**Journal of Current and Advance Medical Research**
*January 2021, Vol. 8, No. 1, pp. 59-64*
*http://www.banglajol.info/index.php/JCAMR*
ISSN (Print) 2313-447X
ISSN (Online) 2413-323X
NLM Catalog ID 101673828
DOI: https://doi.org/10.3329/jcamr.v8i1.52596

**ORIGINAL ARTICLE**

# Immunophenotypic Pattern and Treatment Outcome after Completion of Induction Remission in Children with Acute Lymphoblastic Leukemia

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[**Received on:** 1 November 2020; **Accepted on:** 20 December 2020; **Published on:** 1 January 2021]

## Abstract

**Background**: Acute lymphoblastic leukemia (ALL) is presented with different immunophenotypic pattern. **Objective:** The purpose of the study was to evaluate the immunophenotypic pattern of ALL and also, to recognize the frequency of different ALL subtypes and treatment outcome after induction remission therapy. **Methodology:** This prospective study was conducted in the Department of Paediatric Hematology and Oncology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from June 2017 to July 2018 for a period of one year. Newly diagnosed admitted cases of ALL aged 1 to 17.9 years were included. Immunophenotyping from aspirate marrow samples were done in a special hematology laboratory. Patients were monitored during induction remission period with physical examination and required investigations. **Result**: Among 87 analyzed patients, 81 patients (93.1%) were B-cell ALL and 6 patients (6.9%) were T-cell ALL. After completion of induction remission therapy 61 patients had undergone complete remission and among them B cell ALL were 56(69.1%) and T cell were 5(83.3%) (P=0.464). None of the patient had partial response or induction failure. Complication were developed in 53(60.91%) patients during induction therapy. Most common cause of death was septicemia (22/26). Death was more in patients who had total WBC count >50X$10^9$/L (p=0.017) and received regimen B (p=0.031). **Conclusion:** B cell ALL was more common and most of the patients had undergone complete remission after induction remission therapy. [*Journal of Current and Advance Medical Research, January 2021;8(1):59-64*]

**Keywords:** Acute lymphoblastic leukemia**;** immunophenotype; induction remission

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**Cite this article as:** Diba F, Karim MA, Soma SA, Chowdhury I, Mamun S, Mondol MNI. Immunophenotypic Pattern and Treatment Outcome after Completion of Induction Remission in Children with Acute Lymphoblastic Leukemia. J Curr Adv Med Res 2021;8(1):59-64
**Funding:** This study has been performed without any funding from outside else.
**Conflict of Interest:** There was no conflict of interest to any of the authors of the study.
**Contributions to authors:** Questionnaire development, Data collection: Diba F, Karim MA, Soma SA; Data analysis: Diba F; Manuscript writing: All authors are involved; Manuscript revised: Chowdhury I, Mamun S

## Introduction

Acute lymphoblastic leukemia is the most common malignancy in children. It accounts for 72% of all cases of childhood leukemia. Approximately 4900 children are diagnosed with ALL each year in United States, with an incidence of 3 to 4 cases per 100,000 white children[1]. In the Department of Pediatric Hematology and Oncology, BSMMU, 455 newly diagnosed childhood malignancy were registered in a year 2012 and among them 58% were ALL[2]. ALL classified as B-lymphoblastic leukemia and T-lymphoblastic leukemia. B-precursor cell accounts for 80% ALL cases. T-cell accounts for 15-20% of ALL cases and mature B-cell accounts for 1 to 2% of ALL cases[3].

Diagnosis of acute lymphoblastic leukemia (ALL) is made by integrating the study of cell morphology, cytochemistry, immunophenotype and cytogenetics. Morphological bone marrow examination is the first step for the primary diagnosis of ALL with an accuracy of 86.8%[4] leaving a diagnostic dilemma.

Leukemic cell of different type express characteristic nuclear, cytoplasmic and cell surface antigen which is known as the immunophenotype of the cell. Immunophenotype is achieved by means of labelled antibodies that recognize specific cellular antigens by flowcytometry. In an individual patient, the role of immunophenotyping may be confirming diagnosis, identifying prognostic differences, staging a disease and detecting an aberrant immunophenotype[5]. It is essential to consider all information that is available cytology, histology, immunophenotype and genetic characteristics in order to make an accurate and precise diagnosis. The purpose of the study was to evaluate the immunophenotypic pattern of ALL and also to recognize the frequency of different ALL subtypes and treatment outcome after induction remission therapy.

