ORIGINAL ARTICLE

FREQUENCY OF CD34 EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKAEMIA AND ITS CORRELATION WITH CLINICOPATHOLOGICAL CHARACTERISTICS: A SINGLE CENTRE EXPERIENCE FROM PAKISTAN

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Background: This study was carried out to determine the frequency of CD34 positivity in acute lymphoblastic leukaemia (B-ALL) in our population and to report its association with the clinicopathological profile at the time of diagnosis. **Methods:** The cross-sectional study was conducted at National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan, from March 2020 till December 2020.Newly diagnosed patients were selected, from both genders and all age groups. Relevant history and findings of physical examination were recorded. Immunohistochemistry was done on trephine biopsy and molecular studies were carried on bone marrow aspirates or peripheral blood samples. **Results:** Out of 105 patients enrolled, 67 (63.8%) were males, with a male to female ratio (M: F) 1.8:1. Of the total patients, 62 (59.04%) were above 15 years of age. CD34 was expressed in 73 (69.5%) cases. Lymphadenopathy, splenomegaly, and hepatomegaly were separately noted in context to CD 34 expression in 22 (66.6%), 24 (64.8%), and 14 (58.3%) patients, respectively. CNS disease was seen in a total of 3(2.75%) subjects, in which 2 (66.6%) of the patients had CD34 expression. Total 81 patients in our study fall into the high-risk group out of which CD 34 expression was seen in 58(71.6%) subjects. Cytogenetic analysis, BCR-ABL p190, and MLL gene rearrangement were investigated in all participants. Cytogenetic analysis revealed an abnormality in 20 (19%) cases out of which 13 (17.8%) cases were from CD34 positive group. **Conclusion:** Our study reported CD34 expression in more than two-thirds of cases. High-risk disease was significantly associated with CD34 expression.

Keywords: Acute lymphoblastic leukaemia; Cytogenetics; CD34 expression; BCR-ABL; Hyperdiploidy

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INTRODUCTION

Acute lymphoblastic leukaemia is a neoplasm of hematopoietic precursors in which there is clonal proliferation, accumulation, and infiltration of tissues by neoplastic cells. It has two peaks; one at the age of 1–5 years and the second peak at the age ≥50 years.[1] The incidence of acute lymphoblastic leukaemia increases steadily with increasing age.[2] A variety of surface differentiation antigens are found on leukemic cells, which are also present on normal lymphocyte precursors. Because of this, it was thought that ALL cells originate from the normal lymphoid precursors and are arrested at earlier stages of development into either B or T-Cell lineage.[3]

Hematopoietic stem cell surface protein cluster of differentiation 34 is normally expressed in the very early stage of development among all the cell lineages including myeloid and lymphoid pathways. However, when abnormal blasts are present, they tend to keep expressing this antigen in later developmental stages of cell maturation.[4] In adult ALL CD34 expression is associated with poor prognosis, as compared to paediatric ALL. Clinical significance and disease prognosis are dependent on CD34 expression among ALL. Therefore, the need to evaluate CD34 presence at the time of diagnosis has crucial clinical value. The common antigen of acute lymphoblastic leukaemia that is CD10 positive B-Cell ALL expresses CD34 positivity in almost two-thirds of cases of B-ALL. On the contrary precursor B cell ALL which are in the very early stage of development, and are CD10 negative, are usually CD34 negative.[5] It is a common observation that markers of immaturity like CD34 are commonly found in AML rather than ALL and TdT is commonly found in ALL. In acute myeloid

leukaemia one of the poor prognostic factors is CD34 positivity. In paediatric ALL, CD34 is correlated with favourable outcome but in adults, CD34 is linked with inferior prognosis.[6] These differences between childhood and adult ALL regarding the clinical impact of CD34 expression might be related to particular genetic markers of good prognosis such as t (12; 21), hyperdiploidy in children, and of bad prognosis t (9; 22) in adults.[7] Usually translocation 1;19 is found to be associated with CD34 negative ALL. CD34 positive ALL frequently have translocation 9;22 and 4;11.[8] In this study, we intended to identify and associate the expression of CD34 in ALL with immunohistochemistry, cytogenetic and molecular findings in our population.

