Cytopathological and Bacteriological Diagnostic Performance of GeneXpert in Clinically Suspected Tuberculous Lymphadenitis in a Tertiary Care Hospital, Bihar (India)

Imtiaz Ahmad[1], Rajiv Kumar[2], Chandan Kumar Poddar[3], S. K. Shahi[4]

[1]Associate Professor, Department of Pathology, Vardhaman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar, India. [2]Associate Professor, Department of Pathology, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India. [3]Research Scholar, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India. [4]Professor, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India

ABSTRACT

BACKGROUND

Tuberculosis is a prehistoric disease and confirmation of its existence was seen in the Vedas. The oldest of them (Rigveda, 1500 BC) calls the infection yaksma. It claimed millions of lives in Europe and was called `The White Plague'. Robert Koch has written that tuberculosis killed one third of Europeans of middle age.

METHODS

This is a descriptive study. Tuberculous lymphadenitis isolates from sputum samples of clinically suspected cases of tuberculosis seen in Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar and Associated Hospital of Bihar between March 2019 and March 2020 were included in the study. The study protocol was approved by the hospital ethics committee.

RESULTS

A total of 200 fine-needle aspirated specimens from lymph nodes were integrated in the study. Out of 200 cases, 90 aspirates were reported as cytomorphology suggestive of tuberculous lymphadenitis. Compared to the reference standard, Xpert MTB/RIF correctly identified 88 out of 90 TB cases (sensitivity, 97.80%). The possible false-negative result had a prolonged transit interval of 9 days before Xpert MTB/ RIF testing, which may have affected the result. Xpert MTB/ RIF was positive in two cases, with negative cytomorphology and culture (specificity, 95.65%). The cytomorphology from one of the false-positive results showed a necrotizing suppurative lymphadenitis, which is consistent with TB. However, no organisms could be identified on microscopy or culture. The cytomorphology of the other false positive result showed an epithelial inclusion cyst, and the reason for this false-positive result remains unknown.

CONCLUSIONS

FNAB is a simple procedure which can be performed in an outpatient setting by clinicians or nursing staff after a short training period (21, 22). It is ideal for use in resource-limited settings, including more remote and rural areas (22). Specimen collection is simple and safe. With the use of a transport vial, virtually no sample preparation is required and there is minimal risk of contamination. Furthermore, the transmission risk to the operator may also be reduced. Combining FNAB and rapid genotypic diagnosis using automated systems should greatly improve access to appropriate diagnosis and treatment for patients with tuberculous lymphadenitis.

KEYWORDS

Tuberculosis Lymphadenopathy, Fine Needle Aspiration Cytology, GeneXpert, Ziehl-Neelsen Stain

Corresponding Author:
Dr. Rajiv Kumar,
Associate Professor,
Department of Pathology,
Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India.
E-mail: chandan_mmb@yahoo.com

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BACKGROUND

Tuberculosis is an extremely prehistoric disease and confirmation of its existence was seen in Tuberculosis in first non-European evolution is found in the Vedas. The oldest of them (Rigveda, 1500 BC) calls the infection yaksma.[1] Tuberculosis in Europe during 18th and 19th centuries. During developed rebellion it claimed millions of lives in Europe and so was called as `The White Plague'.[2] Robert Koch was written that tuberculosis killed one third of Europeans of middle age. According to WHO, tuberculosis stationary kills three million people every year in underdeveloped countries?[2] India is the country with the maximum trouble of TB. As per the Global TB report 2017, the sketchy incidence of TB in India was approximately 28, 00,000, accounting for one-fourth of the world's TB cases.[3] It is approximate that about 40% of the Indian population is spoiled with TB bacteria; the vast majority have latent TB rather than TB disease.[4] Tuberculous lymphadenitis is the most extensive extrapulmonary manifestation of tuberculosis (TB)[5,6] and the majority of cases have no active lung association. Tuberculous lymphadenitis is diagnosis of morphologically on fine needle aspiration cytology of lymph node. Fine needle aspiration cytology is now extensively utilised as a first line diagnostic procedure in the diagnosis of palpable masses, including peripheral lymphadenopathy. Its assessment in the diagnosis of mycobacterial lymphadenitis in adults is well documented.[7] In general, tuberculous (TB) lymphadenitis is diagnosed using conformist methods such as histopathology on source of caseous necrosis and granuloma formation. The possibility of acid-fast bacilli (AFB) identification in tissue section are less because xylene and formalin affect the sensitivity of Ziehl- Neelsen (ZN) technique to detect *Mycobacterium tuberculosis* in histopathology sections.[8]

