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Original Article

Assessment of Immunomarker Profiling in Bone Marrow Trephine Biopsy (BMTB) for Lymphoma Diagnosis

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ABSTRACT

Lymphomas are characterized by clonal abnormality of the lymphatic system resulting in malignant neoplasms, classified into Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL). The immunophenotyping and genetic features of the lymphomas play a major role in its classification. **Objective**: To determine the immunohistochemical profiles for multiple types of Lymphoma by using the primary (CD30, CD20, CD3) and secondary (CD15, CD5, CD10, Ki67, BCL6,) panel of immunomarker. **Methods:** This cross-sectional study was done over a period of 1 year from 1st January 2023 to 31st December 2023. A consecutive sampling technique was used. Bone marrow aspiration, and trephine biopsy samples were taken from each patient. Immunohistochemical (IHC) profiling was done on Trephine biopsy to diagnose Lymphomas. **Results:** Out of 57 lymphoma cases, 41 were male and 16 were female patients. The most affected age group was 45-60 years. Among total cases, 43 (75.4%) were of NHL while only 14 (24.6%) cases were of HL. Among 43 cases of NHL, 38 (88.4%) were found of B-cell type while only 5 (11.6%) were of T-cell origin. CD30 (85.7%) was the most expressed immunomarker in HL while CD20 (92%), CD3 (60%), CD5 (47%), and Ki67 showed the highest positivity rate in NHL. IHC was found to be significant by statistical analysis (p-value < 0.05). **Conclusions:** In addition to morphological findings, another crucial step in lymphoma diagnosis is the selection of relevant immunomarkers after clinicopathological correlation with the patient. Therefore, based on our experience, we suggest the use of a limited, cost-effective immunomarker panel for optimal diagnosis of lymphomas and subtypes.

INTRODUCTION

Lymphomas are characterized by the clonal abnormality of the lymphatic system in which the architecture of lymph nodes may be destroyed because of metastasis or due to malignant neoplasia which initiates in the lymph nodes itself [1, 2]. From a hematopathological point of view, malignant lymphomas are defined as the presence of a homogenous population of neoplastic cells as well as a tumor growth pattern which may be either a nodular or follicular pattern or it could be a diffuse pattern of infiltration [3]. From an immunological perspective, lymphomas are expanded clonal proliferation of lymphocytes or B or T cell types of lymphocytic series. Generally, lymphoid tissue-associated malignant neoplasms are classified into two main classes including Hodgkin lymphoma and Non-Hodgkin lymphoma [4]. The distinguishing feature of Hodgkin lymphoma from other lymphomas lies in their exceptional cellular composition including atypical large neoplastic cells mainly Reed-Sternberg and Hodgkin (HRS) cells along with their variants and nonneoplastic reactive cells. According to classification by the World Health Organization (WHO 2016), four different histological variants including nodular sclerosis, lymphocyte depleted, lymphocyte rich, and mixed cellularity were proposed for classical Hodgkin's lymphoma [5]. The immunophenotyping and genetic features of the lymphomas play a major role in its classification. The most common markers useful for the confirmation of classical Hodgkin lymphoma included

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CD30+, CD45+, and CD15+ [6]. On the other hand, non-Hodgkin lymphomas represent various categories of lymphoid malignancies linked with multiple causes. It is estimated that NHL accounted for 5.1% of entire malignant neoplasms and 2.7% of cancer-related fatalities [7]. The origin of NHL may be either B or T cell types. According to WHO 2016 classification, NHL comprises usual and usual subtypes including CLL/SLL (small lymphocytic lymphoma, mantle cell lymphoma (MCL) MALT lymphoma, and follicular lymphoma (FL). Other categories include Diffuse Large B cell lymphoma (DLBCL) and Burkitt lymphoma (BL) [8]. The literature revealed that the distribution of the subtypes of lymphomas varies geographically either within or between the countries. Another study reported a lower frequency of mantle cell lymphoma and follicular lymphoma in Asian countries as compared to the Western population [9]. It was also reported that in the Indian population, the most common subtypes of B-cell NHL were found to be DLBCL [10]. It was also found that the cases of lymphomas associated with Natural killer cells or peripheral T cells were more prevalent in Western countries as compared to Asian countries [11]. Immunophenotyping by immunohistochemistry plays an important role in the diagnosis of the disease along with two other important tools like genetic profiling and morphological examination. The most common markers used for the differentiation of B and T cell lymphomas include CD3, CD30, and CD45 [12]. Lymphoma is included in the top five most prevalent cancers in Pakistan and the disease burden is high with huge financial implications in an under-resource country like ours.

