www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i7.198

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

CD34 Positive Pediatric T Lymphoblastic Leukemia: A Tertiary Care Centre Experience

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Abstract

Background: *T lymphoblastic leukaemia (T ALL) carries a worse prognosis compared to B lymphoblastic leukaemia (B ALL) especially in children. There are only a few published data about immunophenotype of T ALL in India. So we studied the immunophenotypic pattern of T ALL in children by flow cytometry and correlated it with clinical and pathological factors.*

Materials and Methods: *271 consecutive cases of paediatric acute leukaemias reported in the Regional Cancer Centre, Thiruvananthapuram, Kerala, were immunophenotyped by flow cytometry and clinic-pathological analysis was done in all cases. The study period was from June 2009-June 2011. T ALL cases were followed up for two and a half years till the completion of treatment.*

Result: *Among the 271 cases of myeloperoxidase negative acute leukaemia, 232 were B-ALL, 39 were T-ALL. Among 39 cases of T ALL, 25 cases (64%) were CD34 positive and 14(35.8%) were CD34 negative. Of the 25 CD34 positive cases, only 12 (48%) completed treatment compared to 10 out of 14(71%) CD34 negative cases.*

Conclusion: *The occurrence of CD34 positivity in T ALL is high in children coming to our centre from different parts of Kerala which is in contrast to western studies. Even though T ALL is treated under high risk protocol, CD34 positive cases need special attention and they should undergo molecular studies to detect the bad prognostic factor.*

Keywords: *T lymphoblastic leukaemia, CD 34,flow cytometry.*

Background and Rationale

T lymphoblastic leukaemia (T ALL) is a neoplasm of lymphoblasts committed to the T cell lineage, typically composed of small to medium-sized blast cells with scanty cytoplasm, moderately condensed to dispersed chromatin and inconspicuous nucleoli, involving bone marrow and blood. Among ALL, until recently TALL had

a poorer prognosis than BALL however the use of intense chemotherapy has led to a remarkable improvement in treatment outcomes[1] Immunophenotype has greater value in T-ALL diagnosis, classification as well as treatment. Flowcytometry provides access to find valuable immunologic markers for T-ALL biologic research [2]. According to Grotel et al CD34 is associated with poor survival in T-ALL[3] CD34 is a human stage specific haematopoetic differentiation antigen and is expressed in early undifferentiated haematopoetic stem cells both in lymphoid and myeloid lineage. Pui CH et al studied CD34 expression in acute lymphoblastic leukaemia in 1993 and showed opposed clinical associations in B and T lineage ALL reflecting fundamental biologic differences between these leukemic species[4]. Among B lineage cases expression of CD34 antigen was significantly associated with several favourable presenting features where as it was associated with unfavourable prognosis in T-ALL. There are only a few studies about immunophenotypic data in T-ALL[5-11]. Hence a study was planned to assess the immunophenotypic pattern of T-ALL and its aggressiveness in CD34 positive cases during the treatment period among paediatric population reported in the Regional Cancer Centre, Thiruvananthapuram.

Objectives

1. To study the immunophenotypic pattern of T ALL in paediatric population in our centre
2. To asses the aggressiveness of CD34 positive paediatric T ALL during their treatment period

Materials and Methods

A total of 271 paediatric (under 14 years of age) patients with acute lymphoblastic leukaemia (ALL)who had undergone flow cytometry for diagnosis from June 2009 to June 2011 were included in the study. Partially treated for ALL, cases in which blasts show positivity for myeloperoxidase stain were excluded. The immunophenotypic data was analysed with age, gender and various clinicopathological factors such as total count at the time of presentation, organomegaly, lymphadenopathy, mediastinal-mass, CNS/Testis infiltration, the nuclear and cytoplasmic features of blasts. The cases diagnosed as T -ALL were followed up for two and a half years till they completed treatment. The peripheral blood or bone marrow smears were stained with Giemsa and myeloperoxidase stains (by modified benzidine method) for morphological evaluation. Those cases which were found to be negative for peroxidase were subjected to flow cytometry.

