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Original Article

Diagnosis and Subtyping of Acute Lymphoblastic Leukemia by Using Immunohistochemistry in Bone Marrow Biopsy Specimens

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ABSTRACT

Background: Acute lymphoblastic Leukemia (ALL) is a hematological malignancy characterized by neoplastic proliferation and accumulation of lymphoid precursors known as blasts. It has two types; B cell ALL and T cell ALL, depending on type of lymphoblast involved. We used selective panel of immunohistochemical (IHC) markers to confirm the diagnosis and lineage of ALL.
Objective: This study was carried out to see the utility of immunohistochemistry (IHC) for diagnosis and lineage confirmation of acute lymphoblastic leukemia (ALL) with minimum selected panel of IHC markers and to know the pattern of B and T cell ALL among the patients referred to our department.
Methodology: It was a prospective descriptive study conducted at the pathology department of Pakistan Institute of Medical Sciences (P.I.M.S). Fifty-six suspected and untreated cases of ALL of both genders and all ages were included in the study. The panel of IHC markers used to confirm both B and T cell ALL were TdT, for B cell ALL CD10, CD20, CD 79a and for T cell ALL CD3 was used. The data was entered and analyzed in SPSS version 20.
Results: Out of total 56 patients of ALL, 43 (77%) patients had B cell ALL, 10 (18%) patients had T cell ALL. On IHC two (3%) patients had biphenotypic and 1 (2%) patient had Burkitt Leukemia. The age range was from 9 months to 69 years and peak age group was 6-10 years. Overall male to female ratio was 1.5:1. FAB type ALL-L2 was most common type in all age groups. There was male predominance in both B and T cell ALL, but this difference was more marked in T cell type with male to female ratio 4:1.CD 10, CD 20 and CD 79a are more helpful markers in B cell ALL. For T cell ALL CD 3 is the highly specific and sensitive marker. **Conclusion:** Based on our results, we came to this conclusion that IHC is a good and reliable method for immunophenotyping to diagnose and subtype ALL with limited resources and in conditions when paraffin embedded trephine biopsy is the only material available for diagnosis.
Key words: Acute lymphoblastic leukemia (ALL), Immunohistochemistry (IHC).

Introduction

Acute Lymphoblastic Leukemia (ALL) is a hematological malignancy caused by malignant transformation of lymphoid precursors which divide and produce clones of abnormal cells known as blasts. There are two major types of ALL, B cell ALL and T cell ALL depending on type of lymphoblast involved.[1,2,3]
ALL can occur at any age but it is the most common leukemia of childhood.[4] The disease shows two age peaks; 2 - 3 years and then after 40 years of age.

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It is more common in males than in females. In children B cell ALL is more common i.e 85% and T cell ALL is 15%. Incidence of T cell ALL is relatively higher in adults i.e 25%.[1,4] In Pakistan there is no proper cancer registration system thus limited data is available about frequency and incidence of the ALL.[5]
Clinical features of ALL are mostly related to bone marrow failure due to infiltration by blast cells and are similar in both children and adults [6,7].
ALL has been categorized by three main classifications, namely; French-American-British (FAB) group Classification, Proposed World Health Organization Classification of Acute Leukemia, European Group for the Immunological Classification of Leukemia (EGIL).[8]
The French-American-British (FAB) group classifies ALL in three subtypes L1, L2 and L3 on the basis of

morphology of the blast cells. The important morphological features considered were cell size with uniformity of blast population, cytoplasm, presence or absence of nucleoli.[9,10] The European Group for the Immunological Classification of Leukemia (EGIL) classifies acute leukemia into myeloid, lymphoid (B lineage, T lineage) or biphenotypic on the basis of immunophenotyping alone.[8] The WHO classification of lymphoid and myeloid neoplasm uses morphology and cytochemistry in addition to Immunophenotypes, cytogenetics, molecular genetics and clinical features for classification into various subtypes.[11]

Diagnostic work up for ALL includes blood counts, peripheral blood film and bone marrow examination along with morphology, cytochemistry and immunophenotyping. Cytogenetics and molecular genetics (mostly PCR-based techniques and sequencing) also play an important role in diagnosis, classification and detection of minimal residual disease.[6, 12,13] Bone marrow aspiration is recommended in all patients. It is important for blasts count, morphology and cytochemistry. Trephine biopsy is recommended in situations like dry tap, diluted aspirate, or for diagnostic procedures like immunohistochemistry[1].

