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## FLOW CYTOMETRIC AND DEMOGRAPHIC ANALYSIS OF T CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN PAKISTANI POPULATION

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**Background:** This study was carried out to analyze the proportion of T cell acute lymphoblastic leukemia (TALL) among all acute lymphoblastic leukemia (ALL) in Pakistani population and its correlation with the demographic features. Accuracy of cell surface markers used in flow cytometric analysis of the leukemic cells was also determined. **Methods:** Data of 209 consecutive cases of acute lymphoblastic leukemia (ALL) presenting between July 1995 and July 2003 was analyzed. Flow cytometry was performed on all ALL cases using the standard protocols. TALL markers included CD3, CD5 and CD7. **Results:** Proportion of TALL among known ALL Pakistani patients was 17.22%. Mean age of the TALL patients was 17.2 years. Proportion of TALL was higher in adults than in children (21.95% vs. 14.17%). Overall in this study there were more male patients affected by TALL (25/36 or 69.40%) than females (11/36 or 30.60%). The female to male ratio among TALL patients was 1:2.27. However, the proportion (%) of TALL in females was higher than males (18.96% vs. 15.82%) i, e, 1.2:1. CD7 was found to be the most sensitive among both adults & children. It was positive in 94.4% of the TALL cases. **Conclusion:** Proportion of TALL among ALL in Pakistan is similar to that reported in this region, indicating a candidate association with geographical location and socioeconomic status. The reactivity of markers with TALL cells was similar to what we expected based upon literature. However, due to some aberrant and cross reactivity displayed by each marker, we strongly recommend a panel approach including B and myeloid markers to ensure a correct diagnosis of TALL.

**Keywords:** Leukemia, T cell acute lymphoblastic leukemia (TALL), Flow Cytometry

### INTRODUCTION

The hallmark for the diagnosis of acute leukemia, until recent past, has been the morphology and cytochemistry. They provide correct diagnosis in about 80% of the cases. For instance, Jawaid et al. in their series reported that 11% cases of acute leukemias were unidentifiable in terms of their phenotype while 9% were identified incorrectly on morphological basis, all of which were correctly allocated to their lineages after flow cytometric analysis.<sup>1</sup> Thus, major developments in the field of immunology have now brought an era of diagnosing acute leukemias by means of flow cytometry. Flow cytometry is a powerful tool for the investigation of normal and neoplastic cells and their classification at every level. Therefore, it has great prognostic and therapeutic implications. Its ability to measure multiple parameters on individual cells in a suspension at high speed is ideal for the study of leukemic cells.<sup>2</sup> It identifies cell markers by applying monoclonal antibodies against them. The discovery of monoclonal antibodies has made it possible to define the precise stages of differentiation and lineage of hematopoietic cells and has resulted in tremendous advancement in the classification of leukemias.<sup>1-5</sup> Not only acute myeloid leukemia (AML) can be differentiated from the acute lymphoblastic leukemia (ALL) but B-cell or T-cell

lineages can also be determined in the latter<sup>(6)</sup>, which cannot be achieved by morphology and cytochemistry alone. By this method more than 98% of acute leukemia cases can now be precisely allocated to their respective lineages<sup>(1)</sup>. In addition to leukemia phenotyping leukemia associated phenotypic features have been used to detect minimal residual disease in both ALL and AML.<sup>1</sup>

Acute leukemia results from clonal proliferation of immature hematopoietic cells "frozen" at an early stage of differentiation<sup>2</sup> including primitive cells with multilineage potential.<sup>7</sup> ALL and AML are its two major types. ALL is broadly classified as having a T- or B-cell origin, with further division of B-lineage ALL into three distinct subtypes: early pre-B cell, pre-B cell, and B cell. A fourth subtype, transitional pre-B-cell ALL, was recently described and may be associated with a favorable prognosis.<sup>8</sup> T-cell ALL has been subclassified according to the stage of thymocyte differentiation or the stage in the expression of T-cell-receptor protein but this approach has limited clinical usefulness.<sup>8</sup> Therefore, we have limited our discussion broadly to TALL without going into details of subclasses of TALL.

Flow cytometric analysis of leukemic samples started routinely at The Aga Khan University (AKU), Department of Pathology in 1995. Since then, there has been continuous evaluation and

revision of the panel of antibodies used to analyze the leukemic cells. It is among the very few hospitals in Pakistan, which is endowed with the facility, as a major tertiary care referral center with its currently 48 laboratory collection points in all four provinces of Pakistan. Thus, the samples studied at AKU Hospital are a good representative of overall Pakistani population.

