ALL in pre-B with a good prognosis originate from more initial stem cells.

The expression of CD38 was observed in 44 children (86.27%), which included 82.60% of pro-B, 84.62% of pre-B, and 100% of T-ALL. Our results similar to those of Tong et al. [9] did not show a significant difference between subtypes.

The disparities reported between research can be attributed to the limited sample number of patients in many studies, as well as methodological variations in the definition of ALL, [30] geographical distribution [31], and age [32].

5. Conclusion

Altogether our study shows that the immunophenotypic profile of children with ALL is mostly similar to earlier publications in Iran and it is applicable for monitoring and prognosis of the children with ALL in Qazvin City.

Limitation

The small sample size of this study was one of the limitations of this study that was highly related to the willingness of patients to continue treatment in Tehran, the capital of Iran.

Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee of Qazvin University of Medical Sciences authorized this study protocol (IR.QUMS.REC.1398.302).

Table 3. Immunophenotyping findings in children with acute lymphoblastic leukemia (ALL)

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-ALL-ProB</td>
<td>B-ALL-PreB</td>
</tr>
<tr>
<td>CD10 +</td>
<td>21(91.30)</td>
<td>25(96.15)</td>
</tr>
<tr>
<td>CD10 -</td>
<td>1(3.34)</td>
<td>1(3.84)</td>
</tr>
<tr>
<td>CD34 +</td>
<td>18(78.26)</td>
<td>12(46.15)</td>
</tr>
<tr>
<td>CD34 -</td>
<td>4(17.39)</td>
<td>14(53.85)</td>
</tr>
<tr>
<td>CD38 +</td>
<td>19(82.60)</td>
<td>22(84.62)</td>
</tr>
<tr>
<td>CD38 -</td>
<td>3(13.04)</td>
<td>4(15.38)</td>
</tr>
<tr>
<td>PAS +</td>
<td>8(34.78)</td>
<td>17(65.38)</td>
</tr>
<tr>
<td>PAS -</td>
<td>12(52.17)</td>
<td>7(26.92)</td>
</tr>
<tr>
<td>TdT +</td>
<td>19(82.60)</td>
<td>23(88.46)</td>
</tr>
<tr>
<td>TdT -</td>
<td>2(8.7)</td>
<td>2(7.7)</td>
</tr>
</tbody>
</table>

Abbreviations: ALL: Acute lymphoblastic leukemia; CD: Cluster of differentiation molecules; PAS: Periodic acid–Schiff; TdT: Terminal deoxynucleotidyl transferase.

P<0.05 were considered significant.
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Authors' contributions

Conceptualization and methodology: Majid Vafaie and Mohammad Derhami; Validation and project administration: Majid Vafaie; Investigation: Mohammad Derhami; Formal analysis: Hamid Sadeghi; Writing, original draft, review, editing and supervision: Saeideh Gholamzadeh Khoei.

Conflict of interest

The authors declared no conflict of interest.

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