Histomorphologic and Immunohistochemical Study of Lymph-Node Biopsies in Generalised Lymphadenopathy

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ABSTRACT

BACKGROUND

"Lymphadenopathy" refers to nodes that are abnormal in size, consistency, or number. It is designated as generalised" if lymph-nodes are enlarged in two or more non-contiguous areas. Approximately three fourths of patients presenting with unexplained lymph-node enlargement have localised lymphadenopathy, whereas one fourth have generalised lymphadenopathy. In the present study an attempt is made to identify and categorise various neoplastic versus non-neoplastic lesions in patients presenting with generalised lymphadenopathy in a tertiary care centre.

METHODS

A cross sectional study of 100 lymph-node biopsies was conducted. After adequate fixation in 10 % formalin, tissues were routinely processed and stained with haematoxylin and eosin (H & E). Special stains like Ziehl-Neelsen and reticulin were employed whenever indicated. Immunohistochemistry (IHC) was performed for all neoplastic lesions and for some cases of non-neoplastic lesions.

RESULTS

Amongst 100 cases of lymph node biopsies analysed in this study, 71 cases were non-neoplastic and 29 were neoplastic. The most common non-neoplastic lesion observed was granulomatous lymphadenitis (32 cases), followed by reactive lymphadenitis (20). 25 cases of non-Hodgkin lymphoma (NHL) and 4 cases of Hodgkin lymphoma were observed in the neoplastic group. In the age range of 4.5 to 77 years included in the study, major bulk of cases was found in the age group of 11 - 20 years; out of this, 78.9 % were non neoplastic and 21.1 % were neoplastic. It was observed that 100 % of cases in the age group below 10 years were non-neoplastic, whereas in the age group above 70 years, all cases were neoplastic.

CONCLUSIONS

Granulomatous lymphadenitis was the most common lesion observed, probably because of high incidence of tuberculosis in this part of state. Morphological evaluation by light microscopy supplemented by special stains and IHC are necessary for establishing final diagnosis of generalised lymphadenopathy.

KEYWORDS

Generalised Lymphadenopathy, Haematoxylin and Eosin, IHC, Granulomatous Lymphadenitis, Kikuchi Fujimoto Lymphadenopathy, Hodgkin Lymphoma, Non-Hodgkin Lymphoma

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BACKGROUND

The lymphatic system forms one of the most pervasive and important part of our anatomy. Lymphadenopathy is a rather common clinical finding in a primary healthcare setting. It can be an incidental finding in patients being examined for various other reasons, or it may be a presenting sign and symptom of patient's illness. Correlation between clinical finding and laboratory data is essential in arriving at a diagnosis.[1] The microscopic diagnosis of a lymph-node lesion is extremely difficult; probably more errors are made in this field than in any other organ as reticuloendothelial cells possess a remarkable power of reacting to an irritant by proliferation and hyperplasia, so at times it is impossible to decide whether we are dealing with an inflammatory condition or a true neoplasm.[2] The present study is an attempt to identify and categorise various diseases of the lymph node and to determine the frequency of each lesion.

METHODS

This is a hospital based cross sectional study of 100 lymph-node biopsies obtained from the cases of generalised lymphadenopathy in the Department of Pathology, Government Medical College, Thrissur, Kerala from December 2012 - June 2014. Patients with localised lymph-node enlargement were excluded from the study.

Tissue Preparation

The specimens were fixed in 10 % formalin for 24 hours after recording the gross morphological features. The number and size of nodes, shape, colour, consistency, appearance on cut section, presence of necrosis, matting and appearance of capsule were noted. 5 mm thick bits (at least 2) were submitted for processing, 4 – 5 micron thick sections were cut with a microtome and stained with haematoxylin and eosin stain. Special stains like Ziehl-Neelsen (ZN), reticulin were employed wherever indicated. The stained sections were studied by light microscopy.

Immunohistochemical Staining

All cases of lymphoproliferative disorders, diagnosed by light microscopy, were taken up for IHC. The individual NHL cases were classified according to the World Health Organization (WHO) classification based on positive and relevant negative immunophenotypic expression. IHC studies were also carried out in non-neoplastic conditions for demonstrating particular findings that aided in an accurate diagnosis. Panel of antibodies used in a given case was dependent on the morphological evaluation and varied from 3 to 10. Immunohistochemistry was performed according to avidin-biotin peroxidase complex method using positive and negative controls as required.

