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# Study of Clinico-hematological and Immunophenotypic Profile in Adult Patients with Acute Lymphoblastic Leukemia in Eastern India

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors DB and SD designed the study, wrote the protocol, analyses of the study and wrote the first draft of the manuscript. Author SS managed the literature searches, performed the immunophenotypic analysis. Authors SCS and RM observed the clinical findings and patient profile. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** To study clinical, hematological and immunophenotypic profile of adult patients with acute lymphoblastic leukemia (ALL) in a tertiary care centre of eastern India.
**Study Design:** Cross-sectional study
**Place and Duration of Study:** Postgraduate Department of Medicine and Department of Clinical Hematology, SCB Medical College and Hospital, Cuttack, Odisha, India between August 2011 and July 2012.
**Methodology:** Sixty naïve consecutive cases of adult ALL were taken into this study. Secondary and relapsed cases of ALL were excluded. Along with clinical evaluation, full blood count, bone marrow aspiration study, immunophenotyping by flow cytometer was done in all of the cases.
**Results:** Patients between 15 to 24 years constituted 58.3% of cases in the study. Mean age was 26.58 years and median age (50th percentile) was 22 years. Pallor (96.7%) and

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fever (81.3%) were the most common presentations. Bleeding manifestations and fever were significantly associated with B-ALL (*P*<.01). Lymphadenopathy and mediastinal mass were found significantly higher in patients with T-ALL(*P*<.01).On bone marrow morphology, FAB L1 was found in 38.3% and L-2 was seen in 61.7% of cases. Myeloperoxidase stain was negative in all cases.68.3% cases came out as B-ALL and 31.7% as T-ALL after immunophenotyping. CD19 and cytoplasmic CD79a were the most commonly found to be positive in patients with B-ALL whereas CD7 and cytoplasmic CD3 were the most common antigens expressed in those with T-ALL. Anti-MPO was negative in all of the cases. Aberrant myeloid expression was also found in 21.7% cases. There was no significant association of aberrant myeloid expression with B or T-cell immunophenotype (*P*=.32).
**Conclusion:** Clinico-hematological and immunophenotypic profile of ALL patients in this region were almost similar with the reports published from other parts of India but there were a few differences from the Western data. This, however, needs further work from this region of India.

*Keywords: Acute Lymphoblastic Leukemia; Immunophenotype; B-ALL; T-ALL; Aberrant myeloid expression.*

## 1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) encompasses a group of lymphoid neoplasms that morphologically and immunophenotypically resemble B-lineage and T-lineage precursor cells. The WHO International panel on ALL recommends that the previous French American British (FAB) morphologic classification (L1, L2, L3) be abandoned, since this classification has no clinical or prognostic relevance [1]. It instead advocates the use of the immunophenotypic classification. ALL constitutes of two main immunologic types: B cell and T cell. Due to limited resources for flow cytometry (FCM), the FAB classification are being still used in many parts of this country. At the same time, there are paucity of published reports of incidence and subtypes of ALL based on WHO classification from India. A study from Tata Memorial Hospital, Mumbai (TMH) by Gujral et al (2009) had revealed that T-ALL constituted 29.67% cases and all the rest constituted B-ALL among adult patients with ALL [2]. Advani et al. [3] from Mumbai in their study with 250 cases of ALL had found T-cell phenotype to be present in 30.6% cases. Study from Chennai (Madras, 1994) by Rajalekshmy et al showed higher prevalence of T-ALL (44.2%) [4]. The present study was designed to undertake immunophenotyping of adult naïve cases of ALL at a tertiary care hospital in the eastern India. There are no published reports of immunophenotyping in ALL from this part of the country. Our study population consisted of people from Odisha, Jharkhand, Chhattisgarh and some part of West Bengal. Among these, patients from Jharkhand, Chhattisgarh and parts of Odisha are often tribal where as patients from coastal Odisha and West Bengal are of north-Indian-mongoloid mixed descent. Again, people from western Odisha and Chhattisgarh often carry the sickle cell disease gene which is only found in this part of India.