FNAC is accurate, low-priced, quick & simple method that is used for diagnosis of lymphadenopathy. FNAC is the principal line investigation for lymphadenopathy. GeneXpert utilizes a DNA-PCR technique for concurrent detection of *Mycobacterium tuberculosis* and Rifampicin resistance-related mutations. It is fully automated bench top cartridge-based nucleic acid amplification (CB-NAAT) assay for TB finding that includes all the necessary steps of DNA PCR and gives results within 2 hours. The diagnostic accuracy of GeneXpert for pulmonary TB has been reported high[9,10] Patients with a high risk of tuberculosis-like HIV-associated TB patients and extra pulmonary cases in whom ZN Stain smear examination is frequently negative are the most likely to be benefited from GeneXpert.[10]

We wanted to evaluate FNAC findings of extra pulmonary tuberculosis with ZN stain and GeneXpert and assess the difference in results.

METHODS

This is a descriptive study. Tuberculous lymphadenitis isolates from sputum samples of clinically suspected cases of tuberculosis seen in Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar and Associated Hospital of Bihar between March 2019 and March 2020 were included in the study. The study protocol was approved by the hospital ethics committee.

Inclusion Criteria

Inclusion Criteria Patients attending the Department of Pathology, Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar and Associated Hospital of Bihar, between March 2019 and March 2020, having fever, night sweats, cough for more than 3 weeks with sputum, loss of appetite, loss of weight, chest pain, haemoptysis and/or radiological evidence of tuberculosis were included.

Sample Collection

A total of 200 Fine needle aspirates were obtained from patients who presented with signs and symptoms of Tuberculosis after consent was given. Early morning sputum samples were collected in clean, sterile, leak proof, wide mouth containers.

Sample

Respiratory samples included sputum, broncho-alveolar lavage (BAL), endotracheal aspirate, and pleural fluid. Non-respiratory (extrapulmonary) samples included in our study were lymph node biopsy, peritoneal fluid, pus, and gastric aspirates. Each sample was processed for evaluate the FNAC findings of extra pulmonary tuberculosis with ZN stain and GeneXpert.

FNAC material obtained was smeared on slides and directly stained with strong Carbol fuchsine and steamed for five minutes. The slides were then washed with tap water and decolourised with 1% acid alcohol until clear. Subsequent rinsing in tap water followed. Counter staining was done in Methylene blue for three minutes and finally rinsed in tap water and left to air dry. Examination was done using high power (100X) and reported using WHO format. Total 200 clinically diagnosed patients of lymphadenopathy referred to the Department of Pathology, Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar, between March 2019 and March 2020 were included in this study. The variables included in the study were age, sex, and site of lesion. Relevant history and examination of nodes were recorded.

Cytology smears and ZN stain smears were examined in Department of Pathology, Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, and Bihar. Nodes were aspirated after all aseptic measures with sterile disposable 23-G needle attached with 10 cc disposable syringe. Multiple smears were prepared with part of aspirated material; two to three smears were stained with haematoxylin and eosin (H&E) stain and ZN staining was performed on separate slide. All data were grouped and analysed. Smears stained

with H&E stain (Figure 1: Granuloma formation, H&E stain, 40 X.) were examined under microscope for the presence of granuloma, necrosis, Langhans giant cells, plasma cells, lymphocytes, macrophages, and neutrophils. Smears stained with ZN stain were examined under oil immersion objective for AFB. Presence of sheets of epithelioid cells with lymphocytes and plasma cells with or without multinucleated giant cells were diagnosed as granulomatous lymphadenitis, and eosinophilic granular material containing inflammatory cells and necrotic cell debris was defined as caseous necrosis.[11] The TB abscess was described as degenerate caseous necrosis and/or liquefied necrotic material with marked degenerating and viable inflammatory cell infiltration without epithelioid granuloma.[11]

