

## Cytohistopathological Analysis of Cervical Lymphadenopathy: A Prospective Study

Shailendra Yadav<sup>1</sup>, Mukesh Waghmare<sup>2</sup>, Pravin Meshram<sup>3</sup>, Nikhil Charmode<sup>4</sup><sup>1</sup>Assistant Professor, Dept. of Pathology, GMC Medical College, Nagpur<sup>2</sup>Assistant Professor, Dept. of Pathology, GMC Medical College, Nagpur<sup>3</sup>Assistant Professor, Dept. of Pathology, GMC Medical College, Nagpur<sup>4</sup>Assistant Professor, Dept. of Pathology, GMC Medical College, Nagpur

Received: 25-09-2023 / Revised: 23-10-2023 / Accepted: 18-11-2023

Corresponding Author: Dr. Nikhil Charmode

Conflict of interest: Nil

### Abstract:

**Introduction:** When it comes to diagnosing cancer and other disorders, the two-thirds of lymph nodes that are located in the neck are among the most important. In India, FNAC is a crucial diagnostic tool for tubercular lymphadenitis since it is effective, inexpensive, and easy to use. Even in juvenile situations, FNAC shows great specificity and sensitivity because of its rapidity, safety, and reproducibility, which contribute to accurate diagnosis and treatment planning.

**Aim and Objective:** To evaluate the Cytohistopathological characteristics of cervical lymph nodes.

**Methods:** The use of fine needle aspiration cytology (FNAC) was crucial to a prospective study of cervical lymphadenopathy that ran from 2022–2023. The patient group was evaluated comprehensively across many clinical domains, including medical history and cervical lymph node examinations. Cytological diagnoses were obtained using FNAC using sterilized needles. Contributing useful insights to pathology, the research sought to improve the accuracy of cervical lymphadenopathy diagnostics.

**Result:** This study found that the gender-specific distribution of non-neoplastic lesions revealed noteworthy patterns. Men exhibit a higher prevalence of acute and chronic lymphadenitis, whereas tuberculous lymphadenitis is more common in females. Reactive lymphadenitis slightly favours men. Overall, non-neoplastic lesions are notably more prevalent in men (51.14%) than in females (48.86%). The gender-based breakdown of total lesions (n=103) in the study underscores a higher incidence in men. Categorization reveals critical insights into primary and secondary lesion types, emphasizing the necessity for accurate diagnosis for optimal therapeutic strategies.

**Conclusion:** Painful adult neck lumps are prevalent. Travel, animal interaction and trauma are risk factors. Needless excisional biopsy can be avoided by using FNAC. FNAC and other tests reliably diagnose benign cervical lymphadenopathy, enabling conservative treatment in most patients.

**Keywords:** Neck Lumps, Lymphadenitis, Cytological, Tuberculous.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

### Introduction

Approximately two third of the lymph nodes in the body are located in the neck. Given the wide range of etiological factors, the expansion of those lymph nodes is quite a significant substance and serves as a gauge for the spread of cancer and diseases [1]. Although antibiotic treatment normally resolves acute infectious lymphadenopathy on its own, abscess development might happen and necessitate aspiration or incision [2].

Numerous genetically different and widely distributed sub-lineages of tuberculosis have emerged, each displaying unique disease phenotypic traits that have been shown in animal models and epidemiological research [3]. This may help to describe the wide range of clinical and

pathological manifestations of the illness in those who are unable to manage their infections, together with the variability of the host immune response as well as drainage. Numerous investigations have shown the high specificity and sensitivity of Fine Needle Aspiration Cytology (FNAC) [4].

Cervical lymphadenopathy is the most frequent concern that patients bring in ENT OPD. The cause might be everything from simple irritation to malignancy and tuberculosis; occasionally, it can be non-specific [5].

In India, one of the most typical reasons for swollen cervical lymph nodes is tubercular lymphadenitis. It is to be distinguished from other differential diagnoses, for which Fine Needle

Aspiration Cytology (FNAC) is one of the most trustworthy, economical, and essential diagnostic methods [6]. Depending on drainage of lymphatic system, the immune system responds by producing swollen lymph nodes. Neck masses are a common cause of worry in adults, children, and newborns [7].

