



## Histopathological study of Lymph node lesions

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### Abstract:

Lymph node biopsy and histopathological examination (HPE) is an extremely important investigation helpful in differentiating between reactive and neoplastic states including lymphomas, sarcomas, histiocytosis and leukemias. Lymph node biopsy is also helpful in differentiating between primary and secondary malignant lesions of the lymph node. The present study was carried out in the Department of Pathology, Kannur Medical College, Kannur, on patients with clinically palpable lymph nodes i.e. either localised or generalised lymphadenopathy. The cervical lymph node was excised in preference to axillary and inguinal nodes. Among the non-neoplastic lesions tuberculous lymphadenitis (34.2%) and among the neoplastic lesions, metastatic carcinomas (19.90%) were the most common. Lymphomas comprised approximately 4% of the cases. Non-neoplastic lesions were most common in the 20-30 year age group whereas neoplastic lesions were most common in the 51-60 year age group. HPE on properly selected and excised lymph nodes enables one to arrive at an accurate diagnosis.

**Key words:** Lymph node, Biopsy, Non-neoplastic, Neoplastic, Metastasis, Lymphoma

### Introduction:

Lymph nodes are organised to detect and inactivate foreign antigens arriving via the environment, that is, the skin, respiratory tract and gastrointestinal tract. Lymph nodes contain most of the peripheral components of the immune apparatus: mononuclear phagocyte elements throughout (as phagocytic cells in the cortex and sinuses, and antigen processing cells in the follicular and interfollicular areas), B-cells, mainly in primary or secondary follicular centres, and various T-cells, primarily in the paracortex. The cortex is the outer, convex portion of the node in which B-cell lymphoid follicles are scattered. Primary follicles are formed of aggregates of small lymphocytes with round or slightly irregular nuclei, condensed chromatin and scant cytoplasm. The secondary follicle is composed of centroblasts (large non-cleaved cells with vesicular nuclei, multiple nucleoli and scant amphophilic cytoplasm) and centrocytes (small cleaved cells with

angulated or folded nuclei, fairly dense chromatin, indistinct nucleoli and scant cytoplasm). These follicular centre B-cells are intermingled with tingible body macrophages, follicular dendritic cells and few intrafollicular T-cells. The follicles are surrounded by mantle zone and marginal zone composed of medium sized cells with moderate amount of pale to clear cytoplasm. The paracortex (T-cell rich) is just deep to and between the follicles whereas the medulla (plasma cell rich) is in the deep portion of the node. The efferent lymphatics leave the node at the hilum.<sup>1</sup>

Lymph nodes are arranged in groups along the lymphatics. The major groups of lymph nodes include occipital, posterior and anterior auricular, cervical, axillary, epitrochlear, inguinal, pulmonary hilar, mediastinal, intraabdominal and retroperitoneal. Some locations are more frequently associated with certain causative agents which are therefore considered suggestive of the cause of

lymphadenopathy. Poor selection of biopsy site, poor selection of lymph node to be sampled, and improper removal of the lymph node can cause misdiagnosis of lymph node pathology.<sup>2</sup>

Lymph nodes may be the only site of disease; however, most nodal disease is related to abnormalities in the organ associated with the abnormal node. Nodal diseases are complex because of the large number of diseases reaching nodes via lymph and because of the inherent complexity of the immune system and its own diseases.

Nodal biopsy is deceptive in its apparent simplicity. All nodes are not affected equally in patients; disease often increases nodal fragility, and removal of intact nodes in these cases requires skill and patience.

Lymph node biopsy can also be used to differentiate among lymphomas, carcinomas, histiocytosis & leukemias. Lymph node biopsy is useful to differentiate between primary & secondary malignant lesions of the lymph node.<sup>3</sup>

The aim of the present study was to differentiate between inflammatory (reactive) and neoplastic states of the lymph nodes, to find out the frequency and incidence of various lesions with respect to age, sex and lymph node involved, as well as histopathological subtyping and grading of malignant tumours in order to assess the prognosis.

#### **Aims and Objectives:**

1. To find out the frequency of various lesions affecting the lymph nodes
2. To detect the incidence of the various lesions with respect to age, sex and lymph nodes involved
3. To find out the histopathological subtype of tumours in case of malignant lymphomas.
4. To determine the histopathological grading of malignant tumours in order to assess the prognosis.

