Microbiological evaluation of clinically suspected cases of tubercular lymphadenopathy by cytology, culture, and smear microscopy – A hospital-based study from Northern India

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ABSTRACT

Introduction: Over the past few years, the incidence of extrapulmonary tuberculosis (EPTB), particularly of tubercular lymphadenitis (TBLN), is on the rise. TBLN, which contributes to 20–40% of EPTB cases, often poses a diagnostic and therapeutic challenge for clinicians more so in resource-constrained settings where laboratory confirmation is not available. In this study, we aimed to study if fine-needle aspiration cytology (FNAC) combined with Ziehl–Neelsen (ZN) staining and mycobacterial culture could improve the diagnostic accuracy in patients clinically suspected of TBLN. **Materials and Methods:** This cross-sectional study involved 120 patients (>12 years of age), clinically suspected of peripheral TBLN. Direct examination of the samples with ZN staining and culture on Lowenstein–Jensen (LJ) slants and Bactec MGIT 960 vials (MGIT 960 medium) was performed on previously collected fine-needle aspirates. **Results:** Out of total 120 patients included in study, 43.3% were males and 56.7% were females. Maximum numbers of cases were observed in age group 13–21 (56%). On ZN staining, 21.7% samples were found positive, whereas FNAC findings were suggestive of tuberculosis (TB) in 455 patients. Culture on LJ media showed 33.3% samples to be positive, whereas Bactec MGIT 960 system showed positivity of 35%. Out of 54 samples suggestive of TB on FNAC, only 30 (55.6%) were found positive on Bactec culture. Also out of 66 samples which were not suggestive of TB in FNAC, 12 (18.2%) were found positive in Bactec culture. **Conclusion:** Accurate diagnosis of TBLN requires a multifaceted approach involving microbiology, pathology, radiology, and clinical presentation of the disease. FNAC and ZN staining along with the culture can result in better diagnostic yield and will be helpful in reducing the burden of TB.

Keywords: Acid-fast bacilli, MGIT, *Mycobacterium tuberculosis*, tubercular lymphadenitis, ZN staining

Introduction

Tuberculosis (TB) is an airborne infectious disease, killing nearly two million people worldwide every year.[1] The "captain of all these men of death," TB has been a scourge of the human mankind from the time immemorial, not only due to its effects as a medical problem but also by its impact as a social and economic tragedy.[2] Despite availability of drugs since 1940s, TB has continued to plague mankind and remains the second most common infectious etiology of death after human immunodeficiency virus. The burden of disability and death due to TB is immense in developing and low socioeconomic regions.[3] Presently, the WHO estimates that one-third of the world's population is infected with *Mycobacterium tuberculosis*. In

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2017, there were 10 million new cases of TB and it accounted for 1.3 million deaths.[4] Over 95% of TB deaths occur in low and middle-income countries and 75% of cases being the most economically productive age group (15–54 years). The six countries that stand out as having the largest number of incident cases in 2017 were India, China, Indonesia, Philippines, Pakistan, and Nigeria. India accounts for 23% of the global TB incidence with an estimated 2.2 million cases reported in 2014.[4]

TB can be pulmonary or extrapulmonary tuberculosis (EPTB). EPTB comprises one-fifth of the TB cases and over the past few years there has been a continuous upsurge in the incidence of EPTB particularly tubercular lymphadenitis (TBLN), which constitutes approximately 20–40% of EPTB.[5-8] TBLN, commonly referred to as scrofula or king's evil, is the most common manifestation of EPTB.[9] It remains a diagnostic and therapeutic challenge for clinicians as, the common symptoms for TB, namely, fever, cough, weight loss, fatigue, and night sweats, are absent most of the times and patient often presents only with enlarged lymph nodes (LN), which can also be seen in metastasis of various carcinomas, in lymphoproliferative disorders, or in variety of infections as well. A high index of clinical suspicion coupled with fine-needle aspiration cytology (FNAC) and Ziehl–Neelsen (ZN) staining of the LN aspirates for acid fast bacilli (AFB) plays a vital role in the diagnosis,[10] but the nonspecific/inconclusive cytological findings and poor sensitivity of ZN staining (particularly in paucibacillary samples) often make the diagnosis difficult.[11] Mycobacterial culture being gold standard method is useful for definitive diagnosis.[12] Moreover, it is also important to differentiate tuberculous from nontuberculous mycobacterial lymphadenitis as the treatment modalities for both are different.[13]

With this background, we aimed to study if FNAC combined with ZN staining and mycobacterial culture could improve the diagnostic accuracy in patients clinically suspected for TBLN.

Materials and Methods

This cross-sectional study was conducted in Department of Microbiology, Pathology, TB and Chest Clinic of Maulana Azad Medical College and associated Loknayak Hospital, a major tertiary care center in New Delhi for a period of 2 years from January 2015 to December 2016.

