

Original Research Article**EVALUATION OF FINE NEEDLE ASPIRATION CYTOLOGY TECHNIQUE FOR
EARLY DIAGNOSIS OF TUBERCULAR LYMPHADENITIS IN PATIENTS
PRESENTING AT A PRIMARY CARE SETTING IN INDIA.****ABSTRACT**

Aims: The aim of the present study is to evaluate whether FNAC helps in detecting tubercular lymphadenitis (TB LAN) at an early stage, in a primary care setting. Demographic and cytomorphological profile of TB LAN in this part of the country is also analyzed.

Study design: The study is a retrospective analysis of all FNAC procedures carried out in patients of lymphadenopathy (LAP). Cases diagnosed as TB LAN are analyzed further.

Place and Duration of Study: All patients of LAP presenting at Ama Diagnostic Centre, Lucknow, Uttar Pradesh, India, between August 2008 to December 2015.

Methodology: FNAC procedure was carried out on 780 patients of whom 762 cases yielded adequate material. Of these, 245 cases were diagnosed as TB LAN based on cytomorphological details and Zeihl Neelsen staining.

Results: TB LAN is the second most common cause of lymphadenitis. Lymph nodes of head and neck region are involved most frequently in TB LAN. Majority of TB LAN patients are in 2nd and 3rd decade of life. The disease tends to occur at a younger age in this part of the country.

Conclusions: Cytomorphological profile of the aspirates indicates that use of FNAC helps in detecting TB LAN at an earlier stage of the disease. Majority of the cases of TB LAN are seen between 10-30 years of age. It also occurs at an earlier age in this part of the country. In about 90% of cases TB LAN tends to involve lymph nodes of the head and neck region.

Keywords: Tuberculosis of lymph nodes, Lymph node aspiration cytology, Lesions of lymph node.

1. INTRODUCTION

Lymphadenopathy is a commonly encountered problem in our country because of high incidence of acute and chronic infectious diseases and the rising trend of malignant disorders. The general recommendation regarding management of lymphadenopathy (LAP) of recent onset in case of children is to manage the patient on conservative lines for a period of two weeks. Further investigations are warranted only when LAP fails to regress within 4-6 weeks. The rationale behind this proposal is the fact that in majority of instances, LAP during childhood is reactive in nature and after conservative treatment, shows early resolution [1]. However, this proposal may not always hold true in our country because LAP in children of reactive nature, can persist for a longer duration, due to widespread nutritional deprivation and the resultant immune deficiency. Recurrent episodes of acute reactive lymphadenitis - as may occur in under nourished kids - often leads to chronic lymph node enlargement. Also because of poor economic status, many patients often do not seek early treatment. This produces a confounding clinical picture making it difficult to distinguish - on clinical examination alone - between chronic reactive LAP and chronic lymphadenitis due to specific disorders like tuberculosis (TB). For LAP of adults over 40 years of age, the recommendation states that it should be investigated as early as possible, especially when it involves supraclavicular, posterior cervical or deep seated lymph nodes. This is based on the fact that in adults, comparatively a higher percentage of enlarged lymph nodes show metastatic deposits [2].

From last three decades, fine needle aspiration cytology (FNAC) is being used astutely all over the world for investigating LAP. In our country, the procedure can be of great relevance in distinguishing chronic non-specific reactive LAP from chronic lymphadenitis of a more specific nature like TB. The aim of the present study is to evaluate whether use of FNAC in a primary care setting can help in detecting TB LAN at an early stage of the disease. This would lower the cost of management of a patient with TB LAN. Demographic and cytomorphological profile of TB LAN in this part of the country is also analyzed.

2. MATERIAL AND METHODS

2.1 Study Area

The present study is a retrospective analysis of FNAC carried out for diagnostic purpose in patients of LAP in Pathology section of Ama Diagnostic Centre, Lucknow, Uttar Pradesh, India. The period of study is from August 2008 to December 2015. FNAC procedure was carried out in a total of 780 patients.

2.2 Sample Collection

After explaining the procedure to the patient, consent to undergo the procedure was taken. All risks associated with the procedure – however minor – were explained to the patient. Consent was obtained from the parents, if the patient was under 16 years of age. Following thorough cleansing of the site of aspiration with 70% alcohol and povidone iodine solution, sample was collected using a 10 ml syringe attached to 21 or 23 gauge needle. Multiple air-dried and/or wet-fixed smears were prepared.