## Methodology

This study was design as non-randomized, single centered prospective cohort study. This study was conducted in the Department of Paediatric Hematology and Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from June 2017 to July 2018 for a period of one year. All children presented acute lymphoblastic leukemia with the age group of 1 to 17.9 years who were admitted in pediatric Hematology and Oncology department of BSMMU for induction therapy during the study period were selected as study population. Diagnosis of ALL was done on the basis of history, physical examination and morphological examination of PBF and Bone marrow aspirate. Written and informed consent was obtained before enrollment in the study from a parents or guardian. Prior to the commencement of the study, the thesis protocol was approved by Institutional review board BSMMU, Dhaka. Data were collected using a preformed data collection sheet. Demographic data regarding age, sex, socioeconomic status had been collected from guardian or parents. Medical data regarding initial presentation at diagnosis, treatment received before admission were compiled. Clinical information about pallor, temperature, pulse, blood pressure, respiratory rate, bleeding manifestation, bony tenderness, lymphadenopathy and other systemic clinical parameter had been taken. Immunophenotyping from aspirate marrow samples were done in a special hematology laboratory at department of pediatric Hematology & Oncology using essential antibody panel. Immunophenotyping was performed on CYTOMICS FC-500 flowcytometer using CXP software. The cells were analyzed with the most appropriate blast gate using the combination of forward and side scatters. An antigen was considered positive when the expression is at least 20% of the gated cell. Prior to initiation of therapy routine baseline investigations was done which includes serum electrolytes, uric acid, inorganic phosphate, calcium, SGPT, creatinine, LDH, PT, aPTT and Chest x-ray. Cytospin examination of CSF was done to determine the CNS status. Proper hydration and alkalization was done 24 hours before the start of chemotherapy and continued for 5-7 days through induction remission period. General supportive management like allopurinol, phosphate binder, oral care, anal care etc. was given in all patients. All patients of acute lymphoblastic leukemia were treated with modified UKALL 2003 protocol, regimen 'A'or'B' according to risk stratification. Regimen-A was given to patients aged 1-9 years and initial total WBC count <50000/ $mm^3$. Regimen-B was given to patients aged 10 years or above and initial total WBC >50000/$mm^3$. Remission of induction therapy in ALL had been given for 35 days. Regimen A included 3 drugs that were vincristine, L-asparaginase, dexamethasone, and IT/TIT (intrathecal methotrexate, hydrocortisone, cytarabine). In regimen B along with this daunorubicin was added. Regular follow-up of the patients was done both clinically and with laboratory investigations during the induction period. If the child developed fever during the course of therapy, proper evaluation was done by

physical examination and laboratory investigation including septic screening. Empirical broad-spectrum antibiotic was started and modified as per institutional febrile neutropenia guide line. Bone marrow samples was examined morphologically on D28 of induction therapy and response was evaluated. All collected data were entered in to an SPSS data base form. Statistical analysis was performed by using SPSS (Statistical Package for the Social Sciences) for windows version 22. Descriptive statistics (numbers and percentage) was calculated for all variables and statistical analysis was applied to find associations between variables using Chi-square test. A p-value <0.05 and confidence interval was set as 95% level was considered as significant.

## Results

A total number of 87 ALL patients were recruited for this study. In this study most of the patients were 1 to 9 years of age (87.4%) and remaining were 12.6% cases. Mean age 5.78±0.33 years (Table 1).

**Table 1: Demographic characteristics of the study patients (n=87)**

| Age Group | Frequency | Percent |
|---|---|---|
| 1 to 9 Years | 76 | 87.4 |
| 10 to 17.9 Years | 11 | 12.6 |
| **Total** | **87** | **100.0** |
| Mean±SD (Range) | 5.78 ± 0.33 (1.1-17.3)Years | |

Representing the expression of lymphoid antigen in ALL patient was recorded. Most common antigen in B cell ALL were cCD79a (positive in 100% cases), CD10 (positive in 97.5%) and CD 19 (positive in 96.3% cases). In T cell ALL most common antigen were CD 3 (positive 100% cases), CD7 (positive in 100% case), CD5 (positive in 83.3% cases). CD value 20% considered positive (Figure I).

