ORIGINAL ARTICLE

DIAGNOSTIC SIGNIFICANCE OF BONE MARROW TREPHINE BIOPSY IN DIFFERENT TYPES OF LYMPHOMA

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Background: Bone marrow trephine biopsy is a critical diagnostic tool for detecting lymphoma. This study was conducted to evaluate the usefulness of bone marrow trephine (BMT) biopsy of lymphomas followed by immunohistochemistry. **Methodology:** This cross-sectional retrospective study was done at Department of Pathology (Haematology), Pak Emirates Military Hospital, Rawalpindi from Apr to Jun 2023. Through non-probability consecutive sampling, bone marrow aspiration and trephine biopsy of 43 patients were assessed to see the morphology of cells and pattern of involvement of bone marrow. Immunohistochemistry was also applied on the trephine to confirm the diagnosis and know the origin of these cells. Statistical analysis was done using SPSS-22. Quantitative data was presented as Mean±SD and qualitative data was presented as frequency and percentage. Chi-square test was applied and $p \leq 0.05$ was taken as statistically significant. **Results:** There were 32 (74.4%) male, and 11 (25.6%) female patients. On bone marrow aspiration 16 (37.2%) had non-Hodgkin's Lymphoma, 11 (25.6%) had Chronic Lymphocytic Leukaemia, 2 (4.7%) had diffuse Large B-Cell Lymphoma, 2 (4.7%) had Acute Lymphoblastic Leukaemia, 1 (2.3%) had T-Cell, and 2 (4.7%) showed others diagnosis like Myelodysplastic syndrome. Six (14.0%) showed inconclusive results and 3 (7.0%) had reactive bone marrow. Thirteen (30.2%) patients showed non-Hodgkin's Lymphoma B-Cell in both aspirate and trephine, 3 (7.0%) had Chronic Lymphocytic Leukaemia, and 1 (2.3%) showed T-Cell, ($p<0.001$). **Conclusion:** Bone marrow trephine biopsy plays a pivotal role in the diagnosis of various types of lymphoma and enhances precision of diagnosis.

Keywords: Immunohistochemistry, lymphoma, Trephine biopsy

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INTRODUCTION

Lymphomas are heterogeneous group of malignancies that arise from the clonal proliferation of B cell, T cell and natural killer (NK) cell, subsets of lymphocytes at different stages of maturation. Lymphoma represents approximately 5% of malignancies.[1] Lymphomas are broadly classified into Hodgkin lymphoma and non-Hodgkin lymphoma.[2] Different diagnostic modalities are used for the diagnosis of lymphoma in bone marrow including bone marrow aspiration and bone marrow trephine biopsy augmented by immunehistochemistry.[3]

Lymphoid infiltrates in bone marrow trephine biopsies are most commonly encountered as part of diagnostic or staging work up for leukaemias/ lymphomas or as an incidental finding in a sample taken for neoplastic or non-neoplastic bone marrow pathology.[4] Bone marrow trephine biopsy is invasive but well-tolerated and should be considered the first-line diagnostic test in patients with suspected lymphoproliferative disorders.[5] The findings of bone marrow trephine biopsy can be confirmed by application of specific immunohistochemistry markers on the biopsy specimen. Further staging can be done with incisional and excisional biopsy of the relevant enlarged lymph nodes. The involvement of bone marrow by lymphomas, predicts the disease evolution and prognosis.[6]

The clinical presentation of lymphomas shows vast variation, it can be indolent or highly aggressive. Lymphadenopathy in supra diaphragmatic region is mostly Hodgkin lymphoma but non-Hodgkin lymphoma can present anywhere in the body.[7] The typical systemic symptoms of fever, unexplained weight loss, and night sweats are most important in advanced disease. The route of dissemination of lymphomas can be through blood, lymphatic system and body cavities.[8] There are also certain viruses which are also associated with lymphomas like Epstein Bar Virus (EBV), Human Papilloma Virus (HPV), Human Immune Deficiency Virus (HIV) and Hepatitis B virus.