IHC Panel Employed in the Study

The panel of antibodies used were monoclonal antibodies to CD 3, CD 4, CD 5, CD 8, CD 10, CD 15, CD 20, CD 23, CD 30, CD 45, CD 68, CD1A, BCL-2, BCL-6, Cyclin D1, ALK-1, Tdt, Ki 67 and S100 and polyclonal antibodies to myeloperoxidase (MPO).

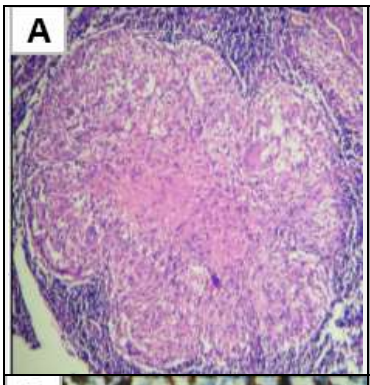


Figure 1a. Granuloma with Central Necrosis [400X.H&E]

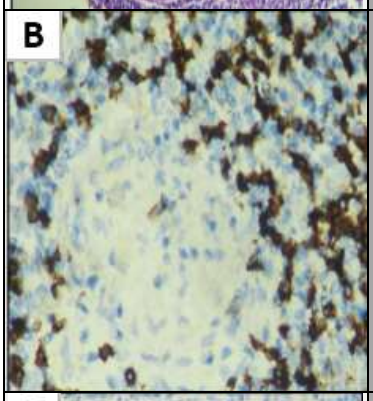


Figure 1b. Lymphocytes around Granuloma Expressing CD4 Positivity [400X]

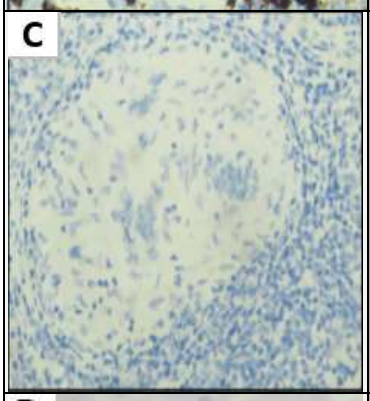


Figure 1c. CD20 Negativity Reveals Absent B Lymphocytes [400X]

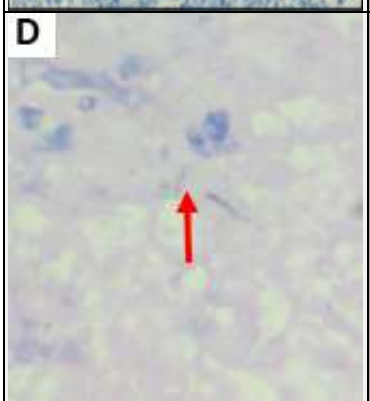


Figure 1d. Area of Necrosis Demonstrating Acid Fast Bacilli [Arrow] (Ziehl-Neelsen Stain) [1000X]

Figure 1. TB Lymph-Node

Tuberculosis (TB) lymph-node: A) Granuloma with central necrosis [400X. H&E] B) Lymphocytes around granuloma expressing CD 4 positivity [400X]. (C) CD 20 negativity reveals absent B lymphocytes [400X]. (D) Area of necrosis demonstrating acid fast bacilli [arrow] (Ziehl-Neelsen stain) [1000X]

