Original Article

Correlation of cytomorphological patterns and acid-fast *Bacilli* positivity in tuberculous lymphadenitis in a rural population of southern India

Suresh Masilamani, P. Arul, C. Akshatha

Department of Pathology, Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur, Perambalur, Tamil Nadu, India

Address for correspondence:
Dr. P. Arul, Department of Pathology, Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur, Perambalur - 621 113, Tamil Nadu, India. E-mail: drarul3@gmail.com

Abstract

Background: One of the most common causes of lymphadenopathy in India is tuberculosis. It can be diagnosed by a minimally invasive procedure known as fine-needle aspiration cytology (FNAC), and thereby unnecessary surgical interventions are avoided. **Aim:** This study was undertaken to evaluate cytomorphological patterns of tuberculous lymphadenitis including human immunodeficiency virus-positive cases, to correlate the acid-fast *Bacilli* (AFB) positivity with cytomorphological patterns and also to find out overall AFB positivity. **Materials and Methods:** In this study, a total of 212 cases of cytologically proven tuberculous lymphadenitis were retrieved and analyzed retrospectively between March 2012 and March 2015 for three different cytomorphological patterns (epithelioid granuloma without necrosis [pattern A], epithelioid granuloma with necrosis [pattern B], and necrosis without epithelioid granuloma [pattern C]) and bacillary loads on Ziehl-Neelsen stain (ZN) for AFB. **Results:** Pattern A through C was observed in 40 (18.9%), 102 (48.1%), and 70 (33%) cases, respectively. AFB positivity was found in 2 (5%) cases of pattern A, 62 (60.8%) cases of pattern B, and 54 (77.1%) cases of pattern C. The highest percentage of AFB positivity (64.7%) was observed in aspirate containing purulent/pus and caseous/cheesy material. The overall AFB positivity was seen in 55.7% (118/212) cases. On grading of AFB positivity, Grade 1+ was observed in 29.7%, Grade 2+ was observed in 28.8%, and Grade 3+ was observed in 41.5% cases. **Conclusion:** FNAC is a sensitive, simple, convenient, safe, minimally invasive procedure to diagnose tuberculous lymphadenitis. Study of both cytomorphological patterns and ZN staining for AFB can improve the diagnostic yield. Regardless of the presence of granuloma, ZN stain must be employed whenever infective pathology is suspected.

Key words: Cytomorphological patterns, fine needle aspiration cytology, tuberculous lymphadenitis, Ziehl-Neelsen stain

INTRODUCTION

Tuberculosis (TB) is thought to be one of the oldest human diseases and the history is almost as old as mankind.[1] The European kings of the middle ages imparted the royal touch to cure the "King's evil" to which mycobacterial lymphadenitis referred.[2] India has the highest TB burden accounting for one-fifth of the global incidence and

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.166121

How to cite this article: Masilamani S, Arul P, Akshatha C. Correlation of cytomorphological patterns and acid-fast Bacilli positivity in tuberculous lymphadenitis in a rural population of southern India. J Nat Sc Biol Med 2015;6:S134-8.

nearly 40% of the Indian population is infected with the TB bacillus.[3] Since the mid-1980s, both in developing and developed countries, there is increasing incidence of extrapulmonary TB has been noted.[4] In India, extrapulmonary TB accounting 20% of all TB cases and its prevalence in the country varies between 8.3% and 13.1% in different districts according to cohort analysis by the Central TB Division, Ministry of Health and Family Welfare in 2002.[5] In India and other developing countries, TB lymphadenitis is the most common form of extrapulmonary TB and it comprises 35% of cases.[6] Infection with the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) is associated with an increased frequency of both pulmonary and extrapulmonary TB especially lymphadenitis.[7,8]

TB lymphadenitis is usually more common in females and in younger age groups in contrast to pulmonary TB which is more common in males and in the older age group and a peak age of onset of 20-40 years.[9,10] There are various diagnostic modalities available for the diagnosis of TB lymphadenitis viz fine-needle aspiration cytology (FNAC), histopathological examination excised lymph nodes, culture, Ziehl-Neelsen (ZN) staining for acid fast *Bacilli* (AFB), imaging studies, and molecular tests. Even though culture is considered as gold standard for the diagnosis, FNAC to be used as the initial diagnostic test in suspected cases of TB as it has excellent sensitivity and specificity, and also it is a simple and less expensive outpatient diagnostic procedure.[11] The present study was undertaken to evaluate cytomorphological patterns of tuberculous lymphadenitis including HIV/AIDS positive cases, to correlate the AFB positivity with cytomorphological patterns and also to find out overall AFB positivity.