Xpert MTB/RIF

The Xpert MTB/RIF RT-PCR assay was performed in accordance with the manufacturer's instructions described in the package insert. Briefly, clinical samples were added to sample reagents using a ratio of 3:1. The specimen container was tightly closed and then manually agitated twice within a 15-min period at room temperature to inactivate possible contaminating bacteria. A 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was then added to the Xpert MTB/RIF cartridge. All specimens that were MTB culture positive and Xpert MTB/RIF negative as well as specimens that were MTB culture negative and Xpert MTB/RIF positive were tested twice for confirmation of results. The last result obtained was used for the final analysis.

Performance calculations, including test sensitivity, specificity, and predictive values, were done using Statistical version 8 to compare the diagnostic performance of the Xpert MTB/RIF test to the reference standard (as positive cytology (cytomorphology consistent with mycobacterial infection and direct visualization of the organism on ZN and/or Papanicolaou stain induced fluorescence microscopy) and/or culture positive for *Mycobacterium tuberculosis*).[12,13]

Statistical Analysis

Data were collected in an excel sheet and were analyzed using the Statistical Package for Social Sciences Version 12.0 (SPSS Inc. Wacker Drive, Chicago, IL USA). A p-value of <0.05 was considered significant. The p-value was applied to compare sensitivity and specificity of Xpert MTB/RIF to the gold standard culture method for identification of TB in our study.

RESULTS

A total of 200 fine-needle aspirated specimens from lymph nodes were integrated in the study. Out of 200 cases, 90 aspirates were reported as cytomorphology suggestive of tuberculous lymphadenitis. The age ranged from 15 to 75 years, with the mean age of 46.5 years. Male preponderance was illustrious accounting for 57.78% (52/90) of cases (Table 1, Figure 1).

Age (Years)	Male	Female	Total	%
15-25	05	07	12	13.33
26-35	14	10	24	26.67
36-45	12	9	21	23.33
46-55	11	07	18	20.00
56-65	07	03	10	11.11
66-75	03	02	05	05.56
Total	52	38	90.00	100
%	57.78	42.22	100.00	100

Table 1. Age and Sex Wise Distribution of Cases of Lymphadenopathy

47% (42/90) of the cases with suggestive cytomorphology of tubercular lymphadenitis werein the range of 18-35 years of age. Depending upon cytomorphological features, cases of granuloma 74 (82%), necrosis 67 (74%), acute inflammation 24 (27%), lymphoid background 19 (21%), giant cell 14 (15%) were found in tuberculous lymphadenitis. In present study, the most common site of involved lymph nodes was of the cervical region in 74% (67/90) of the cases (Table 2).

Site	Number and Percentage
Cervical lymph node	61 (67.78%)
Axillary lymph node	08 (08.89%
Supraclavicular lymph node	06 (06.67%)
Submandibular	02 (02.23%)
Submental	07 (07.78%)
Chest	03 (03.33%)
Inguinal lymph node	02 (02.23%)
Other	01 (01.1%)
Total	90 (100%)

Table 2. Site Distribution of Tubercular Lymph Node

TB lymphadenitis was found in 50 (50%) cases, inflammatory lymphadenitis other than tuberculosis in 45 (50%), and malignant lymphadenopathy in remaining 7 (8%) cases, consisting 2 (02%) cases of primary malignancy (i.e., lymphoma) and 9 (10%) of metastasis to lymph node. Of 90 cases of lymphadenitis, ZN stain was found to be positive for AFB in 42 (47%) cases (Table 3). The cytology suggestive of tuberculous lymphadenitis was found in 90 (45%) cases out of total 200 cases.

