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A MORPHOLOGICAL AND CLONALITY EVALUATION OF BONE MARROW INVOLVEMENT IN NON-HODGKIN'S LYMPHOMA IN A TERTIARY CARE HOSPITAL

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ABSTRACT **INTRODUCTION** Bone marrow aspiration and biopsy has been widely used in different hematological malignancy . In patients newly diagnosed with lymphoma, examination of the bone marrow is essential for staging, treatment, response to therapy and follow up. A bone marrow examination can also be performed in patients in whom a clinical suspicion of lymphoma is present. **METHODS** This was a prospective study that included 55 diagnosed case of Non-Hodgkin's Lymphoma who came for evaluation for bone marrow involvement. The morphological and incidence pattern with the type of clonality which was correlated with immunohistochemical expression of CD3 and CD20 was evaluated from Bone marrow study. **RESULTS** Non-Hodgkin's Lymphoma highest incidence was found to be present in the age group of 51-60 years with a male predominance. Small lymphocytic lymphoma was most common lymphoma coming for Bone marrow study accounting for 36% and was also most common sub-type infiltrating the marrow which was seen in 40% of cases . 36.4% of NHL had marrow infiltration with a male dominance. Bone marrow involvement of diffuse type was most common seen in 50% of cases. Bone marrow infiltration by B-cell Non-Hodgkin's Lymphoma was more commonly seen in 85% than that of the T-cell which was 15% which was further confirmed by immunohistochemistry by using CD20 and CD3. **CONCLUSION** Bone marrow involvement showed a male predominance having most common diffuse pattern of involvement. B cell lineage of Non-Hodgkin's Lymphoma was common compared to T cell lineage. Bone marrow study is essential for the disease diagnosis, treatment and follow up.

KEYWORDS : Bone marrow, Non-Hodgkin's Lymphoma, Small lymphocytic lymphoma

INTRODUCTION

Non-Hodgkin´s lymphoma(NHL) is heterogeneous group of malignant lymphoproliferative disorders. Non-Hodgkin's lymphoma (NHL) are characterized by neoplastic transformation of lymphoid cells and have a high potential for spread to various tissues throughout the body especially bone marrow, liver, spleen, lungs, and brain etc. Bone marrow(BM) biopsy is an essential part of diagnostic workup in NHL and the patients presenting in stage IV disease with infiltration of bone marrow and / or other tissues manifest poor prognosis and response to treatment[1,2].

The technique of bone marrow aspiration has been universally accepted and widely used [3].However, bone marrow biopsy as a diagnostic procedure is being increasingly used in recent years. Trephine biopsy permits an assessment of pattern and extent of infiltration, which is of both diagnostic and prognostic relevance. Sometimes, trephine biopsy may demonstrate lymphoma when no abnormal cells have been detected in blood and bone marrow aspiration smears. Immunohistochemistry increases the accuracy of diagnosis and is helpful in confirming the lymphoma those are missed out in BM biopsy and can classify different sub-types of NHL.

Aim of the Study

(1) To Study the Bone marrow aspiration and Bone marrow biopsy findings in various Non-Hodgkin's lymphomas.
(2) To confirm the infiltration of lymphoma into bone marrow and to know the pattern of involvement.
(3) To classify the clonality as B-cell or T-cell lineage by immunohistochemical markers CD3 and Cd20.

MATERIALS AND METHODS

The present study was carried out in the department of pathology of Hi-Tech Medical College and Hospital for a period of 2years from August 2014 to August 2016 which includes 55 diagnosed case of NHL who came for evaluation for bone marrow involvement. After obtaining informed consent from the patients,bone marrow aspiration and trephine biopsy was performed at posterior superior iliac spine as it is the most suitable and safe site for both aspiration and biopsy.

Bone marrow smears were prepared immediately following aspiration. A minimum of six smears were done and then stained. BM aspirate were stained with Leishman's Stain. The BM trephine biopsy was performed soon after the aspirate. Touch imprints were made from the trephine biopsy prior to placing in fixative. The imprints were fixed and stained using the same method as for aspirate smear. After the imprints were made, the core specimen was placed into a container with appropriate fixative. The biopsy core was fixed in 10% buffered formalin for 8 to 24 hrs and decalcified in 14% EDTA for 16 -24 hrs. After routine processing and paraffin embedding, Haematoxylin and Eosin (H&E) sections were studied.