A total of 120 patients >12 years of age, clinically suspected of peripheral TBLN with no history of antitubercular drug intake, were included in the study. Patients with central lymphadenopathies (abdominal, thoracic), <12 years of age, or patients on antitubercular treatment were excluded.

The study was approved by the Institutional Ethics Committee. The study protocol was explained to the enrolled patients and their written informed consent was obtained.

FNAC was done with 22–23-ga needle and 20-ml disposable syringe with a detachable syringe holder and the obtained material

was divided into three parts: One was used to prepare two air-dried smears and stained with ZN[14] and May–Grunwald–Giemsa[15] stains, respectively. The second part of the aspirated material was used for culture and was processed by digestion, decontamination, and concentration. The *N*-acetyl-l-cysteine and sodium hydroxide method (NALC/NaOH) was used for digestion and decontamination. Thereafter, specimens were concentrated by centrifugation at 3500*g* for 15 min and resuspended in 2 ml of sterile phosphate buffer saline (PBS [pH 6.8]). The processed specimen was then inoculated on two Lowenstein–Jensen (LJ) slants and into Bactec MGIT 960 vials (MGIT 960 medium). The LJ slants and MGIT vials were monitored for 8 and 6 weeks, respectively, before discarding them as negative. The third part of the specimen was collected in an Eppendorf tube containing sterile PBS (pH 6.8) and was stored at −20°C for further studies.

Inoculated LJ media were examined daily for 5–7 days to detect rapidly growing mycobacteria and for any contamination. As soon as any growth was evident, showing typical rough, tough, and buff colonies of *M. tuberculosis* [Figure 1], smear was made and stained with ZN staining for confirming AFB. All the positive cultures were further examined for the rate of growth, pigmentation, and other biochemical properties, namely, niacin production, nitrate reduction, and catalase test for confirmation of *M. tuberculosis*. Development of canary yellow color was taken as positive for niacin production test, whereas no color change was taken as negative [Figure 2a]. For nitrate reduction test, development of red or pink color was taken as positive and no color change as negative [Figure 2b]. For catalase test, production of bubbles was measured [Figure 2c]. All the isolates with niacin production, positive nitrate reduction, and catalase positive were labeled as *M. tuberculosis*. The MGIT vials once flagged positive by the automated system were confirmed by ZN staining to confirm the presence of AFB. Cultures found AFB positive by microscopy were identified by MGIT TBc identification test (TBc ID; Becton Dickinson, Sparks, MD), a rapid immunochromatographic test that detects *M. tuberculosis* complex MPT 64 protein.[16,17]

Figure 1: Lowenstein Jensen media slant showing typical rough, tough, and buff colonies of *Mycobacterium tuberculosis*
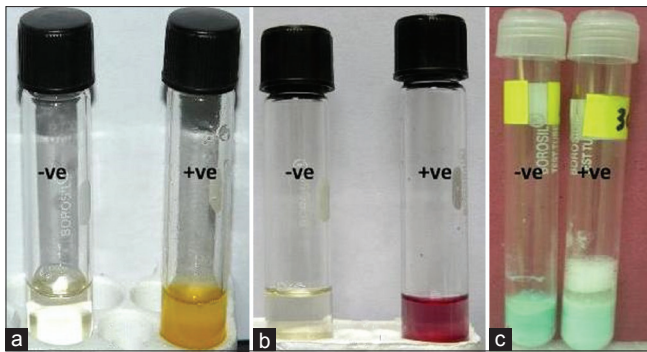
Figure 2: (a) Niacin production test, (b) nitrate reduction test, and (c) catalase test for *Mycobacterium tuberculosis*

The diagnosis of TBLN was made when the following criteria were met: the presence of epitheloid cell granuloma with or without multinucleated giant cells and caseation necrosis, and/or ZN smear positivity for AFB, and/or positive culture for mycobacteria.

Quality control of the LJ media, MGIT media, and reagents used was carried out using standard H37RV strain of *M. tuberculosis* and in-house strain of *Mycobacterium gordonea* (a rapid grower). Sterile distilled water was used as a negative control.

The data were analyzed by using Statistical Package for Social Sciences version 11 and the prevalence of organisms was determined and expressed in percentage.

Results

Out of the total 120 patients included in study, 52 (43.3%) were males and 68 (56.7%) were females with male-to-female ratio of 1:1.3. The mean age of the patients was 23.6 years. Maximum number of cases were observed in the age group 13–21 (56%). Table 1 depicts the age and sex distribution of the study group (*n* = 120).

