Study of infiltration patterns of lymphomas in bone marrow trephine biopsy and immunohistochemical analysis in a tertiary care institute

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Abstract---Lymphomas are malignant neoplasms of lymphoid cells present usually as solid mass in the lymphoid organs of the body. They have a tendency to metastasize to different parts of the body and bone marrow is one of the most frequent sites. They infiltrate particular parts of the bone marrow and hence form various different patterns of infiltration. Each lymphoma has its characteristic one or more patterns of infiltration. Recognition of such a pattern can often help in diagnosis of such lymphoma. This was a prospective descriptive study of one year duration enrolling known cases of lymphoma diagnosed in a tertiary care center and subsequently bone marrow trephine biopsy was done and studied for pattern of infiltration. Also immunohistochemistry was applied to differentiate B cell and T cell lymphomas. Paratrabecular pattern was the most common pattern seen solely or in mixed in the majority of the

lymphoma cases. Also the frequencies of B cell lymphoma with CD-19 positivity were higher than T cell lymphoma with CD-3 positivity. Recognition of pattern of infiltration especially when no lymphomatous cells are identified on blood and or bone marrow aspiration smears are very helpful in diagnosis of lymphoma. The application of immunohistochemical markers on bone marrow biopsy smears can aid in diagnosis and sub-classifying lymphomas in the broad category of B cell and T cell group.

Introduction

Lymphomas are malignant lymphoid cells neoplasm characterized by initial involvement of lymphoid tissue like lymph nodes, spleen and thymus and often present as a solid mass. Lymphomas have been classified into two major groups that are Hodgkin lymphomas (HL) and Non Hodgkin lymphomas (NHL). Non Hodgkin lymphomas are far more common than Hodgkin lymphoma and characterized by the proliferation of precursor or mature B cells, T cells and/or NK cells. [1, 2] Though lymphomas are solid nodal or extra nodal masses of lymphoid cells, they have a tendency to metastasize to different sites like blood and bone marrow. Bone marrow involvement is most frequent by lymphomatous metastasis and sometimes patient present due to symptoms of bone marrow involvement rather than due to lymphoma itself due to the fact that indolent lymphomas have higher tendency to infiltrate into bone marrow than aggressive lymphomas. [3, 4] Each lymphomas have characteristic pattern of infiltration depending upon the part of bone marrow is involved by lymphomatous cells. Six main patterns of lymphoma infiltrating bone marrow have been identified- Focal random, Paratrabecular, Interstitial, Diffuse, Intrasinusoidal and Nodular**.** These patterns help distinguish benign from malignant lymphoma and also help characterize various types of lymphomas. No single pattern is unique to any lymphoma entity but it is possible to make some helpful generalizations. [5, 6] Bone marrow trephine biopsy is needed to visualize these patterns under the microscope and it also has the added advantage of uses of immunohistochemical analysis of lymphomas.

Materials and Methods

This prospective descriptive study of one year duration (2012 to 2013) was carried out in the department of pathology in a tertiary care institute. The entire patients above 10 years of age newly diagnosed with lymphoma either by cytology or histopathology were enrolled in the study group. After proper informed consent and taking full aseptic precaution bone marrow aspiration was done and later bone marrow biopsy was performed from right posterior superior iliac spine using Jamshidi biopsy needle. After obtaining a biopsy core of approximately 6cm in length it was immediately put in fixative solution and after overnight in a decalcifying solution for 2-3 days and finally sent to histopathology section for further processing and block and slide making. Two slides were made, first

stained with H&E stain and second unstained for application of immunohistochemistry (IHC) later on. Slides stained with H&E stain were seen under microscope alongside Leishman stained bone marrow aspiration slides for better visualization and identification of lymphoid cells. Different patterns of infiltration were noted in cases where infiltration was present. Finally IHC with CD-19 and CD-3 was applied on the unstained section of positive NHL cases to further sub-classify them in B cell and T cell groups.

Immunohistochemistry:

Paraffin sections were dewaxed and rehydrated. After antigen retrieval by heat method in appropriate buffer as per standard protocol, IHC staining for CD3 & CD19 was done.

