(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 6, Issue 2 , Page No: 120-133
March-April 2023

IJMSCR

Role Of Fnac In Diagnosis Of Etiological Profile Of Lymphadenopathy

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Lymph nodes are common site of metastasis for different cancers. Thus the clinical recognition and urgent diagnosis of palpable lymphadenopathy is of paramount importance specially to differentiate between inflammatory lesions or metastatic or primary neoplastic tumor. Inclusion of FNAC in the diagnostic protocol as a first line non-invasive investigation seems to be quite useful to confirm or exclude- for example, metastatic disease – especially if a known history of malignancy is present. The results of FNAC compare favourably with those of tissue biopsies and in some situations the aspirate has qualities of a biopsy. Suspicious or doubtful situations should be resolved by surgical biopsy and further by immunohistochemistry and molecular techniques whenever required.

The aim of the present study is to highlight the role of FNAC in diagnosis of etiological profile of lymphadenopathy and to find out the accuracy of FNAC in comparison to biopsy.

Keywords:

Introduction

Lymphadenopathy is one of the commonest clinical presentation in clinical practice. There are approximately 800 lymph nodes in the body and no fewer than 300 of them lie in the neck. (38) Lymphadenopathy is an abnormal increase in size and/ or altered consistency of lymph nodes. It is a clinical manifestation of regional or systemic diseases and serves as an excellent clue to the underlying disease. (50)

Enlargement of lymph node may result from the proliferation of lymphocytes intrinsic to lymph nodes, due to an infection or a lymphoproliferative disorder or from migration and infiltration of nodal tissue by either intrinsic inflammatory cells or metastatic malignant cells. (56)

Size of a node, to which some pathological significance can be attached, depends on the site. In the cervical and axillary regions a lymph node > 1 cm, in the inguinal region

> 1.5 cm size, is considered significant. Causes of lymphadenopathy are reactive lymphadenitis, infections, malignant lymphoma and leukemias, metastatic malignancies and autoimmune disorder. It is important to differentiate between benign and malignant conditions to decide the further line of management.

The etiological profile varies from country to country and region to region. In developing countries like India, acute upper respiratory infections, suppurative skin lesions and tuberculosis are the major causes of regional lymphadenopathy. (77) It has been stated that any significant lymph node enlargement not subsiding or remaining static in size for >2 weeks after conventional antibiotic therapy should be thoroughly investigated. (14)

The diagnostic modalities that can be employed to the etiology of lymphadenopathy are FNAC and excision biopsy. FNAC is particularly helpful in the work-up of cervical masses and nodules because biopsy of cervical adenopathy should be avoided unless all other diagnostic modalities have failed to establish a diagnosis. (56, 48)

Aspiration of lymph nodes for diagnostic purpose was reported as early as 1904 by Grieg and Gray who used this procedure in the diagnosis of trypanosomiasis. (30, 56)

In 1921, Guthrie attempted to correlate lymph node aspiration cytology with various disease processes. (34, 56) FNAC is nowadays recognized as a rapid diagnostic technique because of its simplicity, early availability of results, cost effective procedure, minimal trauma, absence of complications and can be performed in any setting, with results usually available within 24 hours. (42, 39)

With the help of FNAC, preliminary diagnosis of a lesion with an adequate sample can be given within a few minutes when a quick staining and microscopic examination is performed on site. (33)

The diagnostic utility of FNAC of lymph nodes has been described. However, its role in the morphologic assessment of lymphadenopathies does not seem to have been completely established due to the belief by some pathologists that FNAC samples do not supply enough diagnostic material. (24)

In patients with an unknown clinical history of malignancy, a positive FNAC diagnosis may be the first indication of malignancy and be useful in furthering the clinical investigation to search for an occult neoplasm. Since a decision on conservative versus radical management depends on the type of tumor or non-tumorous process, it is of

paramount importance that the FNAC diagnosis of the lymphadenopathy be as accurate as possible. (33)

Diagnosis of lymphadenopathy depends mainly on excision of a gland and histopathological examination. For this, general anaesthesia and hospitalisation are required. Fine needle aspiration cytology, on the other hand, is free from these disadvantages and can safely be used as an alternative or complementary investigative technique. (56)

Excision biopsy which gives definitely the final diagnosis, but open biopsy before appropriate evaluation may be detrimental for a number of reasons. The patient may believe that his disease has been cured and he does not need further therapy, wound infection may delay definitive surgery, and biopsy incision if placed improperly, may compromise the adequacy of the neck dissection performed later. Excision biopsy takes more time than FNAC, delay in management of primary tumors may eliminate the best opportunity of its removal with metastatic nodes. Not all hospital can afford a full- fledged histopathology department.