MATERIAL AND METHODS

After the approval from the Ethical Committee of NIBD, this study was conducted. All acute lymphoblastic leukaemia cases diagnosed at National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan, between March till December 2020, were enrolled. Relevant history and findings of physical examination were recorded on a Performa after taking informed and written consent from patients. The sample size of 105 was calculated by using Raosoft with 12% prevalence[6], 6% margin of error and 94% Confidence interval.

Samples of bone marrow aspirate and trephine biopsy was collected from the posterior superior iliac spine of the patient, films of aspirate were stained with Leishman and Myeloperoxidase stains for morphology and cytochemistry. While the trephine biopsies were fixed by using 10% neutral buffered formalin. Decalcification was carried out by EDTA solution for 24 hours. About 4–5 µm thick sections were cut and stained with H&E. Immunohistochemistry was employed as per the standard procedure.[9] CD45, markers of immaturity (CD34, TdT, CD117), lineage specific marker for B-cell (CD19, CD20, CD79a, PAX-5), T-cell (CD3, CD4, CD7, CD99), and cytoplasmic MPO for myeloid lineage were applied.

For the conventional chromosomal detection bone marrow cells were cultured for 24 hours. Chromosomes were examined at the metaphase stage, as at this stage they were most condensed and visible. To arrest the cells from succeeding to the anaphase stage, the first step is to incubate the chromosomes with Colcemid, which results in the disruption of the spindle fibers. In swollen form the cells were preserved with Carnoy's fixative once treated with a hypotonic solution. The cells were then dropped onto slides and with G-banding technique karyotype was analyzed. According to International Human chromosomes Nomenclature each karyotype was named.[10]

For the BCR-ABL and MLL gene rearrangement molecular test was done, in which RNA was first extracted by LSM (Lymphocyte separation medium) method from either Bone Marrow or peripheral blood and converted to complementary DNA using reverse transcriptase-PCR(RT-PCR). Abnormal genetic changes are amplified from cDNA using a specific primer in a multiplex Polymerase Chain Reaction (PCR) reaction. The DNA products are then visualized on agarose gel after electrophoresis.

All patients were risk-stratified into two groups that are high risk and low/ standard risk, based on Age (≤11 years), TLC (count ≤50x 10^9), Cytogenetic hyperdiploidy, and no CNS disease fall into a standard-risk group and those with Age (≥11 years), TLC count (≥50x 10^9), Cytogenetic abnormality t (9;22), BCR-ABL and CNS involvement were stratified into a high-risk group. In all the patient's lymphadenopathy, hepatosplenomegaly, and CNS involvement, TLC, Platelet, and Haemoglobin count, Ph' chromosome incidence was evaluated.[11]

All results were analyzed and calculated using the SPSS-23. Our data was not normally distributed therefore median and IQR was calculated. Chi-Square test and Mann-Whitney test were applied to see the association with qualitative variables (gender and organomegaly). *p*-value ≤0.05 was taken as significant.

RESULTS

One hundred and five patients with Acute Lymphoblastic Leukaemia (ALL), diagnosed by immunohistochemistry were included in this study. Sixty-seven (63.8%) patients were males, with a male to female ratio (M: F) 1.8:1. CD34 was expressed in 73 (69.5%) of the cases. Sixty-two (59.04%) of the total 105 were above 15 years of age and 43 (40.9%) were below 15 years of age. In the adult group out of 62 patients, 46 (74%) and in the paediatric group, out of 43 patients, 31 (72%) had CD34 positivity on IHC respectively. Lymphadenopathy, splenomegaly, and hepatomegaly were separately noted in context to CD 34 expression in 22 (66.6%), 24 (64.8%), and 14 (58.3%) patients, respectively. CNS disease was seen in a total of 3 (2.9%) subjects, in which 2 (66.6%) of the patients had CD34 expression. The demographical and clinic-pathological parameters observed between CD34 positive and CD34 negative ALL groups were analyzed, however, no