Cytomorphological Picture	AFB Positive Cases	AFB Negative Cases	Total	%
Epithelioid Granuloma with Caseous Necrosis	40	34	74	37%
Necrosis only without Inflammatory Cells	08	08	16	08%
Necrosis with Polymorphs	34	20	54	27%
Neither Necrosis Nor Granuloma	08	48	56	28%
Total	90	110	200	100
%	45%	55%	100	100

Table 3. AFB Positivity in Various Cytomorphological Sub Patterns in Cases of Lymphadenitis

Compared to the reference standard, Xpert MTB/RIF correctly identified 88 out of 90 TB cases (sensitivity, 97.80%) (Table 4). The possible false-negative result had a prolonged transit interval of 9 days before Xpert MTB/ RIF testing, which may have affected the result. Xpert MTB/ RIF was positive in two cases, with negative cytomorphology and culture (specificity, 95.65%). The cytomorphology from one of the false-positive results showed a necrotizing

suppurative lymphadenitis, which is consistent with TB. However, no organisms could be identified on microscopy or culture. The cytomorphology of the other false positive result showed an epithelial inclusion cyst, and the reason for this false-positive result remains unknown. One case had a negative culture result with positive cytology (including mycobacterial identification) and a positive Xpert MTB/RIF test. This patient had been on TB treatment for 1 month at the time of specimen collection, which provides the likely explanation for this discrepant result.

Reference Standard	Sensitivity	Specificity	PPV	NPV
Xpert vs. Reference Standard	97.80	95.65	97.80	95.65
Xpert vs. Culture	97.77	97.80	97.77	97.80
Xpert vs. Smear-, Culture +	100	100	100	100
ZN vs. Culture	48.88	100	48.88	100

Table 4. Diagnosis Accuracy of the Xpert MTB/RIF Test versus Various Reference Standards

(Reference standard is as defined in the text (positive cytology {cytomorphology consistent with mycobacterial infection with direct visualization o cytomorphology of the organism} and/or mycobacterial culture). ZN, Ziehl-Neelsen stain; -, negative; +, positive.)

When setting the results Culture (BACTEC) as the gold standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the FNAC in the diagnosis of TB lymphadenitis results were 96.55%, 96.38%, 95.89% and 96.96% respectively. When compared with of Xpert in the diagnosis of TB lymphadenitis results, the sensitivity, specificity, PPV and NPV of were 95.23%, 99.17%, 96.77% and 98.76% respectively.