Immunophenotyping is used to confirm the diagnosis, lineage, classification and minimal residual disease. In Immunophenotyping, cytoplasmic and nuclear antigens are identified by specific antibodies known as cluster of differentiation (CD) markers. These antigens are developed and expressed during different stages of lymphocyte development. On the basis of these specific antigens ALL is further categorized into two broader subgroups, B and T cell ALL. [14,15] CD markers which are commonly used for diagnosis of ALL are; non-lineage specific markers including TdT, CD 34 , B cell markers including CD10, CD19, CD20, CD22, CD79a, CD45, PAX–5, sIg and T cell markers including CD2, CD3, CD5, CD7, CD45, either CD4 or CD8 [16].

The importance of confirmation of B and T Immunophenotypes is increased in past few years because the treatment of ALL is now modified according to different prognostic factors. B and T cell immunophenotypes are included in favorable and unfavorable prognostic factors which help the oncologist to decide the treatment protocol. For immunophenotyping most laboratories prefer flowcytometry because it gives rapid analysis, highly specific antigens with good preservation.[15,16] Although IHC is an important diagnostic technique in the field of pathology for identification and classification of different tumors but it was not used commonly in diagnosis of acute leukemia. However, in the past few years; great improvement in this technique and introduction of new lineage-specific antibodies made it the preferable choice for the diagnosis of acute leukemia specially ALL in under resourced laboratories. [17-20] In this study we assessed role of IHC in confirmation of diagnosis and detection of B and T cell lineages of ALL with minimal selected panel of IHC markers.

Material and Methods

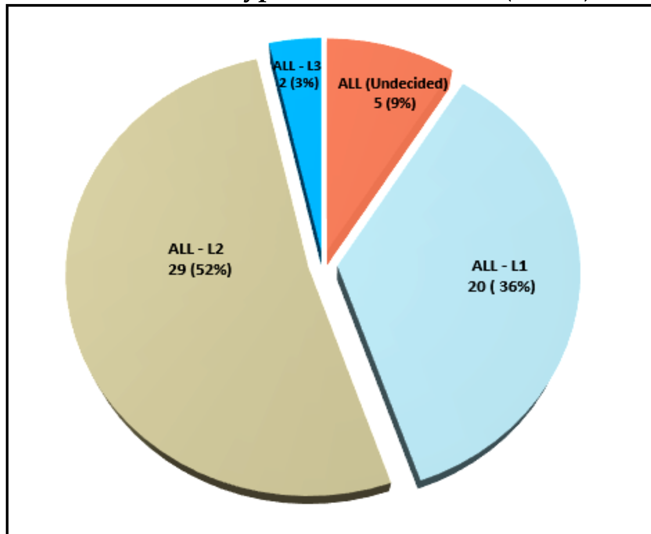
It was a prospective descriptive study conducted in Department of Pathology, Pakistan Institute of Medical Sciences (PIMS) Islamabad. Duration of study was 18 months. Suspected cases of ALL of both genders and all ages were included in the study. These patients were referred to hematology department for bone marrow biopsy. Patients who already received treatment or who were in relapse were excluded from the study. Approval from hospital ethics committee was taken. Bone marrow biopsy was done after taking verbal consent. Results were entered on SPSS version 20. Descriptive statistics were calculated including percentage, mean and ±SD.

Complete blood counts, Peripheral blood film and Reticulocyte count was done in each case. Bone marrow biopsy (Aspiration and Trephine) was done and stained by Wright stain. Bone Marrow aspirate smear was also stained for Iron using Pearl's stain and subjected to special stains like PAS (Periodic acid Schiff), SBB (Sudan black B) and ACP (Acid phosphatase) as per requirement. Bone marrow aspirate was assessed for cellularity and cell lineages. Blast count with morphology and FAB typing was also done. IHC was done on trephine biopsy specimen or bone marrow clot depending upon the quality and quantity of tissue showing infiltration. Results were interpreted under the light microscope along with appropriate staining of negative and positive controls. The result of IHC markers was noted according to British Committee for Standards in Hematology. Out of 200 cells differential count if more than 10% blasts cells were positively stained, then it was taken as positive result for that particular marker. The IHC markers used for B and T cell ALL were TdT, (for B cell ALL) CD10, CD20, CD 79a & (for T cell ALL) CD3. Ki-67 was applied only in those cases that had ALL – L3 morphology. The antibodies used, were of Leica Company (Germany).