The essential way of lymphoma diagnosis is morphology, immunohistochemistry, and flow cytometry.[9] Furthermore fine-needle aspiration and core needle biopsy are often part of the initial evaluation. The Ann Arbor staging system was used for staging Hodgkin lymphoma, and was later adapted for non-Hodgkin lymphoma. It was further modified by incorporating positron emission tomography/computed tomography. Positron Emission Tomography-Computed Tomography (PET-CT) is used for fluorodeoxyglucose-avid lymphoma subtypes.[10]

This study aimed to evaluate patients having lymphoma with fine needle aspiration, trephine biopsy

and immunohistochemistry applied on the cut sections, in early stages as by the initial sign and symptoms with minimum intervention.

METHODOLOGY

This cross-sectional study was conducted in the Department of Pathology (Haematology) Pak Emirates Military Hospital, Rawalpindi during Apr to Jun 2023. We collected retrospective data of last 3 years of the patients, suspected to have lymphoma based on their history and clinical examination. Their bone marrow aspiration and trephine sampling had been done. Sampling was done using non-probability consecutive sampling technique. Informed consent had been taken from the patients. The study protocol was approved by the Ethical Review Committee of the Institution (ERC/ID No. 266).

All the patients presenting with complaints of (fever, weight loss and night sweats) along with physical findings of hepatosplenomegaly and lymphadenopathy were included. Their complete blood counts, peripheral film, bone marrow aspiration and trephine biopsy were done, documented and analysed. Patients with dry tap and inadequate sample obtained were excluded from the study.

Blood sample of 5 mL was taken in EDTA tube for complete blood counts and peripheral blood film examination. CBC was performed by automated haematology analyser. Bone marrow examination was done. With trephine needle bone marrow biopsy was taken. The specimen was embedded in wax and cut sections was prepared and slides were stained. If necessary, immunohistochemistry was applied to make a diagnosis. The retrospective data of 3 years was used to evaluate bone marrow trephine biopsy for lymphomas.

Statistical analysis was done using SPSS-22. Quantitative data was presented as Mean±SD and qualitative data was presented as frequency and percentage. Chi-square test (for comparison of qualitative variables) was applied and $p \leq 0.05$ was taken as statistically significant.

RESULTS

A total of forty-three patients were included in the study, 32 (74.4%) patients were male and 11 (25.6%) were female. The mean age of the patients was 58.67±16.16 years range from 14 to 85 years. Mainly the patients had history of weight loss and fever. (Table-1).

The mean of TLC was 28.18±55.16×10^9/L, RBC 3.48±0.86×10^{12}/L, Hb 9.63±2.27 g/dL, PCV 0.9±0.07 fL, MCV 85.26±12.32 fL, MCH 27.93±3.59 pg, MCHC 32.25±2.49 g/dL, Platelet Count 144.62±128.21×10^9/L and ESR was 52.39±25.69 mm at the end of 1st hour. (Table-2).

The CD20 marker was positive in 33 (76.7%) patients, followed by Ki67 in 24 (55.8%), CD3 in 18 (41.8%), LCA in 17 (39.5%), Pan Ck in 17 (39.5%), BCL-2 in 11 (25.6%), CD30 in 7 (16.3%), CD10 in 4 (9.3%), BCL-6 in 3 (7.0%), CD5 in 1 (2.3%), and C myc in 1 (2.3%) case. Oct-2 was not positive in any patient. (Table-3).

On bone marrow aspiration 16 (37.2%) patients had Non-Hodgkin's Lymphoma, 11 (25.6%) had Chronic Lymphocytic Leukemia, 2 (4.7%) had Diffuse Large B-Cell Lymphoma, 2 (4.7%) had ALL, 1 (2.3%) had T-Cell, and 2 patients (4.7%) showed others diagnosis like MDS. On trephine biopsy 29 (67.4%) patients had Non-Hodgkin's Lymphoma, 8 (18.6%) had Chronic Lymphocytic Lymphoma, 3 (7.0%) had Diffuse Large B-Cell Lymphoma, 1 (2.3%) had T-Cell, and 2 (4.7%) showed Inconclusive results. (Table-4).

