Cytomorphological patterns of tubercular lymphadenitis and its comparison with Ziehl-Neelsen staining and culture in eastern up. (Gorakhpur region): Cytological study of 400 cases

ABSTRACT

Background: Tuberculous lymphadenitis is most common cause of lymphadenopathy in developing countries. Although enormous literature is available on various aspects of the disease including cytological patterns and its incidence in others parts of India and in other countries, only limited literature is available regarding its incidence and morphological spectrum on cytology in eastern parts of Uttar Pradesh in Gorakhpur region.

Aim: The present study was undertaken to estimate the incidence of tuberculous lymphadenitis in our settings along with its morphological spectrum on cytology as well as to determine the utility of culture of fine needle aspirates in addition to cytology and Ziehl–Neelsen (ZN) staining.

Material and Methods: Four hundred cases of superficial lymphadenopathy were subjected to fine needle aspiration cytology (FNAC), and in case, smears were stained with Hematoxylin and eosin (H and E), Giemsa, and ZN stain and categorized into three cytomorphological patterns. All the aspirates were inoculated on two sterile Lowenstein Jensen (LJ) medium.

Result: Out of 400 cases of consecutive lymph nodes aspirated, 180 cases (45%) showed features of tuberculous lymphadenitis. Smears revealed epithelioid granulomas with caseous necrosis in maximum cases (40%). On statistical analysis, difference between group I and group II was found to be significant (P < 0.05); while comparison between groups II and III as well as between groups I and III was found to be statistically insignificant. Overall, acid fast bacilli positivity was seen in 51.6% of the cases.

Conclusion: FNAC has been proved very safe, highly sensitive, and first line investigation in diagnosing tubercular lymphadenitis. The sensitivity can be further be increased by complementary cytomorphology with acid fast staining. Diagnostic accuracy can further be increased by culture.

Key words: Culture, FNAC, patterns, tubercular lymphadenitis, ZN stain

Introduction

Tuberculosis continues to be the biggest health problem in developing countries with enormous social and economic implications. India has the highest burden of tuberculosis in the world, an estimated 2 million cases...
annually, and accounts for approximately one-fifth of the global incidence.[1] In India, 1000 people a day or one per minute die of tuberculosis.[2] During the past decade, the clinical pattern and presentation of tuberculosis has changed dramatically. Much of the traditional learning about this disease is no longer true and tuberculosis has become a new entity.[3] Tuberculous lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis and constitutes approximately 20–40% of extrapulmonary tuberculosis.[4] It remains a diagnostic and therapeutic challenge because it mimics other pathologic processes and yields inconsistent physical and laboratory findings. Fine needle aspiration cytology (FNAC) plays a vital role in solving these issues, nowadays being recognized as a rapid diagnostic technique because of its simplicity, cost effective, early availability of results, accuracy, and minimal invasion. Standard diagnostic algorithm for TBLN in India recommends FNAC along with Ziehl–Neelsen (ZN) staining for acid fast bacilli (AFB) in clinically suspected patients.[5] However, this has the disadvantages of nonspecific findings on cytological examination and poor sensitivity by ZN smears.[6] Mycobacterial culture being a gold standard method could be useful as a definitive diagnosis.[7] Although enormous literature is available on various aspects of disease including cytological patterns and its incidence in others parts of India and in other countries, only limited literature is available regarding its incidence and morphological spectrum on cytology in eastern parts of Uttar Pradesh in Gorakhpur region. In our population with limited resources and high tubercular disease burden, presence of epithelioid cell granuloma is considered as an evidence of tubercular lymphadenitis. With this background, the present study was undertaken to estimate the incidence in our settings along with its morphological spectrum on cytology and to determine the utility of mycobacterial culture of fine needle aspirates in addition to cytology and ZN staining.