2.3 Laboratory Procedure

Smears were first stained by May Grunwald Giemsa (MGG) and Papanicolaou technique for preliminary evaluation. If indicated, Zeihl Neelsen stain for acid fast bacteria and Gram staining were also done. All cases were initially segregated into different disease categories as per criteria given in standard text books of cytology. Cases of TB LAN were analyzed further. Diagnosis of tubercular lymphadenitis and their sub-categorization was done based on following criteria:

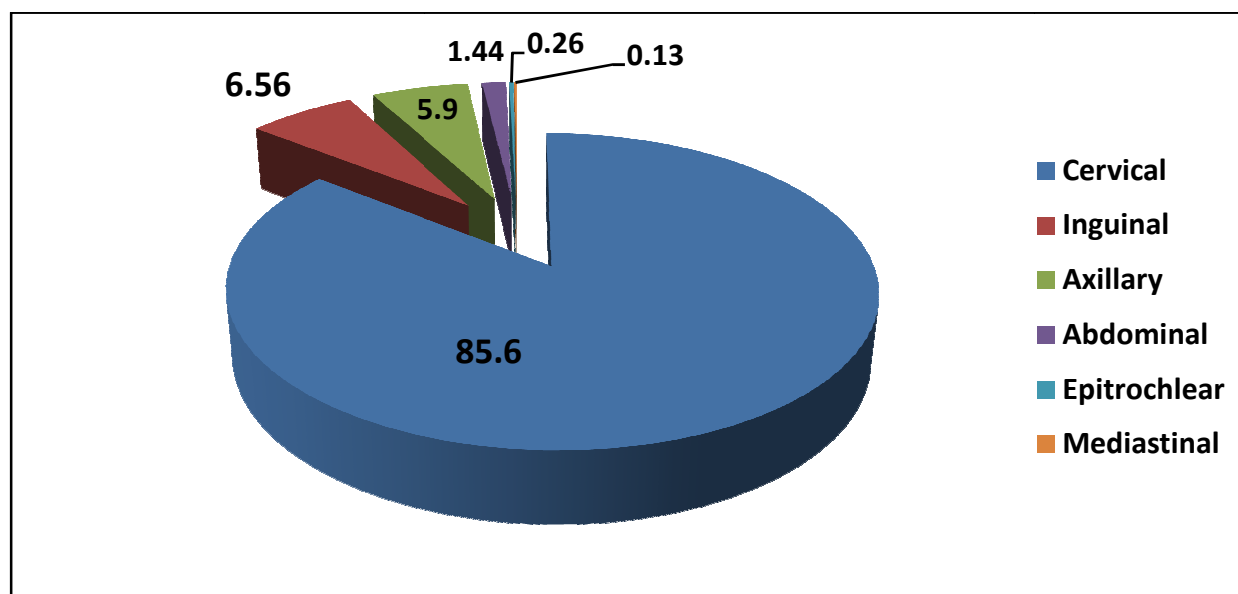
- Group 1: Epithelioid cell granulomas + caseous necrosis.
- Group 2: Presence of caseous necrosis only +/- demonstration of AFB
- Group 3: Epithelioid cell granulomas against an inflammatory background +/- demonstration of acid fast bacteria.
- Group 4: Suppurative necrosis + demonstration of acid fast bacteria (AFB) on Zeihl Neelsen stain (ZN stain).

Cases showing caseous necrosis only, were also classified as tubercular, because previous studies indicate that very few diseases in India - apart from tuberculosis - produce caseation necrosis.

RESULTS

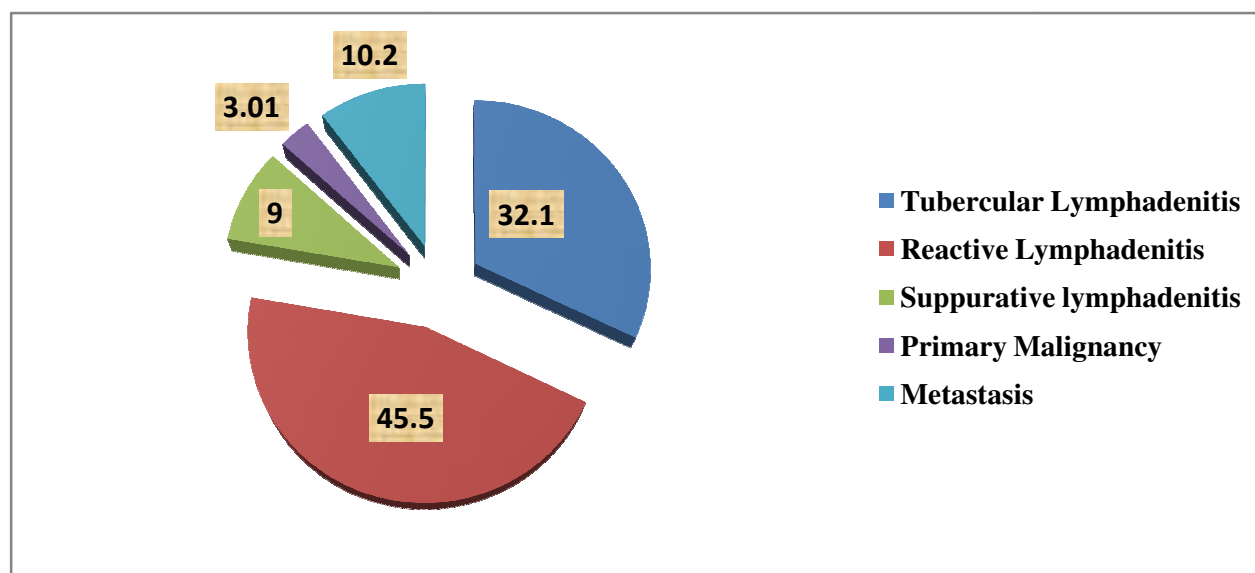
Over the period of 7 years and 5 months, a total of 780 patients of LAP underwent fine needle aspiration procedure for diagnostic purposes. Adequate material was obtained in 762 patients. The anatomical distribution of lymph nodes aspirated is shown in figure 1. It is seen that cervical lymph nodes were the commonest group to be involved by a disease process (85.6% of cases) followed by inguinal (6.56%) and axillary group (5.9%) of lymph nodes.