Primary antibody:
1) Anti CD3 (T-cell) – Biogenex
Product code: AM322-5M
Preparation: mouse monoclonal antibody in Phosphate Buffer Solution (PBS) with carrier protein and preservative (ready to use)
2) Anti CD19 (B-cell) – Biogenex
Product code: AM264-5M
Preparation: mouse monoclonal antibody in PBS with carrier protein and preservative (ready to use)

Secondary antibody:
Super Sensitive™ Polymer – HRP IHC Detection System
Contents: (Peroxidase block, Protein block, Super enhancer, DAB chromogen, Substrate solution:polymer HRP, DAB buffer)

Results

Total 40 cases of newly diagnosed lymphoma (Hodgkin lymphoma and Non Hodgkin lymphoma) were enrolled into the study in which majority belonged to male sex. Out of 40 cases only 13 patients showed infiltration on bone marrow biopsy including 12 cases of NHL and one case of HL. In many cases more than one type of infiltration (mixed pattern) was seen. Majority of the cases (7 cases) showed Paratrabecular pattern (58.33%) either alone or in combination with other patterns followed by diffuse pattern (5 cases / 41.67%). (Table 1/ Fig 1) None showed Intrasinusoidal and nodular pattern. Out of 12 positive NHL cases, 10 cases showed positivity with CD-19 (83.33%) and 2 cases (16.67%) were positive with CD-3 thus sub-classifying them into B cell NHL and T cell NHL respectively. (Table 2/ Fig 2)

Discussion

Bone marrow examination is an essential part of evaluation in lymphoma patients as it has both diagnostic and prognostic value. [7] Bone marrow examination can be performed by bone marrow aspiration or bone marrow trephine biopsy. Though aspiration is an easy and quick process, biopsy is more sensitive and also gives additional topographic information and can be used for application of IHC. Both

bone marrow aspiration and biopsy are complementary to each other as consideration is given to both to the pattern of infiltration and to cytological characteristics to reach right diagnosis.[8] In present study paratrabecular pattern was most commonly found in various lymphomas either alone or as mixed pattern. A much higher incidence of diffuse marrow infiltration was seen in other studies (71.4%, 15out of 21 cases, E J Lim et al, 33.3% by Ishtiaq J et al and 14.6% by Sultan S et al) compared to this study.[9, 10, 11] In this study diffuse pattern was second most common. This disagreement may be due to differences in the frequency of lymphoma cases that were included in the study as each lymphoma has a characteristic pattern of infiltration. Interstitial infiltration can occur both in neoplastic and in reactive conditions however paratrabecular infiltration and a 'packed marrow' (diffuse infiltration) are almost always indicative of neoplasia. [12] None cases in this study showed Intrasinusoidal or nodular pattern which again may due to type of lymphomas and also because, they may easily be missed in early stages. [13] Uses of IHC on trephine biopsy slides have increased diagnostic accuracy and also it helps in proper categorization of lymphomas. In this study we used CD-3, a pan T cell marker and CD-19, a pan B cell marker to divide lymphomas into B cell and T cell types. As in most studies we also found B cell lymphomas far more common than T cell lymphomas with B cell: T cell being 5:1. [14, 15]

Conclusion

Every lymphoma patient should be evaluated by bone marrow examination as it has diagnostic, prognostic as well as therapeutic implication. Mere presence of lymphoma cells in bone marrow put the patient in an advanced stage (Stage IV) as it predicts that malignant cells have already disseminated, making prognosis poor. It also most of the time helps to diagnose and to know the type of lymphoma that helps in therapeutic decision making.