Children are commonly encountered with cervical lymphadenopathy, a typical presentation in the outpatient section of otolaryngology. Enlargement of the lymph nodes may result from iatrogenic causes, infections, autoimmune illnesses, and other ailments [8]. When evaluating nodules and masses, Fine needle aspiration cytology (FNAC) provides a rapid, effective technique for the head and neck area. When compared to the golden norm of excisional lymph node biopsy, the specificity and sensitivity of FNAC in the paediatric age range are shown to be 94% and 100%, respectively [9]. In certain investigations, the diagnosis of metastatic cancer by FNAC was reported to be 100% successful. There is less research on the general population, even though limited research has been done on the clinicopathological manifestation of childhood cervical lymphadenopathy [10].

The histological analysis of biopsy material has long been the focal point of the pathology field. A cutting-edge and innovative diagnostic technique called fine needle aspiration cytology (FNAC) has just been available in the later part of the century. The first individual to describe the recovery of tumour cells using a needle to aspirate cells for microscopic analysis was Kun M (1847) [11].

The name FNAC was used to highlight its simplicity and to separate aspiration and exfoliative cytology with clarity. Aspiration is used to harvest diagnostic material and cytologic analysis from tissues that don't naturally loose cells [12]. An interdisciplinary strategy, involving communication and interventions between the physician, radiologist, and pathologist, is necessary for this operation to be as successful as possible. To extract tissue for microscopic analysis, FNAC is a speedy, relatively simple, safe and reproducible procedure. It also facilitates prompt interpretation of the material [13]. The patient's anxiety can be reduced by doing this operation in opd or by omitting the use with a local anaesthetic. It also enables prompt and effective treatment planning. A prospective study of FNAC of cervical lymph nodes was suggested to assess the precision and efficacy of FNAC in identifying the existence of cervical lymphadenopathy [14].

## Method

### Research Design

A prospective research study on cervical lymphadenopathy, using fine needle aspiration cytology (FNAC), was carried out at our Hospital. The research was conducted from November 2022 to October 2023, lasting for one year. The patient population included referrals from many clinical areas, such as Ear, Nose, and Throat (ENT), General Surgery, and Medicine. Patients had extensive clinical examination, including a full evaluation of their medical history. Data was collected on the length and commencement of complaints, as well as the existence of symptoms such as fever, chills, night sweats, weight loss, hoarseness of voice, difficulty in breathing, difficulty in swallowing, coughing, bouts of bleeding, discomfort, weakness, and any relevant medication use. An exhaustive evaluation of cervical lymph nodes was conducted, taking into account variables such as location, quantity, level, dimensions, texture, mobility, boundary, skin condition above the node and clumping. An assessment was also conducted on the presence of generalized lymphadenopathy. Before performing FNAC, the subjects were given informed permission and rigorous aseptic measures were followed throughout the process. The procedure of fine needle aspiration was performed with a sterile needle with a gauge size of either 21 or 23, which was connected to a syringe with a capacity of 10 millilitres. The smears were immediately collected using 95% ethyl alcohol as fixatives and then stained using the Haematoxylin, Papanicolaou stain. In addition, several slides were dried using air and then treated with May Grunwald Giemsa (MGG) and Leishman-Giemsa (LG) stains. The cytological diagnoses were determined by analyzing the stained slides. Histopathological correlation was conducted for patients in whom excision was done. The primary objective of the research was to improve comprehension and diagnostic precision in instances of cervical lymphadenopathy, hence providing significant contributions to the area of pathology.

### Inclusion and Exclusion criteria

#### Inclusion

- Ages 18–70 were relevant to the research goals.
- Cervical lymphadenopathy patients diagnosed clinically.
- The ENT, General Surgery, and Medicine departments recommend patients.

#### Exclusion

- Persons beyond the study's age range.
- Initial clinical examinations identify patients with cervical swellings not caused by lymphadenopathy.
- Participants would not prefer to provide informed permission for the research.

- Insufficient medical history information made it impossible to analyze the start and duration of symptoms or other essential facts.

**Statistical Analysis**

The study used SPSS 27 software for conducting statistical analysis. To assess the procedure's effectiveness, the results of the cytological and histological analyses were correlated using Bivariate Correlation. The clinical and pathological parameters were examined, and their significance in making a diagnosis was also assessed. MS Excel was used for creating graphs and other calculations. The continuous data were expressed as standard deviation while the discrete data were expressed as frequency and its respective percentage. Statistical computations were performed where appropriate, and a p-value of  $\leq 0.05$  was considered to be significant.