## 2. MATERIALS AND METHODS

The study was conducted between August 2011 and July 2012 in the Post Graduate Department of Medicine and Department of Clinical Hematology, S.C.B. Medical College, Cuttack, Odisha. Sixty naïve consecutive cases of adult ALL (≥15 years) were taken for study. Secondary and relapse ALL cases were excluded. All the patients were hospitalized

for proper diagnosis and necessary individual consent were taken before proceeding for the study. Detailed history taking and clinical examinations were done in all the cases. Hematological parameters were detected by fully automated 5 part cell counter (SYSMEX XT 2000-I). Peripheral smear examination was done to find out the presence of leukemic blasts. Bone marrow aspiration was performed from right iliac crest under strict asepsis with 5 ml of 2% xylocaine infiltration anaesthesia. Smears were analyzed by staining with Leishman stain and Myeloperoxidase (MPO). The FAB criteria were also used to diagnose and sub classify ALL morphologically. Flowcytometric analysis was performed on a FACS calibur flow cytometer (Becton Dickinson, CA, USA) and Cell Quest Pro software was used to acquire and analyze the data. It was done using either bone marrow sample or peripheral blood. The immunophenotypic panel began with the SSC (Side scatter)/CD45 dot plot. Gating was done with CD45 positive cells. The minimum primary screening panels were selected as per the recommendations of the national meeting on "Guidelines for Immunophenotyping of Hematolymphoid Neoplasms by Flowcytometry" held in Mumbai in 2008 [5, 6]. Surface markers used for B cells were CD19, CD10; for T cells were CD7, CD5 (Fig. 1); for myeloid cells CD13, CD33, CD117. Other markers like CD34, HLA-DR were also tested as per recommendations. Additional cytoplasmic markers i.e. specific for B cells cCD79a, for T cells cCD3 and for myeloid cells cAnti-MPO were taken.

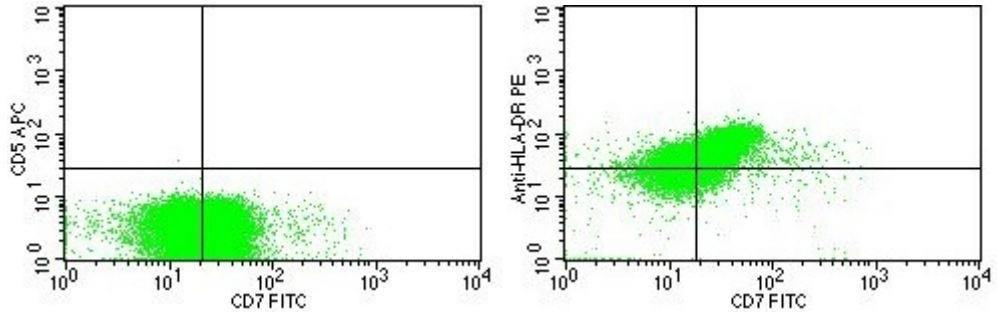


**Fig. 1. Flowcytometric Scatter Plot**

Antigen expression was considered positive according to the bright or dim positivity of the blast cells after reacting with a particular antibody. Statistical analysis was done using SPSS software version 19 (IBM) and Instat3 software. Frequency distribution, chi square tests and logistic regression were mainly done for statistical evaluation.

## 3. RESULTS

In this study, 81.7% of all cases were male. Among cases with T-ALL, 94.7% were male while cases with B-ALL, 75.6% were male. Patients aged between 15-24 years constituted majority (58.3%). Mean age was 26.58 years and median age (50th percentile) was 22 years (Fig. 2). The youngest patient was of 15 years of age and the oldest one was of 72 years of age.