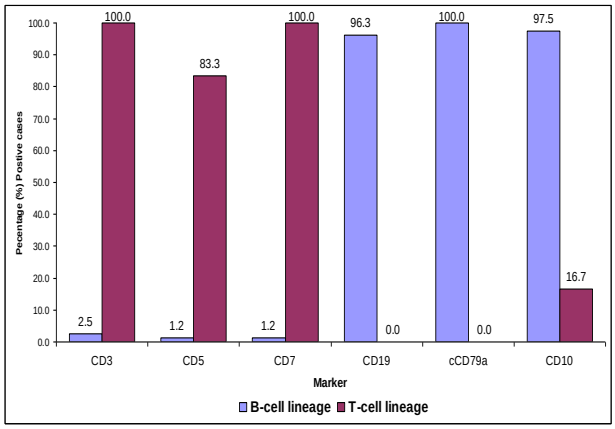


**Figure I: Representing the expression of lymphoid antigen in ALL patients**

Treatment outcome of the study subject observed. Among 87 cases 61 patients had undergone complete remission among them 56 (69.1%) were B cell and 5 (83.3%) were T cell ALL. 26 patients died among them B cell ALL were 25(30.9%) and T cell was 1(16.7%). Most common cause of death was septicemia (Table 2).

**Table 2: Treatment outcome of B cell and T cell ALL**

| Treatment Outcome | B-cell lineage | T-cell lineage |
|---|---|---|
| Complete Remission | 56(69.1%) | 5(83.3%) |
| Partial Remission | 0(0.0%) | 0(0.0%) |
| Death | 25(30.9%) | 1(16.7%) |
| Induction Failure | 0(0.0%) | 0(0.0%) |
| **Total** | **81(100.0%)** | **6(100.0%)** |

*Chi-square test was performed to see the level of significance; p value=0.464

Association between outcome and treatment regimen shows among 87 study subjects 55 patients received regimen A among them 43(78.2%) patients had undergone complete remission and 12(21.8%) died. Regimen B was received by 32 patients among them 18(56.25%) had undergone complete remission and 14(43.75%) died (P value 0.031) (Table 3).

**Table 3: Association between Outcome and Treatment Regimen**

| Outcome | Treatment regimen | |
|---|---|---|
| | **Regimen A** | **Regimen B** |
| Complete remission | 43(78.2%) | 18(56.25%) |
| Partial remission | 0(0.0%) | 0(0.0%) |
| Died | 12(21.8%) | 14(43.75%) |
| Induction failure | 0(0.0%) | 0(0.0%) |
| **Total** | **55(100.0%)** | **32(100.0%)** |

*Chi-square test was performed to see the level of significance; p value=0.031

Among 81 B cell ALL patients 63% (51) patient developed complication during induction and 37% (30) patient did not. In 6 cases of T cell ALL 33.3% (2) patient developed complication and rest 66.7% (4) were free from complication (P value 0.151). Most common complication was septicemia (54.3% in B cell ALL) followed by wet bleeding,

tumor lysis syndrome, Dyselectrolytemia and hyperglycemia (Table 4).

**Table 4: Association between Complications and Pattern of Immunophenotype**

| Complications | B-cell lineage | T-cell lineage |
|---|---|---|
| Yes | 51(63.0%) | 2(33.3%) |
| No | 30(37.0%) | 4(66.7%) |
| **Total** | **81(100%)** | **6(100%)** |

OR (95% CI)= 3.40 (0.58 to 19.69); P value=0.151

Association between outcome and treatment regimen. Among 87 study subjects 55 patients received regimen A among them 78.2% (43) patients had undergone complete remission and 21.8% (12) died. Regimen B was received by 32 patients among them 56.25% (18) had undergone complete remission and 43.75% (14) died (P value 0.031) (Table 5).

**Table 5: Association between Outcome and Treatment Regimen**

| Outcome | Treatment Regimen | |
|---|---|---|
| | **Regimen A** | **Regimen B** |
| Complete remission | 43(78.2%) | 18(56.25%) |
| Partial remission | 0(0.0%) | 0(0.0%) |
| Died | 12(21.8%) | 14(43.75%) |
| Induction failure | 0(0.0%) | 0(0.0%) |
| Total | 55(100.0%) | 32(100.0%) |

*Chi-square test was performed to see the level of significance; p value=0.031

## Discussion

Immunophenotyping has become an indispensable diagnostic tool for identification of cell lineage of leukemia, disease classification and patient management and for disease monitoring of acute leukemia. During the study, a total 87 patients of ALL in the Department of Paediatric Haematology and Oncology, BSMMU were studied to see the pattern of immunophenotyping and induction outcome.

