Section: Pathology

Original Research Article

CYTOMORPHOLOGICAL SPECTRUM OF LYMPH NODE LESIONS BY FINE NEEDLE ASPIRATION CYTOLOGY- A RETROSPECTIVE STUDY IN A TERTIARY CARE CENTRE

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Abstract

Background: Lymph nodes are small organs that serve as immune checkpoints in our body. They often get enlarged due to various causes. Enlarged nodes can present with a spectrum of symptoms related to the cause, which can be an underlying malignancy at times. Fine Needle Aspiration Cytology (FNAC) is a common and rapid out-patient procedure that can be used to diagnose the aetiology of lymphadenopathy. It can avoid unnecessary medical interventions in infectious or inflammatory conditions. Histopathological examination of the lymph node biopsy is the gold standard method for diagnosing causes of lymphadenopathy but is painful, invasive and in-patient procedure. The objectives of the present study were to describe the various diagnoses observed in Fine Needle Aspiration Cytology of lymphadenopathy and to determine the validity of Fine Needle Aspiration Cytology with respect to histopathology in diagnosing lymphadenopathy. **Materials and Methods:** This is a retrospective study done at the Department of Pathology, MIMS, Mandya during March 2022 to May 2022. The study period was January 2020 to September 2022. The records of patients presenting with lymphadenopathy for FNAC to the Department were retrieved and analysed. The records of patients who underwent histopathological examination of lymph node biopsy were also retrieved and analysed. The details such as age, gender and site were collected from the registers maintained at the department. The slides were re-analysed. **Results:** A total of 33 cases of FNAC with histopathological correlation were available. Eighteen cases (54.5%) were benign and 15(45.45%) were malignant on FNAC. Discordance was seen in 6 cases. The sensitivity of FNAC in diagnosing malignancy was 92.85%, specificity 95%, positive predictive value 92.85% and accuracy 94.11%. **Conclusion:** FNAC is helpful preoperatively as it differentiates between benign and malignant lesions in most cases. However, specific diagnosis may not always be possible. Biopsy is the gold standard but FNAC has high specificity in diagnosing malignant tumors and prevents unnecessary extensive, radical surgery for benign lesions where treatment with antibiotics and anti-inflammatory drugs or simple excision would suffice.

INTRODUCTION

As components of peripheral or secondary lymphoid organs, lymph nodes are an important part of immune system.[1] Lymphadenopathy is an abnormal increase in size and /or altered consistency of lymph nodes which can occur in any age group. The causes can range from a simple infection to a malignancy affecting the lymph node. The most common cause of lymphadenopathy in India is tuberculosis.[2] Tuberculosis is one of the endemic infections rampant in our country and can mimic a malignancy and can lead to unnecessary interventions if not diagnosed accurately. Lymphadenopathy is one of the commonest reasons of presentation to the hospital. At times, lymphadenopathy can be the sign of an underlying malignancy. Thus, a proper clinical and cytological examination of the affected lymph node becomes necessary. The procedure of Fine Needle Aspiration Cytology (FNAC) gives a valid option as it is

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minimally invasive, economical, less time consuming, causes mild discomfort and provides a definitive diagnosis majority of the times. It helps to diagnose not only neoplastic conditions but also inflammatory and infectious conditions and thus can help in preventing invasive procedures such as biopsy.

Biopsy for histopathological examination is the gold standard method to arrive at a diagnosis but the procedure is invasive, expensive and time consuming. This study aims to determine the various patterns of lymphadenopathy observed on FNAC at a tertiary care centre and its utility in comparison to histopathological examination.

Objectives of the Study

- To describe the various diagnoses observed in Fine Needle Aspiration Cytology of lymphadenopathy.
- To determine the validity of Fine Needle Aspiration Cytology with respect to histopathology in diagnosing lymphadenopathy

MATERIALS AND METHODS

This is a retrospective study done on cases of lymphadenopathy referred for FNAC and histopathological examination at a tertiary care centre at Mandya, Karnataka from January 2020 to September 2022. The study period was 2 months from the approval obtained by the Institutional Ethics Committee, bearing the number MIMS/IEC/2023/577 and dated 27/3/2022. FNAC was performed using a sterile 22-gauge needle and sterile disposable syringe wherever required. Multiple smears were made on sterile glass slides, half of which were air dried and the other half were fixed using 100% isopropyl alcohol. The air dried smears were stained using Rapid-Malarial Parasite (Rapid-MP) Romanowsky stain and the wet smears were stained using Haematoxylin and Eosin stain. The adequacy of the aspirate was checked by staining with Rapid-MP stain which has a staining time of less than 2 minutes, and the procedure of FNAC was repeated in the same setting wherever the sample was inadequate. For histopathological examination, after receipt of the biopsy sample, fixation of the specimen was done in 10% Neutral Buffered Formalin. After tissue processing, 4 µ to 5 µ thick tissue sections were taken. The sections were stained using Hematoxylin and Eosin stain. The slides were examined under a microscope to arrive at a final diagnosis.