On microscopic view, topography of NHL infiltrating the bone marrow or lymphocytosis was evaluated both in BM aspiration and biopsy slide. Some of the cases which were negative in aspiration were positive in biopsy as lymphoma can be focal in distribution pattern. All the BM biopsy showing infiltration with lymphoma cells and/or lymphocytosis with atypical lymphoid cells were subjected for immunohistochemistry (IHC) evaluatation with CD3 and CD20.

RESULTS

A total of 55 BM were obtained with Male to Female ratio was 2:1. The age of the patients varied from 10 year to 80 years. The maximum incidences of cases were seen in the age range of 51-60 years (table 1).

TABLE - 1 DISTRIBUTION OF CASE ACCORDING TO AGE AND SEX

AGE GROUP (Years)	SEX		Total(n)	Percentage
	MALE	FEMALE		
11- 20	1	0	1	2%
21-30	6	5	11	20%
31-40	4	0	4	7%

41-50	6	6	12	22%
51-60	12	4	16	29%
61-70	7	3	10	18%
71-80	1	0	1	2%
Total	37	18	55	100%

The present study had major clinical symptoms like lymphadenopathy which was seen in 58% of cases, fever and splenomegaly was seen in 40% of cases. Hepatomegaly was seen in 20% of cases.

Small lymphocytic lymphoma(SLL) was most common lymphoma coming for BM study accounting for 36% followed by Diffuse large B cell lymphoma(DLBCL) 24%, follicular lymphoma 9%, , Lymphoplasmacytic lymphoma 7%, Anaplastic large cell lymphoma 5%, Peripheral T-cell lymphoma 5%, Mantle cell lymphoma 4%, Marginal zone lymphoma 4%, Mucosa-associated lymphoid tissue lymphoma 4% and Plasmacytoma 2% .

BM biopsy slide revealed that 20 cases showed positive bone marrow involvement and 35 cases showed negative infiltrations to BM. But in BM aspiration 16 cases were positive and 39 cases were negative . This disparity was further confirmed by IHC. Two cases showed lymphocytosis in BM aspiration which was negative in BM biopsy.

In the present study out of 20 cases of BM involvement 13 were male and 7 were female which showed male dominance . The male to female ratio of BM involvement was 1.86:1. The most common age group for male was 51-60years followed by 31-40years and 41-50years. For female the most common age group was 41-50years followed by 51-60years.

The predominant pattern of BM involvement was of diffuse type which was 50% followed by interstitial 25%, focal 10%, mixed 10% and paratrabecular 5% (table 2).

Table 2 FREQUENCY OF PATTERNS OF BONE MARROW INFILTRATIONS IN NHL

BM INFILTRATION	FREQUENCY(n)	Percentage
DIFFUSE	10	50%
INTERSTITIAL	5	25%
FOCAL	2	10%
PARATRABECULAR	1	5%
MIXED	2	10%
TOTAL	20	100%

A total of 20 cases of NHL were infiltrating to bone marrow were taken for IHC with CD20 and CD3. 16 cases showed membrane positivity to CD20 which were negative to CD3 confirming as B-cell lineage. Similarly 3 cases showed strong membrane positivity to CD3 and negative to CD20 showing that they were of T-cell lineage (table 3). One case of plasmacytoma which showed negative to both CD20 and CD3 but it is of B-cell lineage. So totally 17(85%) were of B-cell lineage and 3(15%) were of T-cell lineage.

Table 3 FREQUENCY OF CD20 AND CD3 IN NHL CASES

IHC MARKER	POSITIVE(n)	NEGATIVE(n)
CD20	16	4
Cd3	3	17

DISCUSSION

Bone marrow examination is an important investigation in the diagnosis of various hematological disorders. It is safe and relatively easy test to perform. BM biopsy is essential for diagnosis in cases of dry tap and blood tap in bone marrow aspirations and in focal involvement of bone marrow. Only a biopsy provides a complete assessment of marrow architecture and pattern of distribution in case of infiltrative disorders.[4] Bone marrow trephine biopsy is superior to bone marrow aspiration in determining the bone marrow involvement by Non-Hodgkin's lymphoma.[5]

NHL has propensity to disseminate especially to the bone marrow. BM involvement by lymphoma indicates stage IV disease and trephine biopsy is the most preferable method to detect marrow infiltration. Bone marrow biopsy is an integral part of staging workup for Non-Hodgkin lymphoma at the time of initial diagnosis as well as after therapy. Thus the procedure is essential as a pre-treatment and prognostic protocol of NHL and the patients found to have marrow infiltration are labeled as having stage IV disease.

