Leukaemia is a prevalent malignant disease globally, ranking fifteenth in 2018 and having a higher prevalence in industrialized nations. However, underdeveloped nations have a higher death rate [1]. According to the American Cancer Society, 178,520 Americans are anticipated to be diagnosed with leukaemia, lymphoma, or myeloma in 2020. Although both sexes are equally afflicted, men are more prone to get it [2]. The age-standardized incidence rates for leukaemia were 6.1 and 4.3 per 100,000 in the US in 2018 [1]. Malignant clonal diseases of the hematopoietic system known as acute leukaemia can affect one or many cell-lines. Due to the considerable replacement of bone marrow by undifferentiated, immature and aberrant hematopoietic cells in these diseases, there are less erythrocytes and platelets in the peripheral circulation. These illnesses can be classified as lymphoid, myeloid, mixed, or undifferentiated depending on the origin of the aberrant hematopoietic cells involved [3]. The etiology of these illnesses is still unclear, despite substantial advancements in acute leukaemia therapy. A variety of environmental and genetic risk factors for the development of acute leukemia have been hypothesized, but none have been proven. A new "two-hit" theory suggests that acute lymphoblastic leukaemia develops due to a combination of hereditary factors and infection exposure [4, 5]. Genetics play a significant role in leukemia development, with several
proposed genetic influences. Increased leukemia risk is related to the processes of leukemogenesis as well as genetic variables in normal hemopoiesis and the development of acute leukemia [6, 7]. Similarly, leukemia development is linked to various environmental factors, including exposure to cancer-causing substances like chemicals, infections, radiation, poisons, hydrocarbons, pesticides, alcohol, tobacco, and illegal drugs [8]. The most prevalent form of cancer in children is acute lymphoblastic leukemia (ALL), which makes up roughly 25–30% of all cancer diagnoses in this age group. Between the ages of 0 and 14, ALL has a yearly incidence of around 4.6 cases per 100,000 persons in the United States, with a peak incidence at 2 to 5 years. Females are somewhat more likely than males to have ALL in the first year of birth [3]. The peak periods for acute myeloblastic leukemia (AML) are early infancy and later in life. Approximately AML affected 7.7 children between the ages of 0 and 14 years during 2005 and 2009. The first year of life in pediatric age group is the time of its greatest incidence rate, which subsequently steadily drops until the age of four. Under one year of age, children encounter the disease at a rate of 18.4 per million [9]. Leukemia is diagnosed and categorized using a variety of methods, such as cytomorphology, histomorphology, cytochemistry, multi-parameter flow cytometry, chromosomal analysis, FISH, and molecular methods. The primary approach for diagnosing, categorizing, staging, and tracking the development of the disease and its response to treatment is immunophenotyping by flowcytometry [10]. Considering that each subtype has a different treatment option and prognosis, flow cytometry's ability to discriminate between them is crucial[11].

METH O D S
The Ethical Committee of the School of Allied Health Sciences, The University of Child Health sciences, The Children's Hospital, Lahore approved this descriptive study design, based on total of 101 pediatric patients. One hundred and one requests sent to the Department of Immunology during the period of July 2021 to February 2022 for the immunophenotyping of hematological malignancies by flow cytometry were included in this study. Using data from Narang et al., (2015), the sample size was estimated while retaining the power of study at 90% and its level of significance at 5% [12]. The samples were sent from patients ranging in age from 6 months to 15 years, of both genders. All the patients were divided into three groups of <5 years, 5-10 years and 11-15 years. On peripheral blood or bone marrow aspirate immunophenotyping was carried out using a dual laser BD FACS Canto II flow cytometer (BD Biosciences, California USA). Whole blood was lysed using BD FACS lysing solution to produce mononuclear cells. Monoclonal antibodies (MoAbs) that have been fluorescein-conjugated (BD Biosciences USA) have been absorbed on monocyte, myeloid cell, T & B cells, or immature precursor cell antigens in various combinations. These MoAbs included phycoerythrin (PE), allophycocyanine (APC), peridinin chlorophyll protein (PerCP), and fluorescein isothiocyanate (FITC). Samples containing 20% or more blast cells reacting with a specific antibody were deemed positive. Data from the study were analyzed using the Statistical Package for Social Sciences (SPSS) version 26.0 for Windows. Percentages and frequencies were used to express the data. All statistical tests were regarded as two-sided and statistical significance was defined as a p-value of equal to or less than 0.05.

R E S U L T S
Data of one hundred and one patients for the immunophenotyping by flowcytometry was analyzed in the immunology department. The age distribution of acute leukemia is depicted in Figure 1. B-ALL was the most common leukemia in all age groups with 45 (56.9%) in the age group of <5 years, 23(31.9%) in 5-10 years, and 8(11.1%) in the age group of 11-15 years. T-ALL and AML were most common 7(53.8%) %) and 7 (43.8%) respectively in the age group of 5-10 years.

Figure 1: The age distribution of acute leukemia in pediatric population. B-ALL was the most prevalent leukaemia in all age groups, T-ALL and AML were same in the age groups other than 11-15 years in which AML was more frequently present Figure 2 shows the gender distribution of acute leukemia. Out of 101 patients, 67 (66.3%) were male and 34 (33.6%) were female. On further analysis of gender distribution in each type of leukemia males were more as compared to females.
Immunophenotyping of Acute Leukemia in Pediatric Patients

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Figure 2: Gender distribution of acute leukemia in pediatric population. All types of acute leukemia were more frequent in males as compared to females.