Although open biopsy with histological examination of excised tissue still remains the gold standard for diagnosis of lymph node enlargement, yet FNAC has now become an integral part of the initial diagnosis and management of patients presenting with lymphadenopathy. This simple technique has gained wide acceptance since it offers a high degree of accuracy, leading itself to outpatient diagnosis and thus reducing the cost of hospitalization.

Aims And Objectives

1. To study the etiological profile of lymphadenopathy as per Fine Needle Aspiration Cytology.
2. To study the etiological profile of lymphadenopathy as per Histopathological Examination.
3. To find out the accuracy of Fine Needle Aspiration Cytology in the diagnosis of lymphadenopathy.

Material And Methods

Place of study: Department of pathology, SMS Medical College and attached hospitals, Jaipur, Rajasthan.

Study design: accepting α error 0.5 and power 80% for 79.1% sensitivity for lymphadenopathy (seed article), sample size was calculated 34, by study size version 2 statistical software.

Study duration: from September 2011 onwards

Sample size: Fine Needle Aspiration Cytology and histological study of minimum of 34 cases, upto maximum of 50 cases if possible.

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Sampling technique: each and every case of lymphadenopathy coming for histopathological examination.

Inclusion Criteria

1. Patients with various age, irrespective of their sex having enlarged lymph nodes of surgical etiology at different parts of body.
2. Patients who need to be treated surgically and not medically after diagnosed by FNAC.

Exclusion Criteria

1. Patients treated medically after FNAC
2. Patients with inconclusive FNAC or histopathology results
3. Unfit for surgical procedures
4. Lymph nodes inaccessible to FNAC procedures.
5. This study will be carried on patients with any palpable enlarged lymph nodes.
6. The material for study will comprise of
    a) Cytosmear made from material obtained by fine needle aspiration using a 23-25gauge needle and 10 ml syringe with the help of plunger.
    b) Biopsy from surgically removed tissue.
    c) Immunohistochemical marker study as and when required.

Staining Techniques

1. Staining of cytology smears:

The *May Grunwald Giemsa* staining techniques were used for the staining of cytology smears. The reagents which were used in the staining of cytology smears are: May Grunwald's stain, Giemsa stain, Methanol, Glycerol, Conical flask and Phosphate buffer(pH-6.8).

Preparation of the stains:

1. **Preparation of May Grunwald stain:** 0.3 g of powdered dye was weighed out and transferred to a conical flask of 200-250cc capacity. 100cc of methanol was added and the mixture was warmed to 50ºC. The flask was then allowed to cool at room temperature and was shaken several times during the day. After standing for 24 hours the solution was filtered. It was then ready for use.
2. **Preparation of Giemsa solution:** 1 g of Giemsa powder was dissolved in 54ml of glycerol at 50^0 C and after cooling mixed with 84ml of methanol GR and filtered.

Air dried smears were fixed in a jar of methanol for 5 minutes.

1. Fixed smears were stained as follows: Dry the film in the air.
2. Fix by immersing in a jar of methanol for 15-20 min.
3. Transfer the fixed films to staining jar containing May-Grunwald-stain freshly diluted with an equal volume of buffered water. (15 min)
4. Transfer them without washing to a jar containing Giemsa's stain freshly diluted with 9 volumes of buffered water, pH 6.8.(10-15 min)
5. Transfer the slides to a jar containing buffered water pH-6.8, rapidly wash in 3 or 4 changes of water, and finally allow to stand undisturbed in water for a short time (2-5 min.) for differentiation.
6. Stand the slides upright to dry.
7. Mounted by a rectangular cover glass using DPX mountant.

Inference of MGG staining:

1. Nuclei and basophilic cytoplasmic components appeared blue
2. Neutrophilic granules appeared lilac
3. Red cells were yellowish red.
4. Cytoplasm of neutrophil was pale pink

2. Haematoxylin & erosin staining technique

Paraffin sections were kept into water after the following procedure:

1. The paraffin of the section was melted over gentle flame.
2. Then the slides were kept in a jar of xylene for 5 minutes.
3. Again slides were kept in a Jar of xylene for 5 minutes.
4. Then sections were transferred to absolute alcohol for 5 minutes.
5. Then they were transferred to 80% alcohol.
6. Then were transferred to 50% alcohol.
7. Finally were washed in running water for 2 to 5 minutes.