Most common complaints among the patients were fever 88 (73.3%) and anorexia 88 (73.3%) followed by weight loss 80 (66.7%) and night sweats 34 (28.3%). All the patients presented with lymphadenopathy and the most common site involved was cervical, 78 (65%); submandibular, 20 (16.7%); and supraclavicular, 12 (10%). It was observed that 28 (23.3%) patients had a significant history of the contact with TB patients, whereas there was no such history in 92 (76.7%) patients. Table 2 depicts the common complaints, site of the LN involved, and history of the contact of TB.

On ZN staining, 26 (21.7%) samples were found positive, whereas 94 (78.3%) were negative for the same. The FNAC findings were suggestive of TB in 54 (45%) patients with granuloma with reactive background (16 [13.3%]) and necrosis with granuloma (16 [13.3%]) as the most common findings. The FNAC findings were nonsuggestive of TB in 66 (55%) patients. Table 3 shows the characterization of the FNAC findings. On

Table 1: Age and sex distribution of cases in the study group (*n*=120)

Age group	Number of males (%)	Number of females (%)	Total (%)
13–21	30 (25)	38 (31.7)	68 (56.66)
22–30	10 (8.3)	24 (20)	34 (28.33)
31–39	08 (6.7)	02 (1.7)	10 (8.33)
40–49	02 (1.7)	00 (00)	02 (1.66)
50–59	02 (1.7)	02 (1.7)	04 (3.33)
59–62	00 (00)	02 (1.7)	02 (1.66)
Total	52 (43.3)	68 (56.7)	60 (100)

Table 2: Clinical symptoms, site of the lymph nodes involved, and history of contact of tuberculosis of the study group (*n*=120)

Clinical features	Number (%)
Symptoms	
Fever	88 (73.3)
Anorexia	88 (73.3)
Weight loss	80 (66.7)
Night sweat	34 (28.3)
Site of lymph node	
Cervical	78 (65.0)
Submandibular	20 (16.6)
Supraclavicular	12 (10.0)
Axillary	06 (5.0)
Preauricular	02 (1.7)
Postauricular	02 (1.7)
History of contact of tuberculosis	
Positive	28 (23.3)
Negative	92 (76.7)

Table 3: Characterization of the FNAC findings in the study group (*n*=12)

FNAC findings		Numbers (%)
Suggestive of TB 54 (45%)	Granuloma with reactive background	16 (13.3)
	Necrosis with granuloma	16 (13.3)
	Granuloma with acute suppurative lesion	06 (5.0)
	Necrosis with acute suppurative lesion	08 (6.7)
	Necrosis with reactive background	04 (3.3)
	Granuloma only	04 (3.3)
Not suggestive of TB 66 (55%)	Acute suppurative lesion	42 (35.0)
	Reactive background	24 (20.0)

FNAC=Fine-needle aspiration cytology, TB=tuberculosis

correlation of FNAC findings with ZN staining, the highest percentage of positivity by ZN staining was found with necrosis with reactive background (4/4 [100%]) followed by necrosis with granuloma (12/16 [75%]) and necrosis with acute suppurative lesion (4/8 [50%]). Table 4 shows correlation of ZN staining with FNAC findings.

Culture on LJ media showed 40 (33.3%) samples to be positive, whereas 76 (63.3%) were culture negative and 4 (3.3%) were contaminated. Mycobacterial cultures on Bactec MGIT 960 TB system were positive for 42 (35%) and negative for 78 (65%)

Table 4: Correlation of ZN staining with FNAC findings			
FNAC findings	Numbers (120)	Positive in ZN staining (26)	Percentage
Acute suppurative lesion	42	02	4.7
Reactive background	24	00	0.0
Granuloma with reactive background	16	02	12.5
Granuloma with acute suppurative lesion	06	02	33.3
Necrosis with granuloma	16	12	75.0
Necrosis with acute suppurative lesion	08	04	50.0
Necrosis with reactive background	04	04	100
Granuloma only	04	00	0.0

FNAC=Fine-needle aspiration cytology, ZN staining=Ziehl–Neelsen staining

cases. The mean turnaround time for culture positivity in ZN smear-positive specimens was 27 days on LJ media and 12 days by MGIT 960 system. The same for ZN smear-negative specimens was 32 days and 19 days by LJ and MGIT methods, respectively. On correlation of FNAC findings with Bactec culture, it was seen that out of 54 samples suggestive of TB in FNAC, only 30 (55.6%) were positive on Bactec culture and 24 (44.4%) were found negative. In addition, out of 66 samples, which were not suggestive of TB in FNAC, 12 (18.2%) showed growth of mycobacteria in Bactec culture, whereas 54 (81.82%) were negative for the same. The sensitivity and specificity of FNAC were found to be 71% and 69%, respectively, with a positive and negative predictive value of 56% and 82%, respectively, when compared with the findings of Bactec culture.