Among 87 ALL patients B cell ALL was 93.1% cases and T cell was 6.9% cases. Percentage of T-lineage ALL varies among different studies from 9.7% to 25.5% cases[5-8]. T cell leukemia usually occurred in older age group. In this study T cell ALL is slightly lower than other studies may be due to in present study only 12.6% patients were >9 years of age. Peak age of development of childhood ALL is 2-5 years. Age range of the present study were 1 to17.9 years with a mean age 5.78 years.

Most of the patients (87.4%) were between 1 to 9 years. Age at diagnosis has a strong prognostic value. Pui et al[9] found children aged 1 to 9 years had a better outcome that either infants or adolescence. The inferior outcome of older children may be due to increased intolerance of intensive therapy. In this study there was no statistically significant difference in outcome between the age groups.

This study found that most common antigen in B cell ALL were cCD79a, next CD10 and CD 19. In T cell ALL most common antigen were CD3 and CD7 both positive in 100% cases, next CD5 which met the EGIL criteria for diagnosis of B and T cell ALL. In case of B cell ALL few patient aberrantly present T cell antigen. CD3 positive in 1.2% cases, CD5 in 1.2% cases .and CD7 positive in 1.2% cases. CD value 20% considered positive. Some myeloid antigen CD 13, CD33 were aberrantly expressed in B-cell and T cell ALL patient. In case of B cell ALL CD13 positive in 1(1.2%) case and CD33 positive in 5(6.17 %) cases. In case of T cell ALL 2(33.3%) cases were positive for CD33.Similar pattern of aberrant presentation of myeloid antigen observed by Seegmiller et al[10] and Iwamoto et al[11].

Most of the patients were treated with regimen A (64%) and rest were treated with regimen B. At the end of induction, 71.0% of the patients in the current study had achieved complete remission among them 65 were B cell ALL and 17 cases were T cell ALL. No significant relationship found between pattern of immunophenotype and treatment outcome (p=0.464). All the patient of both B and T cell ALL had undergone complete remission excluding the death. Rana et al[15] observed remission outcome similar to this study. Most of the reported complete remission rate was 87% to 98% cases[5.,16,18-20]. None of this study subjects had partial response or induction failure. Induction failure rate 2.0% to 4.0% found in many studies[15,19,,21-22] and patients with older age, high leukocyte count, T-cell phenotype, Philadelphia chromosome, and 11q23 rearrangement frequently presented with induction failure[21]. In current study there is no patient with induction failure probably due to low percentage of T cell leukemia and number of patient was less in >10 years age group.

Complication developed 53(60.91%) patients during induction and there was no significant relation found between complication and pattern of immunophenotype (p=0.151). Most common complication were septicemia, occurred 44 (54.3%) cases in B cell ALL and 2 patients in T cell ALL.

Other complications were bleeding (1.2%), tumor lysis syndrome (1.2%), dyselectrolytemia (3.7%) and hyperglycemia (2.5%). Complication were more common in patients who received regimen B than regimen A (p =0.04). Rajeswari et al[20] and Hurwitz et a1[23] found similar data regarding infection during induction remission. It was found that the use of Daunorubicin in combination with dexamethasone during induction would lead to enhanced toxicity[24] and substituting of dexamethasone for prednisoone complicates remission induction, and results in a higher incidence of septic episodes[23,25,26.] .This study also suggest the connection because complication was more in patients group who received regimen B.

Infection was more common in this study may be due to majority of the patient did not stick with the advice regarding oral and mouth care, lack of isolation and barrier nursing. This observation also supported by Hossain et al[19]. Most of the common cause of death was septicemia. Among 26 induction death 22(84.61%) patients died due to septicemia which was supported by most of the studies[27-29]. Connor et al[30] found overall 2.4% cases infectious death among 3126 patient who received UKALL 2003 regimen and mostly occur during induction (36/75).

## Conclusion

This study has found that B cell ALL is the more common variant among children. Excluding the patients who died, all the patients have undergone complete remission irrespective of immunophenotype pattern and none of the patient has induction failure. Complications are more common in age group of 10 to 17.9 years and those who have received the treatment regimen B. Death rate is quite high during induction period and the most common cause of death is septicemia.

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