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8. Section were stained in: Meyer's haematoxylin solution for 5 minutes.
9. Rinsed in water and the slides were passed through gentle stream of running tap water.
10. Differentiated with 1 percent hydrochloric acid solution under microscopic checkup.
11. Counter stained with 1 percent solution of eosin for 30 sections.
12. Rinsed and dehydrated 3 times in acetone for 1 minute each time.
13. Mounted in DPX.

3. Ziehl–Neelsen staining technique:

1. Fix the dry smear in methanol for 5 min.
2. Wash with running tap water
3. Flood slide with hot Carbol Fuchsin for 5-7 min
4. Rinse with tap water
5. Decolorize with 20% H2SO4 till smear is decolorized
6. Rinse with tap water until smear takes light pink color
7. Flood slide with Methylene Blue for 2-3min
8. Rinse with tap water
9. Blot dry and mount with DPX

The results of all the three procedures were compared and evaluated taking histopathology as the gold standard.

Observation And Results

In present study, 34 cases of lymphadenopathy admitted in SMS Hospital, Jaipur, were studied. All cases were aspirated and smear prepared. Tissues were biopsied and cytological findings were compared with histopathological examination.

The diagnosis was consistent with histopathology in 32 cases and not consistent in 2 cases. Therefore, an accuracy rate of 88.89% was obtained.

Clinicopathological Analysis:

In present study, commonest causes of lymphadenopathy were *Tubercular Lymphnoditis* (29.4%) and *Metastatic Carcinoma* (29.4%). Reactive Hyperplasia was (20.5%) at second place. Lymphoma constituted 17.6%, and Granulomatous Inflammatory lesion accounted for 2.94% of lymph nodes as shown in Table 1.

Table No. 01: Distribution of cases according to histopathological diagnosis

Histopathological Diagnosis	**No of cases**	**Percentage (%)**
Tubercular lymphnoditis	10	29.4%
Metastatic carcinoma	10	29.4%
Reactive hyperplasia	7	20.5%
Lymphoma	6	17.6%
Granulomatous Inflammatory lesion	1	2.94%
Total	**34**	**100**

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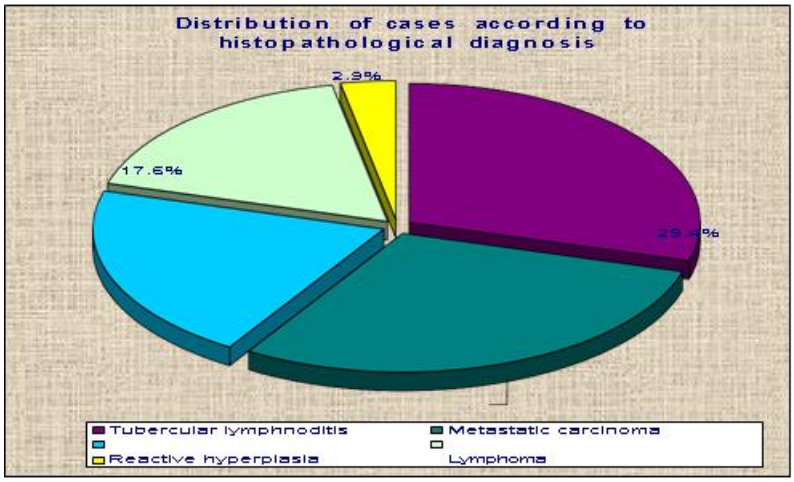
Fig 1. Distribution of cases according to histopathological diagnosis



Out of 34 cases, cytological diagnosis was matched with histopathological diagnosis in 32 cases. One case which was reported as reactive hyperplasia by FNAC was found to be lymphoma on biopsy (Table 2). The other case which was reported reactive hyperplasia on FNAC, turned out to be metastatic carcinoma on histopathology.