Figure 1: Distribution of all cases of LAP in different lymph node groups



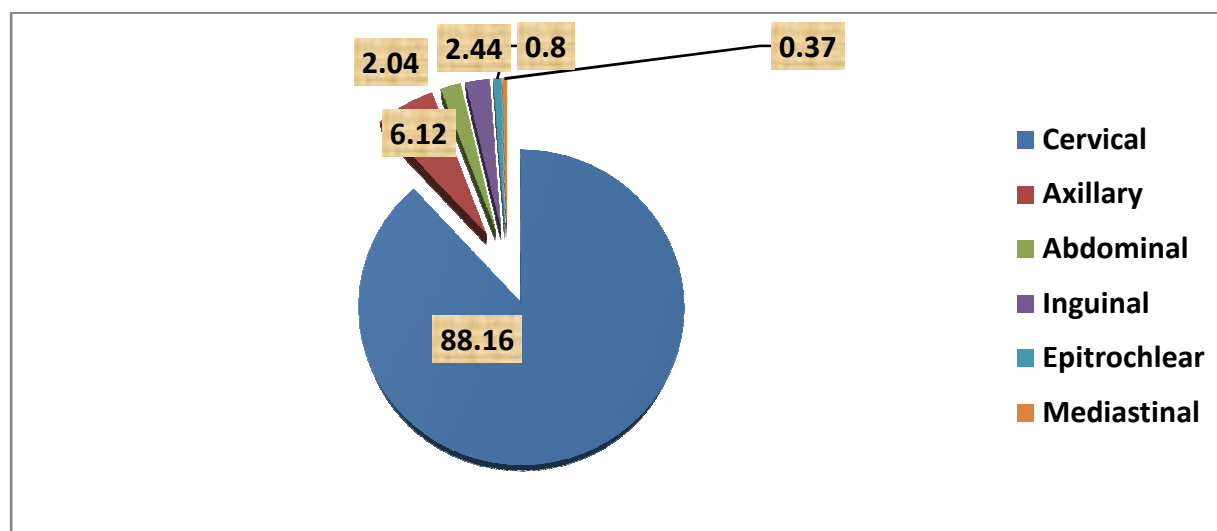
The distribution of disease amongst 762 successful aspirates is shown in figure 2. It is seen that reactive non-specific lymphadenitis was the commonest diagnosis rendered (45.5% of cases) followed by tubercular lymphadenitis (32.1% of cases). A total of 245 cases of LAP belonged to tubercular lymphadenitis (TB LAN) group.

Figure 2: Distribution of disease amongst all cases of LAP



Distribution of TB LAN in different anatomical groups is shown in figure 3. It is seen that cervical group of lymph nodes are involved most often in tuberculosis (88.16% of cases) followed by axillary (6.12% of cases) and inguinal (2.44% of cases) group of lymph nodes.

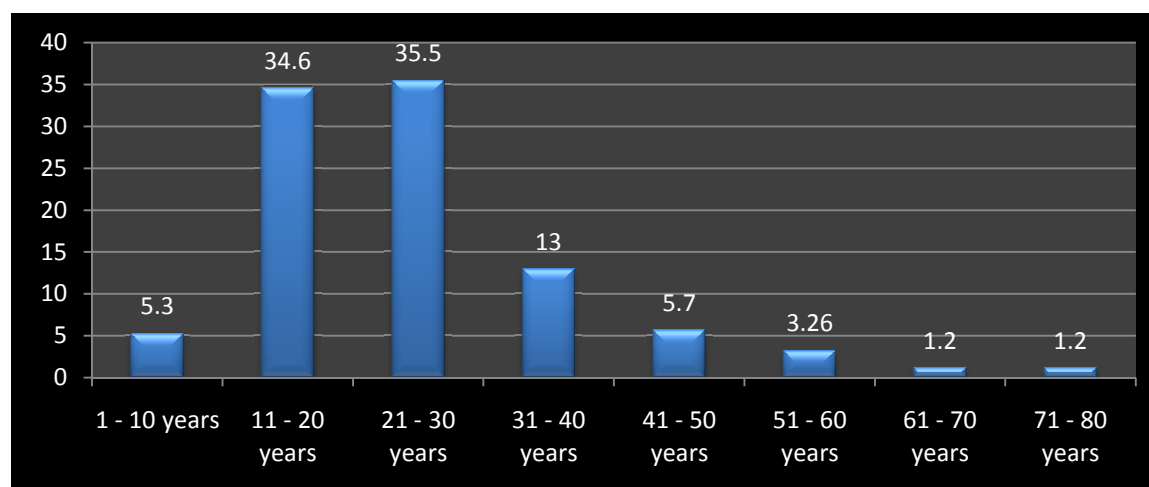
Fig 3: Distribution of tubercular lymphadenitis in different anatomical lymph node groups



The age distribution of TB LAN patients is shown in figure 4. In our series, maximum numbers of cases of TB LAN are seen in second (34.6% of cases) and third decade (35.5% of cases) of life.