Discussion

TBLN is the commonest cause of lymphadenopathy in developing countries and is the most common manifestation of EPTB, contributing to around 25% of all the cases of TB.[3,18] Although it has been stated in various studies that FNAC must be regarded as an accurate and reliable preliminary investigation for patients presenting with lymphadenopathy,[19,20] interpretation of FNAC smears requires a great deal of expertise. As FNAC is a blind procedure and the needle may not always reach the representative area of lesion, relevant history, laboratory reports, and radiological findings should always be taken into account before reporting the FNAC findings.[21,22] Nevertheless in case of any doubt, a repeat aspiration is advised before rendering a diagnosis.[23] It is quite imperative from the results of our study that FNAC combined with ZN staining and TB culture improves the diagnostic accuracy of TBLN.

Maximum numbers of cases were reported in age group 13–21 (56.6%) and the mean age of the patients was 23.6 years. TB primarily affects people in their most productive years of life with important socioeconomic consequences for the household. Mohapatra *et al.* also reported maximum incidence of TBLN in the second and third decades of life and stated that the disease rarely affects patients in their extremes of age. These factors lead to the increased debt burden, particularly for poor and the marginalized sections of the population who are most commonly affected by this disease. In the present study, the female patients

outnumbered the male patients. A female preponderance of TBLN has previously been reported in many Indian studies.[20,24,25] The biological, hormonal, social, environmental, and behavioral differences between men and women may be the probable reason.[2,26,27] Biologically, there is a fundamental difference in the immune system of men and women, and a hormonal influence on immunity can be indicated as the underlying cause for the different patterns of disease in women.[2,26,28] Socially, in developing countries, women often have a low socioeconomic and nutritional status, which can affect the immune response to the disease making them more susceptible to TB.[27] Moreover as TB affects women in their economically and reproductively active years, the impact of the disease is also strongly felt by their children and families. Cultural and financial barriers can act as major obstacles for women seeking care, so they may delay accessing care until the illness is severe.

The most common site of lymphadenitis in our study was cervical LNs (65%) and the most prevalent constitutional symptoms were fever (73.3%) and anorexia (73.3%), the findings which are in tandem with the previous studies.[6,23,24] Apart from the bacterial factors, clinical features are influenced by the host factors, namely, age, sex, nutrition, genetics, family history of the contact, and the immune competence of the patients, which may lead to varied clinical and morphological presentations. Previous studies have shown that a positive history of contact among TBLN patients ranges from 13% to 20%[19,29] and corroborates well with our results where it was 23.3%.

The cytomorphological diagnosis of TB has already been widely studied, and the cytomorphological spectrum of TBLN has been classified mainly into three types, namely, necrotizing granuloma, nonnecrotizing granuloma, and necrosis only.[30-32] We observed necrosis with granuloma (13.3%) and granuloma with reactive background (13.3%) as the most common cytomorphological pattern of all the TBLN cases. In the present study, the ZN staining showed a positivity of 21.7% and was most commonly found in those smears in which necrosis was present. The ZN staining can identify the organism in only 20% of the cases and even in referral laboratories is regarded as insensitive.[33] The low positivity of ZN staining could be attributed to the fact that demonstrating the bacilli in smears requires volumes in range of 1000–10,000/ml of sample, and bacilli may not be detected in the smears at low volumes.[33]

Culture had maximum positivity (55%) in cases diagnosed as TBLN in FNAC findings which is in concordance with the previous studies.[19,34] Moreover, 18.2% of cases were also found to be culture positive which were nonsuggestive of TBLN in FNAC findings, thus proving culture to be a more sensitive and specific method as compared to FNAC. Previous studies have also found that FNAC combined with microscopy and culture improved the diagnostic accuracy.[29,35] We found the MGIT culture method to be superior to LJ culture both in terms of positivity and time to positivity, an observation which is in tandem with the previous study by Rodrigues *et al.*[36]

Data explored from our study clearly indicate that culture along with FNAC and ZN improves the diagnostic accuracy in cases of suspected TBLN. A multidimensional approach, if followed by primary-care physicians for evaluation of such cases, will lead to effective and efficient management of patients and will also be helpful in reducing the overall burden of TB.

Conclusion

Based on the findings of our study, the diagnosis of TBLN requires a multifaceted approach involving microbiology, pathology, radiology, and other clinical specialties for the evaluation of suspected cases. The laboratory findings should always be interpreted along with the clinical pictures to consider a patient for an accurate diagnosis. Addition of culture for *M. tuberculosis* in the diagnostic algorithm along with FNAC and ZN staining surely unveils the diagnostic dilemma in cases of suspected TBLN and can improve the diagnostic accuracy as well as can be helpful in reducing the burden of TB. Further studies are needed to investigate the immune mechanism and its correlation with *M. tuberculosis* so that patients missed for TBLN on FNAC, but are culture positive, can be diagnosed and treated accurately.

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Conflicts of interest

There are no conflicts of interest.

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