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DIAGNOSTIC UTILITY OF IMMUNOHISTO-CHEMISTRY IN SUBTYPING ACUTE LYMPHOBLASTIC LEUKEMIA: A 2 YEARS' EXPERIENCE

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ABSTRACT

OBJECTIVE: To determine the diagnostic importance of immunohistochemistry (IHC) in classifying acute lymphoblastic leukemia (ALL) in a tertiary care center.

METHODS: This cross-sectional descriptive study was done from January-2017 to December-2018 at Rehman Medical Institute, Peshawar, Pakistan. Out of 133 cases diagnosed as precursor lymphoid leukemia, two cases were excluded due to inadequacy of the aspirate smears and 131 cases were included in the study. The immune-phenotype was detected by IHC with immune-markers viz terminal deoxynucleotidyl transferase (TdT), CD34, CD10, CD79a, CD3, myeloperoxidase (MPO), CD117 and paired box protein 1 (PAX1).

RESULTS: Out of 131 cases included 99 (75%) were males and 32 (25%) were females. Mean age of the study participants was 20±16 years (range=16-65 years). Majority of the cases presented with hepatomegaly (n=113/131, 87%), followed by pallor (n=105/131, 80.1%), splenomegaly (n=89/131, 68%) and lymphadenopathy (n=82/131, 63%). Based on IHC, 114 (87.02%) cases were successfully classified to specific subtypes and 17 (13%) cases could not be assigned into any subtype. Eighty-six cases (65.7%) were of Pre-B cell ALL, 17 (13%) cases were T-cell ALL, 8 (6.1%) cases were Pre-T cell ALL while 3 (2.3%) cases were Pro-B lineage.

CONCLUSION: Study concludes that majority of the patients were male and presented with hepatomegaly and pallor. IHC is effective method to sub-classify ALL into various immune-phenotypes in low resource countries where flow cytometry is unavailable. Pre-B cell ALL is common than T-cell ALL.

KEY WORDS: Leukemia (MeSH); Precursor lymphoid leukemia (Non-MeSH); Precursor Cell Lymphoblastic Leukemia-Lymphoma (MeSH); Immunohistochemistry (MeSH); Immunophenotyping (MeSH); Lymphoblastic Leukemia, Acute (MeSH); Leukemia, B-Cell (MeSH); Leukemia, T-Cell (MeSH).

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignancy of the blood, in which there is excessive abnormal replication of the bone marrow white cell precursors.[1,2] Literature suggests different risk factors for the development of ALL, but still a specific cause has not be confirmed so far.[3] ALL had initially been classified as ALL-L1, ALL-L2 and ALL-L3 types by French–American–British group.[4] Then the World Health Organization (WHO) further classified it on the basis of immunophenotyping and genetics studies,[4] which is followed till now.

ALL is one of the most common blood malignancies all over the world.[2,3] The prevalence of ALL is increasing in both the developed and developing countries of the world.[5-8] ALL commonly happens in the children.[2,3] It is known to account for about 80% of all the leukemias in children.[2,3] In Pakistan, the ALL is found to be a common malignancy, especially in child age group.[2] The worldwide prevalence of ALL is reported to be about 3 cases in 100,000 population.[2]

Examination of the bone marrow aspirate and biopsy smears is the most important investigation in making diagnosis of haematological diseases.[1] This is so because it not only helps to reach the definitive diagnosis in most of the cases, but also helps to decide further course of management among the patients. The findings of the bone marrow aspirate and biopsy smears are tailored to the history and other lab examinations of the patient. The final diagnosis is then made and further investigations that should be done are suggested.[1]

Immunohistochemistry (IHC) is an investigation performed to subtype leukemias into different immunophenotypes.[9] This technique was first invented by Alberts Coon in 1940.[9,10] IHC is a procedure that is done on the bone marrow slides. It is performed in order to detect specific protein molecules or antigens that are expressed on the cell surface in the tissue sections.[9] Although, flow cytometry is much more advanced investigation for detection of immunological subtypes of leukmeias. However, it is costly and needs specialized equipments and expertise.[3] So, it is not available in most of the low resource settings.[3] On the other hand, IHC is a cost effective method that needs no specialized equipment and can be used in countries with limited resources. Its use is rapidly increasing in laboratories because it has diagnostic as

TABLE I: CLINICAL FEATURES IN PATIENTS OF ACUTE LYMPHOBLASTIC LEUKEMIA (N=131)