MATERIALS AND METHODS

Overview of study design

The present retrospective study was undertaken at Department of Pathology in our institution, a tertiary care center, conducted over the period of 3 years from March 2012 to March 2015, after obtaining approval from the Institutional Ethical Committee.

Study population

A total of 212 cases with cytologically proven tuberculous lymphadenitis were included in the present study. Patients with nontuberculous mycobacterial infections such as mycobacterium scrofulaceum, mycobacterium avium-intracellulare complex and mycobacterium kansasii, Pulmonary Koch's, Bacillus Calmette-Guerin vaccination causing lymphadenitis, and periodic acid-schiff positive organisms in FNAC smears were also excluded from this study. Nontuberculous mycobacterial infections were excluded by identification of only microabscesses, ill-defined granulomas, noncaseating granulomas and a small numbers of giant cells,[12] and Pulmonary Koch's, Bacillus Calmette-Guerin vaccination causing lymphadenitis were excluded by detailed clinical history collected from medical records.

Collection of data

Clinical data such as site of aspiration, number lymph nodes involved, nature of aspirates (blood mixed material, purulent/pus, caseous, or cheesy) were obtained from patient medical records.

Cytological evaluation

May-Grunwald Giemsa and Hematoxylin and Eosin stained smears were studied for pattern analysis. According to the Das *et al.* study,[13] cytology smears revealing cytomorphological features of tuberculous lymphadenitis were grouped into three categories: Pattern A: Epithelioid granuloma without necrosis [Figure 1a], pattern B: Epithelioid granuloma with necrosis [Figure 1b], and pattern C: Necrosis without epithelioid granuloma [Figure 1c].

ZN stained smears were used for screening of AFB and grading. These positive smears were categorized into three grades; 1+: Smears with occasional *Bacilli*, 2+: Smears with singly scattered AFB, 3+: Smears with a large number of *Bacilli* arranged in bundles detected under ×10 [Figure 1d].[14]

Statistical analysis

All the statistical analysis was performed using IBM SPSS Statistics for Windows (version 20.0, IBM Corporation, Armonk, New York, USA). Correlation between various

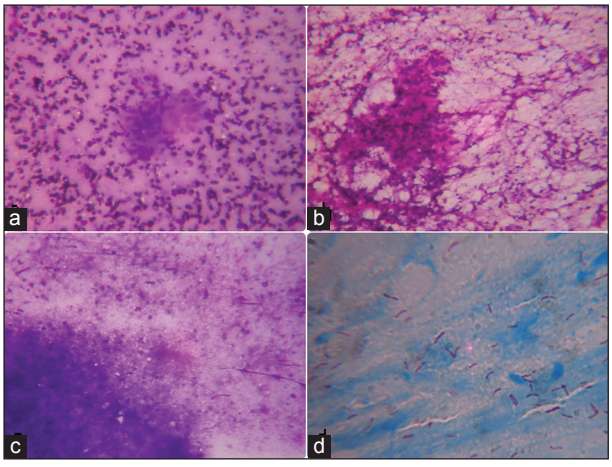

Figure 1: (a) Pattern A: Epithelioid granuloma without necrosis (May-Grunwald Giemsa, ×400). (b) Pattern B: Epithelioid granuloma with necrosis (H and E, ×400). (c) Pattern C: Necrosis without epithelioid granuloma (May-Grunwald Giemsa, ×400). (d) Grade 3+ acid fast **Bacilli** positivity (Ziehl-Neelsen, ×1000)

cytomorphological patterns and AFB positivity was assessed using Chi-square test. The $P \leq 0.05$ was considered for statistical significance.