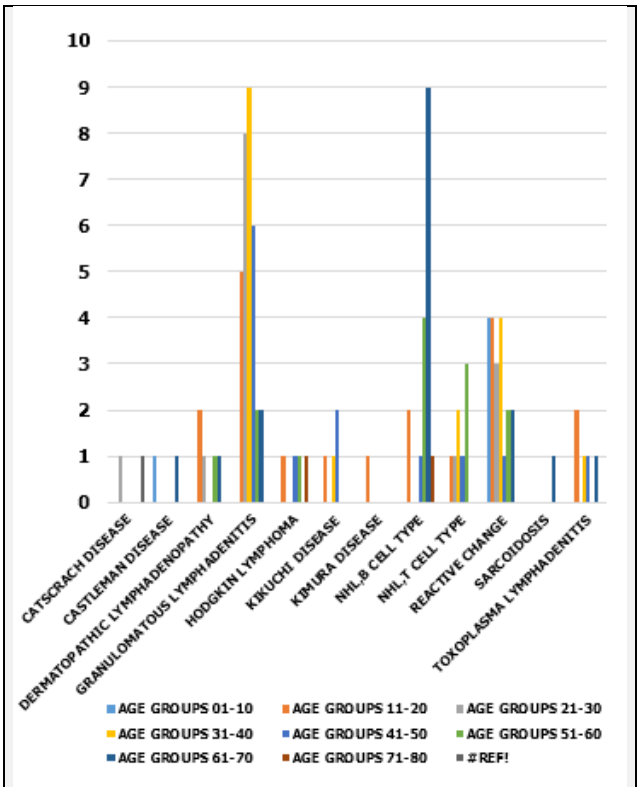
Statistical Analysis

Data was collected, compiled and then analysed using SPSS software version 21. The quantitative and qualitative variables were expressed in terms of percentages.

RESULTS

Granulomatous lymphadenitis was the most common cause (32 %) of non-neoplastic lymphadenitis. Maximum number of cases (9) was found in the age group of 31 - 40 years. 5 cases (1.5 %) showed positivity for acid fast bacilli AFB with ZN stain. IHC demonstrated that CD 4 +ve lymphocytes were more than CD8 + ve cells within the granuloma and CD 20 positive B cells were absent around granulomas (Figure 1).

Reactive lymphadenitis was seen in 20 cases. It was seen more commonly in patients below 30 years with a slight male preponderance of 3:1 ratio. Silver staining for reticulin demonstrated compressed reticulin network around follicle, germinal centre were negative for BCL2 antibody immunostaining.



Graph 1. Age Wise Distribution of Lesions

Dermatopathic lymphadenopathy (5 cases) was seen in less than 30 years of age. Marked paracortical expansion with irregular nodular pale areas adjacent to the subcapsular sinuses composed of interdigitating dendritic cells (S100 +ve) and Langerhans cells (S100 & CD1a +ve) admixed with, histiocytes containing brown pigment (melanin / haemosiderin) in their cytoplasm were taken as key histological findings.