This study aimed to determine the immunohistochemical profiles of multiple types of lymphomas by using different immunomarkers in the patients who visited tertiary care hospital. As a secondary objective, the current study also analyzed the minimum low-cost essential immunomarkers required to reach an appropriate diagnosis of lymphoma with their types and subtypes.

METHODS

The study was conducted at the Department of Pathology, King Edward Medical University/Mayo Hospital Lahore over the study period of 1 year from 1st January to 31st December 2023. Ethical approval (No.329/RC/KEMU) was taken from the Institutional Review Board. It was a cross-sectional study design and samples were collected through consecutive sampling techniques. The sample size was calculated with win-pepi ver: 11.15 to estimate a proportion with confidence level of 95%, acceptable difference = 0.105, and assumed proportion = 0.785 (B-NHL formed 78.5%) [30]. Total 72 samples which fulfilled the inclusion criteria were selected in the study period. Written, informed consent was taken from each of the study participants. Demographic data like age and gender were recorded from all the included cases. Bone marrow aspiration and trephine biopsy were taken from each patient according to the standard PGMIER protocol of bone marrow sample collection [13]. For the processing of bone marrow aspirate, air dried smear slide was stained with May-Grunwald Giemsa stain after fixation. For processing of bone marrow trephine biopsy, formalin-fixed tissue was embedded in paraffin wax, thin sections were cut through microtomy and after de-paraffinization, slides were prepared for hematoxylin and eosin staining procedure for morphological examination were observed by expert Pathologists under light microscope Olympus CX43 five head Microscope using 10x, 20x, 40x and 100x magnification to visualize the lymphoma infiltration pattern of bone marrow. For immunohistochemistry, avidin-biotin peroxidase complex method was used after microwaving was performed for antigen retrieval and washing in TRIS buffer. Different panels of immunohistochemistry markers were selected according to morphological diagnosis. These include CD20, CD23, CD5, BCL6, Ki67, CYCLIN D1, CD25, CD3, CD30, CD79a, CD45, CD10, CD15. Mouse monoclonal antibodies (BioGenex) were used for the binding of these antigens. Lymphomas were identified and categorized according to the infiltration pattern and immunomarkers chosen. For Hodgkin lymphoma: CD30, CD15. For Non-Hodgkin B cell lymphoma: CD20, CD23, CD19 Cyclin D1, Ki67 and BCL6. For Non-Hodgkin T cell lymphoma: CD5, CD30, CD3, CD10, TDT. Interpretation of all cases was done by experienced Pathologists. Results were analyzed using SPSS 28. Percentages and frequencies were calculated for the demographic data, clinical presentation of the patients, and bone marrow infiltration patterns. The chi-square test was used to evaluate qualitative data and validity parameters were calculated.

RESULTS

A total of 72 patients with clinical suspicion of lymphoma were enrolled, out of which 57 cases were diagnosed as lymphomas. Among Lymphoma patients, 41(71.9%) were male while 16 (28.1%) were female. Age distribution showed that the maximum patients were in the age group of 45 to 60 years (44%) followed by the patients from the age group of more than 60 years of age (21%) (Figure 1).

