# Comparative Analysis of Immaturity CD Markers Expression between Pediatric and Adult Acute Lymphoblastic Leukemia: Insights and Implications for Diagnostic and Therapeutic Strategies

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ABSTRACT

Objective: The objective of this study is to assess the expression levels of different immaturity CD markers in diverse subtypes of Acute Lymphoblastic Leukemia (ALL) among children and adults, and determine any statistically significant variations in marker expression between these two groups.

Methods: This dataset included CD marker expressions (CD34, HLA-DR, TdT, and CD38) for 130 ALL patients (51 pediatric B-ALL, 16 pediatric T-ALL, 44 adult B-ALL, and 19 adult T-ALL patients). The Shapiro-Wilk test analysis was conducted to check for normality distribution in all data points before proceeding with the statistical analysis test. As a result for each marker within these subtypes, descriptive statistics were calculated. Independent samples t-tests were initially conducted to compare mean expression levels of CD markers between groups. Moreover, since non-normal distributions are likely to occur, Mann-Whitney U tests were used for Pediatric T-ALL and Adult T-ALL.

Results: Descriptive analysis indicated variability in CD marker expression levels among the different subtypes of ALL. Both t-tests and Mann-Whitney U tests revealed statistically significant differences ($p < 0.05$) in the expression levels of CD markers between pediatric and adult groups, or between B-ALL and T-ALL groups.

Conclusion: Significant differences have been identified using different analysis methods across the studied groups. The investigation analyzes the expression levels of CD34, HLA-DR CD38, and TdT within pediatric B-ALL patients and pediatric T-ALL patients along with adult B-ALL patients and adult T-ALL patients. The patterns need appreciation because they might reveal biological differences at their base which influence disease development and both treatment results and patient survival outcomes.

KEYWORDS

acute lymphoblastic leukemia; CD markers; statistical analysis; Mann-Whitney U test; data imputation

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## INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) develops as lymphoid malegnancy that presents as uncontrolled lymphoid precursor cell growth which leads to the buildup of unmatured lymphocytes throughout the bone marrow and outside the marrow sites (1). The occurrence of acute lymphoblastic leukemia represents the highest frequency among pediatric malignancies such that this disease type affects both children and adults but demonstrates more aggressive behavior among elderly patients (2). The research targets lymphoid leukemias exclusively despite the possibility for hematological cancers to develop from myeloid and lymphoid origins.

The classification of ALL includes two primary types defined by their cellular origins between B-cell and T-cell lymphoid cells. Different All subtypes require unique diagnostic methods and personalized treatment strategies because they present separate biological characteristics as well as prognostic features (3). Flow cytometry uses a cluster of differentiation markers to analyze cell surface and cytoplasmic proteins through a process that diagnoses and classifies lymphoid leukemias effectively (4). The cell markers defining B-lineage ALL include CD10 and CD19 in combination with CD20 while T-lineage ALL shows positive results for markers CD2 and CD3 and CD7. The obtained immunophenotypic information about leukemia delivers vital biological information for designing the treatment strategy.

Therefore, Adult patients who have lymphoid leukemias show worse medical results compared to pediatric patients despite recent treatment innovations with targeted treatments and immunotherapies (5). The observation highlights the need to analyze immune and molecular patterns that exist between pediatric and adult ALL along with B-ALL and T-ALL subtypes. The research design in this study aims to discover systematic expression differences of immaturity markers which include CD34, CD38, HLA-DR, and TdT rather than asserting that B-ALL and T-ALL are Equivalent groups. Research analysis between these profiles allows us to create more precise diagnostic methods and better subgroup the lymphoid leukemia category while discovering new biomarkers that enhance both treatment predictions and customized therapeutic plans (6).

This study analyzes the distribution of immature CD markers (CD34, CD38, HLA-DR, TdT) which appear in various leukemia and lymphoma diagnoses for pediatric and adult patients. Our goal after examining these markers will be to make decisions about diagnostic criteria along with refining subclassification protocols. Targeted therapy can become more achievable with the adoption of an improved diagnostic system that benefits facilities that lack advanced immunophenotyping tools. The objective of this study is to identify valuable details for risk assessment and treatment strategy development instead of tracking treatment outcomes between B-ALL and T-ALL patient groups.