Table No. 02: Distribution of cases according to cytological diagnosis

Cytological Diagnosis	No of Cases	Percentage
Tubercular lymphnoditis	10	29.4%
Metastatic carcinoma	9	26.4%
Reactive hyperplasia	9	26.4%
Lymphoma	5	14.7%
Granulomatous lesion	1	2.94%
Total	**34**	**100%**

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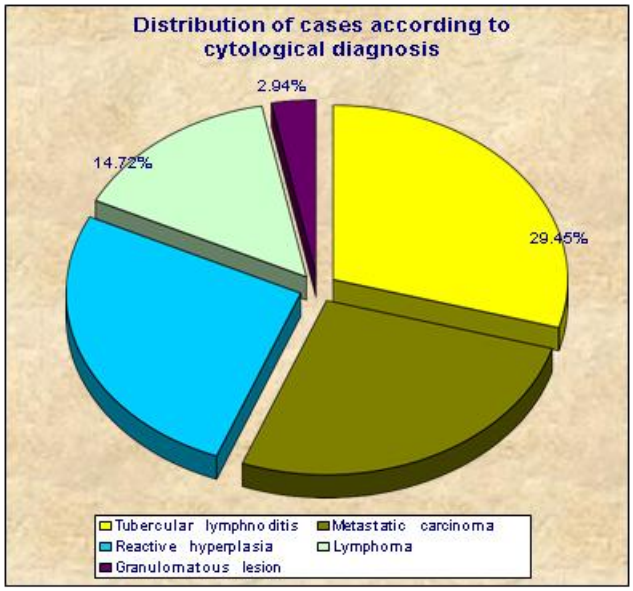
Fig 2. Distribution of cases according to cytological diagnosis



It showed that the 32.3% cases were in 1-20 yrs age group, 41.1% cases were between 21-40 yrs age group and 26.4% cases were in 41-70 years age group. Mean age was 32.67 years (Table 3).

Table No. 03: Distribution of cases according to age group

Age group (years)	Tubercular lymphnoditis	Metastatic carcinoma	Reactive hyperplasia	Lymphoma	Granulomatous inflammatory lesion	Total
1-10	-	-	1 (14.2%)	-	-	1
11-20	4 (40%)	1 (10%)	3 (42.8%)	2 (33.3%)	-	10
21-30	4 (40%)	-	2(28.5%)	2(33.3%)	1 (100%)	9
31-40	2 (20%)	2 (20%)	-	1(16.6%)	-	5
41-50	-	2 (20%)	1(14.2%)	-	-	3
51-60	-	2 (20%)		1(16.6%)	-	3
61-70	-	3 (30%)		-	-	3
Total	**10**	**10**	**7**	**6**	**1**	34

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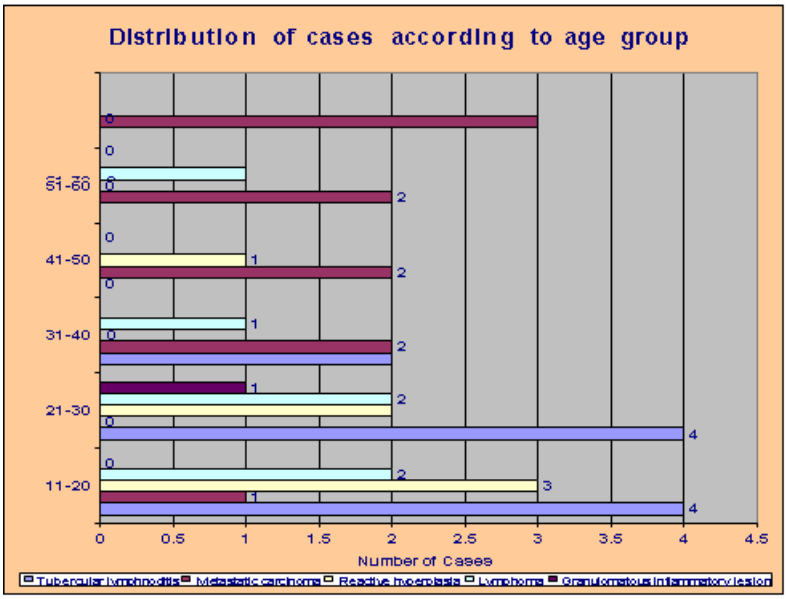
Fig 3. Distribution of cases according to age group



In metastatic carcinoma and lymphoma male preponderance was observed, while tubercular lymphnoditis and reactive hyperplasia show slight female preponderance. 55.8% cases were male and 44.1% cases were female. Male to Female ratio is 1.26:1.

