# BONE MARROW INFILTRATION AND PATTERNS IN PREVIOUSLY DIAGNOSED PATIENTS OF NON HODGKIN LYMPHOMA (A SINGLE CENTRE STUDY)

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## ABSTRACT

***Objective:*** The objective of this study was to determine the frequency of bone marrow infiltration along with various histological patterns of bone marrow infiltration in previously diagnosed patients of Non Hodgkin Lymphoma.

***Study Design:*** Cross sectional study.

***Place and Duration of Study:*** Department of Haematology, Armed Forces Institute of Pathology Rawalpindi. Six months, from Nov 2013 to May 2014.

***Material and Methods:*** This study involved 70 patients diagnosed of Non-Hodgkin Lymphoma.

***Results:*** The mean age of the patients was 51.40 ± 16.50 years and 78.6% of the patients were males and 21.4% were females. Diffuse Large B-Cell lymphoma was the most frequently observed variant (DLBCL, 60%), followed by Follicular (22.9%) and small lymphocytic lymphoma (SLL, 14.3%). Most of the patients were suffering from Stage-IV disease (51.4%), followed by Stage-III (25.7%) and Stage-II disease (22.9%). Bone marrow infiltration was observed in 51.4% patients. The most common pattern of infiltration was recorded as diffuse, observed in 33.3% of patients. The second most common pattern was interstitial which was recorded in 25% cases followed by mix (22.2%) and focal (16.7%) patterns. The frequency of bone marrow infiltration was unaffected by age ($p$=0.497), gender ($p$=0.868) and type of the disease ($p$=0.486).

***Conclusion:*** The most common pattern of diffuse large B-cell Non Hodgkin lymphoma infiltration was bone marrow followed by interstitial tissue.

**Keywords:** Bone marrow, Infiltration, Non-Hodgkin lymphoma.



## INTRODUCTION

Non-Hodgkin lymphoma (NHL) is a diagnosis applied to a group of histologically and biologically heterogeneous clonal malignant diseases arising from the lymphoid system[1]. Bone marrow involvement by NHL points towards stage 4 disease and is associated with poor prognosis[2]. It is distinguished on bone marrow examination which is considered a key component in the workup for staging of NHL. Both bone marrow aspirate and trephine biopsy are performed but trephine biopsy is a preferred procedure for detection of marrow involvement in NHL[3]. Various patterns of infiltration mark the aggressiveness of the disease. They include focal, interstitial, diffuse, mixed and paratrabecular patterns.

The Ann Arbor staging system reveals the anatomic stage of NHL by number of tumour sites (nodal and extra nodal), their location and presence or absence of systemic symptoms. Bone marrow infiltration by Non-Hodgkin lymphoma points towards stage IV disease and is associated with poor prognosis. It is distinguished on bone marrow examination which is considered a key component in the workup for staging of NHL. Various patterns of infiltration mark the aggressiveness of the disease. They include focal, interstitial, diffuse, mixed and paratrabecular patterns. Data describing the frequency of bone marrow involvement and various patterns of infiltration in Non-Hodgkin lymphoma patients in local population is scarce.

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## PATIENTS AND METHODS

It is a cross sectional study. Data were collected from the patients who presented in the department of Haematology at Armed Forces Institute of Pathology, Rawalpindi. The study was completed in one year time.

Patients were selected by non- probability, consecutive sampling. Sample size of 70 patients was calculated by WHO sample size calculator with 9% absolute precision required and 95% confidence level while taking expected frequency of bone marrow infiltration to be 18%. Diagnosed patients of Non Hodgkin lymphoma, of any age and gender were included and patients receiving chemotherapy which could affect the morphology of bone marrow were excluded.

Permission from the institutional ethical committee was obtained and the procedure began after taking informed verbal consent of the patients. Patient's age and gender was recorded following which bone marrow biopsies were performed from posterior superior iliac spine with disposable aspirate & trephine biopsy needles (Surelock bone marrow trephine needle by TSK, sizes 11G-13G) The aspirate was spread and fixed on plain glass slides and examined under microscope but since the study mainly focused on the patterns of infiltration, data were collected from trephine biopsies of average length 2.5 cm each. The specimens of which were placed immediately in 10% formal saline for 24 hours. After fixation, the specimens were decalcified in 4% sulphuric acid for 4-6 hours. It was processed in automatic tissue processor (SAKURA TISSUE-TEK® VIP-5 PROCESSOR). After paraffin embedding (SAKURA TISSUE-TEK® embedding console system); 2-4µm thick sections were cut by rotatory microtome (LEICA RM 2125-RT) and were mounted on glass slides. These slides were stained with haematoxylin and eosin and were examined for bone marrow involvement and histological patterns by using 4x, 10x and 40x power fields. Bone marrow findings of patients were recorded on a predesigned proforma. All the collected data were entered into SPSS version 10. Numerical variables i.e. age were presented by mean ± standred deviation. Categorical variables i.e. gender, types of NHL, stage of NHL, bone marrow involvement and pattern of infiltration in bone marrow biopsy were presented by frequency and percentage. Data were stratified for age, gender, type of NHL to address effect modifiers. Post-stratification chi-square test were applied taking $p \leq 0.05$ as significant.