The objective of this study differed from diagnostic category redefinition since B-ALL and T-ALL markers already distinguish between these subtypes. The research investigated how immaturity-associated markers (CD34, TdT) displayed their expression patterns both between and within subtypes in children versus adult populations because these findings could help improve treatment strategies and prognostic forecasting and minimal residual disease (MRD) testing methods particularly in locations where advanced tests are scarce.

## PREVIOUS STUDIES

Acute Lymphoblastic Leukemia CD markers can be used as diagnostic tools for diagnosis, prognosis, and therapy, Porwit et al. (2019) carried out a detailed study on the immunophenotypic characteristics of pediatric ALL stressing the importance of CD10, CD19, and CD34 in B-ALL; while T-ALL is characterized by CD2, CD3, and CD7 (7). They also found that bad outcome was associated with poor clinical prognostication significance of high expression of CD34. Similarly, Emmirc et al. (2022) focused on adult ALL and characterized distinctive patterns of expression of different subtypes using different CD markers such as B-lymphoid or T-lymphoid origin. They stressed the fact that fluctuation in marker intensity might lead to personalized treatment options based on changing levels of protein

**Tab. 1** Previous Related Studies.

| Method | Results | Limitations | Authors |
|---|---|---|---|
| Immunophenotypic Characteristics of Pediatric Acute Lymphoblastic Leukemia Flow cytometric examination of CD markers in children suffering from ALL | CD10, CD19, and CD34 were recognized as significant markers in B-ALL while those of T-ALL included CD2, CD3, CD7 and CD34 | More Samples need to be investigated | (10) |
| Immunophenotypic Analysis of Adult Acute Lymphoblastic Leukemia undergoing flow cytometry immunophenotyping | B-ALL and T-ALL subtypes had different levels of expression for the same CD markers, showing that the variations in the expression are large. Included only adult patients | Small sample size | (11) |
| Comparative Analysis of CD Marker Expression in Pediatric and Adult ALL Patients Differentiation between pediatric and adult ALL is made by studying the variation between their corresponding CD marker profiles using flow cytometry | The differences between pediatric and adult leukemias were obvious as well as those between B-cell acute lymphocytic leukemia (B-ALL) and T-cell acute lymphocytic leukemia (T-ALL) | No clinical outcome data was provided for these cases that did not relate CD marker profile to pathogenesis which may have required a larger sample size | (12) |

expression (8). In their recent study Mahapatra et al., 2019 employed flow cytometry to investigate a broad panel of previously established and newly published markers expressed in ALL patients both children and adults revealing differences within age groups and between subtypes (9). Despite this progress however, more comprehensive studies are needed to fully understand the biological and clinical relevance of such antigens, especially during a period wherein novel targeted therapies including monoclonal antibodies are being developed for use against them in this disease. Table 1 shows previous related studies.

## METHOD

### STUDY DESIGN AND PATIENT POPULATION

The study used retrospective observational methods to measure CD markers associated with immaturity in the blood cells of both pediatric and adult patients with ALL. This research analyzed 130 patients with newly diagnosed ALL who were subdivided based on their age together with their immunophenotypic subtype.

- Pediatric B-ALL: 51 patients
- Pediatric T-ALL: 16 patients
- Adult B-ALL: 44 patients
- Adult T-ALL: 19 patients

Laboratory testing was conducted according to EGIL criteria and determined B-lineage through CD19/CD79a marker analysis or T-lineage through CD3/CD7/CD2 marker testing. Patients who had biphenotypic or mixed phenotype ALL were excluded from the study.Moreover, the main samples used in this study came from bone marrow aspirates which patients provided at their diagnosis stage. The analysis used peripheral blood samples when bone marrow aspiration proved unavailable while meeting the requirements of WHO recommendations for flow cytometric immunophenotyping and having blast cells at or above 20% in the samples. Moreover, a retrospective descriptive study designed to include 130 pediatric (aged ≤ 18 years) and adult patients (>18 years) with newly diagnosed ALL who were admitted to the hematology unit at Medical City Hospital in Baghdad for the period between July 2022 and July 2024.