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89 **Figure 4: Age wise distribution of tubercular lymphadenitis**



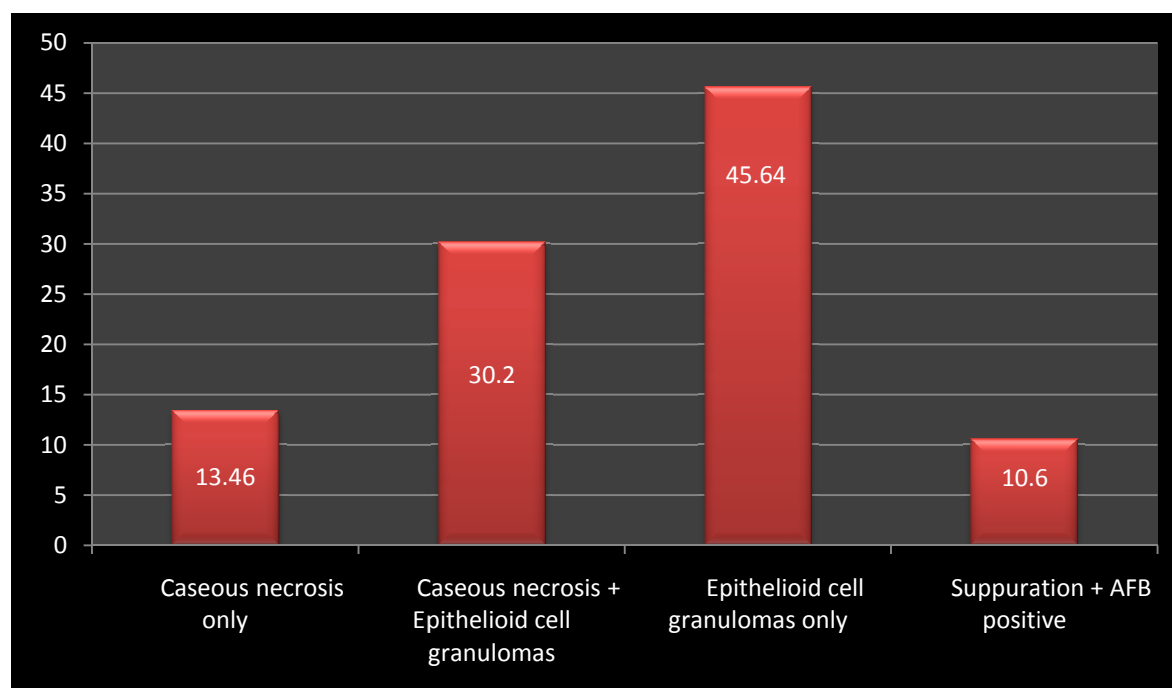
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92 Different cytomorphological features of TB LAN in our series are shown in figure 5. It is observed that
 93 presence of epithelioid cell granulomas against a background of reactive inflammatory cell population
 94 (group 3 patients) was the commonest pattern seen in our series (45.64% of cases).

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96 **Figure 5: - Cytomorphological features seen in tubercular lymphadenitis**



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98 As the cytological picture of group 1 and 2 patients is regarded as diagnostic of tuberculosis, ZN staining
 99 for demonstration of AFB was done mainly for group 3 and 4 patients. AFB was demonstrated in 21.6% of

group 3 cases. As per the protocol of our study, Group 4 cases were classified as tubercular only when they demonstrated AFB positivity.

DISCUSSION

Human body is kept safe from invading microorganisms and rogue circulating neoplastic cells with the help of approximately 600 lymph nodes - tiny sentinel of our fragile health. On provocation, lymph nodes flare up and enlarge, ready to devour the hostile elements. At the same time, swollen lymph nodes act as a warning sign to the patient and his physician. However, bloated lymph nodes - which may represent minor irritants like reactive lymphadenitis or devastating illnesses like metastatic deposits - do not reveal their secrets easily. A clinician confronting lymphadenopathy has to wade through myriad of differential diagnostic possibilities to seek a definitive diagnosis. The problem becomes all the more confounding when lymphadenopathy is deep seated, as the lesion is not easily accessible for microscopic evaluation. To add to this puzzle, are problems of medical resource deficiencies in developing countries, culturally inherent dislike of many patients to go under the knife and often the poor economic status of a patient that precludes use of more advanced diagnostic facilities. In this baffling scenario, FNAC seems to have emerged as a handy contrivance for the beleaguered patient and his physician.