Inclusion Criteria

- All reports of patients who underwent FNAC for lymphadenopathy
- All reports of patients who underwent histopathological examination of lymph node biopsy.

Exclusion Criteria

- All reports of patients who were already under treatment for lymphadenopathy.

Method of Data Collection

All cases of FNAC and histopathology for lymphadenopathy were retrieved from the registers maintained at the department of Pathology. The slides were reviewed and the findings were analysed using descriptive statistics.

RESULTS

A total of 310 patients presented with lymphadenopathy for evaluation by FNAC to the tertiary care centre during the study period. Males (69%) outnumbered the females (31%) and the male to female ratio was 2.2:1. Majority of the patients belonged to the age group of 11 yrs to 20 yrs (27.7%) followed by 21 yrs to 30 yrs (19%). (Fig 1).

Table 1: Distribution of cases received for FNAC according to age group and gender

Age	Females	Males	Total
Under 10 years	34	9	43
11-20 years	7	78	85
21-30 years	10	49	59
31-40 years	18	37	55
41-50 years	17	13	30
51-60 years	5	14	19
61-70 years	4	11	15
71-80 years	0	3	3
81-90 years	1	0	1
Total	96	214	310

Out of 310 cases, 255 cases (82.3%) were seen in cervical group of lymph nodes followed by 32 cases (10.3%) in axillary group, 19 cases (6.1%) in inguinal group and 4 cases (1.3%) were abdominal lymph nodes.

Figure 2: FNAC diagnoses of lymphadenopathy

Category	Diagnosis	Frequency	Percentage (%)
Benign	Reactive Lymphoid Hyperplasia	185	59.7
	Tubercular lymphadenitis	66	21.2
	Acute suppurative lymphadenitis	22	7.1

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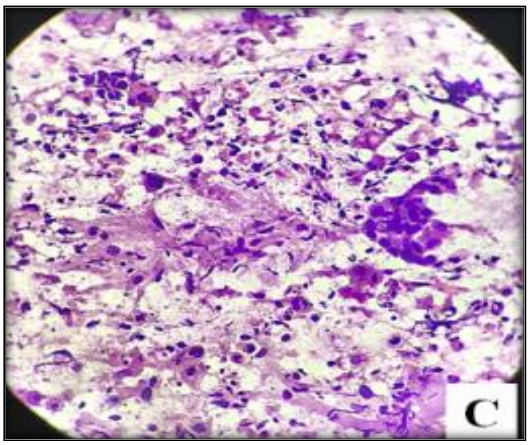
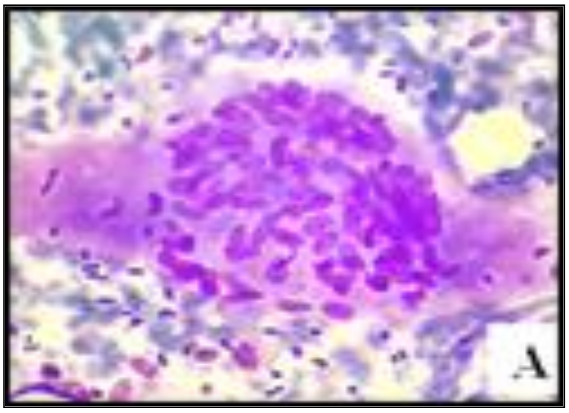
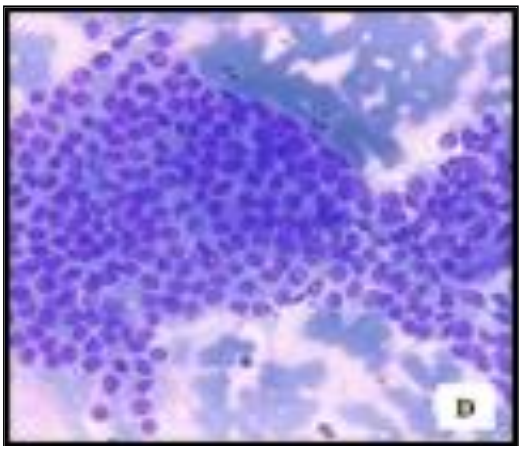
	Toxoplasmosis lymphadenitis	1	0.3
Malignant	Metastatic Squamous Cell Carcinoma (SCC)	21	6.8
	Lymphoproliferative lesion	6	1.9
	Metastatic Adenocarcinoma	5	1.6
	Metastatic Carcinoma (NOS)	2	0.6
	Metastatic Papillary Thyroid Carcinoma	1	0.3
	Hodgkin's lymphoma	1	0.3
	Total	310	100%

