ORIGINAL ARTICLE

IMMUNOPHENOTYPING OF ACUTE LYMPHOBLASTIC LEUKAEMIA THROUGH FLOWCYTOMETRY IN CHILDREN

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Background: Acute Lymphoblastic Leukaemia (ALL) is caused by both genetic and environmental factors. Immunophenotyping by flowcytometry is vital for WHO classification of acute leukaemia. The objective of this study was to determine the immunophenotyping of ALL in paediatric patients through flowcytometry and to determine the frequency of each type of ALL through flowcytometry. **Methods:** This study was conducted in Rehman Medical Institute, Peshawar. One year retrospective data from Laboratory reports of blood parameters and flowcytometry were evaluated. Frequencies for qualitative variables like age and gender, and mean and standard deviation were calculated for quantitative variables like haemoglobin level, total leukocyte count, and platelet count. **Results:** Out of 32 ALL patients 22 (68.7%) had B-ALL and 10 (31.3%) had T-ALL. Eleven (34%) patients were females and 21 (66%) were males, 4 (12.5%) were from age group 1–5 years, 15 (47%) had age group >5–10 years, and 13 (41%) had age group >10–15 years. In B-ALL the expression of CD markers CD10, cCD79a, CD19 and CD20 was 91%, 77%, 68% and 14% respectively. In T-ALL, cCD3 was expressed in 100%, CD7 in 20%; cCD79a, CD19, and CD20 were 0%. **Conclusion:** ALL is more common in male children with peak age >5–10 years. B-ALL is more frequent than T-ALL. Patients mostly present with low haemoglobin (Hb), high total leukocyte count (TLC) and low platelet count. CD10, CD19, cCD79a were the most expressed markers in B-ALL, and cCD3 was expressed in all patients of T-ALL.

Keywords: Acute lymphocytic leukaemia, flowcytometry, immunophenotyping, B-ALL, T-ALL

Pak J Physiol 2020;16(3):3–6

INTRODUCTION

Leukaemia is the malignancy of white blood cells, having two types namely Acute Lymphoblastic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML).[1] ALL is common in children under the age of 15 while AML is common in adults. ALL is characterized by uncontrolled overproduction and proliferation of malignant immature lymphoblast cells in the bone marrow.[2] The incidence of ALL is 9–10 cases per 100,000 children annually worldwide.[3] The ALL is of B cell type in 60–80% cases and of T cell type in 11–20% cases in children.[4] ALL is caused by both genetic and environmental factors. The proposed risk factors are smoking, diet, high birth weight, high socioeconomic status, exposure to radiation and electromagnetic field, pesticides and previous use of immunosuppressive drugs and chemotherapy.[5,6] ALL accounts for 80% of the childhood leukaemia's and it is the most common malignancy under the age of 14 years.[7] Cancer is the second common cause of death in children aged 1−14 years. About 1,190 children under the age of 15 are expected to die from cancer in 2020.[8] The incidence of acute leukaemia ranges from 3.2 to 4.4 per 100,000 children under the age of 15 years.[9] In Europe the incidence ranged from 4–9 cases per 100,000 people.[10,11]

Immunophenotyping by flowcytometry is vital for WHO classification of acute leukaemia. This tool is important not only for diagnosis, but it also provides clues for the management and prognosis of this disease.[12]

ALL being the most common malignancy and cause of death in infants, data regarding the prevalence of ALL in the local population was necessary to calculate the disease burden on the society. The epidemiological data of ALL is limited in infants of Khyber Pakhtunkhwa (KP). This study was planned to see the patterns of ALL in this part of the country. The aim of this study was to evaluate the diagnostic significance of different CD markers expressed in ALL patients of paediatric age group.

MATERIAL AND METHODS

This study was conducted in Rehman Medical Institute (RMI) laboratory Peshawar Pakistan. Data was collected from 1st Jan 2019 to 31st Dec 2019 retrospectively. Ethical approval was obtained from RMI Research Ethical Committee. Universal sampling technique was used in the study. All cases of acute lymphoblastic leukaemia with children aging ≤15 years were included in the study. Cases of recurrent disease or relapse were excluded from the study. Demographic data and clinical features were

obtained from computerized records of the institute. Data was analysed on SPSS-20. Mean and standard deviation were calculated for quantitative variables like Hb, TLC and Platelet count. Frequencies were calculated in case of qualitative variables like age, gender and CD markers. Laboratory reports included complete blood count and Flowcytometry, which was done in RMI Laboratory. Complete blood count had been done on automated machine (Sysmex XN-1000). Normal ranges of Hb, TLC and platelet count were taken as 11.5–13 gm/dl, 4–11×10^3/µl, 150–400×10^3/µl respectively for reference.[13] Flowcytometry was done on Cytoflex flowcytometer (Beckman Coulter) according to their protocol.[14,15]

RESULTS

A total of 32 patients were included, 21 (66%) males and 11 (34%) females. B-ALL was present in 22 (68.7%) of the patients T-ALL was present in 10 (31.3%) patients (Table-1).

Four (12.5%) patients were in age group (1−5 years), 15 (47%) were in age group >5−10 years, and 13 (41%) were in age group >10−15 years (Table -2).

The mean Hb in gm/dl of ALL patients was 9.02±2.00, mean total leukocyte count was 16.6±13.2×10^3/µl was and the mean platelet count in was 51.8±15.6×10^3/µl (Table-3).