In India, for a variety of reasons, TB has been an endemic disorder since a long time. Poverty, malnutrition, high illiteracy rate, lack of universal health care, overcrowding and insanitary living conditions - factors considered to be great allies of this once dreaded disease - are encountered frequently and to varying extent in different parts of this country. Every year 9.4 million new cases of TB are seen world over. India accounts for about 20% of these cases. This translates to 1.98 million new cases of TB every year in India. In HIV negative individuals, extrapulmonary TB (EPTB) constitutes 20% of all cases of TB and nodal TB constitutes 35% of all cases of EPTB. Extrapolation of this data indicates that about 0.12 million new cases of lymph node TB occur every year in India [3].

In recent times, with emergence of drug resistant strains of mycobacteria, treatment of TB has become more complicated. Moreover, management of nodal tuberculosis differs from pulmonary TB. Central Uttar Pradesh is home to many of the factors that promote TB. Being a contagious disease, to eradicate tuberculosis, it is important that the disease be detected at its earliest stage. Whether FNAC can play a role in realization of these efforts, is investigated in our study. As detailed later, it is essential that the implicated strain of mycobacteria in TB LAN should also be identified - as it influences treatment. Fine needle aspiration can contribute in these efforts.

We have also studied the pattern of cytomorphological changes seen in TB LAN and compared our findings with workers from other parts of India. Utilizing already known facts about temporal evolution of tubercular lymphadenitis, an attempt has been made to see whether FNAC can help in detecting the disease at an early stage.

Between, October 2008 to December 2015, a total of 780 patients underwent FNAC procedure for LAP. Adequate material was obtained in 762 patients and they constituted the study group of this series. In our study, lymph nodes of head and neck were the commonest group to be involved by a disease process

(88.16% of cases). Other series from India also indicate that cervical lymph nodes are involved most frequently. Incidence of cervical lymph node involvement, as reported by some other workers ranges from 73- 83% [4 -7]. The obvious reason for greater involvement of cervical group of lymph nodes is their close proximity to anatomical passages of the body that are constantly exposed to environmental infectious agents. They also drain lymphatics from a large part of the body, hence the increased likelihood of acting as a repository for metastatic deposits.

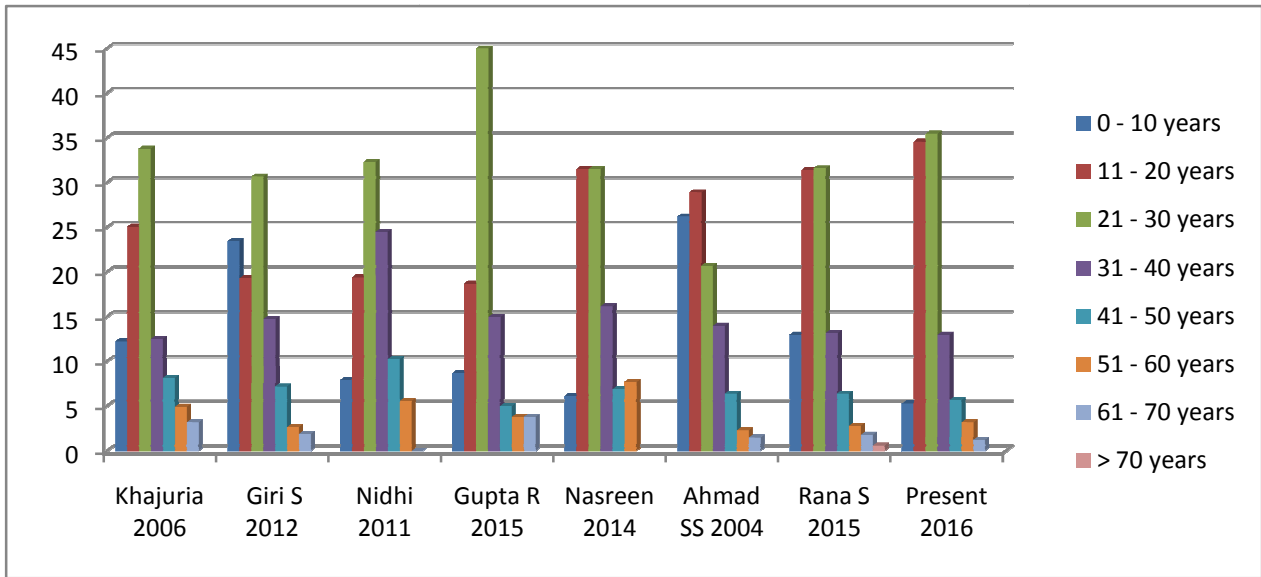
The commonest diagnosis in our series was non-specific reactive lymphadenitis (45.5% of all cases) followed by tubercular lymphadenitis (32.2% of all cases). Our results are in concordance with those of Qadri et al [5], Ahmad et al [7], and Hirachand et al [8] but differ from the findings of Khajuria et al [4], Chand P et al [9] and Paliwal N et al [10], who reported higher incidence of TB LAN compared to reactive LAN.