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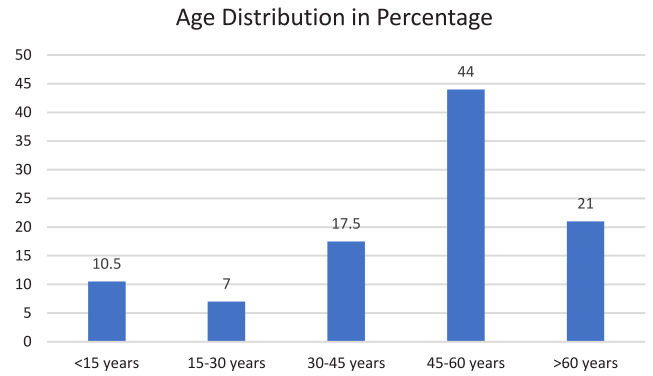

Figure 1: Age Distribution of Lymphoma Cases

CBC findings of the selected cases showed that all the patients were suffering from the cytopenia of cell lines. Maximum patients showed the picture of anemia (31.6%), followed by Bicytopenia (26.3%) and Pancytopenia (22.8%) (Figure 2).

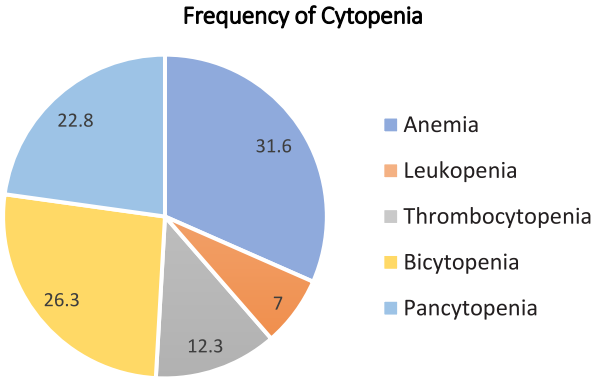

Figure 2: Frequency of the Patients Represented with Different Types of Cytopenia

Clinically, all the patients were affected by organomegaly amongst which maximum patients showed hepatosplenomegaly at 43.9% (n=25), followed by lymph node enlargement at 26.3% (n=15), Splenomegaly at 19.3% (n=11) and 10.5% cases (n=6) showed hepatomegaly. Out of a total 72 enrolled patients with clinical suspicion of Lymphoma, 15 cases were of normal morphology and were thus labeled as Controls. Out of the remaining 57 cases of lymphomas, 43 (75.4%) cases were of Non-Hodgkin Lymphomas (NHL) while only 14 (24.6%) cases were of Hodgkin Lymphomas (HL). Among 43 cases of Non-Hodgkin Lymphomas, 38 (88.4%) were found of B-cell type while only 5 (11.6%) were of T-cell origin. Bone marrow infiltration pattern of the lymphoma cases (Figure 3).

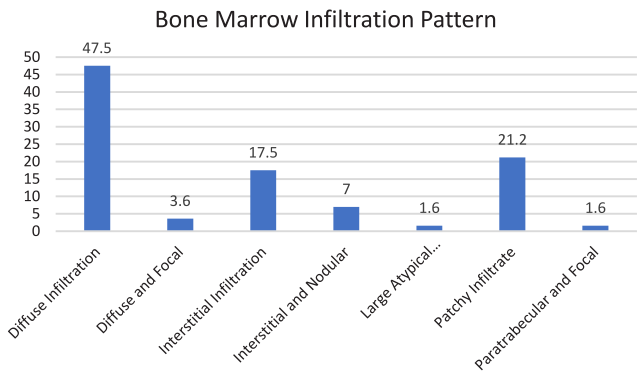

Figure 3: Percentage of Various Bone Marrow Infiltration Patterns in Lymphoma Cases

Immunophenotyping showed CD30 was found to be a more efficient marker for the identification and confirmation of Hodgkin lymphoma. 12 cases (85.7%) out of the 14 total cases of Hodgkin lymphoma were found to be positive for CD30. In the case of non-Hodgkin lymphoma, CD20 was the most efficient marker for the identification of B-cell non-Hodgkin lymphoma while in the case of T-cell CD3 was found to be positive in 3 cases (60%) out of 5 cases. Further categorization of both the T-cell and B-cell non-Hodgkin lymphomas showed their subtypes. A minimum panel of immunohistochemistry for the diagnosis of the above-mentioned lymphomas was further divided into primary and secondary panels. The primary panel included CD20, CD30, and CD3. While CD5, CD10, CD23, BCL6, and Ki67 were nominated as secondary panels. Positive and negative CD markers for the subtypes of B-cell and T-cell Non-Hodgkin lymphoma found in this study were as follows as well a comparison of both bone marrow morphology and immunohistochemistry was done for the identification of Non-Hodgkin lymphoma. p-value of less than 0.05 was considered statistically significant (Table 1).