RESULTS

Out of 212 cases, 118 were AFB positive cases and remaining 94 was AFB negative, but showed cytological features of tuberculous lymphadenitis.

Site and number of lymph node involvement

In the present study, the most common site of involved lymph nodes was cervical lymph nodes in 206 (97.2%) followed by axillary lymph nodes in 4 (1.9%) and inguinal lymph nodes in 2 (0.9%) cases. The most common presentation was single palpable cervical lymphadenopathy in 177 (83.5%) cases followed by multiple unilateral palpable cervical lymphadenopathy in 31 (14.6%) and multiple bilateral cervical lymphadenopathy in 4 (1.9%) cases.

Nature of aspirate

On the basis of appearance of aspirate, blood mixed aspirate were noted more commonly in 179 (84.4%) cases followed by purulent/pus material in 26 (12.3%) cases and caseous or cheesy material in 7 (3.3%) cases. Blood mixed aspirate was predominantly seen in pattern A and B and purulent/pus and caseous or cheesy material were predominantly seen in pattern C.

Cytomorphological pattern, acid-fast *Bacilli* positivity and grading

Of these 212 cases, pattern A was observed in 40 (18.9%) cases, pattern B was observed in 102 (48.1%) cases, and pattern C was observed in 70 (33%) cases. AFB positivity was found in 2 (5%) cases of pattern A, 62 (60.8%) cases of pattern B, and 54 (77.1%) cases of pattern C. The highest percentage of AFB positivity (64.7%) was observed in aspirate containing purulent/pus and caseous/cheesy material. The overall AFB positivity was seen in 55.7% (118/212) cases.

On grading of AFB positivity, Grade 1+ observed in 29.7%, Grade 2+ observed in 28.8% 2, and Grade 3+ observed in 41.5% cases.

HIV positive cases of 10 were included in this study. All the HIV-positive cases showed pattern C and Grade 3+ AFB positivity.

The present study showed significant statistical difference between AFB positivity and cytomorphological patterns ($P < 0.0001$).

DISCUSSION

On clinical examination, cervical lymph nodes (97.2%) were more commonly involved and inguinal lymph nodes were least commonly involved in the present study similar to most of the recently published studies.[15-18] This is because, the organism usually gains access to the cervical lymph nodes through the tonsillar lymphoid tissue.

In this study, most common presentation was single palpable cervical lymphadenopathy in 83.5% cases comparable to Chand *et al.* (81.6%) study,[16] and higher than Nidhi *et al.* (63.3%) study.[15] We observed multiple unilateral palpable cervical lymphadenopathy in 14.6% cases and multiple bilateral cervical lymphadenopathy in 1.9% cases, which were almost comparable to Nidhi *et al.* study.[15] Due to various initiatives by governmental and nongovernmental agencies, the awareness regarding TB has been increased. This might explain the high number of cases presenting with single lymph node enlargement.

According to gross nature of aspirate, blood mixed aspirates were noted in 84.4% followed by purulent/pus material in 12.3% and caseous or cheesy material in 3.3% cases in the current study, whereas, Hemalatha *et al.*[19] observed blood mixed aspirates in 87.3% and purulent to cheesy materials in 12.7% cases. Pattern A and B were predominantly observed in blood mixed aspirate, whereas pattern C was predominantly observed in purulent/pus and caseous or cheesy aspirates in the present study similar to Hemalatha *et al.* study.[19]

Cytomorphological pictures of tuberculous lymphadenitis was described by various authors with minor modifications with different names viz patterns or category.[19,20] We described cytomorphological pictures in our study as patterns. In our study, most common cytomorphological pattern observed was pattern B (48.1%) similar to Hemalatha *et al.* and Gupta *et al.* studies,[19,21] and it was higher than studies done by Nidhi *et al.*,[15] and Chand *et al.*[16] (16.4% and 21.8%, respectively). In the current study, pattern C and pattern A were noted in 33% and 18.9% cases, respectively. This findings were comparable to studies done by Nidhi *et al.* and Hemalatha *et al.*[15,19] The formation of granuloma is dependent on a fairly good immune system within the host. In immunocompromised situations, the immunological interplay between lymphocytes, macrophages, the organism, and various interleukins/cytokines may not be efficient to result in granuloma formation. Hence, when AFB positivity is observed without granuloma formation, other comorbid immunodeficient states should be thought of.