## RESULTS

This study involved 70 patients of Non-Hodgkin lymphoma. The age of the patients ranged from 3 years to 90 years with a mean of 51.40 ± 16.50 years. Majority (65.7%) of the patients were middle aged (26-60 years) while 27.1% were old aged (>60 years) and only a small proportion of patients were young (under 25 years of age). Fifty five (78.6%) of the patients were males and 15 (21.4%) were females.

Diffuse Large B-Cell Lymphoma was the most frequently observed variant (DLBCL, 60%) with frequency of bone marrow infiltration being 47.6%, followed by follicular (22.9%) with 50% bone marrow infiltration and small lymphocytic lymphoma (SLL, 14.3%) with 60% bone marrow infiltration. There were only 2 (2.9%) patients of angio-immunoblastic T-Cell lymphoma and they had 100% bone marrow infiltration.

Most of the patients were suffering from stage-IV disease (51.4%), followed by stage-III (25.7%) and stage-II disease (22.9%).

Bone marrow infiltration was observed in 51.4% patients. The most common pattern of infiltration was recorded as diffuse observed in 33.3% of patients. The second most common pattern was interstitial which was recorded in 25% cases followed by mix (22.2%) and focal (16.7%) patterns, paratarabecular pattern was seen only in 2.8% of the patients.

When cross tabulated the data with age groups, the frequency of bone marrow infiltration was 40% in young, 56.5% in middle and 42.1% in old aged patients, however the difference was

statistically insignificant ($p$=0.497) as shown in table-I.

The frequency of bone marrow infiltration was higher in females (53.3% vs. 50.9%; $p$=0.868) as compared to males, however again the difference was statistically insignificant.

The frequency of bone marrow infiltration is shown in table-II.

## DISCUSSION

A number of studies have investigated the frequency and patterns of bone marrow involvement in patients of Non-Hodgkin lymphoma in various populations and have reported variable results. It can be seen that the frequency of bone marrow infiltration varies from as low as 27% in Australian population[4] to as high as 61.4% in Israel[5]. A study done in USA showed bone marrow involvement in 32% of population[6], and another done in Croatia shows 33.8% involvement[7].

Bone marrow infiltration was observed in 51.4% in our study which matches with those of Kumar et al[8] who observed this frequency to be 55.10% in Indian population in 2009. The most common pattern of infiltration was recorded as diffuse observed in 33.3% of patients. The second most common pattern was interstitial which was recorded in 25% cases followed by mix (22.2%) and focal (16.7%) patterns. Other studies done in Norway shows 34.4%[9] bone marrow involvement and in Iraq shows 33.3%[10] respectively. Jamila & Hassan (2008) in Pakistan observed diffuse infiltration in 52% patients. Out of these 52% patients, 34% showed complete replacement and 18% diffuse interstitial infiltration by lymphoma cells. An additional 10% cases manifested diffuse as well as diffuse interstitial infiltration. In 30% of patients diffuse or diffuse interstitial infiltration was accompanied with focal random or focal paratrabecular infiltration. They concluded that diffuse bone marrow infiltration is the commonest pattern probably because of a relatively late presentation by patients[11]. However Kumar et al[8] in 2009 observed mix to be the most common pattern in French and Indian populations. A possible explanation for this variation can be population and genotypic differences as mentioned by Chen et al[12], Hassan et al[13], Lee et al[14], Malik et al[15] and Bartl et al[16]. The frequency of bone marrow infiltration was unaffected by age ($p$=.497), gender ($p$=.868) and type of the disease ($p$=.486).

Shahid Pervez in 2012 observed overwhelming majority of adult patients with diffuse large B cell lymphoma[17]. Another study done in KEMU Lahore states that bone marrow infiltration is more common in our setup as patients present at a later stage[18].

**Table-I: Bone marrow infiltration in different age group.**

| Age groups | Bone marrow infiltration | | Combine (n) | *p*-value |
|---|---|---|---|---|
| | Yes n (%) | No n (%) | | |
| Young (<25 Years) | 2 (40) | 3 (60) | 5 (100) | 0.497 |
| Middle Aged (26-60 Years) | 26 (56.5) | 20 (43.5) | 46 (100) | |
| Old (>60 Years) | 8 (42.1) | 11 (57.9) | 19 (100) | |
| Total | 36 (51.4) | 34 (48.6) | 70 (100) | |

**Table-II: Types of Non-Hodgkin lymphoma with bone marrow infiltrate (n=70).**

| S. No | Type of Hodgkin lymphoma | Bone marrow infiltration | | Total | *p*-value |
|---|---|---|---|---|---|
| | | Yes n (%) | No n (%) | | |
| 1 | Diffuse Large B-cell Lymphoma | 20 (47.6) | 22 (52.4) | 42 (100) | 0.486 |
| 2 | Follicular Lymphoma | 8 (50) | 8 (50) | 16 (100) | |
| 3 | Small Lymphocytic Lymphoma | 6 (60) | 4 (40) | 10 (100) | |
| 4 | Angio-Immunoblastic T-Cell Lymphoma | 2 (100) | 0 (0) | 2 (100) | |
| Total | | 36 (51.4) | 34 (48.6) | 70 (100) | |

A very important limitation of the current study was that we didn't consider the treatment outcome in relation to bone marrow infiltration status and the pattern of infiltration. Therefore such a study in future is highly recommended.

## CONCLUSION

The most common pattern diffuse large B-cell Non Hodgkin lymphoma infiltraton was bone marrow followed by interstitial tissue.

## CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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