Table 1: Immunohistochemical Profile and Comparison of Diagnostic Ability of Bone Marrow and Immunohistochemistry for Diagnosis of Non-Hodgkin Lymphoma

Diagnosis	Positive markers	Negative markers	NHL identified by BMM	NHL identified by IHC	p-Value
B-cell Non-Hodgkin Lymphoma					
Chronic Lymphocytic Leukemia (CLL) n=9	CD20 CD5, Ki67<20%	CD3 CD10	3	9	p < 0.05
Mantle Cell Leukemia (MCL) n=4	CD20 CD5 Ki67>30% CD23	CD3 CD10	1	4	
Diffuse Large B-cell lymphoma (DLBCL) n=15	CD20 CD10 KI67>80% BCL6	CD3 CD5	4	14	
Burkitt's Lymphoma (BL) n=6	CD20 CD10 BCL6	CD3 CD5	2	5	

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Follicular Lymphoma (FL) n=4	CD20 CD10 Ki67<40%	CD3 CD5	1	4	
T-cell Non-Hodgkin Lymphoma					
Angio Immunoblastic Lymphoma n=2	CD3 CD10	CD30	1	2	
Anaplastic Large T-cell Lymphoma n=1	CD30 CD5 CD2	CD3	0	1	
Lymphoblastic Lymphoma n=2	CD3 CD5 Tdt	CD20 CD4 CD8	0	2	
Total	-	-	11	41	-

NHL: Non-Hodgkin Lymphoma, IHC: Immunohistochemistry, BMM: Bone Marrow Morphology

A contingency table was drawn to check the association of bone marrow morphology and immunohistochemistry with the diagnosis of non-Hodgkin lymphoma. This statistical analysis shows that the p-value is 0.0001. Since the p-value is less than 0.05, this suggests that there is a statistically significant difference between the BM and IHC diagnoses (Table 2).

Table 2: NHL Cases Detected by Bone Marrow Morphology Vs IHC

-	Diagnosis Done NHL (Positive)	Diagnosis Missed NHL (Negative)	Total	p-value (chi sq test)
BMM	11	32	43	0.0001
IHC	41	2	43	

Validity parameters showed sensitivity and specificity of IHC to be 95% and 86% respectively with diagnostic accuracy of 93%. IHC was found to be extremely significant by the chi-square test (Table 3).

Table 3: Diagnostic Utility of IHC in NHL Diagnosis

-	NHL Positive Cases - 43	NHL Negative Cases (Normal Control) 15
IHC Detected	41 TP	2 FP
IHC Not Detected	2 FN	15 TN