**Result**

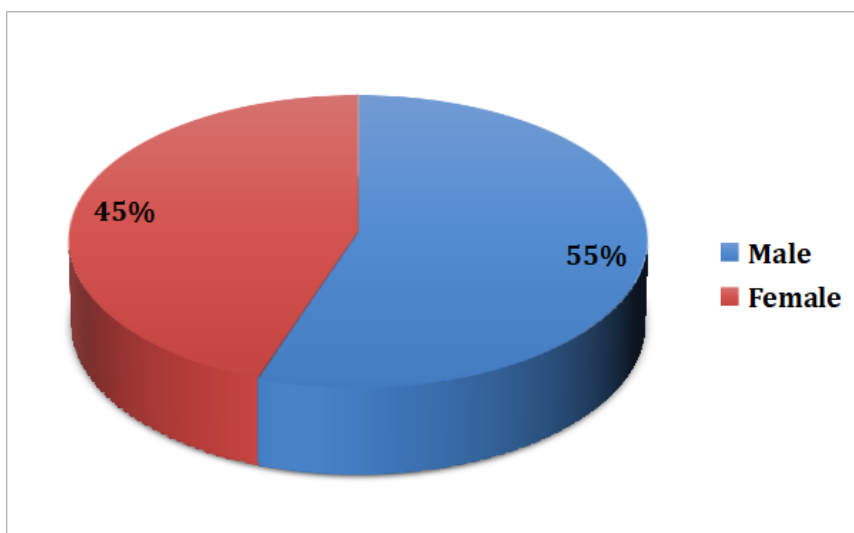
Sex distribution of particular non-neoplastic lesions, classified into distinct kinds, is shown in Table 1. Among the lesions, men were more likely to have acute lymphadenitis (100% male prevalence) and chronic non-specific lymphadenitis (66.67% male prevalence) than females (33.33% female prevalence). In contrast, tuberculous lymphadenitis was more prevalent in females (63.64%) than in men (36.36%). The frequency of reactive lymphadenitis was somewhat greater in men (53.84%) than in females (46.16%). The miscellaneous category did not affect any females whatsoever. In the research, which examined 88 cases in total, the frequency of non-neoplastic lesions was somewhat greater in men (51.14%) than in women (48.86%).

**Table 1: Showing sex-wise distribution of individual non-neoplastic lesions**

Lesions	Sex				Total
	Male	%	Female	%	
Acute lymphadenitis	2	100	-	-	2 (100%)
Chronic non-specific lymphadenitis	18	66.67	9	33.33	27 (100%)
Tuberculous lymphadenitis	16	36.36	28	63.64	44 (100%)
Reactive lymphadenitis	7	53.84	6	46.16	13 (100%)
Miscellaneous	2	100	-	-	2 (100%)
Total	45	51.14	43	48.86	88 (100%)

Table 1 shows the breakdown of total lesions by sex, which includes 103 cases. There were 57 male cases (55.34% of the total) and 46 female cases (44.66% of the total). Lesions are more common in men than in females, according to the general distribution. Although there were more men than

females, the overall number of lesions was 103. This data suggests that there may be a variation in the incidence of lesions depending on gender. It is important to investigate further what variables contribute to this distribution in the group that was analyzed.



**Figure 1: Showing sex-wise distributions of total lesions (n=103)**

Table 2 shows primary and secondary lesions, their types, numbers and percentages. With 1 instance of the particular subtype Nodular Sclerosis, Hodgkin's lymphoma accounted for 11.11% of the primary

lesions. The subtypes Small Cell Type and Diffuse Type were the most common in non-Hodgkin's lymphoma, which had a greater frequency of 88.89%. The percentage of secondary lesions

resulting from adenocarcinoma was 50%, whereas the percentage originating from poorly differentiated carcinoma was 33.33 per cent. It is crucial to differentiate between various kinds of

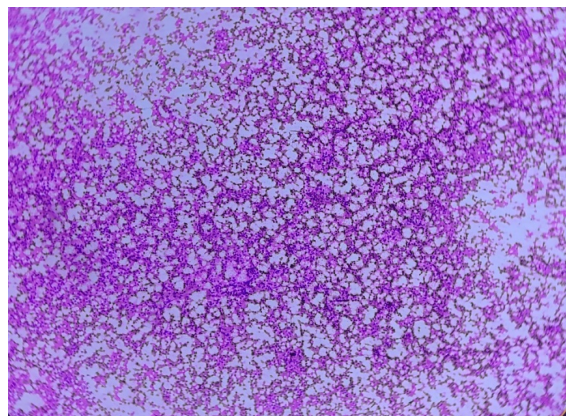
lesions to provide an accurate diagnosis and provide the right treatment since the evidence shows that both primary and secondary lesions are diverse.