## MATERIAL AND METHODS

Details of the flow cytometric analysis of all the leukemic cases that appeared in The Aga Khan University Hospital between July 1995 and July 2003 were obtained from the records of the Department of Pathology. Patients with Acute Lymphoblastic Leukemia were selected and further information was documented on specially designed data entry forms. The data was entered in SPSS 10.0 (Statistical Package For Social Sciences). Analysis was performed using the same software. Fischer's Exact Test and Chi-Square Tests were performed to determine any possible associations between demographic parameters and expression of specific antigens on the TALL cells. P-Value of <0.05 was considered to be the criteria for significance.

Flow cytometry was performed on all the peripheral blood and bone marrow samples by centrifuging mononuclear cells from them on Ficol. These were then stained with fluorescence labeled antibodies (obtained from Becton Dickinson, U.S.A) and run on FACSCAN using cell quest software. The same software was used to analyze the flow cytometry data. CD19, CD20, CD22, CD10, CD3, CD5, CD7, CD13, CD33 and HLA-DR were tested on all the cases. Positivity of a clone for a cell surface marker was accepted if more than 30% of the cells stained with the antibody for the respective marker.

## RESULTS

Data from a total of 209 cases of acute lymphoblastic leukemia (ALL) was retrieved, out of which 127 (60.80%) were children i.e. less than or equal to 15 years and 82 (39.20%) were adults i.e. more than 15 years (Table-1). Out of 209, 158 (75.60%) cases were males and 51 (24.40%) were females (Table1). Thus among ALL patients, adults to children ratio turned out to be 1:1.55 and female to male ratio turned out to be 1:3.1. The mean age of the ALL patients was calculated to be 14.85 years.

The number of patients who were diagnosed to have TALL was 36. Thus the proportion of TALL among acute lymphoblastic leukemia in this study was 17.22%. The mean age of the patients was 17.2 years with a standard deviation of 12.96. The age range of the sample was 44 years (the youngest

patient aged 1 year and the oldest 45 years) with the median age of 15.0 years. Mean age among children was 7.0 years with the standard deviation of 3.6 and that among adults was 27.44 years with a standard deviation of 10.52. Though, equal number of children and adults suffered from TALL, proportion of TALL in patients suffering form ALL was higher in adults than in children (21.95% vs. 14.17%). Thus children to adults ratio of TALL cases in ALL patients was 1:1.55. Figure-1 shows the number of TALL cases in different age groups.

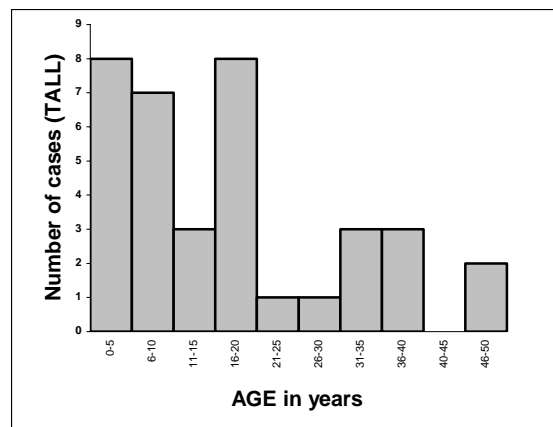


Figure-1: Histogram representing the distribution of TALL among different age groups

Overall in this study there were more male patients affected by TALL (25/36 or 69.40%) than females (11/36 or 30.60%) (Table-1). The female to male ratio among TALL patients was 1:2.27. However, proportion (%) of TALL in females was higher than males (18.96% vs. 15.82%) i.e, 1.2:1 Of all the markers against which the monoclonal antibodies were directed (Figure-2), CD7 was found to be the most sensitive among both adults & children groups and male & female groups. It was positive in 94.4% of the TALL cases (Table2). CD5 came out to be positive in 86.1% of the patients, which is a reasonable sensitivity already expected based on previous studies. CD3 was positive in 38.8% patients. Sensitivity of CD3 might not sound too impressive but it is justifiable by the fact that the samples used in our study were not permeabilised to expose the CD3 antigen, which are primarily present in the cytoplasm. Data was also used to determine if there was any significant difference in the distribution of reactivity in two age and sex groups (Table-2). Except for the significant number of female TALL cases cross-reacting with HLA-DR than male cases, distribution of reactivity was similar in both male-female and children-adult groups.