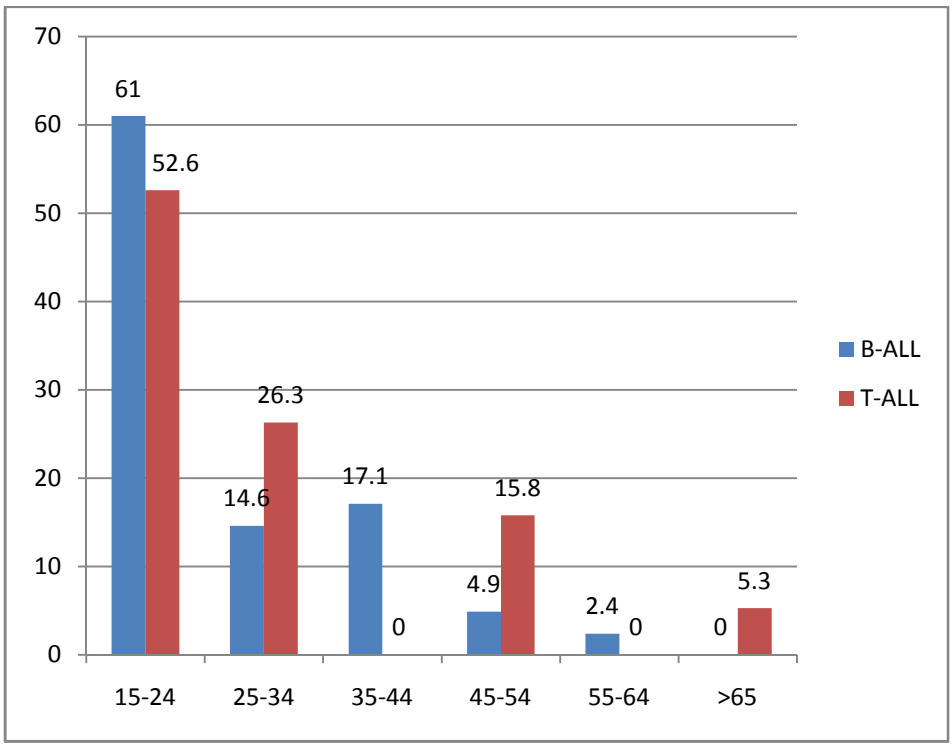


**Fig. 2. Age Distribution (figures above the columns denoting percentages)**

Fever was the most common symptom (81.3%) followed by bleeding manifestations (60%), pain abdomen (40%), bone pain (36.7%). Bleeding manifestations were significantly associated with B-ALL (*P<0.01*). Pallor was the most common sign (96.7%) followed by hepatosplenomegaly (53.3%), lymphadenopathy (53.3%) and sternal tenderness (40%). Mediastinal mass was found in 3.4 % of cases and all of those cases were T-ALL. Testicular swelling was found in 3.4% cases. CNS manifestations, in the form of leukemic blasts in the CSF, were present in only one case (1.7%). Lymphadenopathy was associated with all cases with T-ALL (*P*<0.01), as shown in the Table no 1.

**Table 1. Statistical association between clinical presentations with B-ALL and T-ALL**

| Clinical Presentations | B-ALL (N=41) (%) | T-ALL (N=19) (%) | Total (N=60) (%) | *P* Value |
|---|---|---|---|---|
| Fever | 90.2 | 63.2 | 81.7 | 0.012 |
| Pain Abdomen | 39.0 | 42.2 | 40 | 0.821 |
| Bone Pain | 39.0 | 31.6 | 36.7 | 0.578 |
| Bleeding Manifestations | 78.0 | 21.1 | 60 | <0.0001 |
| Pallor | 97.6 | 94.7 | 96.7 | 0.571 |
| Generalised Lymphadenopathy | 31.7 | 100 | 53.3 | <0.0001 |
| Hepatosplenomegaly | 51.2 | 57.9 | 53.3 | 0.630 |
| Sternal Tenderness | 36.6 | 47.4 | 40 | 0.428 |
| Testicular Swelling | 4.8 | 0 | 3.4 | 0.327 |
| Mediastinal Mass | 0 | 10.5 | 3.4 | 0.002 |
| CNS Manifestations | 0 | 5.2 | 1.7 | 0.139 |