**Table 2: Showing types of primary and Secondary lesions [Neoplastic]**

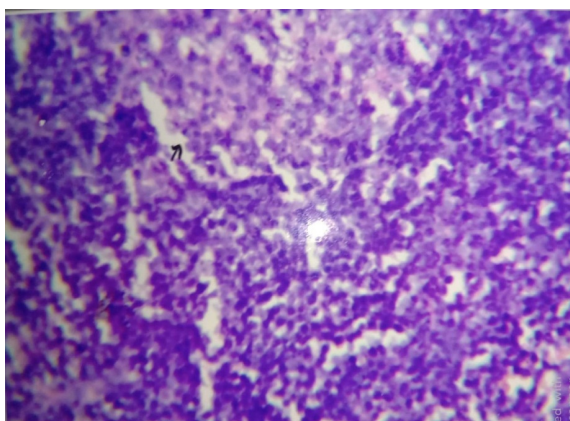
Primary lesions [Neoplastic]			Secondary lesions[Neoplastic]		
Type	No	%	Type	No	%
Hodgkin's lymphoma	1	11.11	Secondaries from adenocarcinoma	3	50
Nodular Sclerosis	1				
Non-Hodgkin's Lymphoma	8	88.89	Secondaries from poorly differentiated carcinoma	2	33.33
Small cell type	6	75			
Diffuse type	1	12.5	Secondaries Ca. larynx (squamous cell Ca)	1	16.67
Mixed type	1	12.5			
Total (Hodgkin's and Non-Hodgkin's lymphoma)	9	100	Total	6	100



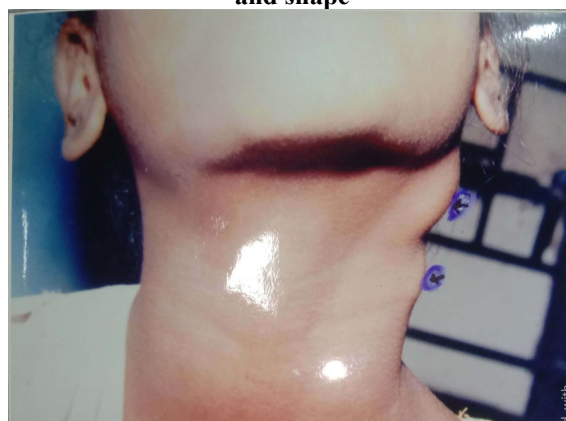
**Figure 1: Showing clinical photograph of reactive lymphadenitis**



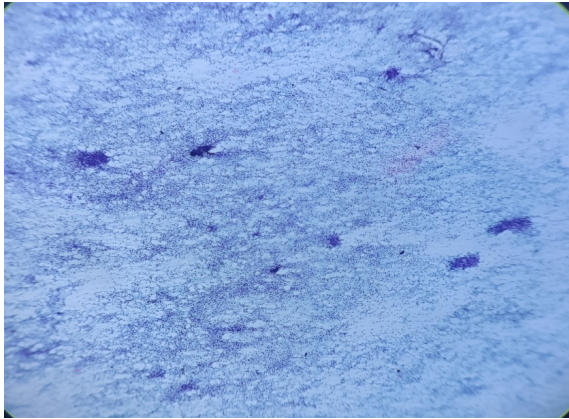
**Figure 2: Microphotograph of reactive lymphadenitis showing cellular aspirate composed of dispersed cells with marked variability in size and shape**



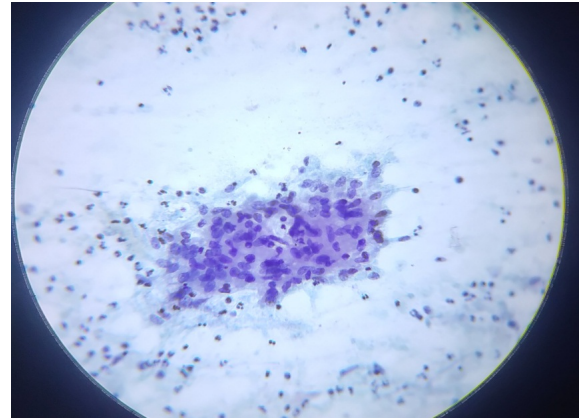
**Figure 3: Microphotograph showing high power view of Reactive lymphadenitis.**