As some cross-reactivity between TALL and myeloid markers is well known, a panel approach

was undertaken in all cases of leukemia phenotyping. This did show weak expression of two myeloid markers CD13 and CD33 on a small percentage of TALL cells (8.3% & 2.7% respectively). HLA-DR is also a useful marker as it is negative on a great majority of TALL cells while usually shows strong reactivity on BALL and myeloid leukemia cells. This does make sense, as normal T lymphocytes also don't express HLA-DR on their surface.

**DISCUSSION**

ALL is diagnosed to 3000-4000 persons in US each year.<sup>7</sup> About 13% of them are diagnosed as TALL<sup>(9)</sup>. Recognition of TALL is also important in the sense of therapeutic and prognostic implications. TALL is known to have a better prognosis<sup>2</sup> unless it is associated with other high risk factors like high

WBC-count, mediastinal mass or CNS disease at diagnosis.<sup>10</sup>

The frequency of TALL in Pakistani patients in this series was calculated to be 17.22%, which is different from that reported in other parts of Pakistan previously. In Rawalpindi, among all leukemia, TALL was reported to be 9.2% in 1990, which was much lower than that reported in other parts of the country e.g. in Lahore, TALL was reported to be 29.1% in 1992.<sup>3</sup> However, our study appears to be the best representative of the Pakistani population as it has the edge of being the most recent, having a greater sample size, collection of samples from all parts of Pakistan, a comprehensive panel of monoclonal antibodies and strict quality control validated by CAP. It is also interesting to note that one of our recent studies on T-cell Non-Hodgkin's Lymphoma (T-NHL) showed a similar frequency i.e. 22.2% among total NHLs in our population.<sup>11</sup>

**Table-1: Demographic distribution of Acute Lymphoblastic Leukemia**

	Total	Children (%)	Adults (%)	Males (%)	Females (%)
<b>ALL</b>	209	127(60.80%)	82(39.20%)	158(75.60%)	51(24.40%)
<b>TALL</b>	36	18(50.0%)	18(50.0%)	25(69.40%)	11(30.60%)

\*p-value < 0.05

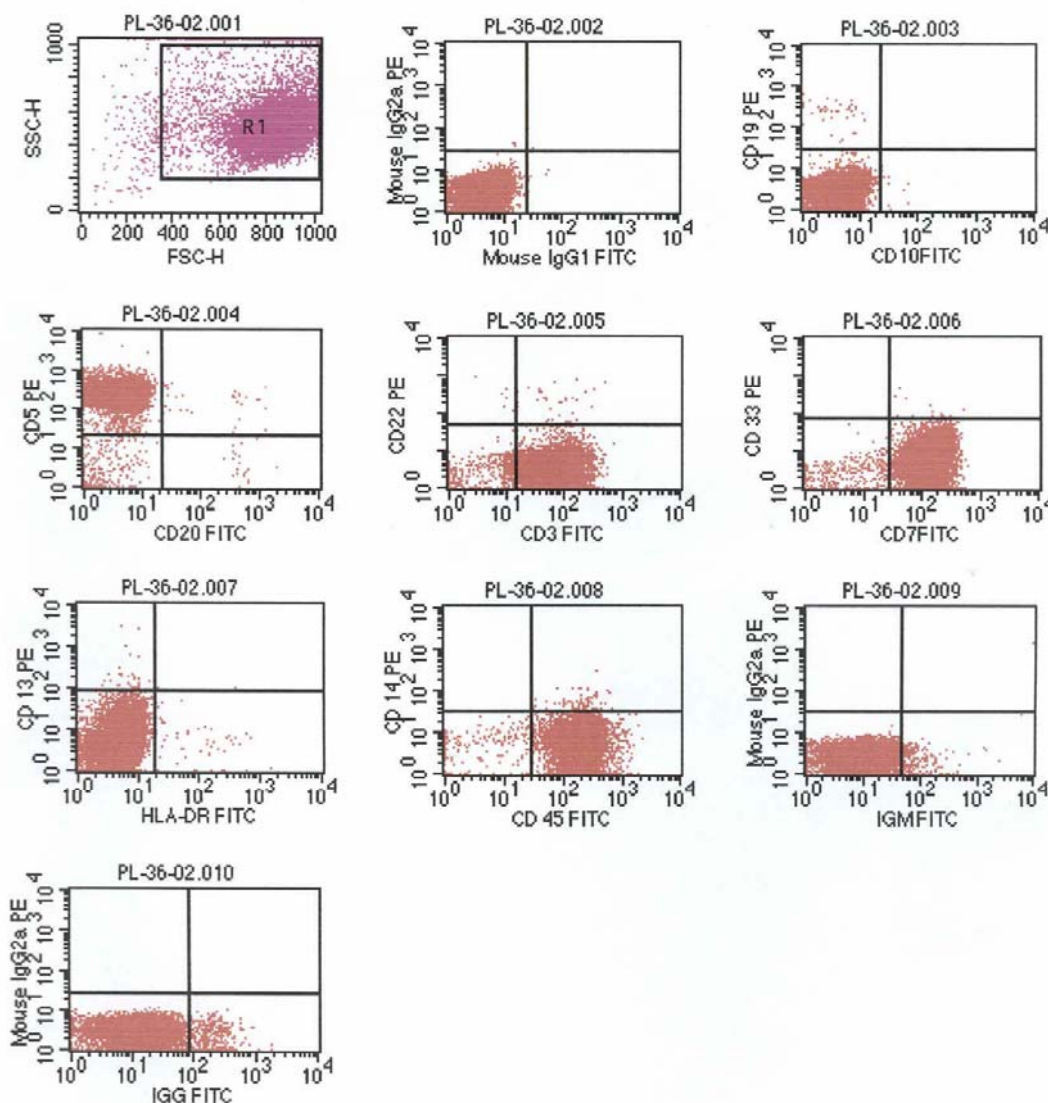
**Table-2: Reactivity of antigens in TALL patients**