Median hemoglobin values for B-ALL and T-ALL were 6.00 and 6.90 g/dl, respectively. Median values of total Leucocyte count for B-ALL and T-ALL were 17.00X103 /mm3 and 16.00X103 /mm3, respectively. Median values of platelet count were 17.00 X103 /mm3 and 36.00 X103 /mm3 respectively. No significant association was found by comparing the hematological parameters in between cases with B-ALL and those with T-ALL. Blast cells were present in peripheral smear in 73.3% cases. Bicytopenia, pancytopenia and relative lymphocytosis were found in 8.3% cases each. Isolated thrombocytopenia was found in 1.7% cases. On bone marrow morphology, FAB L1 was 38.3% and L-2 was 61.7% of cases. All of the cases were found to be MPO stain negative. No L3 case was found. 68.3% cases were diagnosed as B-ALL after immunophenotyping and the rest (31.7%) were T-ALL. No significant association was found between FAB classification and immunophenotypic diagnosis of the cases. Immunophenotypic findings as presented in the table no 2 showed HLA-DR expression was significantly higher with T-ALL than B-ALL (47.4% vs. 12.2%, $p<0.01$) and CD13 was most commonly expressed myeloid antigen. No statistically significant associations was found between aberrant myeloid expression with immunophenotypic or hematological parameters (P=.32).

**Table 2. CD antigen reactivity in patients with ALL**

| CD Markers | B-ALL(N=41) | T-ALL (N=19) | Total (N=60) |
|---|---|---|---|
| cCD3 | 0 | 19 (100%) | 19(31.7%) |
| CD7 | 0 | 18 (94.7%) | 18(94.7%) |
| CD5 | 0 | 12 (63.2%) | 12 (20%) |
| cCD79a | 41 (100%) | 0 | 41(68.3%) |
| CD19 | 39(95.1%) | 3 (15.8%) | 42 (70%) |
| CD10 | 35(85.4%) | 2 (10.5%) | 37(61.7%) |
| CD13 | 5 (12.2%) | 2 (10.5%) | 7 (11.7%) |
| CD33 | 4(9.8%) | 1 (5.3%) | 5(8.3%) |
| CD117 | 1(2.4%) | 0 | 1(1.7%) |
| CD34 | 18(43.9%) | 4 (21.1%) | 22(36.7%) |
| CD45 | 40(97.6%) | 19 (100%) | 59(98.3%) |
| HLA DR | 5 (12.2%) | 9 (47.4%) | 14(23.4%) |
| ANTI- MPO | 0 | 0 | 0 |

## 4. DISCUSSION

There was higher number of male patients in the present study as compared to previous studies like CALGB (Cancer and Leukemia Group B, Chicago, USA), GMALL (German Multicentre study group for Adult ALL), MRC (Medical Research Council, UK), where male gender ranged from 59 to 63% [7,8,9]. Male dominant demographic picture of any disease in any tertiary care hospital is a typical feature in India. The cause behind this might be lower literacy rate amongst the female in India. 58.3% of cases were between 15 to 24 years of age. In SEER (Surveillance, Epidemiology and End Results) Cancer Statistics Review data, adult ALL was relatively higher in the age group of 15-25 years and was similar with our observations [10]. However SEER data also showed a relative rise in incidence of adult ALL after age of 60 years. This was not observed in our study group. Only one patient (1.7%) was above 65 years of age.

Western studies had relatively lower incidence of fever (3 to 56%). Incidence of lymphadenopathy, hepatomegaly, splenomegaly, CNS manifestation as reported in other studies almost correlated well with our study. In previous western studies, lymphadenopathy was present in 40 to 57% cases, hepatomegaly in 24-47%, splenomegaly in 31-56%, CNS

manifestations in 1-7%. Incidence of mediastinal mass was lower in our patients (3.4%) in comparison to previous publications (10-15%). [7,8,9] Previous data from India, by Advani S had shown that hepatosplenomegaly and lymphadenopathy were much more common (95% and 87% respectively) as compared to the data from the west. [3] In the present study the prevalence of hepatosplenomegaly and lymphadenopathy are comparable to the western data. Therefore, there are some subtle differences in the clinical manifestations of ALL in our patients as compared to the developed world, which demands special attention in suspecting the disease in young adults presenting with fever, bleeding manifestations and bone pain. Most of the patients (73.3%) could be diagnosed as acute leukemia from peripheral smear. 8.3% cases presented with pancytopenia and 1 patient (1.7%) presented with only thrombocytopenia. Previous studies like CALGB, GMALL, and MRC found blast cells in peripheral blood in 92 % of adult ALL cases. Our study observed relatively lower incidence of leukemic blast cells in peripheral blood. [7,8,9] 61.7% of cases had bone marrow morphology of FAB L-2 type. Choudhary et al reported FAB ALL-L1 (73.3%) to be more common than FAB ALL-L2. [11] In the present study, 68.3 % cases were B-ALL and 31.7 % were T-ALL. Our study is comparable with this data and also with previous studies [12] (Table No 3).