Results

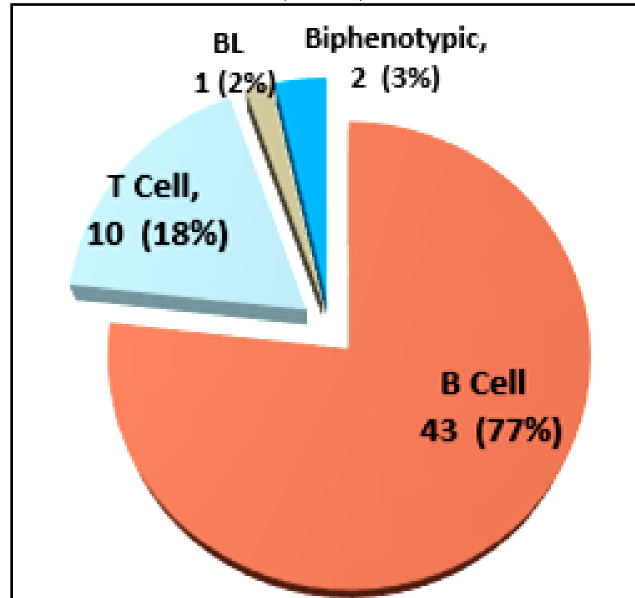
A total of 56 suspected cases of ALL were included in our study. The minimum age of presentation was nine months and maximum age was 69 years while mean age of presentation was 12.5 years. More patients were observed in age group ≤ 15 years (n=43, 76.79%) as compared to age group > 15 years (n=13, 23.21%). Child to adult ratio in our study was 3.3:1. There were more male patients than female in all age groups. Out of total 56 patients there were 34 (60.71%) males and 22 (39.29%) females with over all male to female ratio1.5:1. In both children and adults; FAB type ALL-L2 was most common type. Patients belonged to FAB type ALL L2 were 29 (52%). Patients belonged to FAB type ALL-L1 were 20 (36%) and only two (3%) were FAB type ALL-L3 while Five (9%) cases remained undecided on morphology [Chart 1]. Majority of the patients (n=43, 77%) had B cell ALL while only 10 (18%) patients had T cell ALL [Chart 2]. It was also observed that there was male predominance in both B and T cell ALL, but this difference was more marked in T cell type with male to female ratio 4:1.

Chart 1: FAB Types in ALL Patients (n = 56)



In total 43 cases of B cell ALL, TdT was positive in 34 (79%) cases, CD 10 in 37 (86%) cases, CD 20 in 11 (26%) cases, CD 79a in 21 (49 %) cases and CD 3 was not positive in any case of B cell ALL. In total 10 cases of T cell ALL, TdT was positive in five (50%) cases, CD 3 was positive in all 10 (100%) cases. Rest of all markers were negative in all 10 cases of T cell ALL. Two cases in our study were taken as biphenotypic. TdT, CD 10, CD 79a and CD 3 were positive in one case, while TdT, CD 10 and CD 3 were positive in other case [Table 1]. Ki 67 was applied in two cases which were FAB type ALL-L3 on morphology. In one case, Ki 67 was strongly positive in 100% cells while the other case showed weak positivity.

Chart 2: Pattern of Immunophenotypes in Patients (n = 56)



(BL. Burkitt Leukemia)

Out of total 20 cases of ALL-L1 FAB type, 16 (80%) patients were B cell ALL, three (15%) patients were T cell ALL and one (5%) was biphenotypic on IHC. Out of total 29 patients of ALL-L2 FAB type, 22 (76%) patients were B cell ALL, six (20%) patients were T cell ALL and one (5%) was biphenotypic on IHC. Out of total two patients of ALL-L3 FAB type, one (50%) patient was B cell ALL and one (50%) was Burkitt leukemia on IHC. Out of total five (9%) morphologically undecided patients of ALL, four (80%) patients were B cell ALL and one (20%) was T cell ALL on IHC [Table 2].

Table 1: Pattern of Positivity of IHC Markers (n=56)

IHC Markers	B cell (n=43)		T cell (n=10)		Biphenotypic (n=2)		BL (n=1)	
	n	%	n		n	%	n	%
TdT	34	79	5	50	2	100	0	0
CD 10	37	86	0	0	2	100	1	100
CD 20	11	26	0	0	0	0	1	100
CD 79a	21	49	0	0	1	50	0	0
CD 3	0	0.00	10	100	2	100	0	0

Table 2: Co-relation of FAB Subtypes with Immunophenotypes (n=56)

FAB Type	B cell ALL (43)		T cell ALL (10)		BL (1)		Biphenotypic (2)	
	n	%	n		n	%	n	%
ALL-L1 (n=20)	16	80	3	15	0	0	1	5
ALL-L2 (n=29)	22	76	6	20	0	0	1	4
ALL-L3 (n=2)	1	50	0	0	1	50	0	0
ALL(n=5) (FAB Type Undecided)	4	80	1	20	0	0	0	0