#### **Materials and Methods:**

The present study was conducted in

the Department of Pathology, Kannur Medical College, Kannur. The study was carried out on patients with clinically palpable lymph nodes i.e. either localised or generalised lymphadenopathy. A detailed clinical history including presenting complaints, past and personal history was taken.

In cases where there was only one node enlarged, the excised lymph node, and in case of generalised lymphadenopathy, the cervical lymph node which was excised in preference to the axillary & inguinal nodes formed the material for this study. In case of axillary clearance and excision specimens like intestines, the lymph nodes were carefully located and separated.

The lymph nodes were examined and relevant information regarding the site, number, size, shape, consistency, adherence and mobility of the lymph nodes were noted.

The specimens thus received were fixed in 10% neutral buffered formalin. After fixing for 12-24 hrs, the lymph nodes were cut in the sagittal plane using a sharp fresh blade. The cut surface was inspected and any caseation, necrosis, haemorrhage, calcification, if present were recorded. Bits were taken from the representative areas and when the biopsy was small, the entire tissue was processed.

The lymph node sections were cut to 4 µm thickness. In addition to staining with Haematoxylin & Eosin, Zeihl-Neelsen stain (Z-N Stain), reticulin stain, Periodic Acid-Schiff (PAS stain) and mucicarmine stain were performed when indicated.

#### **Results:**

The present study consisted of 201 cases of lymph node biopsies (Figure I). Out of the total 201 cases, 152 were non-neoplastic lesions (75.62%) and 49 were neoplastic lesions (24.37%).

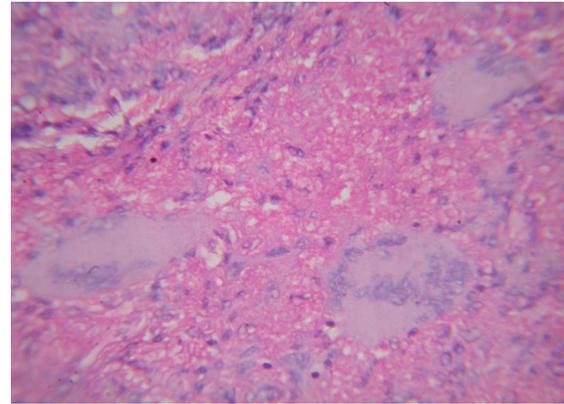
There were 72 male patients and 129 female patients in the present study.

Of the 152 non-neoplastic lesions, 69 cases (34.32%) were of tuberculous

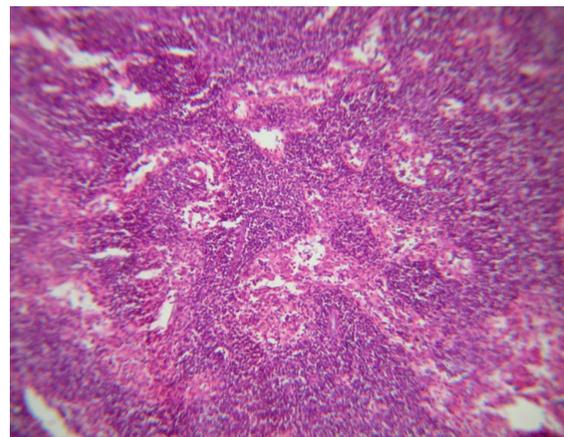
lymphadenitis (Figure II), 39 cases (19.40%) were reactive lymphadenitis, 21 cases (10.44%) were non-specific lymphadenitis, 12 cases (5.97%) were sinus histiocytosis (Figure III), 8 cases (3.98%) were follicular hyperplasias and one case each (0.497%) were dermatopathic lymphadenitis and Langerhan's cell histiocytosis (Figure IV). In one case, the biopsy was inadequate but a few reactive follicles were seen.

30years age group (46 cases- 30.26%). Doubtful cases of tuberculous lymphadenitis with ill-formed granulomas were confirmed by Z-N stain.