Material and Methods

The present study was carried out in the department of pathology over a period of 1 year from June 2014 to July 2015. Four hundred cases of consecutive superficial lymphadenopathy were aspirated for cytological evaluation using 22–23 gauge needle and 10 ml plastic syringe with a detachable syringe holder. In each case, a part of the aspirate was used for preparing three smears at least, one for hematoxylin and eosin (H and E) stain, which was fixed immediately in cytofix containing equal volume of absolute alcohol and ether, one for Giemsa stain, and one for ZN stain. The smears revealing features of tuberculous lymphadenitis were grouped into three categories – (1) Epithelioid granulomas with caseous necrosis with or without Langhans giant cells; (2) Epithelioid granulomas without necrosis with or without Langhans giant cells; (3) necrosis only without epithelioid granulomas. All the aspirates were inoculated on two sterile Lowenstein Jensen (LJ) medium slopes and incubated up to 8 weeks at 37°C. In addition to the demographic profile of tuberculous patients with their present and past treatment history, clinical characteristics of lymph node were also studied. Data have been summarized by descriptive statistics and key proportion expressed with their 95% confidence interval (CI). Fisher exact test was employed for intergroup comparison of categorical variables. All analysis were two tailed. Graphpad prism trial version 6 and IBM SPSS statistics version 23 trial version (IBM Corp, Armonk, New York, USA) of software were used for analysis. The diagnosis of tuberculous lymphadenitis was made when the following criteria were met: the presence of epithelioid cell granuloma with or without necrosis and/or ZN smear positivity for AFB, and/or positive culture for mycobacteria.

Results

Out of 400 cases of consecutive lymph nodes aspirated, 180 cases (45%) showed features of TBLN, and hence, incidence was reported as 45% (CI: 0.400–0.500). Reactive lymphoid hyperplasia, acute suppurative lymphadenitis, metastatic carcinoma, lymphoma, and filarial lymphadenitis with toxoplasma were seen in 37.0%, 7.5%, 7.0%, 3.0%, and 0.5% respectively [Table 1].

The age group of patients ranged from 6 months to 69 years. Maximum cases (74.4%) were in their second to fourth decades of life, with male-to-female ratio of 1:1.5 and mean age of 25.08 years [Table 2].

Out of 180 cases of TBLN, 93 cases (51.6%) showed AFB positivity whereas 87 cases (48.4%) were AFB negative with cytological picture of TBLN. The cervical region was the most common site of involvement (80%), followed by axillary (10.5%) and inguinal lymph node (4.4%). Only 2 cases presented with generalized lymphadenopathy. In our study, among cases with cervical presentation,
majority (51%) had multiple unilateral lymphadenopathy followed by single palpable lymph node; majority cases (51%) had multiple unilateral lymphadenopathy followed by single palpable lymph node (44%) and multiple bilateral cervical lymphadenopathy in (5%) cases. Discharging skin sinuses were seen in association with matted lymph node in 12 cases (8.9%). Grossly purulent material was aspirated in 41% cases, caseous or cheesy material in 25.5% cases, and blood mixed material in 33.5% cases. Out of 180 cases showing cytological picture of TBLN, smears revealed epithelioid granulomas with caseous necrosis in 40% of the cases, followed by necrosis only without epithelioid granulomas 30.5% cases and epithelioid granuloma without necrosis in 29.5% of the cases [Figure 1; Table 3].

On statistical analysis of cytomorphicological findings among 180 cases using fisher exact test, difference between group I and group II was found to be significant ($P < 0.05$), whereas comparison between group II and group III as well as between group I and group III was found to be statistically insignificant ($P = 0.9085$ and $P = 0.0774$, respectively) [Table 4]. Overall, AFB positivity was seen in 51.6% cases, and out of that maximum AFB positivity of smears was present in cases with necrosis only without epithelioid granulomas (78.1%) and least (37.7%) in cases with epithelioid granuloma with caseous necrosis [Table 3].

Tubercle bacilli were demonstrated by smear and/or culture in the two etiological groups as shown in Table 5. A total of 28 cases diagnosed as non-TBLN by cytology were AFB culture positive. Thus, a total of 208 out of 400 cases were found to be of tubercular etiology by FNAC, microscopy, and culture together. Significant statistical differences was seen among the cytology pattern and AFB culture positivity. The $P$ value was <0.0001, which was highly significant. On comparing group I smears with that of group II using Fisher’s exact test, the difference in AFB positivity between the smears was statistically significant ($P < 0.05$).