Clinical Feature	Frequency (n=131)	Percentage
Hepatomegaly	113	86.3
Pallor	105	80.2
Splenomegaly	89	68
Lymphadenopathy	82	62.6
Fever	70	53.4
Weight Loss	47	35.9

TABLE II: DIFFERENT IMMUNOPHENOTYPES OF ACUTE LYMPHOBLASTIC LEUKEMIA AS DETERMINED BY IMMUNOHISTOCHEMISTRY (N=131)

Subtype of Acute lymphoblastic leukemia		Frequency (n=131)	Percentage	Immuno-markers status
B – cell Acute lymphoblastic leukemia	Pro-B ALL	3	2.3	CD34+, TdT+, CD79a+, CD10-, MPO -, CD117-
	Pre-B ALL	86	65.6	CD34+ , TdT+ , CD79a+, CD10+, MPO - , CD117-
T – cell Acute lymphoblastic leukemia	Pre-T ALL	8	6.1	CD34+ , TdT+ , CD79a-, CD10-, cCD3+, MPO -, CD117-
	Mature T-ALL	17	13	CD34+ , TdT+ , CD79a-, CD10-, sCD3+, MPO - , CD117-
Cases that could not be assigned into any lineage		17	13	CD34+, TdT+, CD79a-, CD10-, MPO - , CD117-, CD3-

well as prognostic importance in leukemias.[11]

The antigen molecules present on the surface of blast cells are referred to as the immune-markers. The immune-markers specific for B lineage lymphoblasts are CD19, CD10, CD79a and CD22.[3] The CD3[4] and TdT are the markers of immaturity i.e. their presence confirms that the leukemic cells are the precursor cells that have not matured yet.[3] The makers specific for T-lineage ALL include CD3, CD5, CD7 and CD2.[3] The CD7 is essential for the diagnosis of T-cell ALL.[3]

It is important to specify the immune-phenotypes in cases of leukemia. This is so because the immune-phenotypes tell about the prognosis of the leukemia whether the outcome will be poor or good.[3] The mature B-cell type and T-cell types of ALL are reported to have a poor prognosis in terms of survival.[3] The CD 34 and CD7 markers are also suggestive of a poor outcome in leukemia.[3] On the other hand, the presence or absence of CD19 has not been found to be of any prognostic value so far.[3] So, IHC is of prognostic importance in acute leukemia.[3]

The sub classification of ALL can be done by IHC or flow cytometry. The later one is costly and cannot be used in low income setups. So, the present study was done to determine the diagnostic importance of immunohistochemistry by applying the selective panel of immune-histochemical markers on bone marrow smears and observing its utility in diagnosing different immune-phenotypes of acute lymphoblastic leukemia.

METHODS

This cross sectional descriptive study was done in Rehman Medical Institute, Peshawar, Pakistan from 1st January 2017 to 31st December 2018 (2 years). Ethical approval was taken from the ethical committee of the hospital.

Patients of all ages and both the genders, diagnosed as acute lymphoblastic leukemia based on the review of the peripheral blood and bone marrow smear, were eligible for the study. Out of 133 cases diagnosed as precursor lymphoid leukemia, two cases were excluded due to inadequacy of the aspirate smears and 131 cases were included in the study.

Those cases whose aspirate smears were not adequate or dilute were excluded from the study. The trephine biopsy slides were stained with immune-histochemical stains and evaluated by the consultant hematologist. The results were recorded and analyzed. Leukemia was diagnosed when there were more than 20% lymphoblasts on peripheral blood film or aspirate smear.[1,2,12] The minimum panel of immune-markers used included C34, TdT, CD10, CD79a, PAX1, CD3, CD117 and myeloperoxidase (MPO). The immuno-markers which confirmed the B-lineage of lymphoblasts include CD10 and PAX1. In B lineage ALL, if CD10 was positive, it was diagnosed as pre-B ALL. If CD10 is negative, then it is pro-B lineage ALL. The T lineage immune-markers used was CD3.[3] Immuno-markers used to delineate myeloid lineage included MPO and CD117.[3] Makers of immaturity to confirm the blast nature of malignant cells included CD34 and TdT. Immuno-marker was taken to be positive if more than 20% of the tumor cells expressed the antigen. Data was analyzed using mean and standard deviation for quantitative variables. Qualitative data was analyzed by using frequency and percentage.