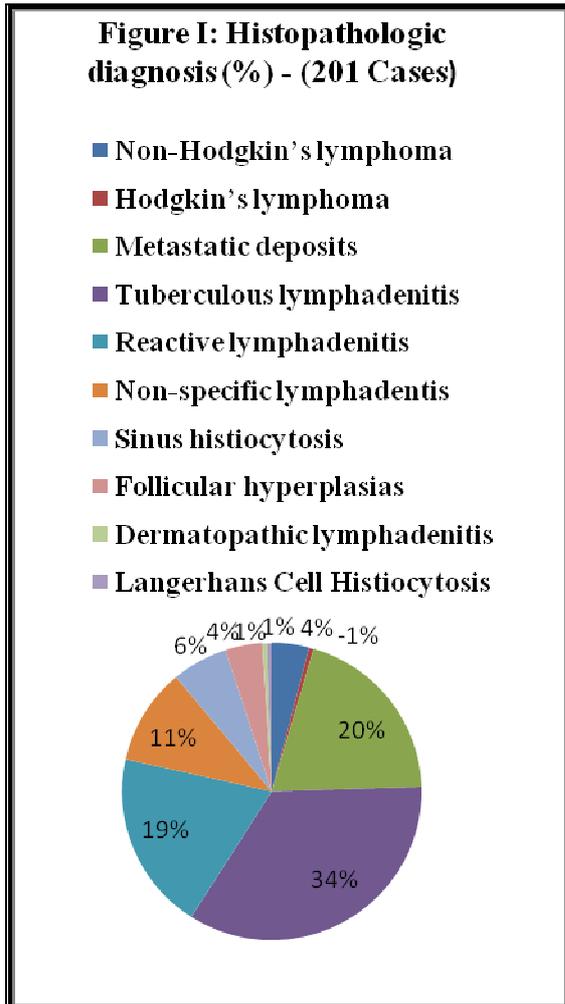
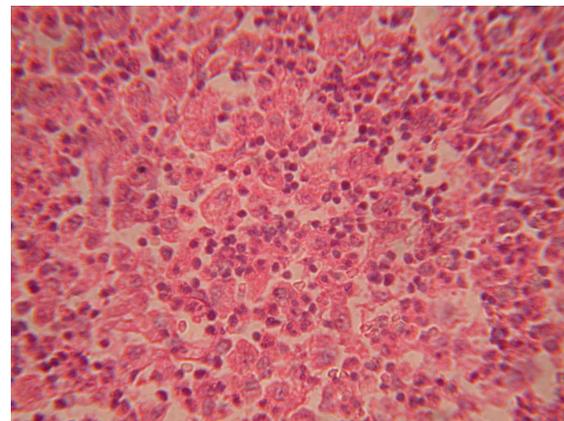
**Figure II: Tuberculous lymphadenitis showing Langhans giant cells (H & E, 40X)**



**Figure III: Sinus histiocytosis – Lymph node (H & E, 10X)**



**Figure IV: Langerhan cell histiocytosis – Lymph Node (H & E, 40X)**



The commonest site of lymph node biopsy among non-neoplastic lesions was cervical lymph nodes in 78 cases (38.80%) followed by axillary lymph nodes in 42 cases (20.89%).(Figure V)

There were 60 male and 92 female patients among the 152 patients with non-neoplastic lesions. The age distribution of non-neoplastic lesions ranged between 1½ months to 70 yrs. The maximum number of non-neoplastic lesions were in the 20–

Out of the 49 cases of neoplastic lesions, 8 cases were non-Hodgkin's lymphoma, 1 case was of Hodgkin's lymphoma (Table I) and 40 cases were metastatic deposits in lymph nodes. Of these, 17 were male patients and 32 were female patients. The age distribution of the neoplastic lesions ranged between 17 and 70 years. The maximum of 13 cases were seen in the age group 51-60years and the lowest number of 2 cases was seen in the age group 11-20 years.

Of the 49 cases, 13 cases presented with cervical lymph node enlargement, 27 cases with axillary lymph node enlargement, 4 cases with perigastric lymph node, 1 case each with inguinal lymph node, preauricular lymph node, lymph node around sigmoid colon and mesenteric lymph node. In one case, the site of biopsy was not mentioned.