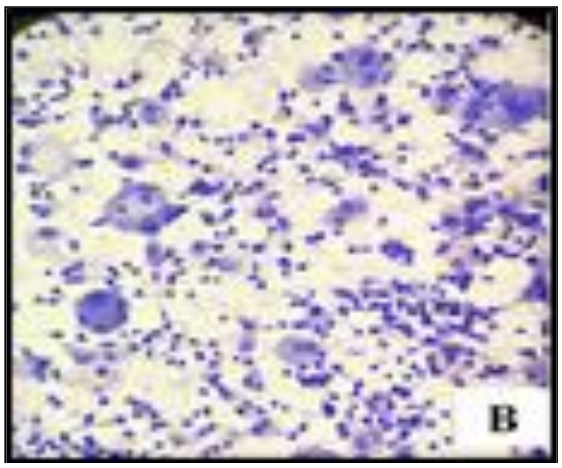
Out of 310 cases, diagnoses favouring tuberculosis were seen in 66 cases (21.2%). Among these 66 cases, 35 cases (53%) showed epithelioid granulomas with caseous necrosis, 20 cases (30.3%) showed only epithelioid granulomas and 11 cases (16.7%) showed only necrosis.

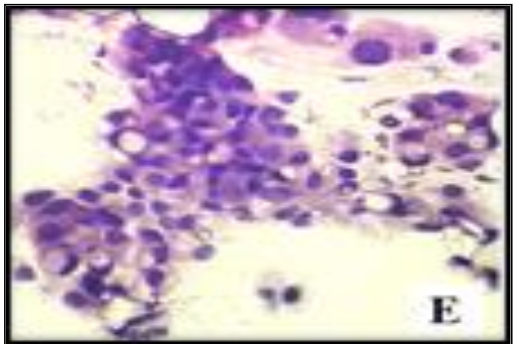
Diagnosis of malignancy was made by FNAC in 35 cases (11.2%) out of which 21 cases (60%) were Metastatic SCC, 6 cases (17.1%) were Lymphoproliferative lesion, 5 cases (14.28%) were Metastatic Adenocarcinoma, 2 cases (5.71%) were Metastatic Carcinoma Not Otherwise Specified (NOS) and a single case (0.3%) of Metastatic Papillary Thyroid Carcinoma and Hodgkin's Lymphoma each were seen.

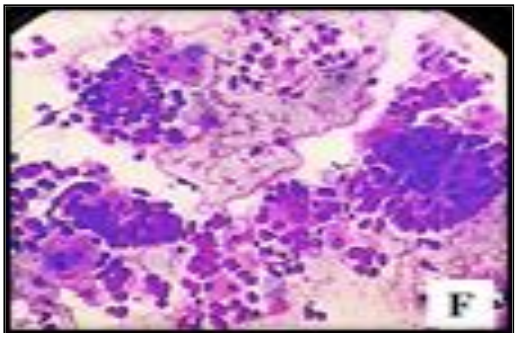












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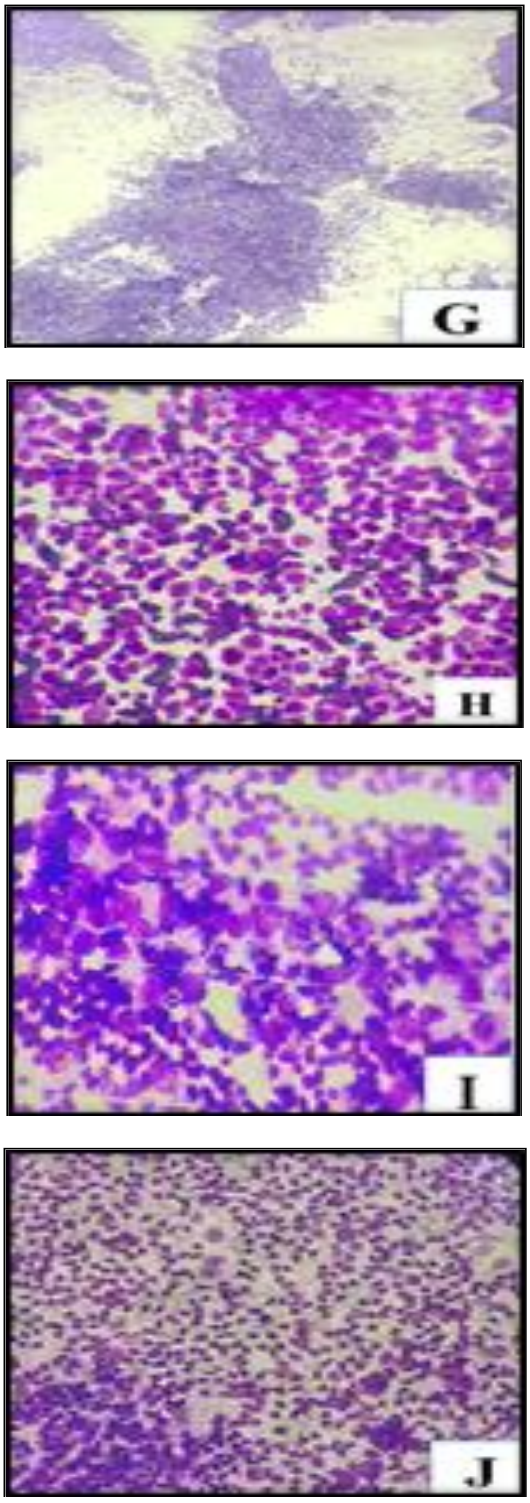

