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Immunophenotyping results from flow cytometric analysis of children with acute leukemia in Ahwaz province

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ABSTRACT

Introduction: Acute leukemia is the most common malignancy in children and acute lymphoblastic leukemia (ALL) accounts for 75% of acute leukemia cases. New treatment protocols have resulted incomplete remission rates up to nearly 100% in children with acute lymphoblastic leukemia. Today, one of the most important prognostic factors in acute lymphoblastic leukemia is intensity of the treatment. Risk stratification is accomplished based on clinical, morphological, immune-phenotypic and cytogenetic findings. The aim of this study was to determine some prognostic factors in children with acute lymphoblastic leukemia.

Methods: In this retrospective study information about age at onset of acute leukemia, sex, initial white blood cell count, FAB-subtype, immunophenotype, and clinical course of newly diagnosed acute lymphoblastic leukemia were extracted from medical records of children admitted to pediatric oncology department of ShafaHospital between-2011and2012.

Results: There were 21 male patients (51.2%) and 20 were female patients (48.8%). The mean age was 4.2 ± 6.34 years,and 24 patients (58.5%) had Arab origins, while 17 patients (41.5%) were of non-Arab ethnicity. Age distribution showed higher incidence of ALL in younger children: 1-4 years 47.5%, 5-9 years 27.5% and 25% in patients >10 years.L2 subtype was more common in our patients 51.2% while L1 subtype was reported 46.3%.Only one patient was reported to be L3 subtype (2.4%), yet we did not detect any significant relation between different age groups and trend for incidence for specific subtype. The number of white blood cell (WBC) at the time of admission was reported as: less than 10,000 cells/cm in 30%, between11-50,000 in 37.5% and >50,000 in 32.5% patients .Organ involvement was present in 47.5%, and central nervous involvement, (proved by positive malignant cells in CSF fluid) was detected in 4.9% of our patients. In our study, HLA-DR was 62.5 % in ALL patients and CD 20 and CD19 was the most common marker in these patients. In our work the most common markers in L1, which was found in 19 patients were reported CD 19, CD33, CD22,CD35, CD20 and CD9. Also the percentage of markers in L2 subtype had a similarity to L1 group.

Conclusion: Conclusion: In this study, FAB-subtype L1 was less than previous studies, while FAB-subtype L2 and pre-B cell immunophenotype was more common than previous studies. Other results were the same as reported in older studies.

Keywords: Acute Leukemia, Children, Immuno-phenotyping, Flow cytometry.

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Introduction

Of all cancers in childhood, leukemias are one of the most important that are prevalent in children with the incidence of 25-30% of all childhood cancers.[1-3] Leukemia is known as the most important group of malignancies in childhood, which in most cases can be cured if diagnosed at the right time.[1-3] The term "leukemia" is described by the abnormal proliferation of undeveloped lymphocytes in bone marrow, peripheral blood and lymphoid tissue.[4] subgroups. Based on the morphological and cytochemical characteristics, leukemia is categorized into acute and chronic leukemia, and acute subtype itself is divided to acute lymphoblastic leukemia (ALL): L1, L2 and L3 and 8 subtypes of acute myeloblastic leukemia (AML).[5] This malignancy accounts for about 8% of all cancers in mankind, and about 50% of known patients with leukemia are represented with acute type, from which 90% are AML.[6] Any organ can be affected by leukemia, and therefore, the symptoms may vary widely from anemia, thrombocytopenia and neutropenia to central nervous system (CNS) involvement. Although any organ van be involved by the disease, the most clinically affected organs in AML are reported to be CNS and skin.[7] The diagnosis of acute leukemia is based on an algorithm, which in first step contains identification of leukemia from other clinically similar disease, and then determining the type (AML or ALL) and in the next step, defining the subtype for proper treatment.[8] The subtype of malignancy is diagnosed by the French –American –British (FAB) criteria by determining the immunohistochemical investigations and its markers and data from investigation of immune markers by flow cytometry method provides knowledge about leukemia subtype , expresses the blast cell lineage and the prognosis of the disease, but it does not command about the distinct therapeutic regimen. Immuno-phenotyping and flowcytometry are known as unique methods for classification of the leukemia subtype worldwide. The information derived by these methods play a vital role in the prognosis of the disease, the patient's survival and response to treatment in clinic.[10-11] Furthermore, previous studies have demonstrated the strong association between data from immunophenotyping and their impact on the application of appropriate treatment regimen , prognosis and survival in the patients.[11-13] To our knowledge, few number monocentric studies have been published about the immunological phenotyping of childhood acute leukemias in our country.[14-15] The present study was conducted to define the immunophenotyping data from children with ALL in Ahwaz to emerge better results from treatment protocols and improved prognosis.