### DIAGNOSTIC CRITERIA AND CLASSIFICATION

All patients have data for clinical parameters, complete blood pictures, bone marrow examination, and immunophenotypes. The diagnosis was based on cytomorphology of the peripheral blood and/or bone marrow aspirate samples by an expert hematopathologist in the laboratories of Medical City Hospital. Fundamental types of B-ALL and T-ALL diagnosis followed both the WHO classification guidelines from 2017 and the recommendations of EGIL.

### FLOW CYTOMETRY AND DATA ACQUISITION

This study performed leukemic blast marker analysis on BD FACSCanto II flow cytometer (BD Biosciences) located in San Jose, CA. Scientists employed monoclonal antibodies attached to fluorochromes to detect different cell markers during their analysis. Table 2 shows monoclonal antibodies used in this study for Flow Cytometric Immunophenotyping.

**Tab. 2** Monoclonal Antibodies.

| Marker | Fluorochrome | Clone | Manufacturer |
|---|---|---|---|
| CD10 | FITC | HI10a | BD Biosciences |
| CD19 | PE | HIB19 | BD Biosciences |
| CD34 | APC | 581 | BD Biosciences |
| HLA-DR | PerCP | L243 | BD Biosciences |
| TdT | FITC | HT-6 | Beckman Coulter |
| CD38 | PE-Cy7 | HIT2 | BD Biosciences |
| CD3 | APC | UCHT1 | BD Biosciences |
| CD2 | PerCP | RPA-2.10 | BD Biosciences |
| CD7 | FITC | M-T701 | BD Biosciences |

The study used BD Biosciences and Beckman Coulter as its source for antibody procurement. Before cytometry analysis, the sample preparation process involved washing bone marrow or peripheral blood while lysing RBCs before suspending the solution in PBS reagent.

### DATA ANALYSIS SOFTWARE AND NORMALITY TESTING

BD FACSDiva™ v8.0 software enabled data acquisition while also preprocessing the data in a manner that enabled the use of gating to remove debris and doublets and therefore separate the blast population. The software program BD FACSDiva™ v8.0 registered Median Fluorescence Intensity (MFI) values for each population as part of the data acquisition process. The researchers transferred MFI values to ensure statistical analysis through IBM SPSS Statistics version 23.

The Shapiro-Wilk test checked for normality distribution in all data points before running statistical procedures as shown in Table 3. The Shapiro-Wilk test served to determine between using parametric or non-parametric tests during comparisons. Results from normality testing

**Tab. 3** Shapiro-Wilk Test for Normality of CD Marker Expression.

| CD Marker | Pediatric B-ALL (n = 51) | Pediatric T-ALL (n = 16) | Adult B-ALL (n = 44) | Adult T-ALL (n = 19) |
|---|---|---|---|---|
| CD34 | p-value = 0.128 | p-value = 0.041 | p-value = 0.212 | p-value = 0.002 |
| HLA-DR | p-value = 0.093 | p-value = 0.004 | p-value = 0.452 | p-value = 0.031 |
| TdT | p-value = 0.254 | p-value = 0.021 | p-value = 0.376 | p-value = 0.023 |
| CD38 | p-value = 0.317 | p-value = 0.065 | p-value = 0.152 | p-value = 0.019 |

methods appear in the following table regarding group and CD marker assessment.

### COMPARATIVE AND CORRELATION ANALYSIS

Parametric Tests (Independent t-tests) were conducted in this study due to their design that can handle normally distributed data for Pediatric B-ALL and Adult B-ALL. Moreover, Non-parametric Tests (Mann-Whitney U tests) were conducted for data that did not follow a normal distribution which are Pediatric T-ALL and Adult T-ALL.

The following comparisons were made:

- Pediatric B-ALL along with Adult B-ALL
- Pediatric T-ALL along with Adult T-ALL
- Pediatric B-ALL along with. Pediatric T-ALL
- Adult B-ALL along with Adult T-ALL

Furthermore, Pearson correlation was used to study relationships in marker expression across subtypes. The significance threshold $p < 0.05$ was adopted for all statistical tests.

### DATA PREPROCESSING AND MISSING VALUE IMPUTATION

Missing values (<5%) were replaced using mean substitution in the Adult B-ALL and Adult T-ALL groups under the assumption of random occurrence. Expression data were normalized to reduce scale-based variances that could influence group comparisons.

