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Frequency of Lymphomatous Infiltration of Hodgkin's & Non-Hodgkin's Lymphoma in Bone Marrow by Bone Marrow Aspiration, Trephine Biopsy and Clot Section.

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ABSTRACT

Background: Lymphomas are malignant neoplasm of lymphoid cells, classified broadly in Hodgkin's and Non Hodgkin's lymphoma and have variable tendency to metastasize to blood and bone marrow depending on their types. **Methods:** We performed bone marrow aspiration and biopsy on known patients of lymphoma diagnosed on Lymph node cytology or biopsy in the Department of Pathology King Georges Medical University along with general blood work up to know the incidence of various lymphomatous infiltrations and also to compare different procedure for their effectiveness. Patients already on treatment, relapsed cases of lymphoma and patients below 10 yrs of age group were excluded from the study. After taking proper history and consent, general examination and bone marrow procedure was done and clot section was also made. **Results:** We found that males were more commonly involved than females in both the type of lymphomas with majority of cases in both lymphoma group showed bimodal age distribution that is below 30 yrs and after 40 yrs. Most common type to infiltrate bone marrow was lymphoblastic lymphoma (75%) followed by large cell types and small cell types with equal frequencies of (66.7%). Bone marrow aspiration, clot section and biopsy did not show any statistical significant differences in the efficacy. **Conclusion:** lymphoma frequently metastasizes in the bone marrow and its frequency depends on the type of lymphoma.

Keywords: Hodgkin Lymphoma (HL), Non Hodgkin Lymphoma (NHL), Bone marrow (BM).

INTRODUCTION

The bone marrow is found within the central cavities of axial and long bones. It consists of hematopoietic tissue islands and adipose cells surrounded by vascular sinuses interspersed within a meshwork of trabecular bone.[1] Peripheral blood (PB) and bone marrow (BM) are relatively frequently involved by lymphomas. Lymphomas are malignant neoplasms characterized by the proliferation of cells native to the lymphoid tissue. They are divided into two broad groups, Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL).[2] In both Hodgkin and non-Hodgkin lymphomas, the evidence of bone marrow involvement indicates the highest Ann Arbor stage (stage IV) by itself, with several therapeutic implications. Therefore, the assessment of eventual bone marrow involvement is recommended in all patients of lymphoma.[3] NHL involves uncontrolled clonal expansion of B and T cells. B-cell NHL constitutes the majority of cases and, of these, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) are the two major subtypes.[4] HL is characterised morphologically by the presence of neoplastic giant cells the Reed Sternberg (RS) cell admixed with a variable inflammatory infiltrate. RS cells express CD30 and CD15 antigens. The incidence of bone marrow infiltration for all types ranges from 30% to 55% in most of the larger studies among adults. The incidence of marrow involvement in Hodgkin's lymphoma is around 38% but varies with the histopathologic type.[5] Compared to aggressive lymphoma, relatively high frequencies have been reported in indo-lent lymphomas, such as mantle cell lymphoma (MCL), follicular lymphoma (FL), and marginal-zone lymphoma.[6]

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MATERIALS AND METHODS

It was a prospective descriptive study of 1 year duration having total 40 patients. All patients of lymphoma proven on biopsy or FNAC, reporting to Pathology Department, KGMU, Lucknow were

enrolled after taking ethical clearance from the committee and written consent from patient/patients attendant. However patients already on treatment, relapsed cases of lymphoma and patients below 10 yrs of age group were excluded from the study.

Data collection

Bone Marrow (BM) Examination

The bone marrow examination was carried out in the departmental laboratory after taking written consent from each of them under all required precautionary measures from the Right posterior superior iliac spine and sternum as feasible. The examination includes Trephine Biopsy and Bone Marrow aspiration along with preparation of Clot sections of the same patient.

Requirements

- Sterilized BM trephine biopsy needle (Jamshidi's needle)/aspiration needle (Salah's needle).
- Dispovan syringes -5 ml and 20 ml.
- Local anaesthetic (LA) – xylocaine 2.0%.
- Betadiene solution.
- Spirit.
- Prewashed and scratch free sections.
- Emergency drugs tray.

Position of patient and preparation

- Bone marrow trephine biopsy and preferably aspiration were carried out on preferably on posterior superior iliac spine with patient in right or left lateral position and knee (contra lateral to the respective lateral position) flexed against abdomen.
- The above mentioned area was exposed and cleaned with spirit swab, followed by betadiene solution.
- After infiltrating the area with 2.5 ml of local anaesthetic up to the periosteum and waiting for 30-60 seconds, first bone marrow biopsy procedure was carried out and then bone marrow aspiration was done.
- Imprint films were made by touching (touch imprint) / rolling (roll imprint) the marrow core on the slide. Rest of the core was put for fixation.
- The films were drawn immediately from the aspirate (ideally 6- 10) and allowed to dry, followed by staining with Leishman's stain.
- The rest of aspirate was put to clot and further processed with formalin (10%) for clot section films.