The present study is a prospective study of 55 already diagnosed case of NHL who presented with fever, lymphadenopathy, splenomegaly and were received in the department of pathology of Hi-Tech medical college and hospital for staging and to know the status of remission on already treated patients. There was no complication during the BM study.

In this study male to female ratio was 2:1 which was similar to study of S. Kumar et al[6]. Study done by Filiz vural et al[7] showed a low male to female ratio of 0.6:1. This disparity can be due to different population of study. The median age of presentation was 47.5years in this study which was closely similar to the study of Yun-Fei Shi et al[8] and S. Kumar et al[6]. The most common age group of the cases was 51-60years followed by 41-50years in the present study.

Lymphadenopathy was the most common presentation seen in 58% of cases, followed by fever, splenomegaly and Hepatomegaly which was also same in the study done by Khalid Hassan et al[9] and Fatma A et al[10]. SLL was the most common subtype of NHL seen in 36% of cases followed by DLBCL which was 24%. This finding was similar to Tarek M.N. El Bolkainy et al[11] where SLL was most common subtype of NHL seen in 40% of cases followed by DLBCL seen in 20% of cases.

BM biopsy was superior to BM aspiration in the diagnosing of NHL .BM biopsy showed NHL infiltration in 20 cases and negative in 35 cases. BM aspiration showed NHL infiltration in16 cases and negative infiltration in 39 cases.Yun-Fei Shi et al[8] in their study showed BM aspiration and BM biopsy agreed in 84.12% of cases which is very much similar to our observation.

In the present study, 20 cases (36.4%) of NHL had BM involvement which was similar to study of Conlan et al[12] having 34.4% and G.Lambertenghi- Deliliers et al[13] where it was 39.5%.

The predominant histological pattern of BM involvement by NHL was of diffuse type accounting for 50% followed by interstitial (25%), focal, mixed and paratrabecular, which was comparable with Malik et al[14] where diffuse type was seen in 46.35 of cases. Khalid Hasan et al[9] also had most common type of diffuse infiltration.

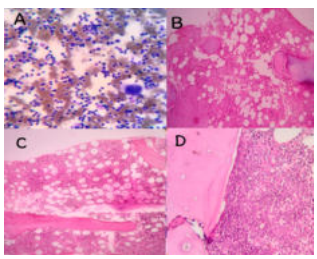

Fig 1 – BM aspiration showing lymphoma cells(A), BM biopsy showing Diffuse pattern (B), Interstitial pattern (C), Paratrabecular pattern (D) of infiltration , all low power magnification

Bone marrow infiltration by B-cell NHL was 85% which was more common than that of the T-cell which was 15%. The finding was comparable with Faris H Jaafer et al15 where the incidence of B-cell type was 88.5% and T-cell NHL was 11.5%. Fatma A et al10 also had nearly same incidence rate.

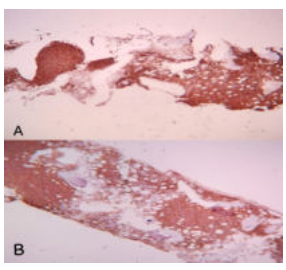

Fig 2- BM biopsy showing membrane positivity to antibody CD 20 (A) and antibody CD 3 (B) , low power magnification

BM study is essential for all and suspected cases of lymphoma for disease stratification and treatment. It is ideal for the clinical set up in

developing countries to have BM study in cases of lymphomas. Immunohistochemistry evaluation should be properly done following the standard protocol. IHC standards have been evolving during the last few decades as the clinical needs for standardization for IHC laboratory testing have been rising. In particular, IHC testing that provides prognostic and predictive information important for stratification of patients for specific therapies.

CONCLUSION

Bone marrow biopsy is a safe and easy procedure with minimal patient discomfort. It is cost effective and does not require sophisticated equipment. Bone marrow examination is an important investigation in the staging of lymphomas. Only a biopsy provided a complete assessment of marrow architecture and pattern of distribution and was useful for special stand Immunohistochemical stains. So BM is more accurate and sensitive compared to BM aspiration IHC of BM slides is required to increase the diagnostic accuracy in NHL as it is likely to be missed in morphological evaluation during BM aspiration or in morphological interpretation BM biopsy. Also IHC can be helpful to subtype the NHL into different categories based upon their antigen expression. Flow cytometry can validate and is an additional potentially powerful tool to compare results of IHC. Thus BM aspiration and biopsy with flow cytometry followed by IHC evaluation is the proper way of evaluating NHL for its major subtypes, staging, treatment protocol, prognosis and follow up.

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