RESULTS

Out of 131 cases included, 99 (75%) were males and 32 (25%) were females. The mean age of the study sample was 20±16 years (range: 16-65 years). Clinical features of the study participants is presented in Table I. Majority of the cases presented with hepatomegaly (113/131, 87%), followed by pallor (105/131, 80.1%) and splenomegaly (89/131, 68%).

Based on IHC, 114 (87.02%) cases were successfully classified to specific subtypes and 17 (13%) cases could not be assigned into any subtype. Hence, the utility of immunohistochemistry in subtyping

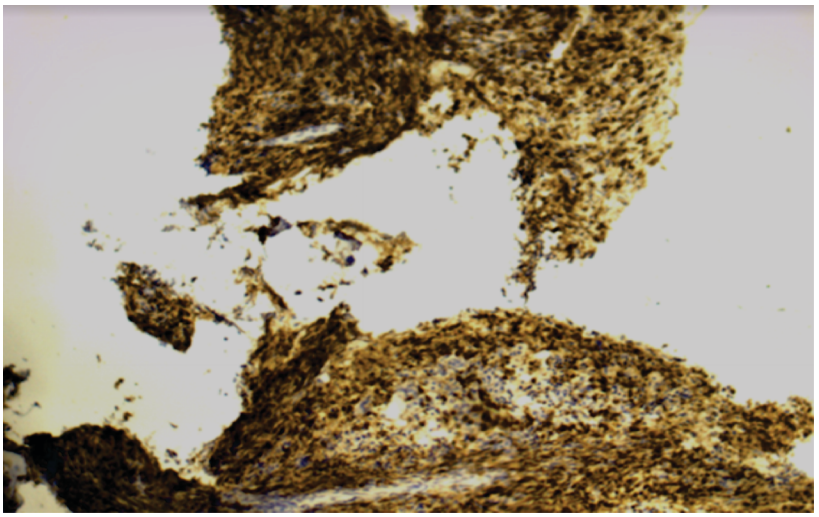

Figure 1: Photomicrograph showing bone marrow trephine biopsy section with positive CD10.

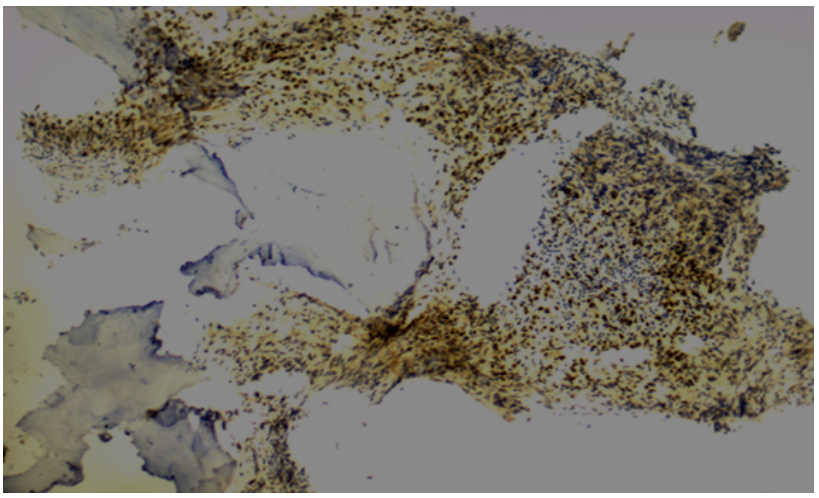

Figure 2: Photomicrograph showing bone marrow trephine biopsy section with positive TdT.

acute lymphoblastic leukemia was 87%. B-lineage ALL & T-lineage ALL were detected in 89 (67.9%) & 25 (19.1%) cases respectively with B:T ratio of 3.5:1. Eighty-six cases (65.7%) were of Pre-B cell ALL, 17 (13%) cases were T-cell ALL (table II).

Figures 1-5 shows the bone marrow trephine biopsy section with positive CD10, positive TdT, negative MPO, positive CD79a and negative CD3 respectively.