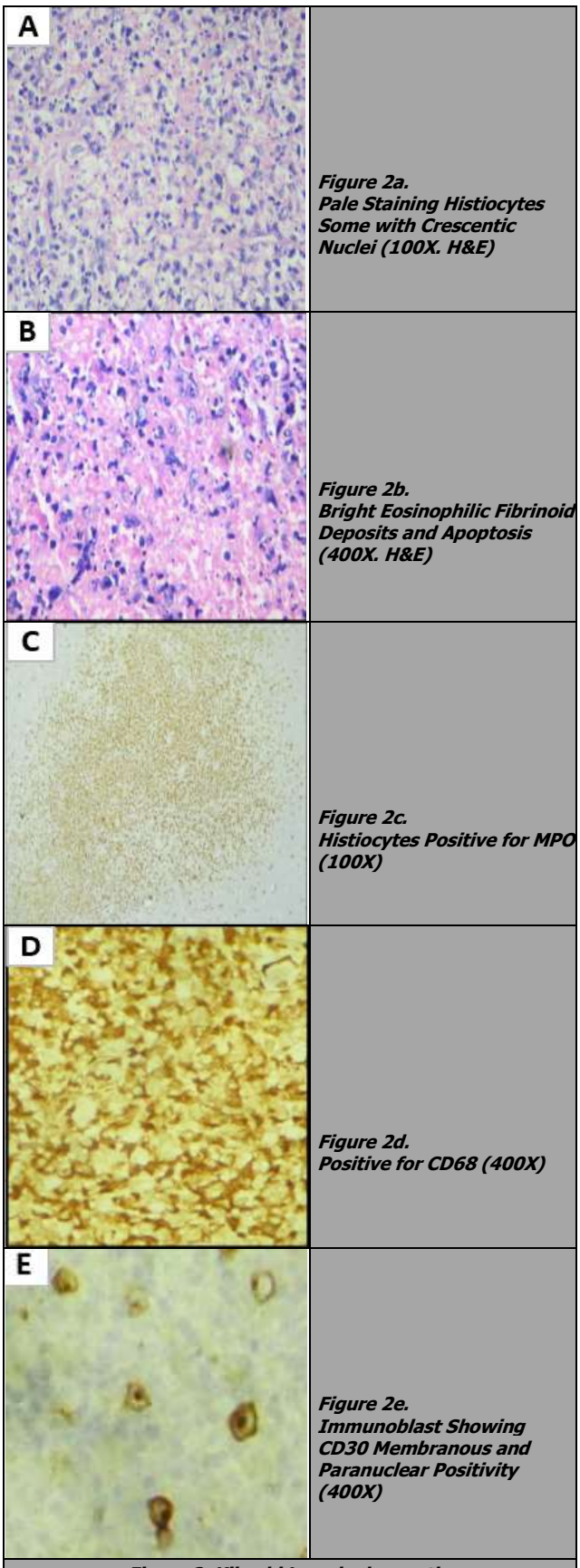


Figure 2a. Pale Staining Histiocytes Some with Crescentic Nuclei (100X. H&E)

Figure 2b. Bright Eosinophilic Fibrinoid Deposits and Apoptosis (400X. H&E)

Figure 2c. Histiocytes Positive for MPO (100X)

Figure 2d. Positive for CD68 (400X)

Figure 2e. Immunoblast Showing CD30 Membranous and Paranuclear Positivity (400X)

Figure 2. Kikuchi Lymphadenopathy

Kikuchi lymphadenopathy: A) Pale staining histiocytes some with crescentic nuclei (100X. H&E) B) Bright eosinophilic fibrinoid deposits and apoptosis (400X. H&E). C) Histiocytes positive for MPO (100X) and (D) for CD68 (400X). E) Immunoblast showing CD 30 Membranous and paranuclear positivity. (400X)

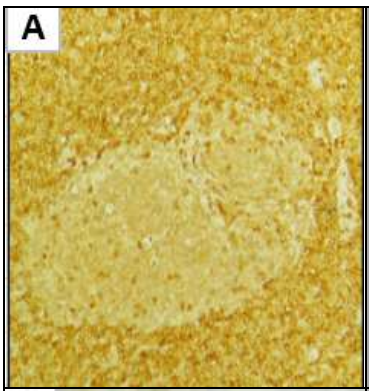


Figure 3a. Expanded Mantle Zone Showing BCL2 Positivity [400X]

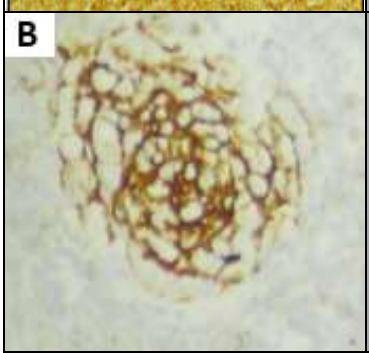


Figure 3b. Concentric Rings of Follicular Dendritic Cells Expressing CD 23 Immunostain [400X]

Figure 3. Castleman Disease Hyaline Vascular Type

Castleman disease hyaline vascular type: (A) Expanded mantle zone showing BCL2 positivity [400X]. (B) Concentric rings of follicular dendritic cells expressing CD 23 immunostain. [400X]

Toxoplasma lymphadenitis accounted for 5 % of lesions; most common age group affected was 11 - 20 years with slight male predominance. Follicular hyperplasia and presence of microgranulomas were most consistent findings. Kikuchi disease was observed in 4 persons with equal M:F ratio with common age being 41 - 50 years. (Figure 2). Out of 2 cases of Castleman disease, one was hyaline vascular sub type and other was plasma cell subtype. (Figure 3). There was one case each of cat-scratch disease, Kimura disease and sarcoidosis.

Out of 29 neoplastic lesions, 25 (86.2 %) were non-Hodgkin lymphoma and 4 (13.8 %) were Hodgkin lymphoma.

In cases of Hodgkin lymphoma, age group ranged from 11 - 80 years and male to female ratio was 1:1. Commonest histologic subtype observed was lymphocyte rich (50 %) followed by mixed cellularity and unclassifiable (25 % each). Half of the cases had stage III disease at the time of diagnosis.