In the present study 14 known cases of HIV were also included. Out of these, 12 cases (85.71%) showed necrosis only without epithelioid granuloma and 2 cases (14.28%) showed caseous necrosis with epithelioid granuloma. AFB positivity was seen in all the cases.

**Discussion**

Superficial lymphadenopathy is very common clinical finding, etiology of which can be suspected by clinical signs and symptoms. However, a morphological diagnosis is essential to start antituberculous treatment in cases of TBLN. FNAC is a simple, noninvasive, cheap tool with high sensitivity in diagnosing tuberculosis in developing countries such as India and can replace excision biopsy. TBLN can be seen in patient ranging from early to advanced age. In our study, the youngest patient was 6 months and the oldest was 69 years old. Majority of patients (74.4%) were in their second to fourth decades of life with a male-to-female ratio of 1:1.5 and mean age of 25.08 years. Similar pattern of age distribution was reported by Gupta et al., Paliwal et al., Ergete et al., Purohit et al., Chand and Gupta. However, Mahapatra reported maximum patients in second decade followed by third decade. In the study of Gupta et al., the younger was 5 months old and oldest was 95 years old, whereas Paliwal et al. reported the youngest patient aged 4 years old with

**Table 2: Distribution of cases of tubercular lymphadenitis according to age and sex**

| Age groups (years) | Males number (%) | Females number (%) | Total number (%) |
|-------------------|------------------|--------------------|------------------|
| 0-10 years        | 15 (8.3)         | 15 (8.3)           | 30 (16.7)        |
| 11-20 years       | 14 (7.7)         | 22 (12.2)          | 36 (20.0)        |
| 21-30 years       | 20 (11.1)        | 42 (23.3)          | 62 (34.5)        |
| 31-40 years       | 11 (6.1)         | 25 (13.9)          | 36 (20.0)        |
| 41-50 years       | 07 (3.88)        | 02 (1.1)           | 09 (5.0)         |
| 51-60 years       | 02 (2.2)         | 02 (1.1)           | 06 (3.3)         |
| >60 years         | 00 (0)           | 01 (0.5)           | 01 (0.5)         |
| Total             | 71 (39.4)        | 109 (60.5)         | 180 (100)        |

**Table 3: Distribution of cases according to cytomorphological patterns and AFB positivity**

| Features                                      | Number | Percentage | AFB Positivity |
|-----------------------------------------------|--------|------------|----------------|
| Epithelioid granulomas with caseous necrosis  | 72     | 40.0%      | 30 (37.7%)     |
| Epithelioid granulomas without caseous necrosis| 53     | 29.5%      | 20 (41.6%)     |
| Caseous necrosis only without epithelioid granulomas | 55     | 30.5%      | 43 (78.1%)     |
63 years old as the oldest. Females suffered more from TBLN compared to males. Fatima et al. along with other authors also reported female preponderance. However, Ahmad et al. and Rajsekharan et al. reported higher incidence in males. High incidence in females may be due to poor nutritional status and overall lower standard of living in developing countries.

In our study, out of the total 400 consecutive lymph node aspirations from patients referred to pathology department, 180 smears showed cytomorphological features suggestive of TBLN. Thus, the incidence reported was 45%. Ahmad et al. reported incidence of 38%; Gupta et al. and Tilak et al. reported 34.6% and 38.8% incidences, respectively, in their study. Comparative high incidence noted by us may be because our institute is a referral centre catering to a large population, and incidence rate also vary according to geographical region.

Clinically, in our study, cervical region was the most commonly affected region involved in 80% of the cases. This is in concordance with Bezabith et al. who observed cervical involvement in 74.2% of the cases. Several authors also reported cervical lymph nodes as most common lymph node involvement. Multiple unilateral lymphadenopathy was the most common presentation (51%), which corroborated with the results reported by Aggarwal et al., Gupta et al., and Ahmad et al. However, Paliwal et al. and Chand reported single palpable lymph node as the most common presentation. Sharma et al. observed similar pattern of findings among a study conducted among pediatrics age groups.