**Figure 4: Showing clinical photograph of tuberculous lymphadenitis**



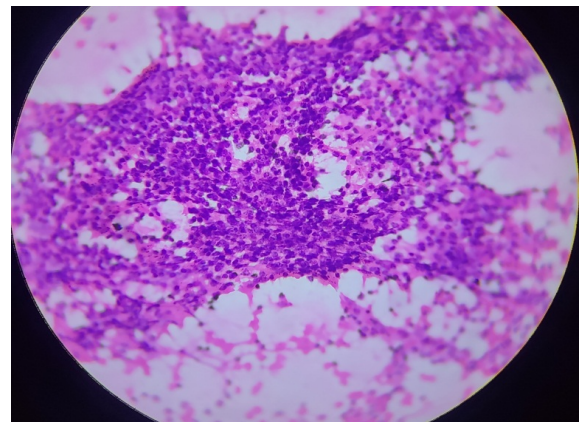
**Figure 5:** Microphotograph of slide prepared from FNA of Lymph node and shows epithelioid granulomas with pale amorphous background



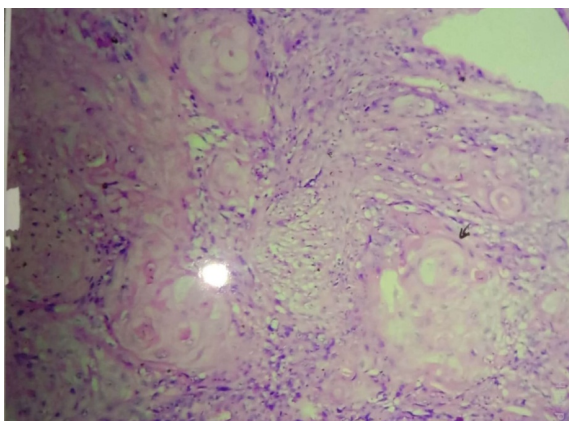
**Figure 6:** Microphotograph of FNA lymph node shows cluster of epithelioid histiocytes and single forms mixed with lymphocyte[GRANULOMA] [40x]



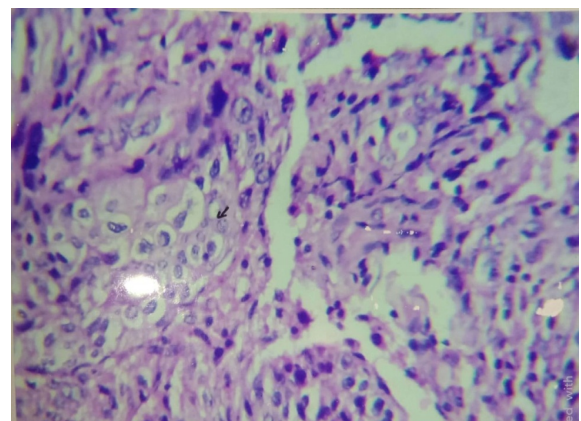
**Figure 7:** Showing clinical photograph of metastatic lymphadenopathy from squamous cell carcinoma larynx.



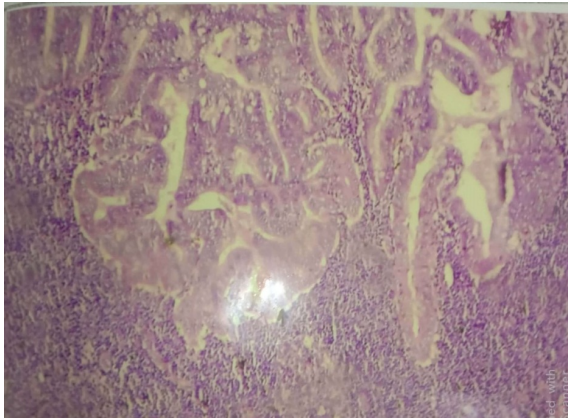
**Figure 8:** Microphotograph of aspirate from lymph node with secondaries of squamous cell carcinoma showing atypical keratinizing squamous cells



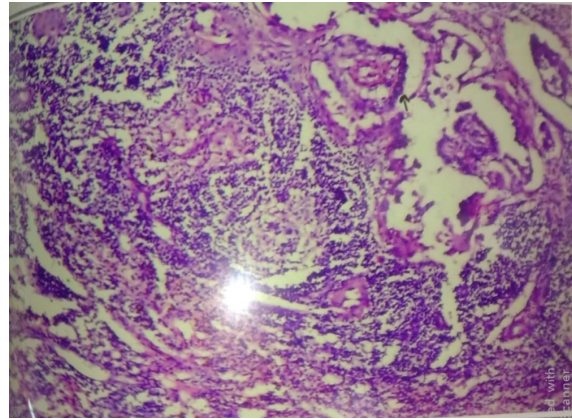
**Figure 9:** Microphotograph of squamous cell carcinoma from carcinoma larynx metastasize to lymph node showing well-formed keratin pearls