Criteria for diagnosis of TB LAN have been mentioned above. Cases showing only epithelioid cell granulomas, without AFB positivity, were also categorized as tuberculosis. Although many diseases can show granuloma formation, in our country tuberculosis is the predominant cause. Also such patients – many of them cannot afford more expensive investigations - are often given a short therapeutic trial of anti-tubercular drugs. After the trial period, non-responders are investigated further [11, 12]. Some studies from India have demonstrated that patients of granulomatous lymphadenitis, who were negative for tubercle bacilli on culture, responded to anti- tubercular treatment and showed clinical improvement [13].

In our series, cervical group of lymph nodes are involved most frequently by TB LAN (88.16% of cases). Similar data is reported in some other series also, viz. Chand P - 70% [9], Giri S - 51.89% [14], Gupta R - 70% [15], Rana - 83.6% [16].

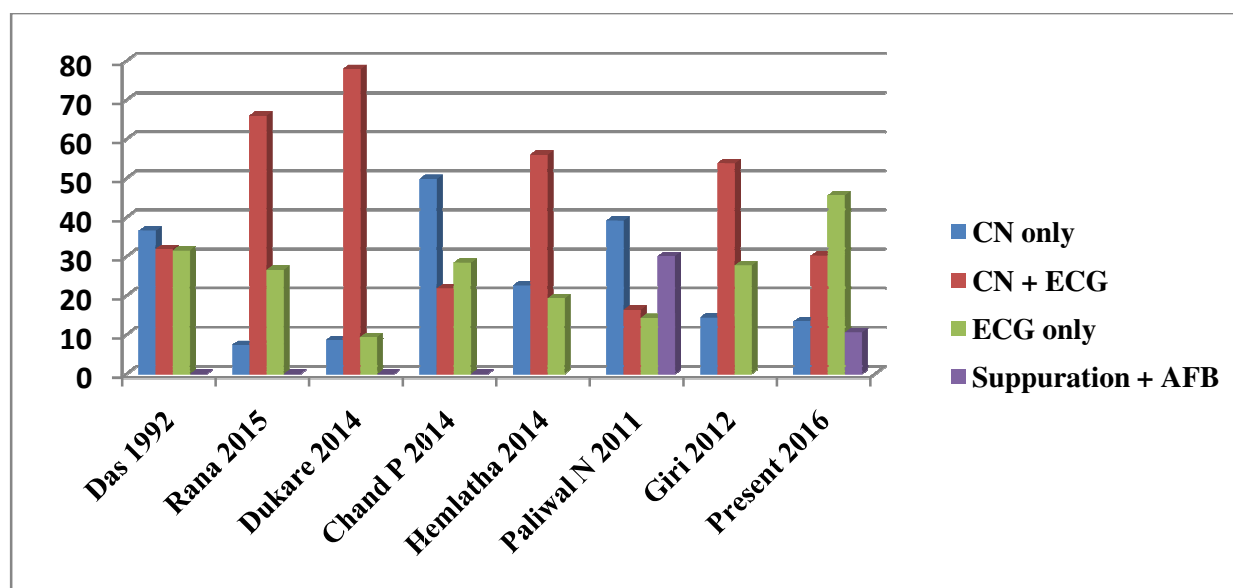
In our series, TB LAN was seen most frequently in the third decade of life (35.5% of all cases) closely followed by second decade of life (34.6% of cases). Thus majority of cases of TB LAN (70.1% of all cases) are seen in between 11 to 30 years of age. Comparison of age distribution of TB LAN patients in our series with some other recent series from different parts of India is shown in figure 6. It is observed that in our series, TB LAN was more or less equally distributed in second and third decades of life. This is similar to findings of Ahmad et al [7], Rana et al [16] and Nasreen et al [17], but at variance to those of Paliwal [10], Giri [14] and Gupta [15] who found higher incidence of TB LAN in third decade of life. In our series, the reason for high incidence of TB at an earlier age can possibly be attributed to high population, overcrowding, low literacy rates and low nutritional and immune status of patients in this part of the country.

Figure 6: Comparison of age distribution of TB LAN in different series



Cytomorphological patterns of TB LAN seen in our series were shown in fig 5. Other authors have also described similar cytomorphological features in TB LAN with or without minor variations [9], [10], [14], [16], [18], [19], [20]. Comparison of cytomorphological features of TB LAN in our series with some other recent studies from different parts of India is depicted in figure 7. It is seen that in our series a larger percentage of cases had presence of only epithelioid cell granulomas (45.64% of all cases). This can be explained when we consider the dynamics of evolution of histopathological changes in tuberculosis. During initial phases, tubercle bacilli multiply within macrophages that accumulate in lymph nodes. Also, epithelioid cell granuloma formation begins. At this stage, little cell death occurs. After two to four weeks, macrophages develop the ability to destroy the bacilli. At the same time, macrophages and other cells are also destroyed by the by-products of type 4 hypersensitivity reaction which leads to caseation necrosis [21]. Hence, presence of epithelioid cell granulomas alone indicates an early stage in evolution of the disease. Nearly half of the patients in our series show presence of epithelioid cell/ epithelioid granulomas only. This suggests that utilizing FNAC in a primary care setting helps in early detection of the disease. Many of the other studies referred to earlier were carried out at secondary or tertiary care centres. As the patient usually presents later to these medical facilities, it may explain the variation in the pattern of cytomorphological profile seen in those series.