The maximum of 3 cases (33.3%) of lymphomas were in the 61-70 year age group; of the 9 lymphoma cases, 7 were males.

Non-Hodgkins lymphomas were predominant in the 21-30 years & 51-70 years age group (2 cases each). Six of the 8 cases of non-Hodgkin's lymphomas were in males. All cases of non-Hodgkin's lymphomas showed increase in the reticulin fibres within the lymph node (Figure VI).

A single case of Hodgkin's lymphoma was encountered. The patient was male, aged

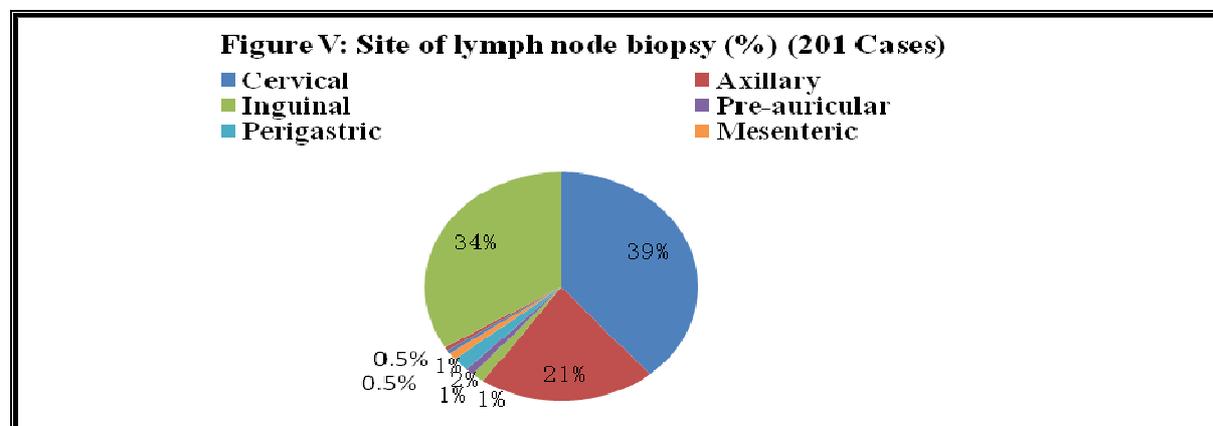
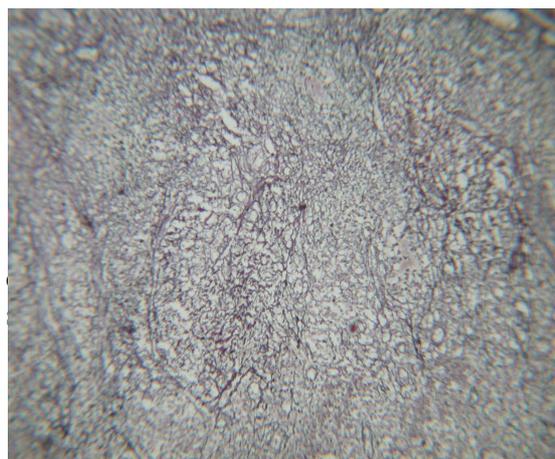
65 years with generalised lymphadenopathy.

**Metastatic deposits in lymph nodes:**

Of the 40 cases of metastatic deposits in lymph nodes, a maximum of 12 cases were in the 51- 60 years age group. A single case of mucoepidermoid carcinoma showed positive with Periodic Acid Schiff staining. Mucicarmin staining was positive in 6 cases of metastatic adenocarcinoma.

The site of biopsy included axillary lymph node (26 cases), cervical lymph node (6 cases), perigastric lymph node (3 cases) and 1 case each of inguinal, mesenteric, lymph node around sigmoid colon, preauricular lymph node and in one case, the site of biopsy was not mentioned.