Most common cytomorphological pattern in our study was epithelioid granulomas with caseous necrosis in 40% cases. Gupta et al. also reported epithelioid granulomas with caseous necrosis as the most common cytomorphological pattern (52–55%). However Paliwal et al. reported necrosis only without epithelioid granuloma as the most common cytomorphological pattern in 39.2% patients. Gupta et al. observed epithelioid clusters with or without Langhans giant cells with necrosis, whereas Chand reported caseous necrotic material with epithelioid cell granulomas and giant cells as the most common cytomorphological pattern.

Overall AFB positivity was seen in 51.6% of the cases, out of which the highest positivity was seen in smears revealing necrosis only with or without epithelioid cell granulomas (78.1%). Gupta et al. reported overall AFB positivity of 65% with maximum positivity (75%) in necrosis with polymorphs and with or without epithelioid granulomas. Gupta et al. reported AFB positivity in 19.6% among 138 cases. However, studies by various authors reported maximum AFB positivity in necrosis only without epithelioid granulomas. In our study, 28 cases out of 220 diagnosed nontubercular on FNAC were AFB culture positive. Thus, 180 out of 400 cases were diagnosed as tubercular by FNAC alone, as against 208 cases when combined with microscopy and culture. Talwar et al. found that FNAC when combined with microscopy and culture improved the diagnostic accuracy. Fifty-four cases out of 100 were diagnosed as tubercular by FNAC alone, as against 60 when combined with microscopy and culture.

In the present study, out of 14 human immunodeficiency virus (HIV) positive cases, 12 cases (85.71%) showed necrosis

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### Table 4: Statistical comparison between different cytomorphological groups

| Groups | Features                          | Positive | Negative | Using Fisher exact test (P) |
|--------|----------------------------------|----------|----------|---------------------------|
| I      | Epithelioid granuloma without necrosis | 53 (29.5%) | 127 (70.5%) | P<0.05, df = 1 (significant) |
| II     | Epithelioid granuloma with caseous necrosis | 72 (40.0%) | 108 (60.0%) |                       |
| III    | Caseous necrosis only without epithelioid granuloma | 55 (30.5%) | 125 (69.5%) |                       |

df: Degree of freedom, CI: Confidence interval

### Table 5: Demonstration of tubercular bacilli by smear and/or culture

| Smear/culture for AFB | Cytopathological diagnosis of tuberculosis | Nontubercular group on cytopathology |
|-----------------------|-------------------------------------------|--------------------------------------|
| Smear positive alone  | 15                                        | -                                    |
| Culture positive alone| 38                                        | 28                                   |
| Smear and culture positive | 78                                      | -                                    |
| Smear and culture negative | 49                             | 192                                  |
| Total                | 180                                       | 220                                  |
only without epithelioid granuloma and 2 cases (14.28%) showed caseous necrosis with epithelioid cell granuloma. AFB positivity was seen in all the cases whereas in study conducted by Masilamani et al., all the HIV positive cases \( n = 10 \) showed necrosis only without epithelioid granuloma with Grade 3+ AFB positivity.\[^{22}\]