Fig 3: A: Granulomatous lymphadenitis: Cluster of epithelioid cells on a background of reactive lymphoid cells; Rapid MP, 40X
3.B: Toxoplasma lymphadenitis in an immunocompromised patient: Scattered histiocytes and other lymphoid cells with numerous bradyzoites in the cytoplasm; H&E, 10X
3.C: Metastatic SCC: Scattered keratinised malignant squamous cells on a background of tumor diathesis; H&E, 10X
3.D: Metastatic Papillary Thyroid Carcinoma: Tumor cells arranged in papillary fronds with nuclei displaying longitudinal grooves and intranuclear cytoplasmic inclusions; Rapid MP, 40X
3.E: Metastatic Adenocarcinoma: Multilayered sheets of malignant epithelial cells with many signet ring cells, in a background of scattered lymphoid cells; H&E, 10X
3.F: Metastatic Adenocarcinoma: Tumor cells with papillary and acinar arrangement on a background of lymphoid cells; H&E, 40X
3.G: Metastatic Carcinoma (NOS): Sheets of epithelial cells with pleomorphic nuclei containing vesicular chromatin, prominent nucleoli and moderate to abundant cytoplasm on a background of lymphoid cells.
3.H: Lymphoproliferative lesion: Sheets of large lymphoid cells with hyperchromatic nuclei, occasional nucleoli and scant cytoplasm admixed with few mature lymphocytes in background; Rapid MP, 40X
3.I: Lymphoproliferative lesion: Sheets of large lymphoid cells with large hyperchromatic nuclei containing 1-2 prominent nucleoli and scant to moderate amphophilic cytoplasm; Rapid MP, 40X
3.J: Hodgkin's lymphoma: Scattered Reed-Sternberg giant cells amidst reactive lymphoid cells; H&E, 40X.

Cartridge Based Nucleic Acid Amplification Test (CB-NAAT) was done on all 310 cases and was positive for Mycobacterium tuberculosis bacteria in 30 cases (9.7%). Among these 30 cases, FNA showed reactive lymphoid hyperplasia in 46.7% (14 cases), granulomatous lymphadenitis in 40% (12 cases) and necrotising lymphadenitis in 13.4% (4 cases).

Histopathological correlation was available in 33 cases, which are tabulated below.

Figure 4: Comparison of FNAC diagnoses and histopathological diagnoses

FNAC diagnosis	Histopathology diagnosis
Reactive lymphoid hyperplasia (8)	Reactive lymphoid hyperplasia (6)
	Granulomatous lymphadenitis (1)
	Hodgkin's lymphoma (1)
Granulomatous lymphadenitis (8)	Granulomatous lymphadenitis (7)
	Toxoplasmosis lymphadenitis (1)
Necrotising lymphadenitis (2)	Kikuchi's lymphadenitis (1)
	Tubercular lymphadenitis (1)
Acute suppurative lymphadenitis (1)	Reactive Lymphoid hyperplasia (1)
Lymphoproliferative lesion (6)	Small Cell lymphoma (2)
	Diffuse Large B-Cell Lymphoma (1)
	Follicular lymphoma (1)
	Lymphoblastic lymphoma (1)