194 **Figure 7: Comparison of cytomorphological features of TB LAN in different series**



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197 AFB positivity rate is low in our study possibly due to similar reasons. At least 10^3 to 10^5 bacilli/ml of
198 sample should be present for a positive ZN stain [10]. Earlier stages of TB LAN are known to have low
199 AFB load. Higher percentage of lymphocytes, epithelioid cells and giant cells limit proliferation of *M.*
200 *tuberculosis*. Conversely, caseous necrosis is associated with proliferation of tubercle bacilli and high
201 AFB load [18].

202 Nodal tuberculosis is common not only amongst residents of the Indian subcontinent, but also in
203 immigrants from this region to more advanced economies. Important factors suggested for this
204 predisposition are cultural practices like - close communal living and the habit of spitting in open (thus
205 favouring oropharyngeal exposure), genetically determined tropism of mycobacterium towards lymph
206 nodes of this population group and possibly BCG vaccination. Genetic differences in the virulence of
207 organ tropism of different strains of *M. tuberculosis* have been demonstrated in other geographic locales
208 [22]. Whether the same phenomenon exists in India needs to be investigated.

209 Tubercular cervical lymphadenitis (TBCLN) can be produced by *M. tuberculosis* (MTB) or by non-
210 tubercular mycobacteria (NTM). MTB cervical lymphadenitis can be a local manifestation of a more
211 widespread systemic disease or it can be due to reactivation of organisms spread earlier from lungs
212 through lympho-hematogenous route. In contrast, NTM cervical lymphadenitis is purely a local disorder
213 caused by bacteria gaining direct access into lymph nodes through oropharyngeal mucosa, salivary
214 glands, tonsils and gingiva [3]. Many cases of NTM TB are resistant to routine anti-tubercular treatment.
215 Surgical removal of lymph nodes has been proposed as a first line treatment in NTM adenitis and as a
216 last resort management in systemic disease caused by drug resistant MTB [23].

217 It is our view that since a case of TBCLN may represent either 'NTM adenitis' or 'reactivated MTB
218 adenitis' with quiescent primary disease', it is imperative that the two entities are distinguished at the very

first instance - because the treatment differs. Current recommendation for diagnosis of NTM infection states that the specimen should be obtained directly from the diseased organ [24]. Hence FNA can be utilized for providing samples for diagnosis and sensitivity assays directly from the affected lymph nodes with minimal invasion and discomfort to the patient.

A word of caution, especially relevant for physicians working in a primary care setting is that a patient with FNAC diagnosis of reactive lymphadenitis should be kept on follow-up when clinically warranted. TB LAN evolves through five stages. During the first stage, lymph nodes show non-specific features of reactive hyperplasia [3] and the diagnosis of TB can be missed sometimes [14]. Studies show that cases which were initially diagnosed on histological biopsy as reactive lymphadenitis, went on to develop tuberculosis after six months of follow up [25].

CONCLUSION

FNAC can serve as a primary investigative modality for early diagnosis and management of TB LAN in resource poor settings in our country. It helps in early diagnosis and management of patients of tubercular lymphadenitis. About 32% of all cases of lymphadenopathy are due to tuberculosis. 70% cases of TB LAN are seen between 10-30 years of age and it also occurs at an earlier age in this part of the country. In about 90% cases TB LAN tends to involve lymph nodes of the head and neck region. Utility of FNAC in management of TB LAN is also discussed.

CONSENT

The study is a retrospective analysis of a laboratory investigative procedure. No personal data of any patient is being presented. An informed consent of all patients was obtained prior to performing the investigative procedure.

REFERENCES

1. Twist CJ, Link MP. Assessment of lymphadenopathy in children. *Pediatr Clin North Am*. 2002; 49:1009-1025.
2. Richner S, Laifer G. Peripheral lymphadenopathy in immune-competent adults. *Swiss Medical Weekly*. 2010; 140: 98-104.
3. Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Ind J Med Res*. 2004; 120:316-353.
4. Khajuria R, Goswami KC, Singh K, Dubey VK. Pattern of lymphadenopathy on fine needle aspiration cytology in Jammu. *JK Science*. 2006; 8(3):157-9.
5. Qadri SK, Hamdami NH, Shah P, Lone MI, Baba KM Profile of lymphadenopathy in Kashmir valley: A cytological study. *Asia Pacific J Cancer Prev*. 2012; 13:3621-3625.
6. Pandit AA, Candes FP, Khubchandani SR. Fine needle aspiration cytology of lymph nodes. *J Postgrad Med*. 1987; 33:136-136.