Fig 4. Distribution of cases according to sex

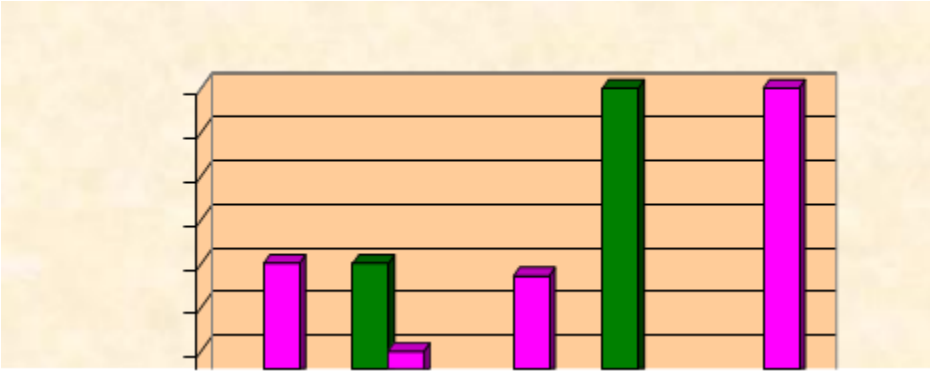


Table No. 04: Distribution of cases according to sex

Histological diagnosis	Male (%)	Female (%)
Tubercular lymphnoditis (n=10)	4 (40%)	6 (60%)
Metastatic carcinoma (n=10)	6 (60%)	4 (40%)
Reactive hyperplasia (n=7)	3 (42.8%)	4 (57.1%)
Lymphoma (n=6)	6 (100%)	-

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Granulomatous inflammatory lesion (n=1)		-	1 (100%)	

Correlation between cytological and clinical diagnosis was noted in 79.41% cases of lymphadenopathy. Out of 10 cases of Tubercular Lymphnoditis, 8 cases were diagnosed clinically as tuberculosis. In case of Metastatic Carcinoma, in 8 out of 9 cases clinically diagnosed were Metastatic Carcinoma. Out of 5 cases of Lymphoid neoplasm, clinical diagnosis was made in 4 cases. Accuracy of clinical diagnosis in relation to cytology was 79.41% as shown in Table 5.

Table No. 05: Co- relation between clinical diagnosis and cytological diagnosis

Clinical diagnosis	**Cytological diagnosis**					
	Tubercular lymphnoditis	**Metastatic carcinoma**	**Reactive hyperplasia**	**Lymphoma**	**Granulomatous inflammatory lesion**	**Total**
Tubercular lymphnoditis	8	1	2	-	-	**11**
Metastatic carcinoma	1	8	-	1	-	**10**
Reactive hyperplasia	1	-	6	-	-	**7**
Lymphoma	-	-	1	4	-	**5**
Granulomatous inflammatory lesion	-	-	-	-	1	**1**
total	**10**	**9**	**9**	**5**	**1**	**34**

Diagnosis in all 34 cases was confirmed by histopathology. Out of 34 cases, cytological diagnosis was matched with histopathological diagnosis in 32 cases. One case which was reported as reactive hyperplasia by FNAC was found to be Hodgkin lymphoma of mixed cellularity type on biopsy. The other case which was reported reactive hyperplasia on FNAC, turned out to be metastatic carcinoma on histopathology (Table 6).

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Table No. 6: Co-relation between cytological diagnosis and final diagnosis

Histopathological diagnosis		Cytological diagnosis				
		Tubercular lymphnoditis	Metastatic carcinoma	Reactive hyperplasia	Lymphoma	Granulomatous inflammatory lesion
TB	10	10	-	-	-	-
Metastatic Ca	10	-	9	1	-	-
RH	7	-	-	7	-	-
Lymphoma	6	-	-	1	5	-
Granulommatous inflammatory lesion	1	-	-	-	-	1
Total	**34**	**10**	**9**	**9**	**5**	**1**

Sensitivity= 94.12%, Predictive value of positive test= 94.12%, Accuracy= 88.89%

P value was >0.05. However, FNAC was well correlated with histopathology, but due to less number of cases studied, it could not be proved to be statistically significant.