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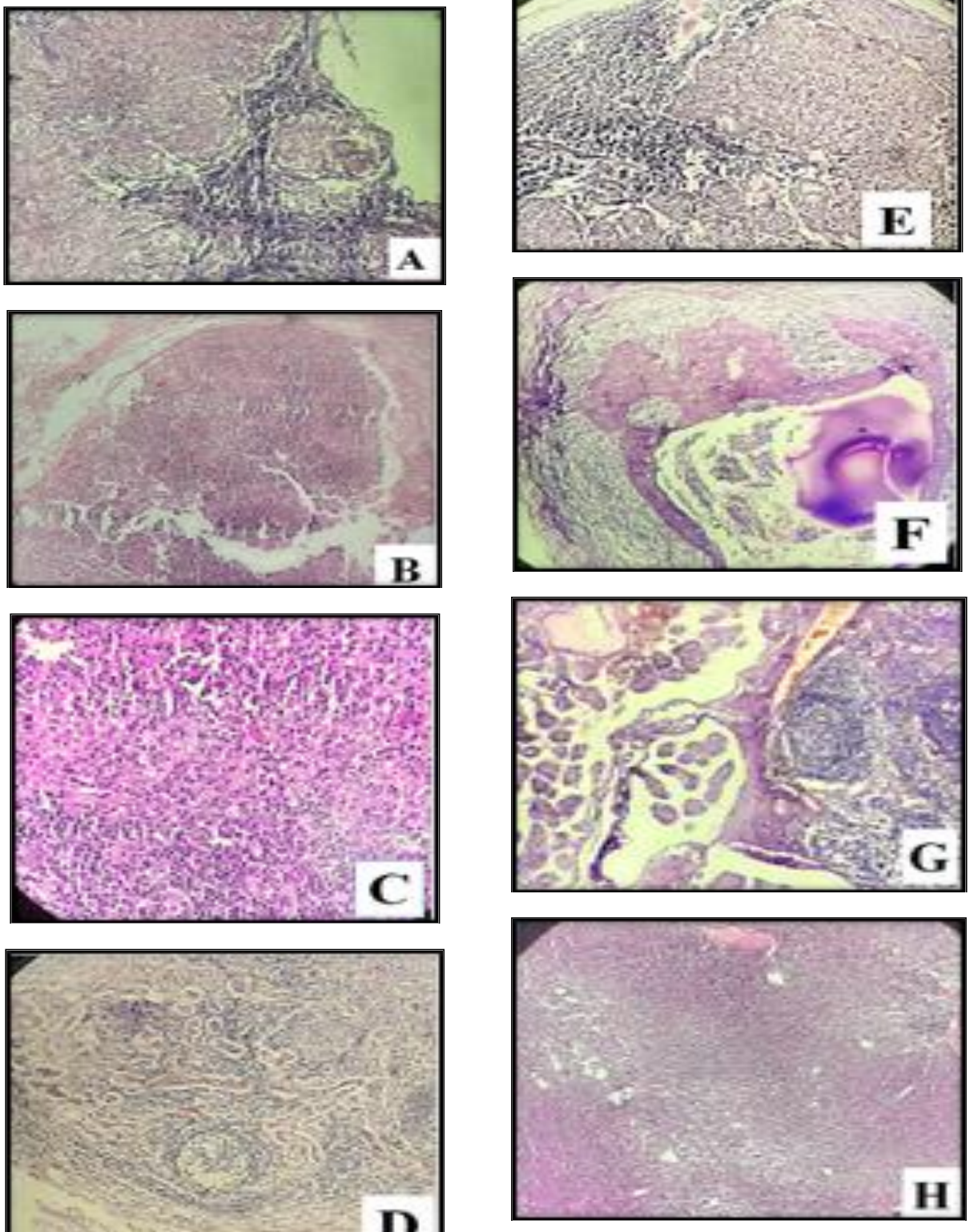
	Reactive Lymphoid hyperplasia (1)
Metastatic Squamous Cell Carcinoma(4)	Metastatic Squamous Cell Carcinoma(4)
Metastatic Adenocarcinoma (3)	Metastatic Adenocarcinoma from colon (1)
	Metastatic Infiltrating Ductal Carcinoma from breast (1)
	Metastatic Adenocarcinoma from stomach (1)
Metastatic Papillary Thyroid Carcinoma (1)	Metastatic Papillary Thyroid Carcinoma (1)

Histopathology images [Figure 5]