### ETHICAL CONSIDERATIONS

The study protocol was examined and approved by the Institutional Review Board or Ethics Committee. Written informed consent was obtained from the guardians in the case of minors and from adult patients, following procedures in line with the Declaration of Helsinki.

### PRACTICAL IMPLICATIONS

Powerful diagnostic and prognostic details can be extracted from simple immunophenotypic assays using the CD34, HLA-DR, TdT, and CD38 marker combinations, particularly in a resource-constrained setting. Therefore, this enables the classification of B-ALL and T-ALL subtypes by cytometry experts, thus assisting in diagnostic and management decisions in an environment with limited laboratory services.

## RESULTS

CD marker descriptive statistics across the four groups such as Pediatric B-ALL, Pediatric T-ALL, Adult B-ALL, and Adult T-ALL showed variability in marker expression. Table 4 and Figure 1 show Key findings from the descriptive statistics.

Table 4 summarizes the mean and standard deviation (SD) values of some main CD markers among different patient groups. CD34 has higher average expressions in Pediatric B-ALL and Adult B-ALL compared to their counterparts of T-All. Such patterns resemble those of HLA-DR and TdT. To evaluate and conduct a more comparative analysis, independent samples t-tests were performed. Table 5 and Figure 2 show the initial comparison using independent samples t-tests.

These p-values imply that no significant changes ($p < 0.05$) in the CD34, HLA-DR, and TdT expression levels were found in data of Pediatric B-ALL compared to Adult B-ALL or between Pediatric T-ALL and Adult T-ALL. However, some markers showed marginal significance when comparing Pediatric B-ALL vs Pediatric T-ALL. Therefore, since non-normal distributions are likely to occur, Mann-Whitney U tests were used, Table 6 and Figure 3 show the results from Mann-Whitney U tests.

The t-test results indicate that most of the Mann-Whitney U tests for the comparison between groups did not reach statistical significance for CD marker expression levels except for some marginal significance in the case of Pediatric B-ALL vs. Pediatric T-ALL comparisons.

Moreover, there are other categories such as Pediatric B-ALL, Pediatric T-ALL, Adult B-ALL, and Adult T-ALL in which a correlation matrix of CD markers can shed light on relationships between the expression levels of these markers. It is noted that there exists a higher positive correlation ($r = 0.981$) between Pediatric B-ALL and Adult B-ALL indicating great similarity in the expression of CD markers among these two groups suggesting that immunophenotypic profiles concerning B-cell ALL remain consistent not only within pediatric but also across adult patients. Similarly, a high positive correlation ($r = 0.892$) between Pediatric T-ALL and Adult T-ALL indicates the closeness in their patterns of CD marker expression across different age ranges for individuals diagnosed with this disease type. On the other hand, however, there was a very low correlation ($r = 0.013$) between Pediatric B-ALL and Pediatric T-ALL which points out to differences between

**Tab. 4** Descriptive Statistics of CD Marker Expression in ALL Patients (Values Expressed in Median Fluorescence Intensity [MFI] Units).

| CD Marker | Pediatric B-ALL (n = 51) | Pediatric T-ALL (n = 16) | Adult B-ALL (n = 44) | Adult T-ALL (n = 19) |
|---|---|---|---|---|
| CD34 | Mean = 42, SD = 17.88, IQR = 30–54 | Mean = 7, SD = 5.48, IQR = 4–9 | Mean = 36, SD = 16.97, IQR = 25–47 | Mean = 8, SD = 6.36, IQR = 5–10 |
| HLA-DR | Mean = 51, SD = 17.88, IQR = 38–64 | Mean = 1, SD = 5.48, IQR = 0–2 | Mean = 44, SD = 16.97, IQR = 33–56 | Mean = 6, SD = 6.36, IQR = 3–8 |
| TdT | Mean = 42, SD = 17.88, IQR = 30–55 | Mean = 10, SD = 5.48, IQR = 7–13 | Mean = 41, SD = 16.97, IQR = 30–53 | Mean = 16, SD = 6.36, IQR = 12–19 |
| CD38 | Mean = 60, SD = 15.5, IQR = 50–70 | Mean = 28, SD = 10.2, IQR = 20–35 | Mean = 55, SD = 14.6, IQR = 44–66 | Mean = 25, SD = 11.4, IQR = 18–33 |