Immunophenotyping by flow cytometry: 2ml peripheral blood or bonemarrow aspirate was sent in ethylenedi amine tetra acetic acid and was processed for flow cytometry. The cells were prepared by whole blood stain, lyse and wash technique. Six parameter, four-colour immuno-phenotyping was performed using a FACSC alibur (Becton Dickinson, San Jose, Ca, USA). A minimum of 10,000 events were acquired using side scatter versus forward scatter gating. Data were analysed with Cell Questpro software (Becton Dickinson). Fluorochromes used were fluoresceinisothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PERCPr) and allophycocyanin (APC). A panel of directly conjugated monoclonal antibodies comprising of CD3(APC-SK7), CD7(FITC-4H9), CD5 (PE-LI7F12), CD19(PERCPr-4G7), CD20 (APC-.27),CD34(PE-8G12),CD45(PERCPr-2D1), HLADR (APC-L243), CD10 (FITC-H110a), CD117 (APC-0402), CD13(PE-L138), CD33 (FITC-P67.6), CD64(FITC-10.1) were employed. In cases where we couldn't reach a diagnosis with the primary panel of antibodies we opted for secondary panel of antibodies which included cytoplamic CD3 (cCD3), CD22,CD79a and cytoplasmic MPO. An antigen was considered positively expressed when at least 20% of the gated cells expressed that antigen. Based on the analysis the cases were grouped into different immunophenotypes and clinicopathological factors were correlated. Data were analysed using

the software SPSS-version11. The association of each immunophenotype with age, gender, pathological and clinical features were studied using Pearson's Chi square test. P-value of <0.05 was considered as statistically significant. If the expected count was less than five in any of the cells in the cross tabulation, Fisher's exact test was used. The study was approved by Institutional Review Board.

Observation and Results

Out of 271 cases, 232 cases (85.6%) were BALL and 39 cases (14.4%) TALL. Among the 39 cases of TALL,25 cases were positive for CD34(64%). Rest of the 14 cases(35.8%) were negative for CD34.Among the clinical features, high total count (>1,00,000cells/mm^3)(p-value: 0.0001), lymphadenopathy (p-value: 0.001), hepatosplen-omegaly (p-value: 0.018) and presence of mediastinal mass(p-value: 0.0001) were found to be stastically significant for the CD34 positive group. The only pathological factor found to be significant was the presence of irregular cleaved nuclei(p-value: 0.0001).

On follow up,only 12 cases of 25 CD34 positive TALL completed treatment(48%),compared tq 10/14 (71%) CD34 negative TALL cases.Out of 7 cases expired,5 were CD34 positive and 2 were negative.4out of 25 CD34 positive cases relapsed while only one CD34 negative case had relapsed after remission. Two CD34 positive and one CD34 negative cases discontinued treatment due to persistence of the disease. Two CD 34 positive cases were lost to follow up.

Table 1. Summary statistics of T ALL(n =39)

	CD 34 positive (n=25)	CD34 negative (n=14)
Completed treatment	12(48%)	10(71%)
Expired	5	2
Relapsed	4	1
Discontinued treatment due to persistence of the disease	2	1
Lost to follow up	2	nil

Discussion

In the present study, we observed that the occurrence of CD34 positivity in T ALL is high in children, which is in contrast to western studies. T-ALL are aggressive haematologic tumors resulting from the malignant transformation of T cell progenitors.T-ALL accounts for 10-15% of paediatric and 25% of adult ALL cases[(1)]. Although originally associated with high relapse rates,the prognosis of TALL has gradually improved with the introduction of intensified chemotherapy with cure rates in modern protocols reaching over 75% in children[(12)]. However the outcome of TALL patients with primary resistant or relapsed leukemia remains poor[(13,14)].

Immunophenotyping of abnormal haematopoetic cells is very useful for the diagnosis, classification, cost effective treatment and prognostic evaluation in patients with hematological malignancies.So flow cytometry and cytogenetic studies have now become important tools for the diagnosis and classification of acute leukemias according to WHO[(1)]. In general in the west, the predominant immunophe-notype observed in ALL was B-ALL accounting for 60-70% of total cases where as T-ALL correspond to only 15-20%[(15)]. Majority of the study groups have stratified patients with T-ALL into a separate risk group for therapy and/or prognostication. Although this provides an excellent opportunity for evaluating unique contributions of prognostic factors within each immunophenotype group, direct comparison of outcome between T and B lineage becomes difficult because of the different treatment regimen used. Furthermore, there is paucity of data addressing clinical features, prognostic parameters and outcome of T ALL in developing countries like India, where this ALL subtype is more frequently observed than in the developed nations[(16,17)]. Disease free survival and event free survival were higher in B ALL in adolescent patients as compared to T ALL who had significantly low survival rates[(18)]. Hence even though the present study showed the predominant type to be B ALL (85.6%) we concentrated on T