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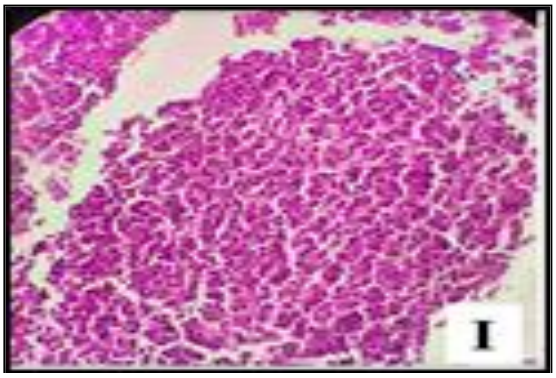
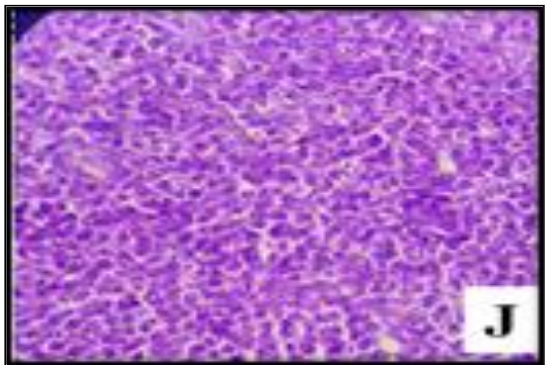




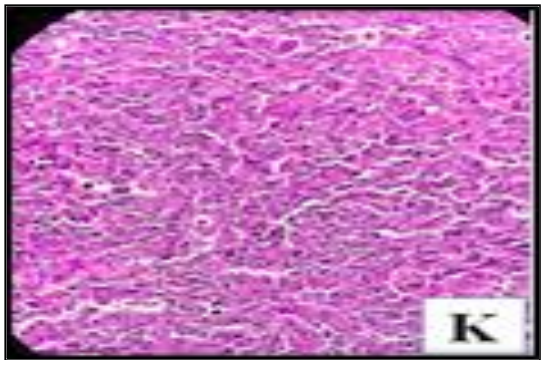


Fig 5: A: Tubercular lymphadenitis: Clusters of caseating granulomas with centrally located Langhans type of multinucleated giant cell, within the lymph node parenchyma, H & E; 10X

5.B: Toxoplasma lymphadenitis: Scattered microgranulomas within the lymph node parenchyma in an immunocompetent patient, H & E; 10X

5.C: Kikuchi's lymphadenitis: pale areas composed of histiocytes, lymphoid cells and karyorrhectic debris. Few histiocytes display curved nuclei, H & E; 40X

5.D: Metastatic Gastric Adenocarcinoma: Acinar and tubular arrangement of glandular cells within the lymph node parenchyma, H & E; 10X

5.E: Metastatic infiltrating ductal carcinoma of breast: Solid nests of tumor cells surrounded by reactive lymphoid cells, H & E; 10X

5.F: Metastatic Squamous Cell Carcinoma: Solid nests of squamoid tumor cells with many abnormal mitotic figures and few dyskeratotic cells surrounded by reactive lymphoid cells,
H & E; 10X

5.G: Metastatic papillary thyroid carcinoma: Tumor cells arranged in papillary pattern with classic Orphan-Annie nuclei and occasional longitudinal nuclear grooves, surrounded by reactive population of lymphoid cells within the parenchyma, H & E; 10X

5.H: Small Cell Lymphoma: Sheets of monomorphic small sized lymphocytes effacing the lymph node architecture, H & E; 10X

5.I: Follicular lymphoma: Neoplastic follicle with absence of polarization and germinal centre comprising of majorly centrocytes, H & E; 40X

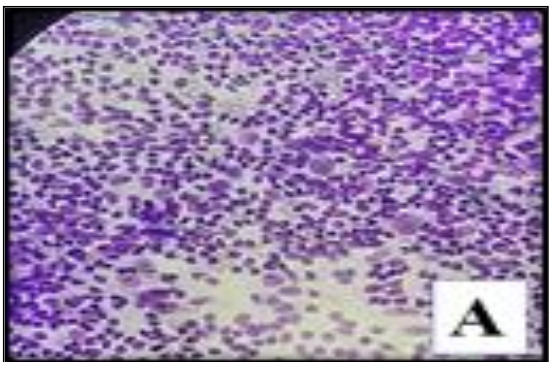
5.J: Lymphoblastic lymphoma: Sheets of monomorphic medium sized lymphoid cells with loss of normal architecture of lymph node, H & E; 40X

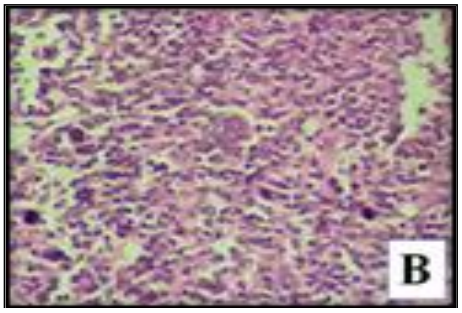
5.K: Diffuse Large B-Cell Lymphoma: Sheets of large, bizarre appearing lymphoid cells with effacement of normal lymph node architecture, H & E; 40X.