Similar to few Indian studies,[13,15,19] we observed more

Table 1: Comparison of patterns of tuberculous lymphadenitis and overall AFB positivity in the present study with other studies

Study	Year	Epithelioid granuloma without necrosis n (%)	Epithelioid granuloma with necrosis n (%)	Necrosis without epithelioid granuloma n (%)	Overall AFB positivity (%)
Ergete and Bekele[20]	1997	7 (3.4)	131 (63.7)	67 (32.7)	71.7
Nidhi *et al.*[15]	2010	25 (14.3)	29 (16.4)	69 (39.2)	71
Chand *et al.*[16]	2013	156 (28.4)	120 (21.8)	85 (15.4)	44.54
Hemalatha *et al.*[19]	2014	29 (19.3)	84 (56)	34 (22.7)	54
Present study	2015	40 (18.9)	102 (48.1)	70 (33)	55.7

AFB: Acid fast *Bacilli*

number of AFB positivity in pattern C (77.1%) followed by pattern B (60.8%) in the current study. Pattern A showed 5% AFB positivity in our study, whereas Nidhi *et al.*[15] and Chand *et al.*[16] studies noted 3.25% and 2.56% AFB positivity, respectively. We found maximum percentage of AFB positivity (64.7%) in aspirate containing purulent/pus and caseous/cheesy material similar to studies done by various authors.[15,19,22] If the purulent material is aspirated, slides should be stained for ZN staining to rule out tuberculous lymphadenitis, because it can mimic as acute suppurative lesions.[19] The present study showed overall AFB positivity in 55.7% cases comparable to most of the published studies,[16,17,19] and lower than Nidhi *et al.*[15] and Ergete and Bekele[20] studies. Varying AFB positivity has been found by many authors ranging from 35.6% to 55.2%.[19] Slides can be decolorized for ZN staining if cytological features of TB and necrosis present; this may increase the AFB positivity.[15] Yield of AFB positivity can be further increased by doing repeat FNAC of a lymph node.[23] Clinical correlation and microbiological assessment should be done in AFB negative cases showing only epithelioid granulomas without necrosis. Microbiological assessment is necessary in AFB negative cases to confirm the diagnosis of TB as approximately 10,000-100,000 mycobacterial organism/ml of the sample should be present for smear AFB positivity.[15] In the present study, all the HIV positive cases (n = 10) showed pattern C with Grade 3+ AFB positivity, whereas Hemalatha *et al.* study,[19] included 4 HIV positive cases, of theses 3 cases showed numerous macrophages with 3+ AFB positivity and 1 case showed pattern B with 1+ AFB positivity.

On grading of AFB positivity, 29.7% cases showed Grade 1+, 28.8% cases showed Grade 2+, and 41.5% cases showed Grade 3+ positivity in the present study. Hence, it can be argued that the bacterial load in the rural population affected by TB seems to be high.

There was significant statistical difference between AFB positivity and cytomorphological patterns ($P < 0.0001$) similar to Hemalatha *et al.* study.[19] Comparison of patterns of tuberculous lymphadenitis and overall AFB positivity in the present study with other studies is shown in Table 1.