Material and Methods

The study was performed on thirty five patients with documented and newly diagnosed ALL patients referred to the Shafa hospital, Ahwaz, Iran between September 2011 and September 2012. Peripheral blood samples were gathered from the patients, and the diagnosis of ALL was based onmorphology and immunophenotyping. The written consent form was signed by the patients and their parents, and the study was approved by the ethic committee of Ahwaz university of medical sciences.Peripheral blood samples preparation was done by dilution until the number of white blood cells reached up to less than 10 $x10^3$/ml, and then a glass slide was prepared for microscopic investigation was prepared.

Monoclonal antibodies (CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD19, CD20, CD22, CD33, CD34, HLA-DR, and TdT) and PBS were added to 35 µl of cell suspension to reach a total volume of 50 , then washed twice and re-suspended in 300-500 µl of bone marrow samples. The suspensions were then incubated in the dark for 15 minutes at room temperature. Following incubation, 16.6 µl of paraformaldehyde (4%) was added to the cell suspensions and incubated for 4 minutes at room temperature, in the dark. One ml of lysing solution was added for 10 minutes at room temperature, also in the dark. Cells were then centrifuged at 1500 rpm for 5 minutes, and supernatant was discharged. For cytoplasmic stainingcell suspensions were incubated for 15 minutes at room temperature, then washed twice and re-suspended in 2 ml PBS containing 1% FCS. Cells were then centrifuged at 1500 rpm for 5 minutes. The supernatant was discharged, and 250 µl PBS was added and the tubes were wrapped with aluminum foil. Samples were analyzed in a Bartech four color flowcytometer. A positive signal was recorded if 20% or more of the cells reacted with the given monoclonal antibody and samples that gave a positive

signal on < 20% cells were recorded as 'low expression'.

Data were analyzed by SPSS Ver.20. We used Pearson correlation coefficientand Mann-Whitney test were define significant relationbetween two variables. The Chi-square test was also used to determine therelation between two categorical data. A p value of <0.05 was regarded as significant.

Results

Of 41 studied patients, 21 patients were male (51.2%) and 20 were female patients (48.8%). The mean age was 4.2 ± 6.34 years (between 1 to15 years),and 24 patients (58.5%) had Araborigins,while 17 patients (41.5%) were of non-Arab ethnicity.

Age distribution showed higher incidence of ALL in younger children: 1-4 years 47.5%, 5-9 years 27.5% and 25% in patients >10 years.L2 subtype was more common in our patients (51.2%) while L1 subtype was reported 46.3% (**Table 1**). Only one patient was reported to be L3 subtype (2.4%), yet we did not detect any significant relation between different age groups and trend for incidence for specific subtype (P_value=0.9). The major part of our patients had a Pre-B cell ALL (48.7%), though 20.5 % had early Pre-B cell and 17.9% were reported with T cell. The amount of patients with B-cell and Bi-lineage was 7.7% and 5.1%, respectively. The most common CD markers in each morphological subtype are shown in **Table 2**.

The number of white blood cell (WBC) at the time of admission was reported as: less than 10,000 cells/cm in 30%, between11 -50,000 in 37.5% and > 50,000 in 32.5% patients. Organ involvement was present in 47.5%, and central nervous involvement, (proved by positive malignant cells in CSF fluid) was detected in 4.9% of our patients (**Table 1**).

Table1. Demographic and patients characteristics of the patients with ALL.