**Table 3. Incidence of subtypes of ALL in the Indian Subcontinent during last two decades**

| Author | Year | Place | B-ALL (%) | T-ALL (%) |
|---|---|---|---|---|
| Rajalekshmy KR [4] | 1994 | Tamilnadu, India | 55.8 | 44.2 |
| Advani SH [3] | 1999 | Mumbai, India | 69.4 | 30.6 |
| Khawaja et al. [13] | 2005 | Abottabad, Pakistan | 78.1 | 21.9 |
| Gujral S. et al [2] | 2009 | Mumbai, India | 70.4 | 29.6 |
| Present Study | 2012 | Cuttack, India | 68.3 | 31.7 |
| Western World [14] | | | 75 | 20 |

Immunophenotypic profile of our patient group correlates well with the western study done by Renate Thalhammer Scherrer et al. with 325 cases of adult ALL, though they found less expression of CD13 and CD33 in their patients with B-ALL. [12] In Tata Memorial Hospital, Mumbai in 2009 Gujral S et al although observed that CD7 and cytoplasmic CD3 were positive in all patients with T-ALL and CD19 and cytoplasmic CD79a were positive in all cases with B-ALL. The prevalence of CD5, CD10 and CD34 markers were higher and expression of HLA-DR was lower than observed in this study [2]. Tata Memorial Hospital in Mumbai is one of the largest referral hospitals for malignant diseases in our country attending to patients from different parts of India with multi-ethnic origin and the study population in that study was very large (1471 patients). This could be the reason for having difference in expression of CD markers in patients with ALL as compared to our study done in Cuttack with 60 patients only.

No statistically significant association was found between CD34 with B-ALL or T-ALL. Imam Seldom et al. found statistically significant association of CD34 expression with T-ALL [15]. Our observations was similar with the study by Kazunori Nakse, Mary Sartor et al where no statistically significant associations between CD34 expression and ALL immunophenotype were observed [16]. Publication by Wenxiu et al showed aberrant myeloid expression in 39.5% of ALL cases [17]. Similar results were noted by Vitale et al. [18]. The present study showed comparatively lower expression of aberrant myeloid

antigen. No statistically significant association was found between myeloid expressions with immunophenotypic diagnosis of B-ALL and T-ALL. Indian study by Bharat Bhusan et al showed expression of CD33 in 39% cases of adult B-ALL and 33% cases of adult T-ALL which was higher than our observation [19]. This might be due to difference in ethnicity of the patients studied. Kazunori Nakse, Mary Sartor found myeloid antigen expression was more frequent in B lineage ALL than in T - lineage ALL, but this difference was statistically significant only for CD33 expression (12.6%) in B-ALL [15]. In the present study with only 60 patients, relatively more frequent expression of aberrant myeloid antigen was observed in cases with B-ALL than those with T-ALL, but it was not statistically significant for any CD markers.

## 4. CONCLUSION

ALL in adults presented mostly with prolonged fever (81.3%) in contrast to western experience where fever constituted less than 60%. The other clinical features were bleeding manifestations, bone pain, lymphadenopathy and hepatosplenomegaly. Three fourth of the patients with ALL had leukemic blast cells in the peripheral blood smear. After using a panel of CD markers during immunophenotyping by flow cytometer, it was observed that B-cell ALL was more common than T-cell ALL. CD19 and cytoplasmic CD79a were the most commonly found to be positive in patients with B-ALL whereas CD7 and cytoplasmic CD3 were the most common antigens expressed in those with T-ALL. Some cases also manifested aberrant myeloid antigen expressions but such expressions were not statistically significant. The findings were almost similar with the reports published from other parts of India. However, further studies are needed from this part of India.

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## COMPETING INTERESTS

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