statistically significant difference was found between these two groups. Total 81 patients in our study fall into the high-risk group out of which 58 (71.6%) patients were positive for CD34 and 23 (28.3%) were negative for CD34. Table-1 demonstrates the detailed characteristics of all ALL patients and Table-2 demonstrates Clinical and laboratory characteristics in $CD34^{+}$ and$CD34^{-}$ ALL patients. Cytogenetic analysis of 105 patients revealed hyperdiploidy in 5 (4.7%) cases, t (9;22) in 5 (6.8%) cases from $CD34^{+}$ and 5 (15.6%) cases from $CD34^{-}$ groups, and other abnormalities like trisomy 8, t (8;9), t (7;14), i (9), dicentric (7;9) in 1(0.9%) case respectively; these all were CD34 positive (Figure-1).

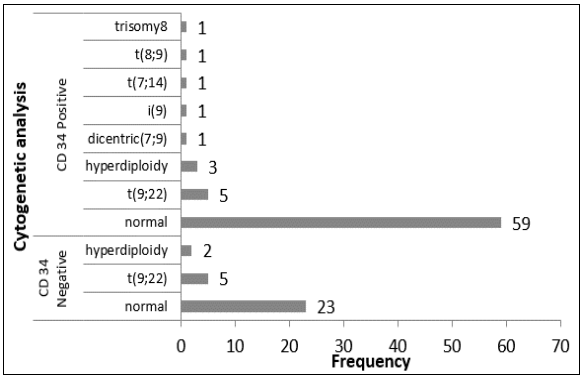

Figure-1: Cytogenetic analysis (N=102)

Note: 3 Cases were not included because of culture failure.

Table-1: Detailed characteristics of all ALL patients.

Characteristics	
Female, N (%)	38 (36.2)
Age in years, median (IQR)	17 (27-10.5)
Hemogram	
Hemoglobin g/dl, median (IQR)	8.7 (10.1-7.4)
Total Leucocyte count $x10^9$, median (IQR)	7.1 (25.6-2.3)
Platelet count $x10^9$, median (IQR)	37 (70.5-15.5)
Absolute Neutrophil count $x10^9$, median (IQR)	0.7 (2.5-.14)
Systemic Involvement	
Liver	
Enlarged	24 (22.9)
Not enlarged	81 (77.1)
Spleen	
Enlarged	37 (35.2)
Not enlarged	68 (64.8)
Lymph Nodes	
Axillary	01 (1)
Cervical	22 (21)
cervical, inguinal	2 (1.9)
Generalized	8 (7.6)
Absent	72 (68.6)
CNS Involvement	
Absent	102 (97.1)
Present	3 (2.9)
Markers	
CD 10	
Negative	7 (6.7)
Positive	98 (93.3)
CD 34	
Negative	32 (30.5)
Positive	73 (69.5)
BRC-ABL	
Negative	95 (90.4)
Positive	10 (9.5)
Risk Stratification	
High	81 (77.1)
Low	24 (22.9)

Table-2: Clinical and Laboratory Characteristics in $CD34^{+}$ and $CD34^{-}$ ALL.

Variables	CD34		
	Negative	**Positive**	***p*-value**
Gender			
Females	11	27	0.798
Males	21	46	
Median Age, years	2	2	0.416*
Median Hemoglobin, g/dl	8.3	8.9	0.182*
Median TLC, $x10^9$	4.77	7.3	0.211*
Median Platelet count, $x10^9$	23.5	39	0.222*
Liver			
Enlarged	10	14	0.175
Not enlarged	22	59	
Spleen			
Enlarged	13	24	0.444
Not enlarged	19	49	
Lymph nodes			
Present	11	22	0.667
Absent	21	51	
CNS Involvement			
Absent	31	71	0.668
Present	1	2	
Cytogenetic			
Normal	23	59	0.541
Abnormal	7	13	
BCR-ABL			
Negative	29	68	0.45
Positive	5	5	
Risk Stratification			
High	23	58	0.395
Low	9	15	

**p*-value was computed by applying Mann -Whitney test

DISCUSSION

In acute lymphoblastic leukaemia (ALL) the role of CD 34 was more clearly defined than in AML although considerable changes had occurred over the last few years; in the early 90's it was thought that CD34 was a marker of all precursor cells from the different hematopoietic lineages as well as a feature of acute leukaemia as these represent neoplasms of immature precursors.[12]