- 255 7. Ahmad SS, Akhtar S, Akhtar K, Naseem S, Mansoor T, Khalil S. Incidence of tuberculosis from study
256 of fine needle aspiration cytology in lymphadenopathy and acid fast staining. *Ind J Community*
257 *Medicine*. 2005; 30 (2):63-65.
- 258 8. Hirachand S, Lakhey M, Akhtar J, Thapa B. Evaluation of fine needle aspiration cytology of lymph
259 nodes in Kathmandu Medical College teaching hospital. *Kathmandu Univ Med J*. 2009; 7:139-142.
- 260 9. Chand P, Dogra R, Chauhan N, Gupta R, Khare P. Cytopathological pattern of tubercular
261 lymphadenopathy on FNAC: Analysis of 550 consecutive cases. *J Clin Diag Res*. 2014; 8(9): 16-19.
- 262 10. Paliwal N, Thakur S, Mullick S, Gupta K. FNAC in tuberculous lymphadenitis: Experience from a
263 tertiary level referral centre. *Ind J Tub*. 2011; 58:102-7.
- 264 11. Dua T, Ahmad P, Vasenwala S, Beg F, Malik A Correlation of cytomorphology with AFB positivity by
265 smear and culture in tubercular lymphadenitis. *Indian J Tuber*. 1996; 43:81- 84.
- 266 12. Paul PC, Goswami BK, Chakarabarti S, Giri A, Pramanik R. Fine needle aspiration cytology of lymph
267 nodes: An institutional study of 1448 cases over a five year period. *J Cytol*. 2004; 21:187 -190.
- 268 13. Chandanwale S, Buch A, Verma A, Shruti V, Kulkarni S, Satav V. Evaluation of granulomatous
269 lymphadenitis on fine needle aspiration cytology – Diagnostic dilemma. *Int J Pharm Bio Sci*. 2012;
270 2(3):278-285.
- 271 14. Giri S, Singh K. Fine needle aspiration cytology for the diagnosis of tuberculous lymphadenitis. *Int J*
272 *Cur Res Rev*. 2012; 4:124-130.
- 273 15. Gupta R, Dewan D, Suri J. Study of incidence and cytomorphological patterns of tubercular
274 lymphadenitis in a secondary care level hospital of Jammu region. *Ind J Pathol Oncol*. 2015; 2(3):
275 161-164.
- 276 16. Rana S, Sharma P, Kajhan S, Singh P, Gill MK, Kumar A. Cytomorphological patterns of tuberculous
277 lymphadenitis: Experience from a tertiary centre in rural Haryana. *Sch. J. App. Med. Sci*. 2015;
278 3(3G):1547-1552.
- 279 17. Nasreen K, Rashid Taj Md. Role of FNAC in tuberculous lymphadenitis. *Indian J Appl Res*. 2014;
280 4(9): 423-5.
- 281 18. Das DK, Bhambani S, Pant JN, Parkash S, Murthy NS, Hedau ST, Sodhani P, Pant CS. Superficial
282 and deep seated tuberculous lesions: fine needle aspiration diagnosis of 574 cases. *Diag*
283 *Cytopathol*.1992; 8(3):211-5.
- 284 19. Hemlatha A, Shruti PS, UdayKumar M, Bhaskaran A. Cytomorphological patterns of tubercular
285 lymphadenitis revisited. *Ann Med Health Sci Res*. 2014; 4(3):393-396.
- 286 20. Dukare SR, Jadhav DS, Gaikwad AL, Ranka SN, Kale PB, D'Costa G. Fine needle aspiration
287 cytology of cervical lymphadenopathy – A study of 510 cases. *Asian J Sci Tech*.2014; 5:537-40.
- 288 21. Quast TM, Browning RF. Pathogenesis and clinical manifestations of pulmonary tuberculosis. *Dis*
289 *Mon*. 2006; 52: 413- 419
- 290 22. Hesseling AC, Marais BJ, Kirchner HL. Mycobacterial genotype is associated with disease phenotype
291 in children. *Int J Tuber Lung Dis*. 2010;14: 1252- 58

- 292 23. Pomerantz M, Madsen L, Goble M, Iseman M. Surgical management of resistant Mycobacterium
293 tuberculosis and other mycobacterial pulmonary infections. *Ann Thor Surg.* 1991; 52: 1108-12.
- 294 24. Katoch VM, Mohan Kumar T. Atypical mycobacterial infections. In: Sharma SK, editor. *Tuberculosis,*
295 *1st Ed.* New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2001 p. 439-51.
- 296 25. Moore SW, Schneider JW, Schaaf HS. Diagnostic aspects of cervical lymphadenopathy in children in
297 the developing world: A study of 1877 surgical specimens. *Pediatr Surg Int.* 2003; 19: 240-244.