Out of 8 cases of reactive lymphoid hyperplasia received for histopathological examination, 1 case (12.5%) was diagnosed as Granulomatous lymphadenitis and 1 case (12.5%) turned out to be Hodgkin's lymphoma. The granulomatous lymphadenitis was missed as a different region within the lymph node was hit during the procedure of FNA and smears showed only reactive population of lymphoid cells with few reactive histiocytes in the absence of caseous necrosis and well-formed granulomas.

In the case of Hodgkin's lymphoma, very few scattered large cells were seen which were interpreted to be reactive histiocytes with prominent nucleoli.

[Figure 6:A & B]





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Out of 8 cases of Granulomatous lymphadenitis diagnosed on FNAC, 1 case (12.5%) turned out to be Toxoplasma lymphadenitis due to presence of many microgranulomas which are characteristically seen in toxoplasmosis.

The only case diagnosed as acute suppurative lymphadenitis on FNAC was found to be reactive lymphoid hyperplasia following biopsy, perhaps due to visualization of few polymorphonuclear leukocytes in the FNA smears.

Among the 3 cases of Metastatic carcinoma received for biopsy following FNAC, 1 case each of metastatic adenocarcinoma from colon, metastatic infiltrating ductal carcinoma breast and metastatic adenocarcinoma from stomach.

Among 6 cases of lymphoproliferative lesion on FNAC, 5 cases (83.34%) showed Non-Hodgkin's lymphoma on histopathological examination, 2 (40%) of which were Small cell lymphoma, one case of Diffuse Large B-Cell Lymphoma, Follicular lymphoma and Lymphoblastic lymphoma each (20%). One case (16.67%) showed reactive lymphoid hyperplasia.

DISCUSSION

Lymphadenopathy is a quite common presenting complaint in our country. Lymph nodes can be enlarged due to many conditions like infection, inflammation or a neoplastic process.

At times, it can be just a mild inflammation due to fever or a small local infection but it can also be due to a more devastating systemic infection such as Tuberculosis or Human Immunodeficiency virus infection or due to a malignant process.

Majority of the patients belonged to the age group of 11 yrs to 20 yrs (27.4%) followed by

21 yrs to 30 yrs (19%). This observation differs from the studies done by Ozlem Ton Eryilmaz.[3], Dr.SasiKanth Uddagiri.[4] and Panduranga Chikkannaiah.[5] who found 31 to 40 yrs to be the common age group in their studies. The males outnumbered the females in the present study which was in concordance to the studies done by Ozlem Ton Eryilmaz.[3], Dr.SasiKanth Uddagiri.[4] whereas Panduranga Chikkannaiah.[5] found that females outnumbered the males in their study.

In the present study, out of 310 cases, 255 cases (82.3%) were seen in cervical group of lymph nodes. This is similar to the findings by Ozlem Ton Eryilmaz.[3] and Mohammed Abdul Nasar et al.[6] who found cervical lymph node involvement in 75% and 64% of cases, respectively. PandurangaChikkannaiah.[5] found 88.8% and H J Ha.[7] found 93.05% involvement of cervical nodes in their study.

The etiological profile of the current study shows that the commonest non-neoplastic cause of lymphadenopathy was Reactive Lymphoid Hyperplasia with 185 cases (59.7%). Ozlem Ton Eryilmaz.[3] also found similar findings with 239/392 cases (61%) of reactive lymphoid hyperplasia and Mohammed Abdul Nasar et al.[6] found 40/72 cases (55.56%) to be reactive lymphadenopathy.

The present study showed that the commonest neoplastic cause of lymphadenopathy was Metastatic Squamous Cell Carcinoma with 21 cases (6.8%). Ozlem Ton Eryilmaz.[3] found 61 cases (15.6%) of metastatic deposits, Mohammed Abdul Nasar et al.[6] found 20 cases (27.78%) of metastatic lymphadenopathy and Panduranga Chikkannaiah found 13%.[5]

In the present study, FNAC was able to diagnose malignancy in 36 cases out of which 21 cases (58.34%) were metastatic SCC, six cases (16.67%) were lymphoproliferative lesion, five cases (13.89%) were metastatic adenocarcinoma, two cases (5.56%) were Metastatic Carcinoma NOS and a single case each (0.3%) of metastatic PTC and Hodgkin's lymphoma.

In the present study, out of 29 cases of metastatic lymphadenopathy, the primary lesion could not be identified in two cases (6.89%). FNAC was able to detect the type of malignancy in 27 cases (93.1%) thus narrowing down the possible site of primary lesions.