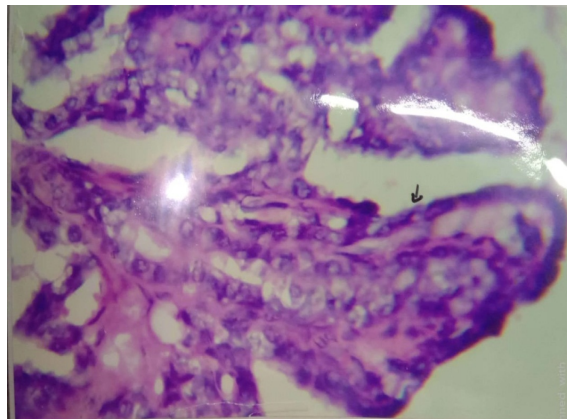
**Figure 10:** Microphotograph showing secondaries from poorly differentiated carcinoma with pleomorphic cells ( H and E stain 400X)



**Figure 11: Microphotograph showing metastasis of adenocarcinoma to lymph node with well-formed glands and rim of residual lymph node seen (H and E stain 40X)**



**Figure 12: Microphotograph showing secondaries of papillary carcinoma of thyroid in lymph node (H and E stain 10X)**



**Figure 13: Magnified view of secondaries of papillary carcinoma thyroid in lymph node (H and E 40X)**

## Discussion

A study was conducted to ascertain the use of a trustworthy diagnostic technique for cervical lymphadenopathy when used in conjunction with the gold-standard histological analysis of the removed lymph node [15]. The FNAC test is a dependable, secure, and precise method for assessing cervical lymphadenopathy in the first stages. Given the high measure of diagnostic accuracy demonstrated by the statistical results in this investigation, it is clear that FNAC has clinical usefulness to cervical lymph nodes [16].

The method of aspiration with a fine needle cytology (FNAC) offers a rapid, efficient, and reasonably priced means of assessing the easily apparent surface masses. Given that inguinal, axillary, and cervical lymphadenopathies are frequently observed clinical issues, we assessed the effectiveness of FNAC for lymphadenopathy evaluation in this study. Without the need for an incisional or transverse biopsy, the cause of lymphadenopathy may be quickly and accurately classified into four groups by FNAC: reactive, inflammatory/infectious, metastatic, and lymphoproliferative [17]. Additionally, the area of

involvement, age, and gender are helpful indicators of the lymphadenopathy's aetiology. It was shown that young persons were more likely to have reactive lymphadenitis, but middle-aged and older adults were more likely to have tuberculous lymphadenitis, metastatic cancer, etc. Comparably, cervical lymph nodes showed higher rates of tuberculous lymphadenitis than axillary & inguinal lymph nodes, although the latter two locations showed higher rates of metastatic cancer [18].

A quick, low-cost outpatient treatment called fine needle aspiration cytology (FNAC) of different lymph nodes is used to diagnose a variety of lymphadenopathy causes. It is employed not only to make the cytological diagnosis of tuberculous lymphadenitis but also for additional ancillary tests such as AFB culture and Ziehl-Neelsen staining [19]. The purpose of the study was to assess the cytopathological structure of FNAC aspirates from individuals who had lymphadenopathy, with a focus on tuberculous lymphadenopathy [20]. A straightforward, reasonably priced method with a high degree of accuracy for identifying tubercular lymphadenitis is FNAC. In underdeveloped nations where TB is more prevalent, FNAC in conjunction with Ziehl-Neelson staining ought to be the initial

course of inquiry for patients including lymphadenopathy, notwithstanding various drawbacks and difficulties [21].

One of the most prevalent clinical manifestations in individuals of all ages is cervical lymphadenopathy [22]. When identifying instances of lymphadenopathy, FNAC is an easy, affordable, and non-invasive medical care that can be administered via an outpatient procedure. FNAC should be used to evaluate the different causes of cervical lymphadenopathy, as well as to connect histological results with lesion distribution according to age and gender [23]. Cervical lymphadenopathy may be diagnosed with high sensitivity, specificity, & diagnostic accuracy using FNAC, a low-risk, low-cost, and fast technique. Granulomatous lesions were more common in non-neoplastic patients.