**Figure VI: Reticulin stain - Increase in reticulin fibres in a case of lymphoma**



**Table I: Clinical and Histopathological diagnosis of 9 cases of lymphomas**

Sl. No	Clinical Diagnosis	Histopathological Diagnosis
1.	Lymphadenopathy	NHL – Small Cell Type (Low Grade)
2.	Secondary from carcinoma caecum	NHL Small non-cleaved cell (High Grade)
3.	Left submandibular sialadenitis	NHL Small lymphocytic lymphoma (Low Grade)
4.	Secondaries	NHL Diffuse small cell type (Low Grade)
5.	Hodgkin lymphoma	NHL Diffuse small cell type (Low Grade)
6.	Lymphoma/ TB	HL Lymphocytic predominance type
7.	TB/Hodgkin lymphoma	NHL Diffuse mixed small and large cell type (Intermediate Grade)
8.	Lymphoma/ TB	NHL Diffuse mixed small and large cell type (Intermediate grade)
9.	TB	NHL Diffuse mixed small and large cell type with predominance of large cells

**Table II: Primary site of malignancy and the Histopathological diagnosis**

Primary site of malignancy	Histopathological diagnosis	Number of cases
Breast	Infiltrating lobular carcinoma	3
	Infiltrating ductal carcinoma	19
	Medullary carcinoma	3
Parotid	Mucoepidermoid carcinoma	1
Thyroid	Papillary carcinoma	1
Lung	Small cell carcinoma	1
Stomach	Adenocarcinoma	4
Colon	Adenocarcinoma	1
Unknown	Squamous cell carcinoma	5
	Adenocarcinoma	1
	Metastatic anaplastic carcinoma	1

The primary site of malignancy included breast in 25 cases, stomach in 4 cases, and parotid, thyroid, lung, and colon in 1 case each. In the remaining 7 cases, the primary site of malignancy was unknown (Table II).

### Discussion:

Lymph node biopsy and histopathological examination is a simple & effective aid in the diagnosis of various lesions of the lymph node. Although FNAC can provide a reliable clue in the diagnosis in many cases, only HPE provides a conclusive proof of disease. Several studies have been conducted to detect the frequency of lesions affecting the lymph nodes in a particular geographical area. Studies on lymph node biopsies by Albasri AM et al<sup>4</sup> and Roy A et al<sup>5</sup> have demonstrated the reliability of biopsy in the diagnosis of

lymphadenopathy. In these studies, a large number of lymph node biopsies were evaluated and frequency distribution with regard to age, sex, type of lesion etc were tabulated.

Out of the 201 cases of lymphadenopathy that were subjected to biopsy, 152 were non-neoplastic lesions (75.62%) and 49 (24.37%) were neoplastic lesions; whereas in the study by Albasri et al<sup>4</sup> the non-neoplastic lesions were 47.1% and neoplastic lesions accounted for 52.9%. Roy A et al<sup>5</sup> in their study recorded an incidence of non-neoplastic and neoplastic lesions of 47% and 53% respectively.

Out of the 152 non-neoplastic lesions, 69 cases were tuberculosis lymphadenitis (34.32%), 59 cases (24.35%) were reactive lymphadenitis (including 12 cases (5.97%) of sinus histiocytosis and 8 cases (3.98%) of follicular hyperplasias), 21 cases were non-specific lymphadenitis (10.44%), 1

case was dermatopathic lymphadenitis, 1 case was Langerhan's cell histiocytosis and in one case biopsy was inadequate. This is in contrast to the study by Roy A et al<sup>5</sup> wherein tuberculosis lymphadenitis comprised 18% of the cases and miscellaneous non-neoplastic lesions comprised (29.0%).

Z-N Staining demonstrated acid-fast bacilli as pink coloured rods in a few doubtful cases of tuberculous lymphadenitis with ill-formed granulomas. Weiss et al<sup>6</sup> in their study on benign lymphadenopathy found reactive follicular hyperplasia as the most common pattern. Reactive follicular hyperplasia needs careful differentiation from follicular lymphoma. Kikuchi disease and autoimmune diseases like systemic lupus erythematosus are among the causes of extensive necrosis in lymph nodes which need to be ruled out by immunohistochemistry.<sup>7,8</sup>

Langerhan's Cell Histiocytosis must be differentiated from dermatopathic lymphadenitis, sinus histiocytosis with massive lymphadenopathy and Hodgkin's lymphoma on cytological examination. Histopathology, however demonstrates the pathognomonic Langerhan's cells.<sup>9</sup>

Among the 49 cases of neoplastic lesions in the present study 40 were metastatic lesions (19.90%), 8 cases (3.98%) were non-Hodgkin's lymphoma and one case was Hodgkin lymphoma (lymphocyte predominant type- 0.49%) which differs from the results obtained by Roy A et al<sup>5</sup> wherein metastatic carcinoma comprised 8.5%, non-Hodgkin's lymphoma comprised 32.1% and Hodgkin lymphoma comprised 12.4% both of which are much higher than in the present study.