In non-Hodgkin lymphoma, maximum number of cases (9) was in the age group of 61 to 70 years with male to female ratio of 3:2. Among all NHLs, 17 cases (68 %) were of B cell type and 8 (32 %) were of T cell type based on immunophenotyping.

DISCUSSION

In this study, we have attempted to classify common causes of generalised lymphadenopathy in 100 lymph-node biopsy specimens. Non-neoplastic lesions accounted for most of the cases of lymphadenopathy.

It was observed that the commonest cause of lymphadenopathy was granulomatous lymphadenitis (32 %). This is attributed to high prevalence of tuberculosis in this geographical area. Among 32 cases, only 5 cases (1.5 %) showed positivity for AFB with Z N stain. Therefore, present study and previous studies prove that histopathology remains one of the most important methods for diagnosing tuberculosis.[3] IHC of tuberculous granulomas were as similar to other studies.[4,5,6]

Out of 20 cases of reactive lymphadenitis, 15 cases (75 %) showed follicular hyperplasia, 4 cases (20 %) showed paracortical hyperplasia and 1 case (5 %) showed sinus histiocytosis, which concurred with previous studies.[7]

Kikuchi Fujimoto lymphadenopathy accounted for 5.6 % of all non-neoplastic lesions, with no female predominance. Immunohistochemically histiocytes were positive for both MPO and CD 68. Variable number of CD 4 +ve and CD 8 +ve T lymphocytes were present at periphery of necrotising areas and in some cases CD 4 cells outnumbered, a few of the cases showed occasional CD 30 positivity in immunoblast and atypical monocytes as were seen in previous studies.[8,9,10,11] The histopathologic findings of toxoplasma lymphadenitis cases in our study are comparable with study by M Eapen et al.[12] The histiocytes in dermatopathic lymphadenitis were negative for CD 68 and positive for S100 and CD1a. 40 % of cases in the study did not have obvious skin lesions.[13]

In Castleman disease, CD 23 expression in follicular dendritic cell network in atrophic follicles and variable BCL 2 expression in mantle zone and negative expression in germinal centre are in agreement with previous study.[14]

All the neoplastic lesions in this study were lymphomas either Hodgkin or non-Hodgkin lymphoma. Immunohistochemistry was applied to accurately phenotype the abnormal population detected by morphology and classified according to WHO classification. Metastasis was not observed as a cause of generalised lymphadenopathy in present study as compared to previous studies. This may be because of early detection rate of cancers in this part of Kerala. Lymphoma constituted 29 % of all cases in the study, Hodgkin lymphoma was seen in 4 / 29 cases (13.8 %) in the age group ranging from 11 - 80 years and M:F ratio was 1:1.

In current study most common subtype was lymphocyte rich (50 %) followed by mixed cellularity (25 %). This is in disagreement with most of the previous studies.[15,16] This disparity may not be of much significance as the number of cases studied is much low in this study compared to previous studies. 50 % of our patients had stage III disease at the time of diagnosis.

Non-Hodgkin lymphoma constituted 25 / 29 cases (86.2 %) of all lymphoid malignancies. NHL was more common in older adults and male to female ratio was 3:2. Lymphoma of B-cell type was more than that of T-cell type. 32 % of all NHLs were found to be diffuse large B cell lymphoma (DLBCL) followed by follicular lymphoma (23.5 %).

Peripheral T cell lymphoma (PTCL) comprised the major bulk of T cell lymphoma (37.5 %). Similar observations were made by Roy et al and Naresh K N et al.[17,18]

CONCLUSIONS

Granulomatous lymphadenitis was the most common lesion observed in our study. The age of patients ranged from 4.5 years to 77 years. To conclude, the varied aetiology of generalised lymphadenopathy observed at our centre, as documented by histopathological evaluation, indicates that there is a need for methodological approach to generalised lymphadenopathy that can disclose the accurate diagnosis. Uncommon aetiological causes should be considered in the differential diagnosis of generalised lymphadenopathy.

Morphologic evaluation by light microscopy supplemented by special stains and immunohistochemistry are necessary for establishing the final diagnosis of generalised lymphadenopathy. Large series studies and long term follow up are necessary for better understanding and substantiating results obtained from the present study.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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