The purpose of the study is to assess cervical lymph nodes' fine-needle aspiration cytology (FNAC) diagnostic accuracy and reliability, with a focus on situations where the cytology & the histology disagree. Overall FNAC cervical lymph node diagnosis with a total discordance rate of 17.8%, accuracy was 82.2%. An experienced cytopathologist should evaluate FNA in patients who have not previously been diagnosed with cancer [24,25]. The interpretation of this test should be made in light of clinical, radiological, as well as laboratory findings. If any of these findings are lacking, additional research is warranted to address the limitations and drawbacks of using cytomorphological features alone [26].

Since fine-needle cytology is less intrusive, more affordable, and simpler than biopsy, it is being used as a preliminary diagnostic tool for swellings of the head and neck. The study set out to assess the findings of cervical lymphadenopathy using fine-needle non-aspiration cytology and look at the factors influencing the non-diagnosis rate [27]. Cervical lymphadenopathy is currently most commonly caused by TB in North Africa. When it comes to the identification of cervical tuberculous lymph nodes that carry the danger of non-diagnostic cytology, fine-needle non-aspiration cytotoxicity is both accurate and safe [28].

### Conclusion

This study concluded that a painful neck lump is a frequent adult complaint. Possible patient-specific risk factors include prior trauma, travel, animal interaction, and history. A physical exam should assess lymph node location, consistency and discomfort. This research found FNAC to be a valuable diagnostic tool for determining the cause of cervical lymphadenitis. Most patients can avoid excisional biopsy. Patients need intensive assessment, including chest X-ray, Montoux test, FNAC, and histological study. The evaluation is

easy, safe, cost-effective, well-tolerated, minimally invasive, and has no recorded problems. This test is effective in identifying cervical lymphadenopathy, particularly when paired with other tests. The majority of cervical lymphadenopathies are benign. When using FNAC as a diagnostic test, most patients may be dealt with conservatively, with surgery very uncommon. Despite extensive research on cervical lymphadenopathy and FNAC, the molecular and genetic mechanisms affecting gender-specific non-neoplastic lesion distribution are still unknown. Exploring these aspects may improve diagnosis and therapy. Future studies should examine molecular markers and genetic differences that cause gender-specific cervical lymphadenopathy patterns. This greater knowledge might improve patient outcomes via focused treatments, tailored treatment regimens, and diagnostic advances.

### References

1. Mili MK, Phookan J. A clinicopathological study of cervical lymphadenopathy. *Int J Dent Med Res.* 2015; 1(5):24–27
2. MacGregor FB. Tumours and cysts of the head and neck. In: Musheer Hussain S (ed) *Logan Turner's diseases of the nose, throat and ear: head and neck surgery*, 11th ed. CRC Press, Boca Raton. 2015.
3. Wirth T, Hildebrand F, Allix-Be'guec C, Wo'lbeling F, Kubica T, Kremer K et al. Origin, spread and demography of the Mycobacterium tuberculosis complex. *PLoS Pathog.* 2008; 4(9):e1000160.
4. Lopez B, Agular D, Orozco H, Burger M, Espitia M, Ritacco V, Barrera L, Kremer K, Hernandez-Pando R, Huygen K, Van Sooligen D. A marked difference in pathogenesis and immune response induced by different Mycobacterium tuberculosis genotypes. *Clin Exp Immunol.* 2003; 133(1):30–37.
5. Pareek M, Evans J, Innes J, Smith G, Hingley-Wilson S, Loughheed KE, Sridhar S, Dedicoat M, Hawkey P, Lalvani A. Ethnicity and mycobacterial lineage as determinants of tuberculosis disease phenotype. *Thorax.* 2013; 68(3):221–229.
6. Rozovsky K, Hiller N, Kopelwitz BZ, Simonovsky N. Does CT have an additional diagnostic value over ultrasound in the evaluation of acute inflammatory neck masses in children? *Eur Radiol.* 2010; 20:484–490
7. Papadopoulos E, Michailidi E, Papadopoulou E, Paspalki P, Vlahakis I, Kalmanti M. Cervical lymphadenopathy in childhood epidemiology and management. *Pediatr Hematol Oncol.* 2009; 26(6):454–460.
8. Bazemore AW, Smucker DR. Lymphadenopathy and malignancy. *Am Fam Physician.* 2002; 66:2103–2110.