CONCLUSIONS

FNAC is a sensitive, simple, convenient, safe, minimally invasive procedure to diagnose tuberculous lymphadenitis. Study of both cytomorphological patterns and ZN staining for AFB can improve the diagnostic yield. Regardless of the presence of granuloma, ZN stain must be employed whenever infective pathology is suspected.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kumar A. Lymph node tuberculosis. In: Sharma SK, Mohan A, editors. Tuberculosis. 2nd ed. New Delhi: Jaypee Brothers Medical Publishers; 2009. p. 397-409.
2. Cantrell RW, Jensen JH, Reid D. Diagnosis and management of tuberculous cervical adenitis. Arch Otolaryngol 1975;101:53-7.
3. Ministry of Health and Family Welfare. TB India 2009: RNTCP Status Report. New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare; 2009.
4. Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. Clin Infect Dis 2009;49:1350-7.
5. Arora VK, Chopra KK. Extra pulmonary tuberculosis. Indian J Tuberc 2007;54:165-7.
6. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res 2004;120:316-53.
7. Aguado JM, Castrillo JM. Lymphadenitis as a characteristic manifestation of disseminated tuberculosis in intravenous drug abusers infected with human immunodeficiency virus. J Infect 1987;14:191-3.
8. Finfer M, Perchick A, Burstein DE. Fine needle aspiration biopsy diagnosis of tuberculous lymphadenitis in patients with and without the acquired immune deficiency syndrome. Acta Cytol 1991;35:325-32.
9. Shubha AB, Sapna H, Dinesh RB. Tuberculosis lymphadenitis presenting a diagnostic dilemma - A case report. Int J Dent Clin 2010;2:48-52.
10. Golden MP, Vikram HR. Extrapulmonary tuberculosis: An overview. Am Fam Physician 2005;72:1761-8.
11. Handa U, Mundi I, Mohan S. Nodal tuberculosis revisited: A review. J Infect Dev Ctries 2012;6:6-12.
12. Kraus M, Benharroch D, Kaplan D, Sion-Vardy N, Leiberman A, Dima H, *et al*. Mycobacterial cervical lymphadenitis: The histological features of non-tuberculous mycobacterial infection. Histopathology 1999;35:534-8.

13. Das DK, Pant JN, Chachra KL, Murthy NS, Satyanarayan L, Thankamma TC, *et al*. Tuberculous lymphadenitis: Correlation of cellular components and necrosis in lymph-node aspirate with A.F.B. positivity and bacillary count. Indian J Pathol Microbiol 1990;33: 1-10.
14. Kumar S, Ferns S, Sujatha S, Jatiya L. Acid-fast staining patterns and their correlation with HIV positivity. Acta Cytol 2005;49:111-2.
15. Nidhi P, Sapna T, Shalini M, Kumud G. FNAC in tuberculous lymphadenitis: Experience from a tertiary level referral centre. Indian J Tuberc 2011;58:102-7.
16. Chand P, Dogra R, Chauhan N, Gupta R, Khare P. Cytopathological pattern of tubercular lymphadenopathy on FNAC: Analysis of 550 consecutive cases. J Clin Diagn Res 2014;8:FC16-9.
17. Bezabih M, Mariam DW, Selassie SG. Fine needle aspiration cytology of suspected tuberculous lymphadenitis. Cytopathology 2002;13:284-90.
18. Sharma S, Sarin R, Khalid UK, Singla N, Sharma PP, Behera D. Clinical profile and treatment outcome of tuberculous lymphadenitis in children using DOTS strategy. Indian J Tuberc 2010;57:4-11.
19. Hemalatha A, Shruti P, Kumar MU, Bhaskaran A. Cytomorphological patterns of tubercular lymphadenitis revisited. Ann Med Health Sci Res 2014;4:393-6.
20. Ergete W, Bekele A. Acid fast *Bacilli* in aspiration smears from tuberculous patients. Ethiop J Health Dev 2000;14:99-104.
21. Gupta AK, Nayar M, Chandra M. Critical appraisal of fine needle aspiration cytology in tuberculous lymphadenitis. Acta Cytol 1992;36:391-4.
22. Ahmad SS, Akhtar S, Akhtar K, Naseem S, Mansoor T, Khalil S. Incidence of tuberculosis from study of fine needle aspiration cytology in lymphadenopathy and acid fast staining. Indian J Community Med 2005;30:63-5.
23. Kumar N, Jain S, Murthy NS. Utility of repeat fine needle aspiration in acute suppurative lesions. Follow-up of 263 cases. Acta Cytol 2004;48:337-40.