Leishman's stain preparation and staining for aspiration smears

- Leishman's stain powder (0.15 g) dissolved in 100 ml of acetone free methanol.
- All the stain must be dissolved (ready to use but will improve on standing).
- Few drops of the stain were poured to cover the film.
- After 30 -60 seconds, buffer solution was added on it and mixed.
- The films were washed after 8- 10 minutes with buffered water/ distilled water.

Fixation, decalcification and processing of trephine biopsy

- Fixation is a complex series of chemical events and differs for different group of chemical substances found in the tissue.
- The aim of fixation includes that the fixed tissue should not change shape or volume during subsequent procedures, should be left in condition which allow, subsequently, clear staining of sections and process of autolysis and bacterial attack should be prevented.
- The fixative used for trephine biopsy specimen, was made by :
  - 40% formaldehyde (100 mL)
  - NaCl (9 g)
  - Distilled water (900 mL)
- The trephine biopsy was kept for overnight in the 15- 20 times volume of the fixative.
- Decalcification is the procedure to remove out the calcium from the specimen, so that further processing and cutting of the specimen can be carried out easily.
- The solution used for decalcification of trephine biopsy specimen, was prepared by
  - 10% Formic acid (20 mL).
  - 40% Formaldehyde (5 mL).
  - Distilled water (90 mL).
- The trephine biopsy specimen was kept for 48 hours in the 15- 20 times volume of the decalcifying agent.
- After appropriate decalcification, the biopsy specimen was put for overnight tissue processing.

❖ The aim of tissue processing is to embed the tissue in a solid medium, firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut and soft enough not to damage the knife or tissue.

❖ Overnight processing schedule was as follows
  - 10% formalin.....8 hours
  - 10% formalin +10%alcohol.....1 hour
  - 100% alcohol.....1 hour
  - 100% alcohol.....1 hour
  - 100% alcohol.....1 hour
  - 100% alcohol.....1 hour
  - 100% alcohol.....1 hour
  - Pure chloroform.....1 hour
  - Pure chloroform.....1 hour
  - Pure chloroform.....1 hour
  - Wax .....1.5 hours
  - Wax .....4.5 hours

Hematoxylin & eosin staining for biopsy and clot sections

Preparation of solution:

Harris hematoxylin	2.5 g
Absolute alcohol	25 mL
Potassium alum	50 g
Distilled water	500 mL
Mercuric oxide	1.25 g

- The hematoxylin (2.5 g) was dissolved in the absolute alcohol (25 ml), and then added to potash

alum (50 g), which had previously been dissolved in the warm distilled water in a 500 ml flask. The mixture was boiled and rapidly brought cool and mercuric oxide (1.25 g) was slowly and carefully added.

- For Eosin preparation, Eosin Y was used.
- Alcoholic eosin powder (1 g) was dissolved in absolute alcohol (100 ml) to prepare 1 % eosin solution.

Method

1. Sections were dewaxed & hydrated through graded alcohols.
2. Sections were stained in an alum hematoxylin solution.
3. Sections were washed well in running tap water for 5 minutes.
4. Sections differentiation was done in 1% acid alcohol for 5-10 seconds.
5. Sections were washed again well in tap water for 8-10 minutes.
6. Sections were stained in 1% eosin Y.
7. Sections were washed again well in running tap water for 1-5 minutes.
8. Sections were dehydrated through alcohols, cleared and mounted with the Canada balsam mounting media.

Results

Nuclei: blue/black; cytoplasm: varying shades of pink.

Histological diagnosis

The biopsies processed after decalcification and clot sections were examined for metastatic cells by H&E staining. Aspiration smeras were examined for lymphomatous infiltration by Leishman staining.

Statistics

The data was entered in Microsoft Office Excel version 2007 and analysed using descriptive statistics and later presented in the form of tables and figures.