Discrepancies between FNAC and histopathological diagnoses:

In the present study among eight cases of reactive lymphoid hyperplasia received for histopathology, one case (12.5%) was found to be Hodgkin's lymphoma. This is similar to the study done by Ozlem Ton Eryilmaz.[3]who found three cases of Non-Hodgkin's lymphoma out of 33 cases of reactive hyperplasia and H J Ha.[7] who found that among 155 cases of benign lymphadenopathy on FNAC, seven cases (4.5%) were metastatic malignancy and five cases (3.23%) were Non-Hodgkin's lymphoma. Sanna Nazir.[8] also found that out of 20 cases of reactive hyperplasia diagnosed on FNAC, three cases (15%) turned out to be lymphoma and two cases (10%) turned out to be metastatic carcinoma.

Out of six cases diagnosed as lymphoproliferative lesion on FNAC, one turned out to be reactive lymphoid hyperplasia (16.67%) in the present study. H J Ha.[7] also found that out of 192 cases diagnosed as metastatic lymphadenopathy on FNAC, 10 cases (5.2%) turned out to be benign on histopathological examination.

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Figure 7: Statistical comparison of our study with other studies

	Our study (n=33)	Ozlem Ton Eryilmaz[3] (n=73) 2023	Dr. SasiKanth Uddagiri[4](n=100) 2022	Sanna Nazir[8] (n=72) 2022	Panduranga Chikkannaiah[5] (n=106) 2021	Mohammed Abdul Nasar[6] (n=100) 2016
Sensitivity	92.85%	87.9%	87.65%	90%	81%	96.4%
Specificity	95%	100%	98%	100%	94.4%	100%
Positive Predictive value	92.85%	100%	-	100%	81.2%	-
Accuracy	94.11%	94.1%	-	-	98.89%	-

In the present study, the p value is less than 0.0001 which is statistically significant at 0.05 significance level.

FNAC is able to diagnose most of the benign lesions as benign and differentiate benign lesions from malignant tumors in majority of the cases. The drawback of FNAC is that specific diagnosis based on architecture of entire lymph node is not possible. However, since majority of the benign lesions are managed conservatively, the exact diagnosis is not required. In cases where the size of the lymph nodes is small or if it is not palpable, employment of radiological guidance using ultrasonography or computed tomography can be helpful. Diagnosing tumors that have metastasized to the lymph nodes on cytological smears

is crucial, as it may be the sole indication for searching for the primary tumor, especially in patients with occult carcinoma.[9]

In the present study and studies done by other authors, we observed that the following factors may lead to an erroneous diagnosis on FNA.[8,10,11]

1. Poor yield: Due to improper technique of aspiration or fibrosis, the yield may be scant.
2. Hemorrhagic aspirate: Since lymph nodes are rich in vascularization, aspiration during acute inflammation may yield blood mixed aspirate and thus lead to dilution of the aspirate. If the proportion of cells of interest is low, they may be missed on examination. In such cases, sedimentation or cell block preparation may be used.
3. Morphological examination: Benign lesions show reactive population of lymphoid cells which also include histiocytes, immunoblasts and centroblasts which at times show atypical changes due to inflammation. These cells may be confused with Reed-Sternberg cells or large malignant lymphoid cells. In the current study, one case of Hodgkin's lymphoma was missed on FNAC because the R-S cells were misinterpreted as reactive histiocytes with prominent nucleoli. One case which was diagnosed as lymphoproliferative lesion on FNAC was found to be Reactive lymphoid hyperplasia on biopsy due to a relatively increased proportion of cells which mimicked mononuclear R-S cells.
4. Necrotic material: Necrotic inflammatory debris may obscure epithelioid cells in cases of tuberculosis or malignant cells in metastasis. Careful examination of the smears should be done.
5. Aspiration from an uninvolved area within a lymph node may lead to an erroneous diagnosis of benign lymphadenopathy and thus, multiple aspirations, proper history and clinical examination are necessary for better results.

Additional techniques such as Ziehl-Neeson stain or CB-NAAT for Acid Fast Bacilli, Immunocytchemistry or Flow Cytometry for neoplastic lesions can be used as adjuncts to improve the diagnostic accuracy of FNAC.

CONCLUSION

When it comes to diagnosing lymphadenopathy, FNAC is an effective method of diagnosis. It is an outpatient procedure which is relatively painless, cheap and can be done within half an hour before the patient leaves the testing area. The present study has highlighted these facts. Histopathology remains the gold standard. The drawbacks in FNAC can be overcome by thorough examination of the patient, expertise in the field of cytopathology and a general knowledge of the endemic causes of lymphadenopathy. Rarer diagnoses should be made with caution or with an adjunct of other ancillary techniques.

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Conflicts of Interest None.

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