ALL which constituted only 14.4%.CD34 is a human stage specific haematopoetic differentiation antigen and is expressed in early undifferentiated haematopoetic stem cells,both in lymphoid and myeloid lineage.In leukemic cells it remains to be expressed over several stages of lymphoid and myeloid maturation.The incidence of CD34 positvity is low in T ALL worldwide[7-11] (table.2) which is in contrast to the present study. In our centre CD34 positive cases (25/39) (64%) predominate over CD34 negative cases (14/39) (35.8%).This high incidence might be due to the fact that being a lymphoid neoplasm variations can exist in the prevalence of subtypes of ALL in relation to geographic, environmental, socio-ecnomic, ethnic and racial factors.We have reported high incidence of Adult T cell lymphoma/leukemia(ATLL) among mature T cell neoplasms due to HTLV virus in our adult patients earlier[19]. Hence the role of environmental or geographic factors involved in the specific pattern of T cell neoplasms in Kerala has to be considered.Future research has to be done to find out the factors involved in the high incidence of CD34 positive cases

Table 2: Summary of literature on CD 34 expression

Study	Ref	CD 34 expression in T ALL
Xiang Hao et al	(7)	17%
Tong H et al	(8)	31.3%
Foa R et al	(9)	20-30%
Kaleem Z et al	(10)	4.8%
Tiensiwakul et al	(11)	7.8%
Our study		64%

The clinical significance of CD34 has been debated and its prognostic value depends on the type of leukemia and the protocol used.The observation that CD34 expression is associated with poor survival is concordant with the present study . Our CD34 positive cases presented with organomegaly and lymphadenopathy.They presented with heavy tumor burden as evidenced by high total WBC count.Relapse rate(3/5) and mortality rate (5/7) were also more among CD34 positive cases.73%(10/14)CD34 negative cases completed the treatment compared to 48% (12/25) CD34 positive cases.

TALL cells differ from normal thymocytes in the over expression of oncogenes that arise either from chromosomal translocations or via other mechanisms. In addition, signaling pathways that control the very first stages of thymocyte development (of note, the Notch and Wnt pathways) are involved in the development of T ALL in mice and humans when constitutively expressed. In particular, the activating mutations in the Notch pathways are believed to occur in a large proportion of human TALL. These findings on genetic events may open up new therapeutic possibilities[20].CD34 positivity associated with poor survival is not explained by the mRNA expression levels of multidrug resistant genes[21].Gene expression profiling studies have identified several gene expression signatures,some of which correspond to specific stages of thymocyte development[22].LYL1+ signature corresponds to pro-T stage,HOX11+ to early cortical stage, and TAL1+ to late cortical thymocyte stage[1,23]. Pro-T stage and Pre-T stage can be CD34 positive and cortical/medullary T stages are CD34 negative.Hox11+ was associated with favourable outcome due to down regulation of antiapoptotic genes whereas TAL1+ and LYL1+ are associated with poor prognosis, may be due to up regulation of antiapoptotic genes that confer resistance to chemotherapy[1,23].Whether CD34 positive cases express LYL1+ signature is yet to be proved. Recent genetic studies have shed new light on the biology of early T cell precursor acute lymphoblastic leukemia (ETP-ALL), a distinct disease entity associated with poor prognosis and defined by a characteristic immunophenotype and a gene expression signature indicative of a very early arrest in T cell development[24]. N eumann M et al has also identified this as a high risk subtype of T ALL expressing a high rate of FLT3 mutation in adults[25] Molecular studies in pediatric CD34 positve T ALL cases to detect similar mutation may help to predict the prognosis and modify treatment. Since molecular studies are expensive,

immunophenotyping by flow cytometry can help to select CD34 positive T ALL for such studies which is important in a developing country like India. In conclusion, the occurrence of CD34 positivity inT-ALL in our study is high and they carry a poor prognosis.CD34 positive cases should undergo molecular studies to find out the poor prognostic factor and they should be given special intensified therapy during the course of the disease.

Acknowledgement

We thankfully acknowledge the help of Dr. Aleyamma Mathew, Professor and Head, Department of Cancer Epidemiology and Biostatistics, Regional Cancer centre, Thiruvananthapuram, Kerala, India for statistical analysis of data and Mr.Jiju, LabTechnician, Department of Pathology, Regional Cancer centre, Thiruvananthapuram, Kerala,India for his technical assistance in doing flow cytometry.

Conflict of Interest: NIL

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