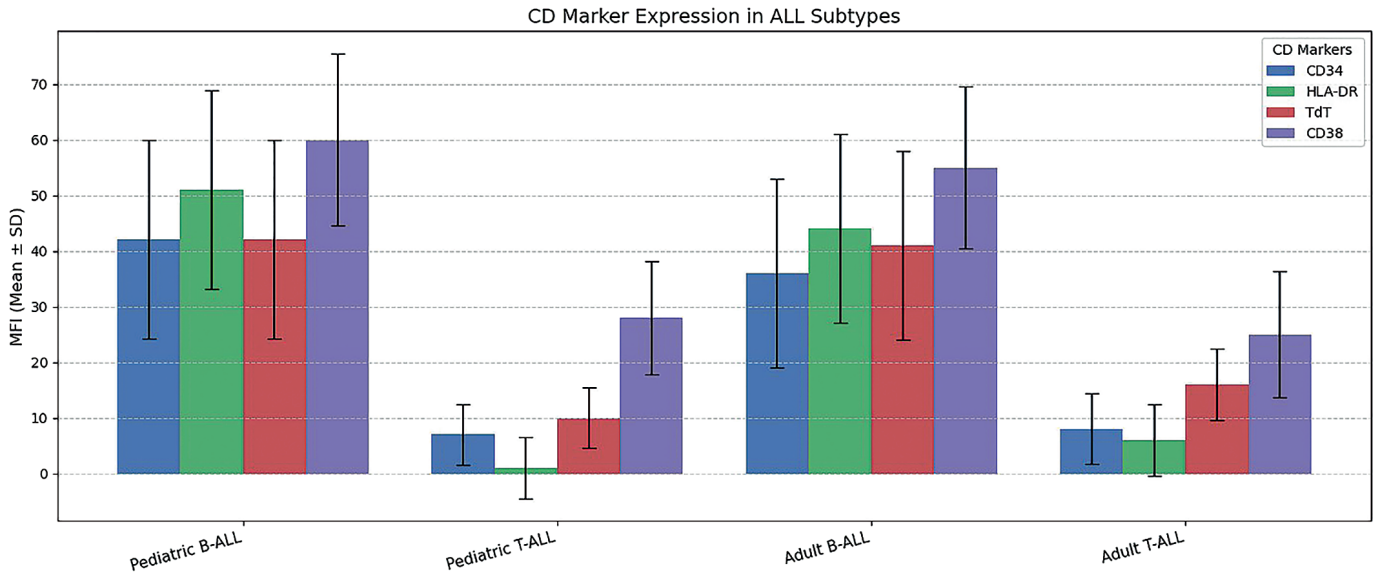


**Fig. 1** Mean ± SD for All CD Markers.

**Tab. 5** Independent Samples T-tests for CD Marker Expression Between Patient Groups.

| CD Marker | p-value (Pediatric B-ALL vs. Adult B-ALL) | p-value (Pediatric T-ALL vs. Adult T-ALL) | p-value (Pediatric B-ALL vs. Pediatric T-ALL) | p-value (Adult B-ALL vs. Adult T-ALL) |
|---|---|---|---|---|
| **CD34** | 0.456 | 0.789 | 0.034 | 0.067 |
| **HLA-DR** | 0.112 | 0.543 | 0.002 | 0.145 |
| **TdT** | 0.678 | 0.123 | 0.021 | 0.054 |
| **CD38** | 0.200 | 0.045 | 0.035 | 0.090 |