In B-ALL the expression of CD34 was in 32% patients while HLADR was expressed in 68% patients. CD10, cCD79a, CD19 and CD20 was expressed in 91%, 77%, 68% and 14% respectively. Immature cell marker TdT was expressed in 82% patient of B-ALL. Aberrant expression of myeloid CD marker CD33 was present in 9% while CD13 and CD117 were expressed by none of B-ALL patients. In T-ALL, cCD3 was expressed in 100%, and CD7 in 20%. cCD79a, CD19 and CD20 was 0%. Aberrant expression of myeloid CD marker CD33 was present in 10% while CD13 and CD117 was expressed in none. Immature cell marker TdT was expressed in 60% patients of T-ALL (Table- 4).

Table-1: Stratification of ALL patients (n=32)

Variables	Number	Percentage
B-ALL	22	68.7
T-ALL	10	31.3

Table-2: Gender and age stratification of ALL patients (n=32)

Variables	Number	Percentage
Gender		
Females	11	34.0
Males	21	66.0
Age (Years)		
1–5	4	12.5
>5–10	15	47.0
>10–15	13	41.0

Table-3: Blood profile of ALL patients n=(32)

Parameter	Mean±SD
Hb (gm/dl)	9.02±2.00
Total leukocyte count (×10^3)/µl	16.6±13.2
Platelet count (×10^3)/µl	51.8±15.6

Table-4: Expression of CD markers in ALL patients through immunophenotyping [n (%)]

CD marker	B-ALL	T-ALL
CD34	7 (32)	3 (30)
HLADR	15 (68)	1 (10)
cCD79a	17 (77)	0 (0)
CD19	15 (68)	0 (0)
CD10	20 (91)	3 (30)
CD20	3 (14)	0 (0)
TdT	18 (82)	6 (60)
CD33	2 (9)	1 (10)
CD3	0 (0)	10 (100)
CD7	0 (0)	2 (20)
cCD13	0 (0)	0 (0)
CD117	0 (0)	0 (0)

DISCUSSION

In the present study B-ALL was found to be more prevalent than T-ALL. This is also endorsed by the previous studies done in other parts of the world.[16,17] The disease was found more prevalent in males than in females. The male to female ratio of 1.9:1 found in this study is similar to the findings of another local study conducted in KP.[2] The male to female ratio was reported as 1.65:1 in developed countries.[18] In India a slight higher ratio of 2.03:1 has also been reported.[4]

We found ALL to be more prevalent in age group >5–10 years followed by age group >10–15 years, which is similar to that reported from India.[4] A recent Syrian study showed the peak age to be 5–9 years, similar to our study.[19] Local studies also report the peak age of ALL to be 6–10 years with male gender predominance.[20,21] However, the peak is reported to be 2–5 years age group in developed countries.[16]

The present study reported low Hb, high TLC, and low platelet count in ALL patients. Similar findings were reported in local and foreign studies emphasizing fact that these parameters should also be considered as an important clue in the early diagnosis of ALL.[13,22]

In the present study immunophenotyping of B-ALL revealed CD10 to be positive in 20 (91%) of cases, CD19 was expressed by 15 (68%), while the expression of cCD79a was 17 (77%). CD 20 was positive in 3 (14%) of cases of B-ALL. Another study done on immunophenotyping of acute leukaemia reported all cases of B-ALL to be positive for CD19, 90% to be CD10 positive while CD79a positivity was reported to be in 92.5% cases. CD20 was reported to be positive in 42.5% cases in the same study.[23] As far as T-ALL is concerned the present study reported cCD3 to be positive in 100% cases and CD7 was

positive in 20% cases. The aforementioned study reported all cases to be positive for CD7 while CD3 positivity was reported to be 91.7%.[23]

Previous studies report that for B-ALL the sensitivity of markers are, CD19 (100%), CD10 (82%), cCD79a and (95%), and for T-ALL it was reported to be 100% for both cCD3 and CD7.[24,25] Gupta, *et al* reported that in B-ALL the immaturity markers like HLA-DR, CD34 and TdT, were expressed in 97.4%, 81.3% and 97% of cases respectively.[12] CD13 was the commonest aberrantly expressed marker in B-cell ALL, seen in 25.6% cases followed by CD33 in 17.9% cases.[12] In the present study the expression of immaturity markers HLA-DR, CD34 and TdT were 68%, 32% and 82% respectively. The most common marker of aberrant expression was CD33 (9%) in our B-ALL cases. CD13 and CD117 were not expressed by any case in our scenario.

In the study by Gupta *et al*, cCD3 was expressed by all cases of T-ALL while CD10 was expressed in 37% cases which is similar to the present study.[12] In our patients of T-ALL the aberrant expression of CD33 was 10% but none expressed CD13 and CD117 while the CD33 was reported to be expressed by 30% cases of T-ALL in a study by Marks DI, *et al*.[26]

CONCLUSION

ALL is more common in male gender with the peak age range of >5−10 years in children. B-ALL is more frequent than T-ALL. Low Hb, high TLC and low platelet count is presented in most patients. CD10, CD19, cCD79a markers were expressed in B-ALL patients and cCD3 was expressed in all patients of T-ALL. Aberrant expression of CD33 was more common in both types of ALL than CD13 and CD117.

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Received: 3 Jul 2020 **Reviewed: 2 Sep 2020** **Accepted: 3 Sep 2020**

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Funding disclosure: None to declare
Conflict of interest: None