Out of the total, 13 (30.2%) patients showed Non-Hodgkin's Lymphoma B-Cell results in both aspirate and trephine followed by CLL (3, 7.0%), and T-Cell (1, 2.3%). There were statistically significant differences between aspirate and trephine reports of the patients ($p<0.001$). (Table-5).

Table-1: Patients' symptoms, red blood cell (RBC) morphology and grading of fibrosis (n=43)

Symptoms	Frequency	Percentage
Fever	10	23.6
Weight Loss	11	25.6
Night Sweats	2	4.7
Fever Plus Weight Loss	9	20.6
Vomiting	2	4.7
Fever, headache, constipation	2	4.7
Swelling in back neck	2	4.7
Abdominal Pain	2	4.7
Urinary Incontinence	2	4.7
COVID	1	2.3
RBC Morphology		
Anisocytosis	38	88.4
Poikilocytosis	32	74.4
Hypochromia	20	46.5
Microcytosis	34	79.1
Elliptocytes	15	34.9
Rouleaux Formation	10	23.3
Target cell	20	46.5
Ovalocytes	3	7.0
Reticulin		
Grade 1	22	51.3
Grade 2	13	30.2
Grade 3	6	13.9
Absent	2	4.7

Table-2: Complete Blood Counts of the patients

CBC	Minimum	Maximum	Mean
TLC (10^9/L)	1.10	298.60	28.18±55.16
RBC (10^{12}/L)	1.78	5.44	3.48±0.86
HB (g/dL)	6.30	14.90	9.63±2.27
PCV (l/L)	0.20	0.45	0.29±0.07
MCV (fL)	28.50	117.40	85.26±12.32
MCH (pg)	19.40	39.90	27.93±3.59
MCHC (g/dL)	27.20	41.60	32.25±2.49
Platelet Count (10^9/L)	10.00	639.00	144.62±128.21
ESR	3.00	130.00	52.39±25.69

Table-3: Interpretation of immunohistochemistry markers (n=43) [n (%)]

Markers	Positive	Negative	Inconclusive
LCA	17 (39.5)	2 (4.7)	24 (55.9)
CD3	18 (41.8)	10 (23.2)	15 (35.0)
CD20	33 (76.7)	9 (20.9)	1 (2.3)
CD30	7 (16.3)	2 (4.7)	34 (79.1)
CD5	1 (2.3)	3 (7.0)	39 (90.6)
CD10	4 (9.3)	13 (30.2)	26 (60.5)
Pan Ck	17 (39.5)	4 (9.3)	22 (51.2)
Ki67 [Low=2, Borderline=7, High grade=15]	24 (55.8)	-	19 (44.2)
Pax 5	2 (4.7)	1 (2.3)	40 (93.0)
Oct-2	0 (0)	1 (2.3)	42 (97.6)
C myc	1 (2.3)	6 (13.9)	36 (83.7)
BCL-2	11 (25.6)	5 (11.6)	27 (62.7)
BCL-6	3 (7.0)	9 (20.9)	31 (72.1)

Table-4: Diagnosis on bone marrow aspiration and bone marrow trephine biopsy (n=43)

Parameter	Frequency (%)
Bone Marrow Aspiration	
Non-Hodgkin's Lymphoma	16 (37.2)
Chronic Lymphocytic Leukaemia	11 (25.6)
Diffuse Large B-Cell Lymphoma	2 (4.7)
ALL	2 (4.7)
T-Cell	1 (2.3)
Inconclusive	6 (14.0)
Reactive	3 (7.0)
Others	2 (4.7)
Bone Marrow Trephine Biopsy	
Non-Hodgkin's Lymphoma	29 (67.4)
Chronic Lymphocytic Leukaemia	8 (18.6)
Diffuse Large B-Cell Lymphoma	3 (7.0)
T-Cell	1 (2.3)
Inconclusive	2 (4.7)