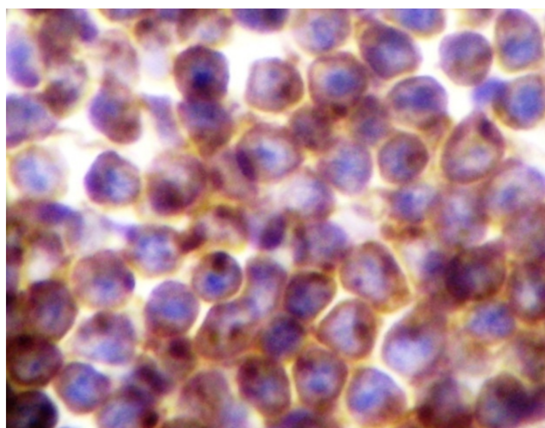


Figure 1: CD10 Positivity at 100X

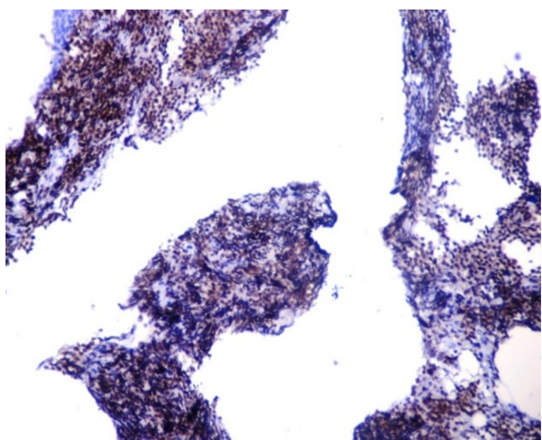


Figure 2: CD 3 Diffuse Positivity at 10X

Discussion

In our study it was observed that among 56 patients; majority of the patients (n=43, 77%) had B cell ALL while only 10 (18%) patients had T cell ALL. It was also observed that there was male predominance in both B and T cell ALL, but this difference was more marked in T cell type with male to female ratio 4:1. In addition two (3%) cases of biphenotypic and one (2%) of Burkitt Leukemia (FAB type ALL - L3) was also observed. A recent study conducted at CMH and Fatima Jinnah Medical College – Lahore, reported 72% patients with B-cell ALL and 28% patients with T-cell ALL in overall patients. [21] Study conducted in Italy included 5202 patients in nine age cohort, reported B-cell ALL in 85.8% patients and T-cell ALL in 14.2% patients.[22] Both these studies show similar result like our study with majority patients having B- cell ALL than T – cell ALL and male predominance. In our study it was observed that TdT was positive in 73% cases and was negative in 27% cases. Among TdT positive cases 79% cases were of B cell ALL and 50% cases were of T cell ALL. A study conducted in College of Medicine, King Khalid University Hospital and King Saud University Medical College - Saudi Arabia showed TdT positivity in 82% of total cases, 95% of B cell and 60% of T cell ALL. TdT was the most commonly positive marker in this study. The sensitivity of TdT in this study was higher than our study[19]. In our study, out of total 43 cases of B cell ALL, CD 10 was positive in 86% cases, CD 20 in 26% cases and CD 79a in 49% cases. None of these markers was positive in T cell ALL. A study conducted at King Hussein Medical Center, Amman – Jordan was reported the highest percentage of CD 10 + cases of pre - B cell ALL.[23] The study conducted in King Khalid University Hospital and King Saud University Medical College - Saudi Arabia reported CD 10 positivity in 65% cases of pre-B ALL and 33% cases of B cell ALL. This study reported CD 10 as second most sensitive B cell marker after TdT.[19] In our study among CD 79a and CD 20; CD 79a was more sensitive B cell marker as it was positive in more cases. CD 20 was equally specific B cell marker as CD 79a, but its sensitivity was comparatively low. It was also observed that CD 79a expression was comparative weaker and focal positive in few cases. In study from Saudi Arabia, CD 79a was positive in 68 % cases of pre-B and 66% cases of B cell ALL while CD 20 was positive in 22% cases of pre-B and 66% of B cell ALL. CD 79 a was positive in one case of T cell ALL while CD 20 was not positive in any T cell ALL case. Specificity of both markers was same but sensitivity of CD79a was more than CD20.[19,24] In a study conducted in Iowa-USA, CD 79a was positive in 85 % cases of B cell ALL while CD 20 was positive in only 6% cases of B cell ALL. This study also described CD 79a as more sensitive marker for B cell ALL than CD 20[25]. In our study out of total 10 cases of T cell ALL, CD 3 was positive in all 10 (100%) cases. CD 3 was observed the