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Figures/Tables

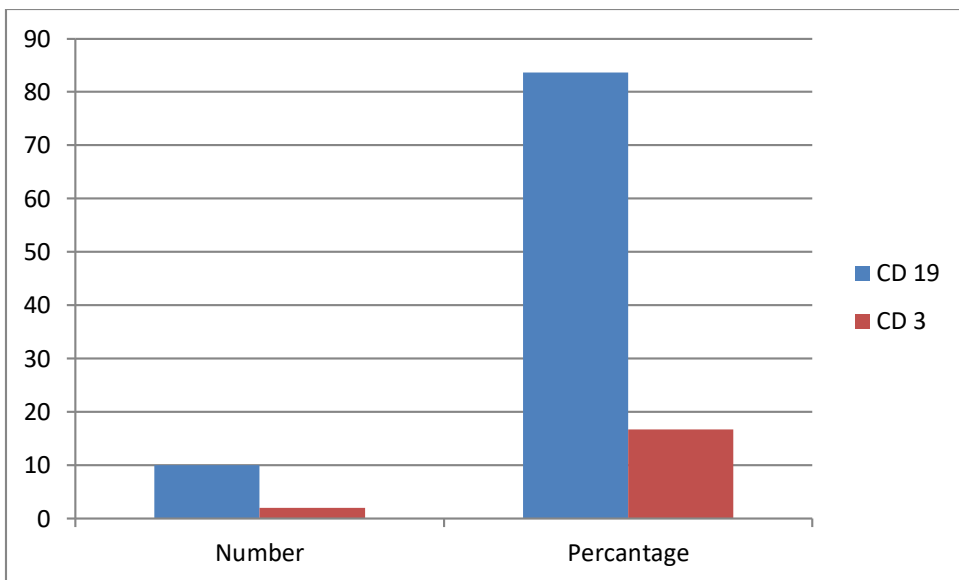**Table 1**: Distribution of pattern of lymphomatous infiltration

Pattern	NON HODGKIN LYMPHOMA (n=12)		HODGKIN LYMPHOMA (n=1)	
	No.	%	No.	%
Paratrabecular	7	58.33	0	00
Diffuse	5	41.67	0	00
Intrasinusoidal	0	00	0	00
Interstitial	2	16.67	0	00
Focal random	2	16.67	1	8.33
Nodular	0	00	0	00

Table 2: Sub typing of NHL cases on IHC

IHC	NON HODGKIN LYMPHOMA (n=12)	
	No.	%
CD19	10	83.3
CD3	2	16.7
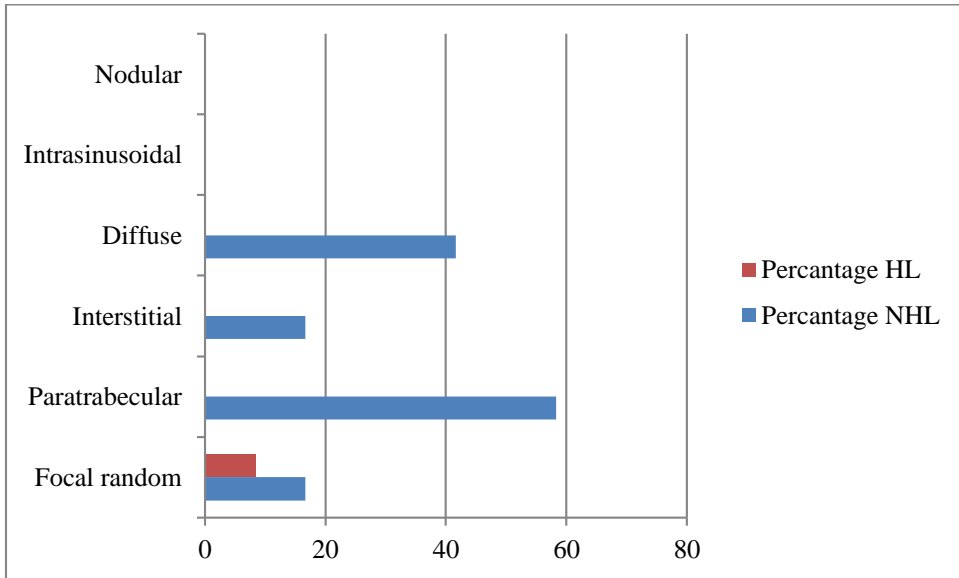
Fig 1 Distribution of pattern of lymphomatous infiltration

Fig 2 Sub typing of NHL cases on IHC