DISCUSSION

It is necessary to classify leukemias based on immunopenotype.[4] This is so because each immunophenotype has its own prognostic significance in leukemia.[4] The FAB group was the first to classify ALL.[4] But it was on the basis of morphology only.[13] The WHO classified the leukemia later on the basis of cytogenetic studies and immunophenotypes.[1,2]

In the present study, it was observed that Pre-B ALL was the commonest subtype of ALL, seen in 66% cases with positive CD34, TdT and CD-79a while negative for myeloid markers like MPO, CD117. The ratio of B –lineage ALL to T-lineage ALL was 3.5:1. So, B lineage ALL was common in this study as compared to T-lineage ALL. Similar findings were reported by a study done in India in 2018, where B cell ALL was seen in 86% cases and T lineage in 14%, making B;T ratio of 6:1 showing that B ALL is common than T ALL.[3] Also, the same study showed that Pre-B ALL was the commonest immunophenotype among B lineage ALL.[3] Similar findings were reported from Japan.[14]

In the present study, it was observed that the immunohistochemistry could successfully assign 87.2% cases of ALL into specific subtype, while the remaining 12.8% cases could not be specified into any immunophenotype. This was so because there was no specific marker positive in these cases apart from positive CD34 and TdT, which are markers of immaturity and hence only tell that its leukemia. So, the utility of immunohistochemistry in the present study was 87% with the limited panel of immunomarkers. This shows that immunohistochemistry is diagnostically important in classifying leukemias. Same findings were presented by Subashchandrabose in 2016 in his study where immunohistochemistry could diagnose 90% of the cases of leukemia cases.[4] Arber in his study was able to subtype 96% cases of acute leukemias through immunohistochemical stains.[15] All this data shows that immunohistochemistry is a diagnostically important investigation to classify and confirm the subtype of ALL.

Immunohistochemistry is a simple investigation that needs no specific equipment.[4] It can be used in cases where hospitals cannot afford the cost of getting equipment, and patients cannot bear the cost of immunophenotypes. Therefore, WHO now suggests that immunohistochemistry can be used as a diagnostic tool for immunophenotypic analysis in low resource settings.[4] Literature suggests panels having different immunomarker for classifying acute leukemias.[4,15] However; every laboratory should develop its own panel of markers. This should be done keeping in mind the finances and subtypes of leukemias that are common in that particular area.[4] The laboratories where bone marrow aspirate and trephine biopsy is done, the immunomarkers are already present and can be easily applied to the slides to determine subtype of ALL. The present cost of applying a single immunomarker on each slide is 1000 Pakistani rupees. The patients can afford up to 3 or even 5 markers at such a price. The European Group for the Immunological Characterization of Leukemias (EGIL) and the British Committee for Standards in Hematology (BCSH) have frequently used

MPO, CD13 and CD33 as the markers of myeloid lineage blasts.[4,12] Of these three markers, the MPO is best detected by immunohistochemistry and has very high specificity for myeloid lineage as compared to other two markers.[4] The EGIS and BCSH have used CD19, CD20 and CD10 to detect the B-lineage lymphoblasts.[4] Of these, CD10 can be detected both by flow cytometry and immunohistochemistry. However, CD10 is also expressed by hematogones. So, CD20 is used to differentiate between blasts and hematogones. So, CD3 is better used in immunohistochemistry.[4] The last to be included in our panel was TdT. This is a marker of immature hematopoietic and lymphoid cells. It is not lineage specific. It is positive in both ALL and acute myeloid leukemia.[4]

LIMITATIONS

The limitation of the study was that the sample was taken from a single center and hence the results may not be truly representing the whole population.

CONCLUSION

Study concludes that majority of the patients were male and presented with hepatomegaly and pallor. Applying a limited panel of immunomarkers on bone marrow smears successfully classifies leukemia into its subtypes. Hence, immunohistochemistry is a good diagnostic investigation to classify leukemias, especially in poor resource countries. Pre-B cell ALL is the commonest subtype of leukemia, followed by T-cell ALL.

RECOMMENDATIONS

Based on our results, we recommend the use of this limited panel of markers for routine evaluation of all acute leukemias. The hematohistopathology labs should extend their immunomarker panels so that they may cover the markers for leukemia subclassification as well.

Further studies should be done including larger number of patients from different hospitals so that the results may be more representative of the whole population in community.