highly sensitive and specific T cell marker. It gives strong expression in most case. In study from Saudi Arabia CD 3 was positive in 70% cases of T cell ALL and was observed as highly sensitive and specific T cell marker[19]. The difference in few results of IHC markers sensitivity with other studies is possibly due to difference in type of antibodies used, difference in processing of tissue and the type of fixative and decalcification material used. In our study two cases showed both B and T cell markers positivity. Out of these two cases, one case showed CD 10 and CD 3 positivity while the other one showed CD 79a and CD3 positivity. They were labelled as biphenotypic. Detailed scoring was not done and additional markers were not applied in these two cases in present study due to limitation of resources. Another study is being carried out in the department to evaluate such cases further. A study conducted in The Aga Khan University Hospital reported biphenotypic ALL in 0.6% patients.[26] In a study conducted in United Kingdom it was observed that 5% patients had biphenotypic ALL.[27] In our study two cases had ALL-L3 morphology and B cell markers positivity on IHC. Ki 67 was applied in both cases. One case showed strong positivity of Ki 67 in 100% blast cells; it was labeled as Burkitt Leukemia (FAB type ALL - L3) on the bases of morphology and IHC; while the other case considered as B cell ALL. Burkitt Leukemia (FAB type ALL - L3) is highly aggressive and gives nearly 100% positivity (at least 95% of tumor cells), on Ki-67 due to the short doubling time of tumor cells. A case report of precursor B cell ALL was presented from Florida – USA in which the blasts showed L3 morphology (Burkitt-like lymphoma), had positivity for TdT, CD10, CD20, MUM-1, PAX5, and BCL 2 but showed weakly positive Ki-67.[28] Another case was reported from China on T-cell ALL which resembled FAB type ALL - L3 on cell morphology. In this case they reported blast cells having typical L3 morphology but on immunophenotyping expressed T cell and AML markers positivity.[29]

Using IHC with above mentioned panel of antibodies and in background of morphological diagnosis we are sure that IHC is an equally good technique for diagnosis and subtyping of ALL in limited resourced laboratories. IHC performed on paraffin embedded tissues shows good results due to improvement in antigen retrieval technique, introduction of automated staining which shortens the time period of staining with improved quality, digital analysis of slides and introduction of new antibodies which are more lineage-specific are the main factors which made the IHC procedure more helpful in research and diagnostic fields of pathology.[18,20]

If we compare IHC from flow cytometry, we will find that IHC is much more economical and more suitable than flowcytometry for laboratories having lesser resources. In addition to this, IHC is also good for morphologic and retrospective analysis with better preservation of antigens. The cytoplasmic and nuclear markers can be more easily detected by IHC as compared to flowcytometry, because it requires membrane permeability.[19] In case of ALL IHC is not only important for diagnostic purpose but it also effects on treatment outcome and overall survival of ALL patients[1, 30]

Conclusion

Based on our results, we came to this conclusion that IHC is a good and comparatively reliable method for immunophenotyping to diagnose and subtype ALL with limited resources and in conditions when paraffin embedded trephine biopsy is the only material available for diagnosis. We suggest the minimal selected panel of IHC markers for B cell ALL is CD 10, CD 20 and CD 79a. CD 10 and CD 79a are more helpful markers in B cell ALL but CD 20 should also be included because it helps in diagnosis of mature B cell ALL cases. For T cell ALL CD 3 is the highly specific and sensitive marker. TdT helps in differentiation of lymphoma and leukemia.

In this study we also conclude that in our set up ALL is more common in children than adults. It is more common in males than in females in all age groups. The peak incidence is in 6-10 years of age. B cell ALL is more common than T cell ALL. CD 10 + B cell ALL is the most common group found in our patients.

Limitations of Study: In our study, we used minimal selected panel of IHC markers. Further categorization of B and T cell ALL, needed extended panel of antibodies which we couldn’t use due to budget limitations. In resolving cases of biphenotypic and bilineage leukemia, IHC has limited value. Flowcytometry and PCR are more helpful in such cases. Detection of immunoglobulin light chains by IHC is difficult due to high background staining. In our study most patients belonged to pediatric age group because mostly patients were referred from Children Hospital, Oncology Department, PIMS. It is therefore suggested that separate studies should be carried out for pediatric and adult populations.

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