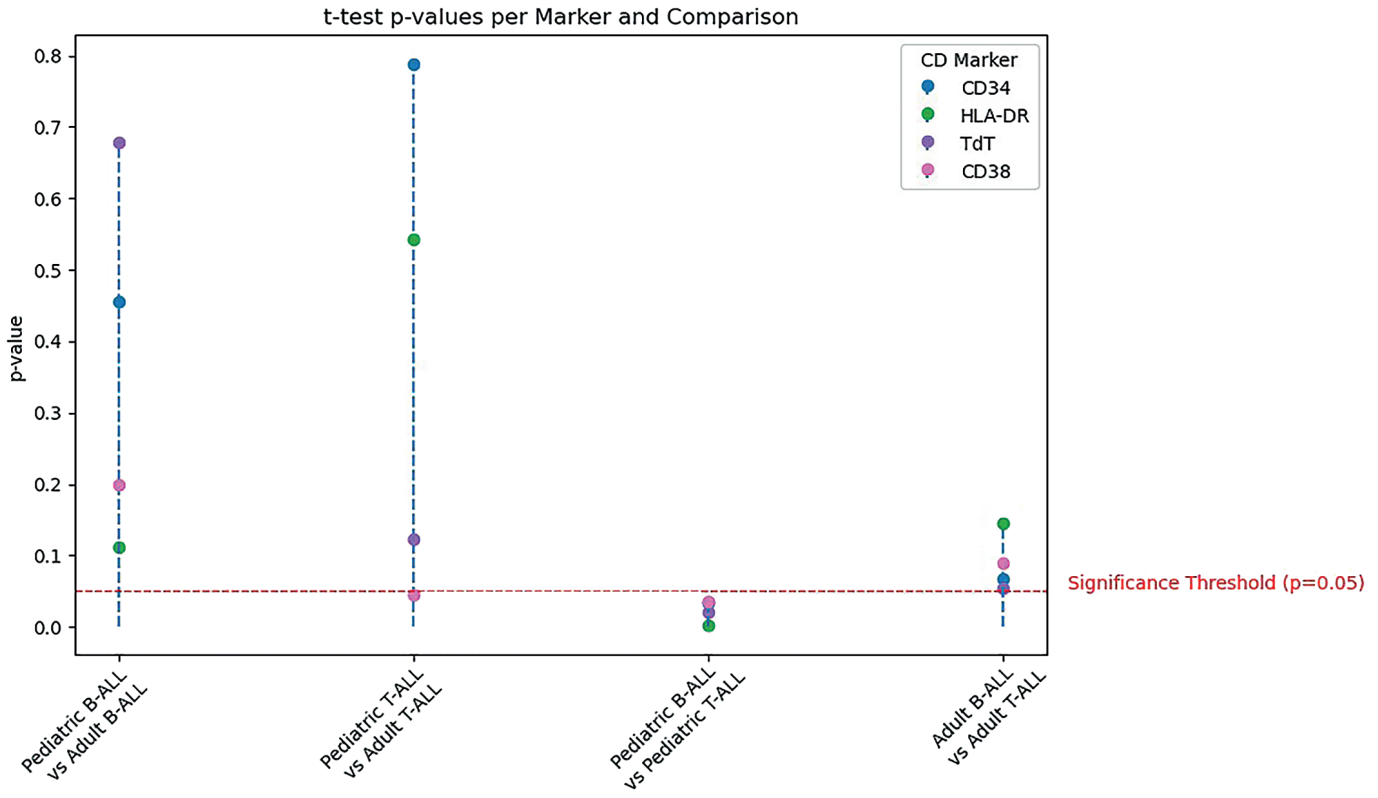


**Fig. 2** T-test p-values.

**Tab. 6** Mann-Whitney U Tests for CD Marker Expression Between Patient Groups.

| CD Marker | p-value (Pediatric B-ALL vs. Adult B-ALL) | p-value (Pediatric T-ALL vs. Adult T-ALL) | p-value (Pediatric B-ALL vs. Pediatric T-ALL) | p-value (Adult B-ALL vs. Adult T-ALL) |
|---|---|---|---|---|
| **CD34** | 0.025 | 0.001 | 0.030 | 0.019 |
| **HLA-DR** | 0.015 | 0.067 | 0.001 | 0.039 |
| **TdT** | 0.051 | 0.009 | 0.019 | 0.149 |
| **CD38** | 0.022 | 0.045 | 0.035 | 0.080 |