9. Layfield LJ. Fine-needle aspiration of the head and neck. *Pathology*. 1996;4(2):409–438.
10. Reddy MP, Moorchung N, Chaudhary A. Clinicopathological profile of pediatric lymphadenopathy. *Ind J Pediatrics*. 2002; 69(12): 1047–1051.
11. Diagnostic role of fine needle aspiration cytology (FNAC) in the evaluation of salivary gland swelling: an institutional experience. Naz S, Hashmi AA, Khurshid A, Faridi N, Edhi MM, Kamal A, Khan M. *BMC Res Notes*. 2015; 8:101.
12. Diagnostic accuracy of fine needle aspiration biopsy in pediatric small round cell tumours. Asim M, Mudassir G, Hashmi AA, et al. *BMC Res Notes*. 2018; 11:573.
13. Diagnostic accuracy of Bethesda system for reporting thyroid cytopathology: an institutional perspective. Naz S, Hashmi AA, Khurshid A, Faridi N, Edhi MM, Kamal A, Khan M. *Int Arch Med*. 2014;7:46.
14. 4. Diagnostic accuracy of touch imprint cytology for head and neck malignancies: a useful intra-operative tool in resource-limited countries. Naveed H, Abid M, Hashmi AA, Edhi MM, Sheikh AK, Mudassir G, Khan A. *BMC ClinPathol*. 2017; 17:25.
15. Role of peritoneal washing cytology in ovarian malignancies: correlation with histopathological parameters. Naz S, Hashmi AA, Ali R, Faridi N, Hussian SD, Edhi MM, Khan M. *World J Surg Oncol*. 2015; 13:315.
16. Diagnostic utility of fine needle aspiration cytology in the evaluation of peripheral lymphadenopathy. Faro RO, Mohammed AZ, Atanda AT. *West Afr J Med*. 2018; 35:162–167.
17. K Das Dilip. Fine-Needle Aspiration Cytology in the Diagnosis of Tuberculosis Lesions. *Laboratory Medicine*. 2000;31(11):625–32.
18. Ruchi Khajuria, KC Goswami, K Singh, VK Dubey. Pattern of Lymphadenopathy on Fine Needle Aspiration Cytology in Jammu. *JK Science*. 2006;190(3):158–60.
19. RR Prasad, R Narasimhan, V Sankran, AJ Veliath. Fine needle aspiration cytology in the diagnosis of superficial lymphadenopathy: an analysis of 2418 cases. *Diagno Cytol*. 1996; 15(5):382–86.
20. PC Paul, BK Goswami, S Chakrabarty, A Giri, R Pramanik. Fine needle aspiration cytology of lymph nodes-An institutional study of 1448 cases over five years. *J Cytology*. 2004; 21:187–90.
21. AK Gupta, M Nayar, M Chandra. Critical appraisal of fine needle aspiration cytology in tuberculosis. *ActaCytol*. 1992;36(3):391–94.
22. Hirachand S, Lakhey M, Akhter J, Thapa B. Evaluation of fine needle aspiration cytology of lymph nodes in Kathmandu Medical College, Teaching hospital. *Kathmandu Univ Med J*. 2009; 7(2):139–42.
23. Bazemore AW, Smucker DR. Lymphadenopathy and malignancy. *American family physician*. 2002 Dec;66(11):2103-110.
24. Young JA. Fine needle aspiration cytopathology. *J of Pathol*. 1993 Jan; 169(1): 109-114.
25. S. Hirachand, M. Lakhey, J. Akhter, B. Thapa Evaluation of fine needle aspiration cytology of lymph nodes in Kathmandu Medical College, Teaching hospital Kathmandu Univ Med J, 2009; 7 (26):139-142
26. V.E. Keith, S.K. Harsharan, G.Z. Jerald Fine needle aspiration biopsy of lymph nodes in the modern era: reactive lymphadenopathies *Pathol Case Rev*, 2007;12(1):27-35
27. P. Adhikari, B. Sinha, D. Baskota. Comparison of fine needle aspiration cytology and histopathology in diagnosing cervical lymphadenopathies. *Australas Med J*, 2011;4: 97-99
28. S.R. Dukare, D.S. Jadhav, A.L. Gaikwad, S.N. Ranka, P.B. Kale, G. D'Costa. Fine needle aspiration cytology of cervical lymphadenopathy – a study of 510 cases. *Asian J Sci Technol*, 2014;5: 537-540.