From 101 cases of acute leukemia that underwent immunophenotyping by flow cytometric analysis, 85 (84.1%) were ALL, and of those 85 cases, 72 (84.7%) were of B cell phenotypes and 13 (15.2%) were of T cell phenotypes. From total of 101 leukemia patients, 16 (15.8%) were found to be AML (Table 1).

**Table 1:** Frequency of acute leukemia on the basis of immunophenotyping by flow cytometry

<table>
<thead>
<tr>
<th>Type of acute leukemia</th>
<th>Frequency (%)</th>
</tr>
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<tbody>
<tr>
<td>B-ALL</td>
<td>72 (84.7%)</td>
</tr>
<tr>
<td>T-ALL</td>
<td>13 (15.2%)</td>
</tr>
<tr>
<td>AML</td>
<td>16 (15.8%)</td>
</tr>
</tbody>
</table>

Most frequent type of acute leukemia is B-ALL in pediatric age group followed by AML and T-ALL.

Immunophenotypic profile of B-ALL showed positivity of cytoplasmic CD79a (cCD79a) and CD19 in 72 (100%) cases, CD10 in 71 (98.6%) cases, CD34 in 50 (69.4%), Anti-Tdt in 66 (88.9%), HLA-DR in 39 (54.2%) and cytoplasmic CD22 (cCD22) was positive in 4 (5.6%) cases. In T-ALL, immunophenotypic profile presented a positive expression of CD2 in 11 (84.6%) cases, cytoplasmic CD3 (cCD3) in 13 (100%), CD5 in 13 (100%), CD7 in 10 (76.9%), CD34 in 7 (53.8%) and Anti-Tdt was positive in 12 (92.3%) cases. Immunophenotyping for AML showed that Anti-MPO was positive in 12 (92.3%) cases, CD10 in 15 (93.8%) cases, CD34 in 14 (87.5%) cases, HLA-DR in 13 (81.3%) and CD11c was positive in 8 (50%) cases. The most common CD markers in B-ALL were cCD79a and CD19 (100%), whereas the most common CD markers in T-ALL were CD3 and CD5 (100%) and in AML were CD34 (93.8%) and CD34 (87.5%). A common marker for B-ALL, T-ALL, and AML was CD34, B-ALL and T-ALL shared the CD marker anti-Tdt whereas AML and B-ALL shared the CD marker HLA-DR (Table 2).

**Table 2:** Immunophenotypic profile of acute leukemia in pediatric population

<table>
<thead>
<tr>
<th>CD Markers</th>
<th>AML (n=16)</th>
<th>B-ALL (n=72)</th>
<th>T-ALL (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>N/A</td>
<td>71 (98.6%)</td>
<td>N/A</td>
</tr>
<tr>
<td>CD19</td>
<td>N/A</td>
<td>72 (100%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

In B-ALL, cCD79a, CD10, CD19, CD34, and Anti-Tdt were present with more than 50% of expression. In T-ALL, CD2, CD3, CD5, CD7, CD34, and Anti-Tdt were positive with more than 50% of expression. In AML, Anti-MPO, CD3, CD34, and HLA-DR was positive with more than 50% of expression. CD19, cCD79a = Most frequent markers in B-ALL.

**CD34, CD123 = Most frequent markers in T-ALL**

**CD34, HLA-DR, Anti-Tdt = Most common CD markers in acute leukemia**

N/A = Not applicable

**Discussion**

In Pakistani pediatric population B-ALL was the most common leukemia in all age groups, where as T-ALL and AML were most frequently present in the age group of 11-15 years. These findings are in accordance with the international data such as in a study B-cell ALL is much more common in all age groups than T-cell disease, accounting for approximately 80% of cases of ALL [13]. Similarly, AML was more frequently present with incidence peak between the ages of 10-14 years [14]. According to the American cancer society AML is significantly more prevalent in the first two years of life and during adolescence, whereas ALL is most prevalent in early infancy, peaking between the ages of 2 and 5 years [15].

According to our findings acute leukemia was more common in boys compared to girls. These results are in favor of another study showing boys were four times at more risk to develop ALL than girls [16]. Similarly, in another study out of 100 acute leukemia patients there were 76 males and 24 females [17]. These results are in line with another study from Pakistan, where males were more likely to have acute leukemia (60%) than females (40%) [18].

In pediatric population of this study ALL was more common than AML. Among ALL, B-ALL was more in children as compared to T-ALL. These findings are in favor of recent Indian data depicting that ALL, constitute 75-80% of childhood acute leukemia [19]. One more study of pediatric...
The present study evaluated the age, gender, and immunophenotypic distribution of ALL in the pediatric population highlighting the frequency of acute leukemia in children. It also reflects the significance of flow cytometry in the diagnosis of leukemia because in order to accurately diagnose leukemia and determine lineage, it is crucial.

**Authors Contribution**

Conceptionalization: FS  
Methodology: FS, AA  
Formal analysis: FS  
Writing-Review and editing: FS, UA, AA

All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

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**REFERENCES**