Table-5: Comparison of diagnosis on bone marrow aspiration and trephine (n=43) [n (%)]

	Aspirate									
Trephine	**Non-Hodgkin's Lymphoma B-Cell**	**CLL**	**DLBCL**	**ALL**	**T-Cell**	**Diluted**	**Reactive**	**Others**	**Total**	***p***
Non-Hodgkin's	13 (30.2)	7 (16.3)	1 (2.3)	1 (2.3)	0 (0)	4 (9.3)	2 (4.7)	1 (2.3)	29 (67.4)	
DLBCL	1 (2.3)	0 (0)	1 (2.3)	1 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7.0)	
CLL	2 (4.7)	3 (7.0)	0 (0)	0 (0)	0 (0)	2 (4.7)	1 (2.3)	0 (0)	8 (18.6)	<0.001
T-Cell	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)	1 (2.3)	
Inconclusive	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.3)	2 (4.7)	

DISCUSSION

Lymphoma is a group of histologically and biologically heterogenous clonal malignant disease arising from the lymphoid system.[11] The clinical presentation ranges from indolent to an aggressive nature. Lymphoma is the most common haematological cancer.[12] Its incidence increases with age, and mostly lymphoma patients are elderly. [13] Environmental toxins like pesticides, tobacco, fertilizers, obesity, genetic factors and autoimmune diseases also play a role in lymphoma aetiology.[14]

As red blood cell morphology shows anisocytosis, poikilocytosis with microcytosis and target cells, all these lead to iron deficiency anaemia which in most of the cases is associated with lymphomas. The variables of complete blood counts show vast variation in the minimum and maximum ranges.

For comparison of aspirate and trephine biopsy in diagnosis of lymphoma, 29 cases were diagnosed as non-Hodgkin lymphoma on trephine biopsy and only 13 patients had the same diagnosis on both aspirate and trephine as Non-Hodgkin's Lymphoma B-Cell. Rest of the 16 patients showed different diagnosis.

Worldwide the lymphoma diagnosis is made on morphology, immunohistochemistry and flowcytometry.[15] The prognosis depends upon the International Prognostic Index, is used broadly for all subtypes of non-Hodgkin lymphoma, and the International Prognostic Score is used for Hodgkin lymphoma.[16] Regarding the treatment of lymphoma, it is the chemotherapy alone or in combination with radiotherapy.[17] In old age patients who are more than 60 years of age at diagnosis have worse outcomes, nevertheless of the staging.[18]

There are certain chemotherapeutic agents which the National Comprehensive Cancer Network (NCCN) recommends avoiding them. As concerned to relapse for non-Hodgkin lymphoma is variable which depends upon the type which is diagnosed.[19] As the most common subtype, diffuse large B-cell lymphoma, has a 40% lifetime relapse rate. For Hodgkin lymphoma lifetime relapse occurs in 10% to 15% of patients with early-stage disease but with advanced stage disease the risk increases to 40%.[20]

In comparison to the international studies Chronic lymphocytic leukaemia, Diffuse large B cell lymphoma and T cell Lymphoma, the Non-Hodgkin B cell lymphoma are the most common then Follicular, Marginal zone, Precursor B cell and least is the Burkitt lymphoma.[21] In our study the pattern is the same; most common is the non-Hodgkin lymphoma, Chronic lymphocytic leukaemias.

Early diagnosis of lymphoma is crucial to prevent metastasis. When disease is spread in the body it is challenging for the oncologist to treat the patient. Treatment decisions are preferably made in a multidisciplinary setting.[22] The prognosis in these patients due to metastasis is really poor. Early diagnosis and confirmation with immunohistochemistry is recommended for the long survival of the patients.

CONCLUSION

Bone marrow trephine biopsy plays a pivotal role in the diagnostics evaluation of various types of lymphoma. Its complementary use along side other diagnostic modalities enhances the precision of lymphoma diagnosis, ensuring optimal patient management.

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