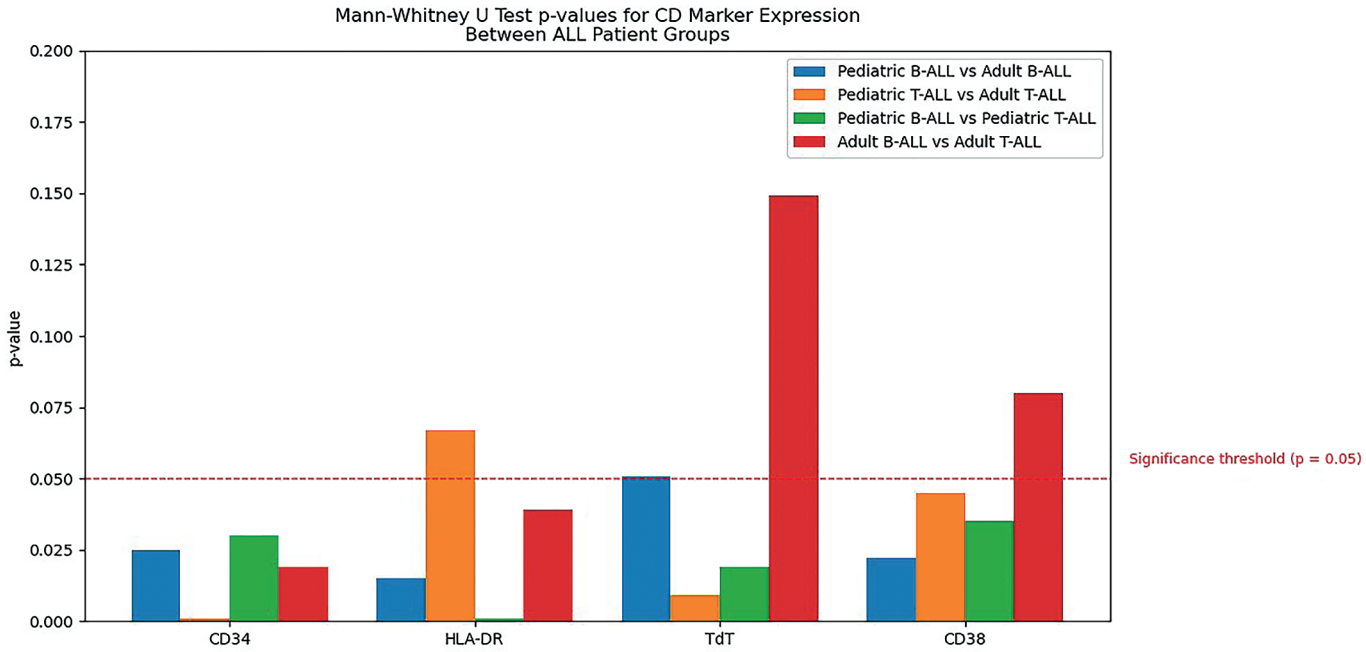


**Fig. 3** Mann-Whitney U Test p-values.

**Tab. 7** Correlation Analysis Results.

| CD Marker | Pediatric B-ALL (51) | Pediatric T-ALL (16) | Adult B-ALL (44) | Adult T-ALL (19) |
|---|---|---|---|---|
| **Pediatric B-ALL (51)** | 1 | 0.013295 | 0.981065 | 0.134304565 |
| **Pediatric T-ALL (16)** | 0.013295 | 1 | -0.1355 | 0.892212673 |
| **Adult B-ALL (44)** | 0.981065 | -0.1355 | 1 | 0.110501866 |
| **Adult T-ALL (19)** | 0.134305 | 0.892213 | 0.110502 | 1 |

those subtypes concerning CD marker expressions among children. Table 5 shows the correlation results analysis.

In addition, a correlation matrix was constructed to evaluate the relationships between the expression levels of these markers across the different patient groups. A very high positive correlation (r = 0.981) was found between Pediatric B-ALL and Adult B-ALL, suggesting that the immunophenotypic profiles of B-cell ALL are remarkably consistent across age groups. Similarly, a strong positive correlation (r = 0.892) was observed between Pediatric T-ALL and Adult T-ALL, indicating that T-cell ALL maintains similar marker expression patterns in children and adults. In contrast, there was an extremely low correlation (r = 0.013) between Pediatric B-ALL and Pediatric T-ALL, underscoring marked differences in CD marker expression between these two pediatric subtypes. Table 7 presents the detailed results of this correlation analysis.