RESULTS

Table 1: Age distribution of the patients

Age (In Years)	Non-Hodgkin's Lymphoma(N=34)'		Hodgkin's Lymphoma (N=6)		P-Value
	No.	%	No.	%	
<30	12	35.3	3	50.0	0.50
30-40	6	17.6	0	0.0	
>40	16	47.1	3	50.0	
Mean±SD	41.97±21.13		35.83±18.04		

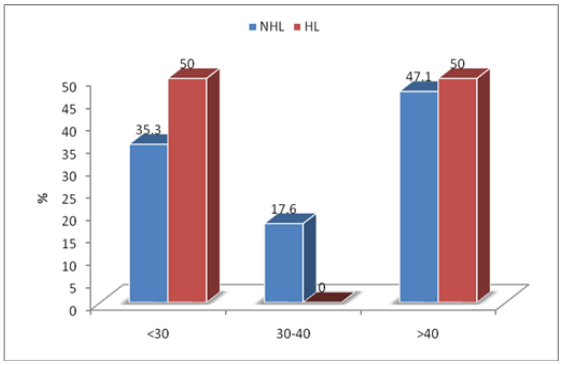

Figure 1: Age distribution of the patients

Table 2: Sex distribution of the patients

Sex	Non-hodgkin's lymphoma (n=34)		Hodgkin's lymphoma(n=6)		p-value
	No.	%	No.	%	
Male	20	58.8	5	83.3	0.25
Female	14	41.2	1	16.7	

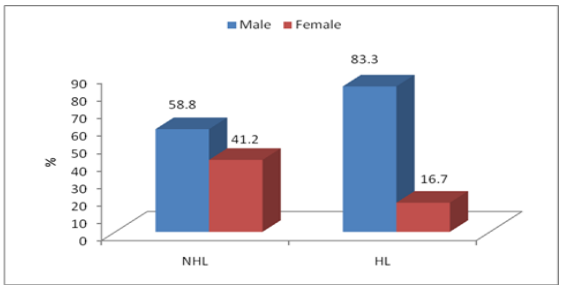

Figure 2: Sex distribution of the

Table 3: Frequency of infiltration by subtypes of lymphomas

Types of lymphoma	No. of patients	Trephine Biopsy Infiltration			
		Positive		Negative	
		No.	%	No.	%
Hodgkin's (Mixed Cellularity)	3	1	33.3	2	66.7
Hodgkin's Lymphoma (Lymphocytic Predominance)	1	0		1	100.0
Hodgkin's Lymphoma (Nodular Sclerosis)	2	0	0	2	100.0
Non-Hodgkin's Lymphoma (Anaplastic Large Cell Type)	3	0	0	3	100.0
Non-Hodgkin's Lymphoma (Angioimmunoblastic Lymphoma)	1	1	100.0	0	0.0
Non-Hodgkin's Lymphoma (Dlbcl)	11	1	9.1	10	90.9
Non-Hodgkin's Lymphoma (Follicular Type)	5	1	20.0	4	80.0
Non-Hodgkin's Lymphoma (Large Cell Type)	3	2	66.7	1	33.3
Non-Hodgkin's Lymphoma (Lymphoblastic Lymphoma)	4	3	75.0	1	25.0
Non-Hodgkin's Lymphoma (Mantle Cell Lymphoma)	2	1	50.0	1	50.0

Patient's age ranged from 13 to 87 years. About half of the patients of Non-Hodgkin's Lymphoma group (47.1%) and 50% of Hodgkin's lymphoma group were >40 years of age. However, 35.3% of Non-Hodgkin's and 50% of Hodgkin's Lymphoma were <30 years of age. More than half of the patients in both Hodgkin's (58.8%) and Non-Hodgkin's Lymphoma (83.3%) were males. Sole case of Angioimmunoblastic Lymphoma showed marrow infiltration (100%) otherwise lymphoblastic

lymphoma type (75% i e. 3 out of 4 cases) was the most common type followed by large cell types and small cell types with equal frequencies of (66.7%) among Non Hodgkin's Lymphoma cases. In Hodgkin's Lymphoma, mixed cellularity was most common type to involve bone marrow (33.3%). The percentage of diagnostic efficacy of marrow infiltration was highest in Trephine biopsy (32.5%) followed by Bone marrow aspiration (27.5%) and Clot section (22.5%) however the differences was not statistically significant.