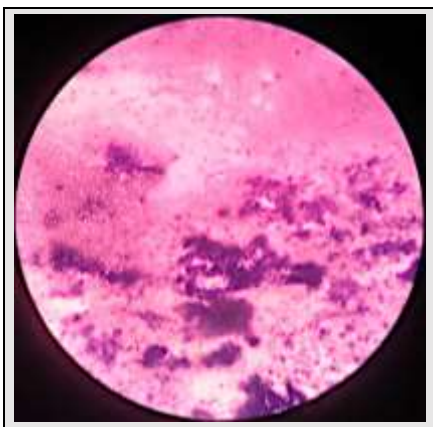

Figure 1. Granuloma Formation, H & E Stain, 40×

DISCUSSION

The *Mycobacterium tuberculosis* complex can affect many organs and systems. It most commonly causes pulmonary tuberculosis, constituting the main source of infection in the community until these patients are diagnosed and treated[14] Clinical presentations of extrapulmonary tuberculosis should be considered in the differential diagnosis of many infectious and non-infectious diseases[15] the diagnosis of tuberculosis by cytomorphology is not new. It is a necrotising granulomatous infection, which cytologically demonstrates the microscopic equivalent of caseous necrosis, granular-appearing necrotic background, together with mature lymphocytes, epithelioid histiocytes and multinucleated Langhans type histiocytes. Several conditions, including mycosis, bacterial and viral adenitis can present the same cytology as does Mycobacterium tubercular adenitis does. Laboratory tests may be essential to establish the cause of such adenopathy correctly, because treatment and prognosis may differ. Demonstration of *Mycobacterium tuberculosis* in fine needle aspirates becomes necessary for an early and accurate treatment. Fine needle aspiration cytology provides a rapid and definitive tissue diagnosis in the superficial lymphadenopathy.[16] India has the highest TB burden as shown in the 2011 World Health Organization (WHO) statistics. The diagnosis of extrapulmonary tuberculosis still remains to be more of a clinical decision. Not many clinically sensitive tests are available in India to assist the treating physician. For accurate diagnosis of *Mycobacterium tuberculosis*, isolation and culture of organism is gold standard, but as *Mycobacterium tuberculosis* is a slow growing organism, culture on conventional Lowenstein- Jensen medium takes 6-8 weeks. Middlebrook medium isolates growth of organism comparatively more rapidly. Mean duration to yield positive culture is about 3 weeks. But for the disease such as tuberculosis, this is too long to wait for results of culture as it is necessary to start treatment at the earliest. Therefore, comparatively rapid diagnostic strategies need to be established for diagnosis of TB lymphadenitis.[17] FNAC is a well-established diagnostic technique for lymphadenopathy evaluation. It is cost effective, safe, minimally invasive, and a rapid method of diagnosing not only TB lymphadenitis but also other pathologies. It also avoids the possible physical and psychological complications of an excision biopsy.[17,18]

AFB were mostly visible in purulent aspirate whether acellular or accompanied by granuloma, and in the absence of ZN staining, case can be misinterpreted as an acute lymphadenitis.[19] When Culture (BACTEC) was taken as the gold standard, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Kappa value the of FNAC in the diagnosis of TB lymphadenitis results were 96.55%, 96.38%, 95.89% and 96.96% respectively. Sensitivity of FNAC was higher and diagnostic accuracy was comparable with other studies.[16]

A recent study by Hillemann et al. demonstrated the effectiveness of Xpert MTB/RIF on extrapulmonary tissue[20] In that study, the combined sensitivity and specificity of 77.3% and 98.2% were reported, respectively. Our study is the first to evaluate the performance of Xpert MTB/RIF in diagnosing tuberculous lymphadenitis through the use of FNAB specimens. Study limitations include the small number of rifampin-resistant cases identified and the fact that the research was conducted in a referral center, as ideally the technique is suited to use in peripheral laboratories to be effective in controlling the disease. A positive aspect of the study is that the patient population is representative of patients presenting with peripheral lymphadenopathy in most areas where TB/HIV are endemic. It is unlikely that our patient cohort had exacerbated disease compared to

patients presenting at primary health care clinics, as these patients are routinely referred from the primary health care clinic to the referral center for FNAB. FNAB is a simple procedure which can be performed in an outpatient setting by clinicians or nursing staff after a short training period[21,22] it is ideal for use in resource-limited settings, including more remote and rural areas[22] Specimen collection is simple and safe. With the use of a transport vial, virtually no sample preparation is required and there is minimal risk of contamination. Furthermore, the transmission risk to the operator may also be reduced. Combining FNAB and rapid genotypic diagnosis using automated systems should greatly improve access to appropriate diagnosis and treatment for patients with tuberculous lymphadenitis.

CONCLUSIONS

GeneXpert has a significant part in diagnosing EPTB. The sensitivity of GeneXpert is additional compared to ZN Stain smear microscopy in the present study. In addition, GeneXpert helps in revealing of Rifampicin resistance which is not possible with FNA and ZN Stain even though FNA is cost effective in the diagnosis of EPTB, combining with GeneXpert has an advantage of detection of FNA missed cases, especially in a suppurative abscess. Moreover, it has a quicker turnaround time (2 hours) compared to culture, which is a gold standard.

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