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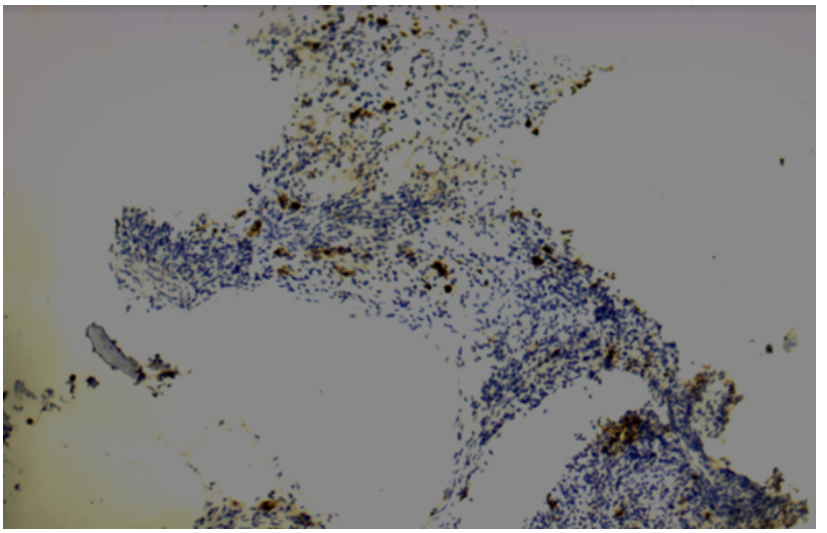

Figure 3: Photomicrograph showing bone marrow trephine biopsy section with negative MPO (myeloperoxidase)

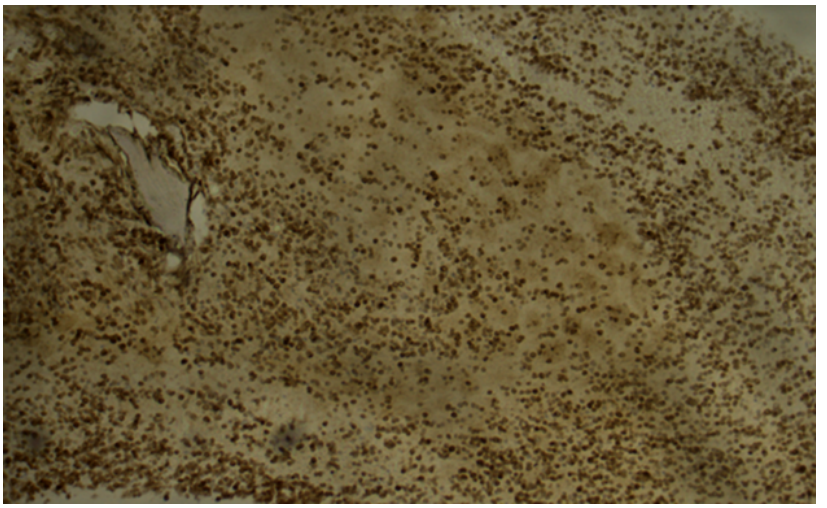

Figure 4: Photomicrograph showing bone marrow trephine biopsy section with positive CD 79a

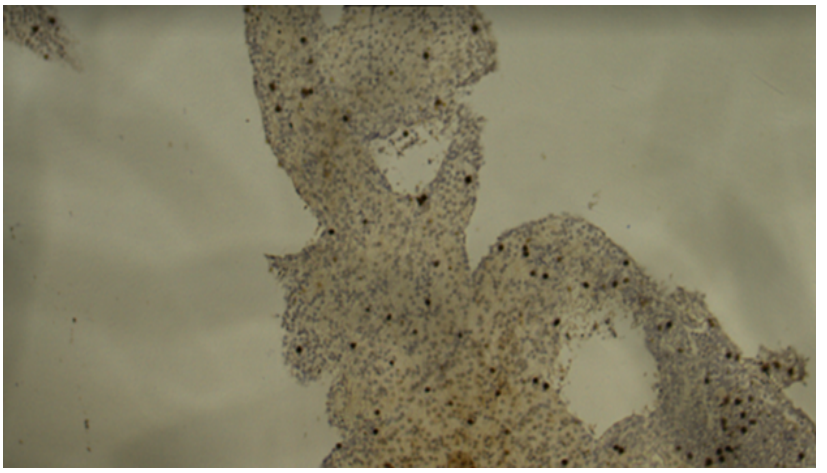

Figure 5: Photomicrograph showing bone marrow trephine biopsy section with negative CD3

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AUTHORS' CONTRIBUTIONS

Following authors have made substantial contributions to the manuscript as under:

NF: Conception and study design, analysis and interpretation of data, critical review, final approval of the version to be published

MIK: Analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

FR: Acquisition of data, critical revision, final approval of the version to be published

SN: Acquisition of data, drafting the manuscript, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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