	Children(%)	Adults(%)	Males(%)	Females(%)	Cases +ive	Sensitivity
<b>CD3</b>	06(33.3%)	08(44.4%)	10(40%)	04(36.4%)	14	38.8 %
<b>CD5</b>	16(88.8%)	15(83.3%)	23(92%)	08(72.7%)	31	86.1 %
<b>CD7</b>	17(94.4%)	17(94.4%)	24(96%)	10(90.9%)	34	94.4 %
<b>HLA-DR</b>	02(11.1%)	02(11.1%)	02(8.0%)	02(18.2 %)	04	11.1 %
<b>CD13</b>	01(5.5%)	02(11.1%)	00(0.0%)	03(27.3 %)*	03	08.3 %
<b>CD33</b>	00(0.0%)	01(5.5%)	00(0.0%)	01(9.1%)	01	02.7 %

\*p-value < 0.05

**Table-3: Proportion of TALL in different regions of the world**

Region	Country	ALL	%TALL
South & South East Asia	Pakistan	209	17.2
	Thailand <sup>17</sup>	38	18.4
	Malaysia <sup>18</sup>	36	22.0
	India <sup>28</sup>	45	17.0
Middle East	Saudi Arabia <sup>24</sup>	163	12.3
Africa	Zimbabwe <sup>14</sup>	75	21.3
	Egypt <sup>19</sup>	124	50.0
Europe	Italy <sup>20</sup>	2038	12.5
	Scotland <sup>21</sup>	498	18.1
	Netherlands <sup>22</sup>	91	19.8
	Germany <sup>26</sup>	1157	13.6
	Germany <sup>27</sup>	54	16.6
	Yugoslavia <sup>39</sup>	54	11.1
America	USA <sup>9</sup>		13.0
	Mexico <sup>13</sup>	402	09.4
	Chile <sup>23</sup>	500	10.0



**Figure-2. Light scatter and fluorescence dot plots of bone marrow from acute TALL. Note strong positivity of blasts for three T cell markers i.e., CD3, CD5 & CD7 and no reactivity with B-lymphoid, myeloid & HLA-DR. This phenotype is consistent with TALL.**

A higher percentage of males were affected than females in our studies as was expected due to previous reports.<sup>9,12-14</sup> About 70% of the TALL patients were males. However, proportion of TALL was higher among females. In males the age group of 16-21 was mostly affected while among females the most vulnerable group was found to be 5-10 years. In our study, ALL affected children more than the adults as is reported previously from Pakistan and abroad.<sup>4,12,14,15</sup> Leukemia is the most common cancer among children in Pakistan.<sup>12,16</sup> In US 2/3<sup>rd</sup> of the patients diagnosed with ALL are children.<sup>7</sup> The correct

diagnosis of phenotype of the leukemic cells is even more critical in children due to therapeutic implications as in US 80% of them are cured as compared to only 30%-40% of adults with ALL.<sup>7</sup> However, in our study proportion of TALL among adults was higher than that in children. Table 3 shows the proportion of TALL in different countries as reported in various studies.