Gomori's reticulin stain revealed reticulin fibres around individual cells in all cases of non-Hodgkin's lymphomas. Non-Hodgkin's lymphomas have a wide range of histological appearances which makes the diagnosis difficult. Timely diagnosis is

important because effective and often curative therapies are available for many subtypes.<sup>10</sup> Pseudolymphoma induced by hypersensitivity to antiepileptic drug diphenylhydantoin which presents with hepatosplenomegaly, generalised or regional lymphadenopathy, and leucocytosis with eosinophilia is an important condition mimicking non-Hodgkin lymphoma.<sup>11</sup>

Hodgkin's lymphoma is one of the most common non-AIDS defining neoplasm in the HIV+ population and is increasing in incidence in patients treated with anti-retroviral therapy. Older patients above 60 years often present with mixed cellular histology unlike in the present study.<sup>12</sup>

In the study by Kamat G C et al<sup>13</sup> the rates of non-Hodgkin lymphoma to Hodgkin lymphoma was 8:1 which was similar to that found in the present study.

In another study by Rahman M A et al<sup>14</sup> reactive hyperplasia comprised (30.89%), (which was higher than that found in the present study), tuberculosis lymphadenitis (33.5%), non-caseous granuloma (1.05%) and miscellaneous non-neoplastic conditions (4.7%). Among the neoplastic lesions 17.28% were lymphomas which included 22 cases (11.52%) of non-Hodgkin's lymphoma and 11 cases (5.76%) of Hodgkin lymphoma giving a ratio of NHL: HL of 2:1 which differs from the present study (NHL: HL= 8:1). Metastatic lesions comprised 12.57% of the cases which was slightly higher than that found in the present study (Table IV).

In the study by Panchal J et al<sup>15</sup> 31.2% cases were tuberculous lymphadenitis, 15.6% were suppurative lymphadenitis, 20% were reactive hyperplasia, 11.1% BCG adenitis, 2.2% Kimura's disease, 15.6% were lymphoma and 4.4% were metastasis in lymph nodes (which is much lower than in the present study).

In a recent study by Vachhani AB et al<sup>16</sup> the incidence of

**Table III: Distribution of lymph node lesions in various studies**

Condition	Roy A et al <sup>5</sup>	Kamat GC <sup>13</sup>	Rahman MA et al <sup>14</sup>	Present study
Tuberculosis	18%	58.19%	33.5%	34.32%
Reactive change	6.8%	30.73%	30.89%	29.35%
Non-specific lymphadenitis	21.6%	2.20%	–	10.44%
Miscellaneous non-neoplastic conditions	0.6%	-	5.7%	1.49%
Non-Hodgkin lymphoma	32.1%	3.27%	11.52%	3.98%
Hodgkin lymphoma	12.4%	0.40%	5.76%	0.497%
Metastatic carcinoma	8.5%	7.37%	12.57%	19.97%

granulomatous lymphadenitis (24%) and lymphomas (2%) were lower, and non-specific lymphadenitis (51%) and metastatic deposits (23%) were higher than in the present study.

In a similar study by Ochicha et al<sup>17</sup> malignancies were higher comprising 43% of peripheral lymph nodes, with lymphomas predominating, accounting for 24 % compared to 4.47% in the present study. Metastasis comprised 19%; the predominant lymph node involved being axillary (38 %) which is similar to that in the present study.

Similar results were also obtained in a cytopathological retrospective study by Lashiram R S et al<sup>18</sup> wherein 48.28% were metastatic tumors, and 27.5% were tuberculous lymphadenitis highlighting the importance of FNAC in the initial evaluation of enlarged lymph nodes.