DISCUSSION

Out of 57 samples, 41 were male and 16 were female patients. The most affected age group was 45-60 years. Among total cases, 43 (75.4%) were of NHL while only 14 (24.6%) cases were of HL. Among 43 cases of NHL, 38 (88.4%) were found of B-cell type while only 5 (11.6%) were of T-cell origin. CD30 (85.7%) was the most expressed immunomarker in HL while CD20, CD3, CD5, and Ki67 showed the highest positivity rate in NHL. IHC was statistically significant (p-value < 0.05) with a sensitivity of 95%. In various studies, geographical variations in the incidence rate and distribution of multiple subtypes of lymphomas are very well documented. Burkitt's lymphoma is reported to be endemic in the African region, while adult T-cell lymphomas and gastric lymphomas are much more frequent in Japan and Italy respectively [1]. In India, a high incidence rate of NHL was reported [14]. In the present study, the prevalence of lymphomas was found to be higher in male as compared to female, which was in accordance with various other studies [15, 16]. Age distribution showed that maximum cases were reported in the age group of 45-60 years. Similarly, to our results, a study reported a median age of 50 years about the prevalence of lymphomas [17]. Padhi *et al.*, also reported that 30 to 50 years was the most commonly affected group with lymphomas [18]. Non-Hodgkin lymphomas were found to be more prevalent than Hodgkin lymphomas in the ratio of 3:1 in our study. A South Indian study also reported a ratio of 3.6:1 concerning NHL to HL, which was in accordance with our results [19]. Similar results were reported in other studies as well [16, 20]. In the present study, B-cell lymphomas were found to be more prevalent than T-cell lymphomas. Various other studies also reported the predominance of subtypes of B-cell lymphomas like DLBCL and Burkitt lymphomas [10, 20]. Another study conducted in Amritsar also reported the predominance of DLBCL, a B-cell NHL among all cases of lymphomas [20]. The most common clinical presentation in the current study was hepatosplenomegaly followed by lymph nodes enlargements. Similar results were also reported in various other studies in which lymphadenopathy was the most common clinical sign examined in lymphoma cases [11, 21]. For the diagnosis of Hodgkin lymphoma, CD30 was found to be the most effective marker. Recently, Fromm and Wood reported the panel of six immune markers including CD3, CD30, CD40, CD20, CD95, and CD 64 for the diagnosis of classical Hodgkin lymphoma among which CD30, CD95, and CD40 were found to be positive in most cases of HL which was in concordance with our results. While other markers represented variable positivity rates [22]. Another study reported similar results of a high positivity rate of CD30 in cases of HL [23]. CD3 and CD5 immune markers were positive in most cases of T-cell NHL. To our results, a study reported the expression of CD3 and CD5 markers in cases of T-cell NHL [5]. In the case of anaplastic large cell lymphoma, expression of CD30, CD5 and CD2 was observed in the present study. Das *et al* also reported similar results [6]. Immunophenotyping of B cell NHL lymphomas showed the positivity rate of CD20, CD10, K167, and BCL2 in subtypes of B-cell NHL. A study conducted in 2021 also reported the strong expression of CD20 in B-cell NHL cases [24]. In cases of Mantle cell lymphoma, expression of CD5 was observed in the current study. Another study reported the high positivity rate of CD5 in cases of Mantle cell lymphoma [25]. Another study in 2020 also reported the expression of CD5, and CD20 immunomarkers in Mantle cell lymphoma and CD10 was a negative marker. This was in accordance with our results. However, they also reported the negative expression of CD23 while in the current study, CD23 was a positive marker in cases of MCL [26]. In the case of Burkitt's lymphoma, expression of CD10, CD20 and BCL6 was reported which was similar to our results. Expression of CD10 was observed in cases of CLL which

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was also in accordance to our results [27]. According to the data reported in 2020, 60% of cases of follicular lymphoma showed expression of CD10 immunomarker. In our study, similar results were reported in which CD10 was a positive marker among cases of follicular lymphomas [26]. Another subtype of B-cell non-Hodgkin lymphoma, Chronic lymphocytic leukemia (CLL) was also identified in the present study. Immunophenotyping of CLL showed expression of CD20 immunomarker. Similar results were reported in 2022, in which differential diagnosis of CLL showed CD20 as a positive marker. Immunohistochemistry of Diffuse Large B-cell lymphoma (DLBCL) was also reported in the same study and the results were positivity of CD20 and CD79a immunomarker which was also in accordance with our results in which CD20 was a positive marker in cases of DLBCL [28]. In the present study sensitivity and specificity of IHC is 95% and 88% respectively with a diagnostic accuracy of 93%. These results suggest that Immunohistochemistry is a good test for diagnosing lymphoma. This is in line with another study done in 2017 [29].

CONCLUSIONS

It was concluded that in addition to morphological findings, another crucial step in lymphoma diagnosis is the selection of relevant immunomarkers after clinicopathological correlation with the patient. Therefore, on the basis of our experience, we suggest the use of limited and effective panels of immunomarkers for optimal diagnosis of lymphomas and its subtypes. CD20 was most expressed marker in case of B cell NHL while T cell NHL showed the expression of CD3 and CD5. CD30 was an effective marker for diagnosis of Hodgkin lymphoma.

Authors Contribution

Conceptualization: MA

Methodology: MA, SH

Formal analysis: MI

Writing-review and editing: SH, RM, HA, NM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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