Gender	
Male	21 (51.2%)
Female	20 (48.8%)
Age group	
1-4 years	47.5%
5-9 years	27.5%
>10 years	25%
Ethnicity	
Arab	24 (58.5%)
Non-Arab	17 (41.5%)
Subtype	
L1	46.3%
L2	51.2%
L3	2.4%
WBC 10 9/L (%)	
<10000	30%
11-50000	37.5%
>50000	32.5%
Immunophenotype	
Pre Bcell	48.7%
Early Pre Bcell	20.5 %
Tcell	17.9%
Bcell	7.7%
Bi lineage	5.1%
Organ involvement	47.5%
CNS involvement	4.9%

Discussion

As mentioned earlier, ALL is one of the most important and known malignancy in childhood, which is responsible for up to 25-30 % of malignancy in children. Male children are almost always involved with higher rates than female,[16-17] and the results from our study suggest similar result with previous studies so far.[18] ALL has a peak of incidence between 1 and 5 years of age, which has been reported before in other studies.[19]

In our study, 46.3% of patients had L1 subtype, which was lower than Children's Oncology Group Statistics report (82%).Moreover,L3 subtype was reported up to 1%, which in our study was 2.4%.[20] The result of a study based on the survey on 738 children with ALL, declared that 86% of the patients belonged to L1 subtype, 13% to L2 and 7% to L3.[21] Since in FAB subtypes for ALL, L1 and L2 are more similar to each other, the difference may be due to the difference in definition .In our study, the most common immunophenotype subgroups was Pre-B cell (48.7%), but other studied studieshave reported this subtype with different range from 18to 20%.[22-23] On

Table2. The number and percentage of Immune markers in each subtype.

	L1	L2	L3
HLADR	10(52.6%)	15(71.4%)	--
TDT	7(36.8%)	9(42.7%)	1 (100%)
CD35	19(100%)	21(100%)	1(100%)
CD34	10(52.6%)	5(23.8%)	--
CD33	19(100%)	21(100%)	
CD22	19(100%)	21(100%)	1(100%)
CD21	1 (5.3%)	--	--
CD20	19(100%)	21(100%)	1(100%)
CD19	18(94.7%)	21(100%)	1(100%)
CD5	4(21.1%)	3(14.3%)	1(100%)
CD7	5(26.3%)	3(14.3%)	1(100%)
CD9	19(100%)	21(100%)	1(100%)
CD10	11(57.9%)	11(52.4%)	1(100%)
CD13	--	1(100%)	1(100%)
CD2	3(15.8%)	2(9.5%)	1(100%)
CD3	5(26.3%)	3(14.3%)	1(100%)

the contrary, these studies have indicated the frequency of early Pre B cell to 63-67%, but in our study we detected this subtype as much as 20.5% CNS involvement was presented in our patients in4.9%. According to the Children's Oncology Group Statistics, 4% of children with ALLpresent with CNS involvement at admission time.[22] The fraction with a WBC count above 50,000 mm3 (32%) was higher than reported from similar studies done in the same region (17%).

Immuno-phenotyping of childhood ALL is necessary for diagnosis and provides useful information about treatment decisions and prognosis of ALL, as well as achieving their morphological information.[23] In our study, HLA-DRwas 62.5 % in ALL patients and CD 20 and CD19 was the most common marker in these patients.In our work the most common markers in L1, which was found in 19 patients were reportedCD 19, CD33, CD22,CD35, CD20 and CD9. Also the percentage of markers in L2 subtype had a similarity toL1 group.The results show similarity to the results from Tong study[24] who reported the most common markers as CD19, CD10, CD22 and CD20with the frequency of 99%, 82.5%, 74.8% and 37.5%,respectively. On the contrary, the result of a study from our country[25] indicated that the most frequent markers in ALL patients were CD7 (11-28%), and CD2 (5-21%) and CD19 (3-14%). CD10 (1-5%) and CD20 (9%). The variety in results from different studies suggests the variation among genetic characteristics of people of each region, whichis based on hereditary and environmental factors.

One of the limitations of our study was the small number of the studied patients in comparison to other studies, and better results could be attained from the study with greater scale and bigger patients.

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