Review of literature (Table-3) shows the proportion of TALL with varied geographical location and socio-economic status. For instance, it is similar in Pakistan, India, Thailand and Malaysia.<sup>17,18</sup>

forming an environmental belt of South & South East Asia. Very high proportions of TALL are reported in Egypt.<sup>19</sup> From Middle East, in reports from Saudi Arabia and Oman, frequency of TALL is lower than the rest of the Asia and somewhat similar to that of western countries in Europe and America.<sup>9,13,20-27</sup> This can be attributed to the similar economical conditions of these countries, as have been suggested by some previous reports.<sup>28</sup> More studies are warranted to probe any association of the proportion of TALL with socio-economical conditions and geographical location.

In our study, sensitivity of different markers was determined as the ratio of true positives to sum of true positives and false negatives. CD7 that turned out to be most sensitive (94.4%) is not totally reliable due to lack of specificity. In previous reports, it was demonstrated to cross react with 14% of non-TALL and 12.76% of BALL.<sup>12</sup> CD5 also came out as a highly sensitive marker (positive in 86.1% of the cases). The high sensitivity of these markers in both children and adults is in accordance with the previous reports<sup>15,17</sup> and are thus the most commonly used antigens for TALL diagnosis.<sup>28</sup>

Positivity of CD3 was 38.8% and like some previous studies<sup>12</sup> wasn't confirmed as the most sensitive marker, which is limited by the fact that it is uncommonly expressed on the surface of T lineage ALL and is almost always present abundantly in the cytoplasm of these cells.<sup>12,29,30</sup> As in our analysis, the cells were not permeabilised; therefore antibodies only reacted with the surface CD3 antigens, thus resulting in a lower sensitivity. However, it is established as a relatively specific marker for TALL.<sup>12,31</sup>

HLA-DR cross-reacted with 11.1% of the TALL cases. CD13 and CD33 cross-reacted with 8.3% and 2.7% cases of TALL. These cross-reacting antigens are usually expressed in AML but occasionally are also found in B or T lineage ALL. Almost same amount of myeloid aberrant expression has also been reported in other studies.<sup>17,32</sup> Therefore they should be interpreted as strongly suggestive of myeloid leukemia only when are expressed in the absence of lymphoid associated antigens.<sup>33,34</sup> Myeloid antigen positive ALL has been reported to respond better to the therapy, thus having a better prognosis than that of myeloid antigen negative ALL.<sup>8</sup>

Lineage infidelity of the flowcytometric markers is an established fact. It refers to the phenotypic aberrations, which include asynchronous antigen expression, antigen over- expression and ectopic phenotype.<sup>32</sup> This infidelity can be intralinear or interlinear<sup>35</sup> as seen in our study, myeloid markers reacting with TALL cells. As mentioned earlier, no marker is ideally specific.

Moreover, many leukemia cells exhibit loss of antigen specific to that particular lineage, therefore it is recommended that classification of leukemia is confirmed with antibody from a second CD cluster that is known to react principally with the suspected cell lineage. However if detection of the confirmatory marker fails, then the suspicion of the lineage proliferation still should not be excluded.<sup>12,29,36-38</sup> Moreover, markers of the other lineages are also applied to rule out the suspicions of their involvement in the disease. Hence, a panel of different antibodies is required to overcome the problem of lineage infidelity, thus, making a reasonably firm diagnosis.

Checking the association of reactivity and cross-reactivity with different demographic parameters is a novel idea, which is tried in this study. This can be helpful in achieving separate panels of antibodies for cases of different demographic backgrounds. Since acute lymphoblastic leukemia are more difficult to study than the myeloid ones due to their heterogeneity, more extensive studies need to be done in this field to achieve a high yielding and reliable set of markers, not only for the diagnosis but also for the study of prognosis and minimal residual disease.

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