**Conclusion**

FNAC has been proved to be very useful, safe, cost effective, highly sensitive, and first line investigation in diagnosing TBLN; patients can be put to treatment immediately without much delay, thus decreasing the infectivity of exposure. The sensitivity can be further be increased by complementary cytomorphology with acid fast staining. Diagnostic accuracy can be further increased by submitting material obtained by FNAC for culture. In conclusion, findings from the present study emphasize a definite need for a combination of tests for diagnosing TBLN.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. World Health Organization Global TB control report. 227, Geneva, Switzerland: WHO; 2008.
2. Chauhan LS. Challenges for the RNTCP in India. J Indian Med Assoc 2003;101:152-3.
3. Canova CR, Kuhn M, Reinhart WH. Problems in the diagnosis and therapy of lymph node tuberculosis in HIV-negative patients. Schweiz Med Wochenschr 1995;125:2511-7.
4. Gupta PR. Difficulties in managing lymphnode tuberculosis. Lung India 2004;21:50-3.
5. Central TB Division (CTD), Directorate General of Health Services, Ministry of Health and Family Welfare Government of India Revised National Tuberculosis Control Programme, (RNTCP), Technical and operational guidelines for tuberculosis Control, Nirman Bhawan, New Delhi. CTD. 2005. Available from http://www.tbcindia.org/documents.asp. [Last accessed on Oct 2006].
6. Verenkar MP, Kannath K, Pinto WM, Rodrigues S, Wisemn PRG. Mycobacteriological study of fine needle aspirates in cervical lymphadenitis. Indian J Tuber 1996;43:187.
7. Rattan A. PCR for diagnosis of tuberculosis. Where are we now? Indian J Tuber 2000;47:79-82.
8. Gupta R, Dewan D, Suri J. Study of incidence and cytomorphological patterns of tubercular lymphadenitis in a secondary care level hospital of Jammu Region. Indian J Pathol Oncol 2015;2:161-4.
9. Palival N, Thakur S, Mullick S, Gupta K. FNAC in tuberculous lymphadenitis: Experience from a Tertiary Level Referral Centre. Ind J Tuber 2011;58:102-7.
10. Ergete W, Bekele A. Acid fast bacilli in aspiration smears from tuberculous patients. Ethiop J Health Dev 2000;14:99-104.
11. Purohit MR, Mustafa T, Morkve O, Siviland L. Gender differences in the clinical diagnosis of tuberculous lymphadenitis: a hospital based study from central India. Int J Infect Dis 2009;13:600-5.
12. Chand P, Dogra R, Chauhan N, Gupta R, Khare P. Cytological pattern of Tubercular lymphadenopathy on FNAC: Analysis of 550 consecutive cases. J Clin Diag Res 2014;8:16-9.
13. Mohapatra PR, Jammeja AK. Tuberculosis Lymphadenitis. J Assoc Phys Ind 2009;57:585-90.
14. Fatima S, Arshad S, Ahmed Z, Hasan SH. Spectrum of cytopathological findings in patients with Neck lymphadenopathy- Experience in a tertiary hospital in Pakistan. Asian Pac J Can Prev 2011;12:1873-5.
15. Ahmad SS, Akhtar A, Akhtar K, Naseem S, Mansoor T, Khalil S. Incidence of tuberculosis from study of fine needle aspiration cytology for lymphadenopathy and acid fast staining. Ind J Comm Med 2005;30:63-5.
16. Rajsekaran S, Gunasekaran M, Bhanumati V. Tuberculous cervical lymphadenitis in HIV positive and negative patients. Indian J Tuber 2001;48:201-4.
17. Tilak V, Dhadel AV, Jain R. Fine needle aspiration cytology of head and neck masses. Indian J Pathol Microbiol 2002;45:23-30.
18. Bezabih M, Mariam DW, Selassie SG. Fine needle aspiration cytology of suspected tuberculosis lymphadenitis. Cytopathol 2002;13:284-90.
19. Aggarwal P, Wali JP, Singh S,Handa R, Wig N, Biswas A. A clinico-bacteriological study of peripheral tuberculosis lymphadenitis. J Assoc Phy Ind 2001;49:808-12.
20. Sharma S, Sarin R, Khalid UR, Singh N, Sharma PP, Behera D. Clinical profile and treatment outcome of tuberculous lymphadenitis in children using DOTS strategy. Indian J Tuber 2010;57:4-11.
21. Gadre DV, Singh UR, Saxena K, Bhatia A, Talwar V. Diagnosis of tubercular cervical lymphadenitis by FNAC, microscopy and culture. Indian J Tuber 1991;38:25-7.
22. 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 Sci Biol Med 2015;6:134-8.

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