The overall concordance rate between clinical and histopathological diagnosis among the 69 cases of tuberculous lymphadenitis was 68.11%, among 9 cases of lymphomas was 33.33% and among the 40 cases of metastatic deposits was 85.0%

#### Sex Distribution:

Out of the 201 cases in the present study, 78 cases (38.80%) were male patients and the remaining 123 were female patients (61.19%) with a male:female ratio of 1:1.57 (M:F). In the study by Albasri AM<sup>4</sup> the M:F ratio

was 1.14:1 and in the study by Rahman M A<sup>14</sup> the M:F ratio was 1:1.2; whereas in the study by Roy A et al<sup>5</sup>, out of the 1010 cases, 63.2% were male patients and 36.8% were female patients with a M:F ratio of 1.7:1.

A statistically significant relationship of sex distribution with observed p-value of 0.008 (significant value  $p < 0.05$ ) was found by the chi-square test ( i.e., females were more prone to both neoplastic and non-neoplastic conditions than males in our study).

#### Site distribution:

The site of lymph node biopsy included cervical lymph nodes in 78 cases (38.80%), axillary lymph nodes in 42 cases (20.89%), inguinal lymph nodes in 3 cases (1.49%) and the remaining from other sites like preauricular, perigastric, mesenteric, lymph node around the sigmoid colon and forearm (Figure V). In 68 cases (33.83%) the site of biopsy was unknown. Whereas, in the study by Rahman M A et al<sup>14</sup> 107 cases i.e. 56% were cervical lymph nodes. Similarly, in the study by Panchal J<sup>15</sup> the majority of the lymph nodes were cervical followed by axillary and inguinal lymph nodes in that order of frequency. Similar results were obtained in the study on benign lymphadenopathies by Weiss LM et al<sup>6</sup>

wherein cervical and axillary nodes were most frequently involved corresponding to the lymph node groups most likely to drain antigens.

### Disease pattern:

In the present study, among the metastatic carcinomas, 5 cases were squamous cell carcinomas (12.5%), 31 cases were adenocarcinomas (77.5%), 1 case anaplastic carcinoma (2.5%), 1 case mucoepidermoid carcinoma (2.5%), 1 case papillary carcinoma (2.5%) and 1 case small cell carcinoma (2.5%); whereas in the study by Saraswat A et al,<sup>19</sup> squamous cell carcinoma accounted for 55.5% which was much higher than in the present study and adenocarcinoma 44.44% which was much lower than in the present study.

The mucin secreting cells in the single case of mucoepidermoid carcinoma were demonstrated by PAS stain which showed intense intra-cytoplasmic magenta coloured vacuoles in cells containing free carbohydrate molecules. Mucicarmin stain was used to demonstrate intra- and extracellular neutral mucins (deep rose to red colour) in metastatic deposits from adenocarcinoma stomach (4 cases), and 1 case each from adenocarcinoma colon and unknown primary site.<sup>2,20</sup>

Fellegara G et al<sup>21</sup> detected 18 examples of benign (heterotopic) epithelial inclusions in axillary lymph nodes in women which should be carefully excluded from metastatic carcinoma. Three categories of these inclusions included squamous, glandular and mixed squamous and glandular type inclusions. Goyal M et al<sup>22</sup> reported a single case of mesothelial cell inclusions within mediastinal lymph nodes presenting as chylous effusion which can be mistaken for metastatic adenocarcinoma, mesothelioma or sinus histiocytosis. About 3% of head and neck cancers present with metastasis from an occult primary neoplasm<sup>23</sup>.

Benign epithelial and non-epithelial inclusions have been found in cervical,

axillary mediastinal, abdominal and pelvic lymph nodes which can be misdiagnosed as metastatic deposit and potentially lead to incorrect tumor staging<sup>24</sup>.

### Conclusion:

HPE when made on properly selected and carefully excised lymph nodes in patients with lymphadenopathy most often enables one to arrive at a definite diagnosis. Further, it may be the only answer where FNAC has not been helpful in the exact diagnosis and subtyping of lymphomas. Immune marker studies may be necessary for definite diagnosis and subtyping. It is also of great value where FNAC has failed to yield any material and also in cases with strong clinical suspicion of metastasis, where the diagnosis has been missed by FNAC even after multiple aspirations.

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