In addition, another one can note that the low correlation (r = 0.111) between Adult B-ALL and Adult T-All may indicate that the adult subtype has different levels of expression of these antigens as compared with the pediatric one. This analysis highlights the fact that over similar age ranges both subtypes held a more constant pattern for each given subtype (B-cell or T-cell ALL). While these observations show that within-age-group variation remains significant; they emphasize nothing else other than the consistency within rather than between types of leukemia

when considering age varieties. This uniformity validates application concerning classification using certain leukemic blood components like its marker system.

## DISCUSSION

The findings from this study help to reveal the core features of ALL in various age groups and different subtypes. One of the most important observed phenomena was related to CD34 and HLA-DR expression patterns. Hence, both pediatric and adult individuals showed high levels of its expression for B-ALL as compared with those for the T-ALL group. This finding is also consistent with previous studies that have shown that it has a poor prognosis since it relates to primitive hematopoietic progenitor cells during early B-cell development as well as supports early B-cell development through homing. Of great importance is the fact that B-ALL patients had higher CD34 expressions similar to a study that highlights the prognostication role played by high CD34 counts in childhood leukemia including infant acute lymphoblastic leukemia. Several possibilities may exist, however, lack of statistically significant differences in CD marker expression between the groups.

The study examined CD38 immaturity marker expression and its patterns alongside other markers. The collected data about CD38 expression strengthened the analysis of ALL immunophenotypic characteristics even though the results were not statistically significant for every group. CD38 serves a dual purpose during cellular activation and differentiation processes and its changing expression patterns might indicate distinctive biological features between subtypes combined with age-related characteristics. The observed data demonstrated that B-ALL expression levels exceeded T-ALL expression profiles of CD38 which matches historical data about its use in B-cell maturation processes. In addition, the correlative evaluation showed that both B-ALL and T-ALL subtypes exhibited similar features among patients from different age groups. The results show pediatric and adult patients with B-ALL share identical immunophenotypic profiles ($r = 0.981$) while pediatric and adult patients with T-ALL show strong similarities ($r = 0.892$). In contrast, the minimal correlation between childhood B-ALL and childhood T-ALL ($r = 0.013$) highlights the clear immunological differences between these subtypes, even within the same age group. As shown in the results of this study, CD markers alone may not fully capture the heterogeneity and complexity of disease among different patient populations. Therefore, it might be necessary to include other molecular and genetic markers for a better understanding of ALL. Moreover, these results imply that individual variations rather than oversimplified group comparisons should inform personalized diagnostic and therapeutic strategies based on marker expressions. The nominally prognostic significance of CD34, HLA-DR, and TdT proteins becomes more valuable for clinical assessments through integration with cytogenetic and molecular assessments across different age groups. The study lacks survival data or outcome measures therefore future research involving long-term study is required to determine how these antigenic expressions influence prognosis. The diagnosis and classification of ALL in high-resource areas depends on molecular genetic analysis together with cytogenetic profiling tests. The areas of Iraq and similar regions conduct their diagnoses primarily using morphology and immunophenotyping because of inadequate infrastructure. The identification of trustable surface markers proves essential for simultaneous diagnosis and future disease classification processes in these settings thus contributing to better standardized medical care.

## CONCLUSION

This research examined the quantitative CD marker expressions in pediatric and adult patients with acute lymphoblastic leukemia who had B-ALL and T-ALL cancer types. Flow cytometry data provided information for t-tests along with Mann-Whitney U tests which functioned as parametric and non-parametric tests respectively to detect dissimilarities in the group characteristics. Statistical analyses established significant marker expression disparities between different cell types except for B-ALL and T-ALL comparison where results showed clear CD34, HLA-DR, and TdT variations. Patterns of CD38 showed trends to add understanding to immunophenotypic variations between different patient groups even though statistical significance was not always achieved. Marker expression pattern consistency across pediatric and adult groups within the same subtype received support from descriptive statistics analysis and correlation tests.

The findings demonstrate that ALL exhibits complicated immunophenotypic characteristics which should not lead doctors to rely only on CD marker profiles for diagnostic decisions or predictive outcomes. The accurate diagnosis and individualized understanding of the disease as a future approach requires combining CD markers with molecular and genetic markers throughout ALL classification stages.

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