The number of adult patients in our study was higher, i.e., 62(59.04%)[13], there is male preponderance, with 63.8% males[14] which is comparable with another study. There is a significant difference between several prognostic factors among childhood and adult ALL. We found expression of CD34 in 73 (69.5%) patients, which is similar to the work done in the past, in which it was reported in 74–83% of cases.[15] Majority of these cases were from the high-risk group rather than standard risk which is opposite to the findings of another study.[16] In children, there were series of favourable prognostic factors like age, total leucocyte count, and less organ involvement. The total leucocyte count in our CD34+ and CD34- paediatric ALL was not greater than 50×10^9/L, which is one of the favourable risk factor in our study which is comparable to the findings of another study, in which TLC count in CD34 negative group was above 50×10^9/L.[17] CNS involvement was seen in a total of three patients (2.8%) and two of them were from paediatric CD34+ ALL group, which is similar to the findings of Cascavilla *et al*.[17] In our set of data, the incidence of lymphadenopathy, hepatosplenomegaly, CNS involvement was observed more in those cases expressing CD34.

One of the strong negative prognostic factors in ALL is Philadelphia chromosome and or the presence of the BCR-ABL fusion gene, in literature incidence of translocation 9;22 was about 3–5% in children and 20–30% in adults with ALL and is associated with a very poor prognosis.[18] In our study, BCR-ABL p190 and t (9;22) were detected in 10 (9.5%) patients out of which$CD34^+$cases were five and four of them were adults. In B-ALL the dominant poor prognostic factor is CD10 negativity which is often correlated with MLL rearrangements and is commonly seen in infants and older adults. In our cohort, no patient in either CD34 positive or CD34 negative ALL group demonstrated MLL rearrangements. In the study seven (6.7%) patients were negative to CD 10 and 98 (93.3%) patients demonstrated Common acute lymphoblastic leukaemia antigen (CD10) which is similar to the findings of another study.[19] The co-expression of CD10 and CD34 was seen in 66 (62.8%) patients which is almost similar to the other study in which it was expressed in 70% of cases.[5]

More than two-thirds of our acute leukaemia patients expressed CD34; the majority of these were from high-risk strata. In literature, this hematopoietic stem cell marker is found to be associated with severe and high-risk diseases in adults. Since we didn't find any statistically significant difference between CD34 positive and CD34 negative group in context with various parameters therefore we cannot predict the clinical course and/or prognosis of leukaemia just based on this one marker. Limitations: Like other epidemiological studies, our study is not without limitations. The limitations of our study are 1) Small sample size. 2) It is a cross-sectional study and therefore no follow-up is done in terms of patient outcome post-treatment. 3) T-cell ALL were not included in this study and 4) it's not a novel study.

CONCLUSION

Majority of the patients in our study were greater than 15 years of age, however, expression of CD34 was not associated with age, and was present in both the age groups. Patients having CD34 positivity, also has more splenomegaly, hepatomegaly and lymphadenopathy as compared to those who were negative for CD34. Association of CNS disease, total leucocyte count, platelet count and cytogenetic and molecular abnormalities was not found to be statistically significant. However, in our study, high risk patients were found to be more CD34 positive than negative which can predicts the poorer outcome. In future, studies with larger cohorts of patients will better define the clinicopathological parameters with CD34 positivity in acute lymphoblastic leukaemia.

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AUTHOR'S CONTRIBUTION

ZAB: Conceived and designed the work. Designed questionnaire. Collected and analyzed the data authored or reviewed drafts of the paper, and approved the final draft. JH: Conceived and designed the work, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft. SW: Conceived and designed the work, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft. MI: Conceived and designed the work, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft. NF: Analyzed the data, prepared the tables and figure. SZ: Analyzed the data, prepared the tables and figure. SS: Conceived and designed the work, analyzed the data, authored or reviewed drafts of the paper.

Tahir Shamsi: Conceived and designed the work, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

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