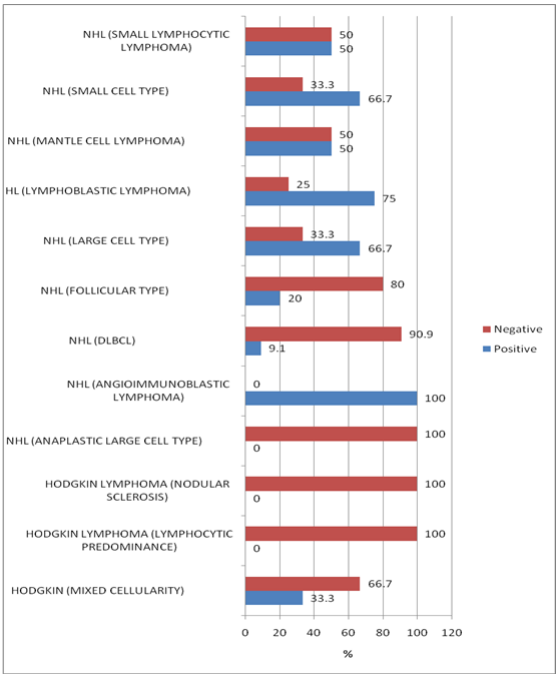

Figure 3: Frequency of infiltration by subtypes of lymphomas

Table 4: Comparison of diagnostic techniques n=40

Diagnostic techniques	Positive		Negative	
	No.	%	No.	%
Bone marrow aspiration	11	27.5	29	72.5
Clot section	9	22.5	31	77.5
Trephine biopsy	13	32.5	27	67.5

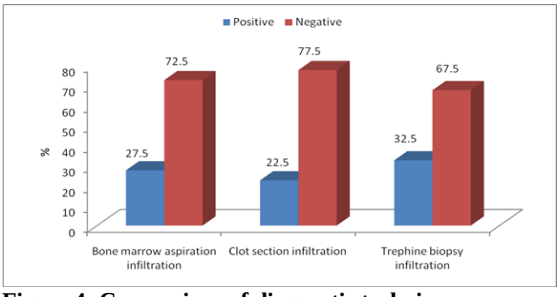

Figure 4: Comparison of diagnostic techniques

DISCUSSION

In our study we tried to detect lymphomatous infiltration of bone marrow by varieties of lymphomas and also noted frequency of infiltration by various types of lymphomas involving bone marrow. A total of 40 patients of lymphomas were included in the study. The groups comprised of 34 patients of NHL (n =34) and 6 patients of HL (n=06). In our study, majority of cases in Hodgkin's lymphoma group showed bimodal age distribution that is below 30yrs and after 40yrs, while the cases of Non-Hodgkin's lymphoma involved all age groups but more commonly above 40yrs which brings this in accordance with most of studies done.[7, 8]

Also our study shows that males are more commonly involved than females in both the type of lymphomas which is in agreement with the studies done by Muzahir et al who observed a 10: 1 male to female ratio. The studies by Cheema et al and P. Geetha et al also showed a slight male preponderance. In our study there were 25 males and 15 females which correlate with majority of above mentioned studies.[9-11]

Incidence of lymphoma infiltrating bone narrow on marrow aspiration in our study was 29.4 % (NHL, 10/34) and a bit less 16.7 % in Hodgkin's lymphoma, 1/6). It was closer to lower side in frequency as compared to study of E J Lim et al done on 47 patients that found 66% lymphoma infiltrating bone marrow, but other studies showed 32% (Conlan MG et al), 35.4% (Subira M et al), and 33.8% (Dominis M et al) infiltration which was closer to what we observed in our study.[12-15]

Frequency wise angioimmunoblastic lymphoma had 100% frequency of infiltration but as there was only a single case of this type we did not consider it. It was lymphoblastic lymphoma that had maximum incidence of bone marrow infiltration (75%) in our study that was also the case with study by E J Lim et al where they found 6 out of 10 patient of lymphoblastic lymphoma had marrow infiltration (60%), followed by large cell types and small cell types with equal frequencies of 66.7% while DLBCL was least common of all types to involve bone marrow.

Arber DA et al observed Follicular lymphoma (30.4%) was the most common type to involve the marrow followed by DLBCL. Hence only few studies are in agreement to our result.[16] This may be due to the number of cases of particular lymphoma cases we got was different from others.

Trephine biopsy was superior to aspiration and clot section in detecting lymphomatous infiltration in bone marrow (35.3%) in our study as depicted in table 4 & fig 4, as compared to 29.4 % & 23.5% on aspiration and clot section respectively. Findings are in agreement with most of studies done before, the reason being that in trephine biopsy provide entire topography in addition to morphology for survey. Mazhar N et al studied on 149 patients and detected 43% patients as having marrow infiltration by bone marrow trephine biopsy while yield on aspiration and clot section was only 3% and 7% respectively.[17]

However most of the study showed superiority of clot section over bone marrow aspiration in detecting marrow infiltration as it provides more marrow fragments for assessment but in our study aspiration was superior may be due technical problems in processing of clots in some cases.

CONCLUSION

Our study showed that males are more commonly involved than females in both the type of lymphomas with majority of cases in both lymphoma group showed bimodal age distribution that is below 30yrs and after 40yrs. Most common type to infiltrate bone marrow was lymphoblastic lymphoma (75%) followed by large cell types and small cell types with equal frequencies (66.7%). Biopsy was superior to aspiration and clot section in detecting lymphomatous infiltration of bone marrow.

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