Table No. 07: Correlation of cytologic and histopathological diagnosis in cases of Metastatic Carcinoma

		Histopathological diagnosis		
		Positive	**Negative**	**Total**
	Positive	9 (true positive)	0 (false positive)	9
Cytological diagnosis	Negative	1(false negative)	0 (true negative)	1
	Total	10	0	10

Sensitivity =90%, Accuracy =90%, Specificity=100% and Predictive value of positive test=100%

Table No. 08: Corelation of cytologic and histopathological diagnosis in cases of lymphoma

		Histopathological diagnosis		
		Positive	**Negative**	**Total**
Cytological diagnosis	**Positive**	5 (true positive)	0 (false positive)	5

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	Negative	1 (false negative)	0 (true negative)	1
	Total	**6**	**0**	**6**

Sensitivity =83.33%, Specificity=100%, Predictive value of positive test=100%, Accuracy =83.33

Out 9 cases of reactive hyperplasia, 7 cases (77.7%) were correlated with finding of histopathology. 2 cases were false positive by FNAC, one was diagnosed as lymphoma and other one as metastatic carcinoma in final diagnosis.

Table No. 09: Corelation of cytologic and histopathological diagnosis in cases of reactive hyperplasia.

		Histopathological diagnosis		
		Positive	**Negative**	**Total**
Cytological diagnosis	**Positive**	7 (True Positive)	2 (False Positive)	**9**
	Negative	0 (False Negative)	0 (True Negative)	**0**
	Total	**7**	**2**	**9**

Sensitivity =100%, **Predictive value of positive test** = 77.78%, **Accuracy** = 77.78%

Table No. 10: Corelation of cytologic and histopathological diagnosis in cases of tuberculosis and granulomatous inflammatory lesion

Statistical values	**Tubercular lymphnoditis**	**Granulomatous inflammatory lesion**
True positive	10	1
True negative	0	0
False positive	0	0
False negative	0	0
Total	10	1

Sensitivity, Specificity, predictive value of positive test of FNAC in tubercular lymphnoditis and granulomatous inflammatory lesion was 100% and diagnostic accuracy also as 100%

Summary And Conclusion

Lymphadenopathy is one of the common clinical presentation of various ongoing disease process

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inside the body. The present study was conducted to find out etiological profile of lymphadenopathy as per cytological and histological examination and to evaluate the accuracy of FNAC in relation to histopathology.

The recent trend in medical practice is toward adopting a diagnostic modality that is both cost effective and minimally invasive. In this regard the diagnostic utility of FNAC seems to be ideal as a first line investigation in evaluating patients presenting with lymphadenopathy. FNAC is a reliable diagnostic method of providing with ease the identification of metastasis in patients with a suspicion of or known malignancy apart from other process.

Fine needle aspiration of all these lesions was done. All cytosmears were stained with hematoxylin and eosin and May Grunwald Giemsa stains. The cytomorphological features were recorded.

The aim of the present study is to highlight the role of FNAC in diagnosis of etiological profile of lymphadenopathy and to find out the accuracy of FNAC in comparison to biopsy. In our study a total of 34 patients were taken for study.

Subsequently the lymph nodes were excised or an open biopsy done and paraffin sections were prepared which were stained with routine hematoxylin and eosin stains. The results of cytomorpholgy were then correlated with histopathology. Out of 34 cases, the diagnosis was consistent with histopathology in 32 cases and not consistent in 2 cases. Therefore an accuracy rate of 88.89% was obtained and summarised as:

1. Cases of lymphadenopathy showed a wide range of age from 10 to 65 years. Mean age was 32.67 years.
2. Tubercular Lymphnoditis and Metastatic Carcinoma were the commonest causes in our study.
3. Commonest cause of lymphadenopathy in children was Reactive Hyperplasia, in adults Tubercular Lymphnoditis and Lymphoma and in older age Metastatic Carcinoma.
4. Immunohistochemistry was performed to know the type of lymphoma and to confirm the diagnosis. Two cases (33.3%) on IHC were found as Hodgkin lymphoma and 4 cases (66.7%) as non Hodgkin Lymphoma.
5. In our study, overall Sensitivity of FNAC was 94.12% and Diagnostic accuracy as 88.89%. Thus, FNAC was well correlated with histopathology.
6. FNAC is a simple and very accurate technique for the diagnosis of lymphadenopathy and may be used as a first line investigation in lymphadenopathy as a screening tool in outpatient clinic.

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