A Study on Tubercular Lymphadenitis with Special Correlation to Rapid Molecular Diagnostic Tests in a Tertiary Care Hospital of Eastern India

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ABSTRACT

BACKGROUND

Tubercular lymphadenitis is the most common extra-pulmonary manifestation of TB that represents 35% of EPTB and 15-20% of all cases of TB. GeneXpert MTB/RIF has the potential to diagnose active TB in non-respiratory specimens from patients suspected of having extra-pulmonary tuberculosis and multidrug resistant tuberculosis**.**

METHODS

This study was conducted in 100 patients on clinically suspected tubercular lymphadenitis admitted to the Department of Pulmonary Medicine, SCB Medical College, Cuttack (from July 2016 to Jan 2018) after obtaining ethical clearance from institutional ethical committee. Pus/aspirate/tissue from lymph node for CBNAAT and liquid culture by MGIT, tissue from lymph node for histopathology were done.

RESULTS

The commonest age group was 16-30 years (48%) followed by 31-45 years (33%). There were 55 females (55%) and 45 males (45%). Fever was the commonest symptom seen in 47 patients (47%) followed by loss of weight in 25 patients (25%). Cervical lymph node was the most common site detected (52%) followed by supraclavicular lymph nodes involvement (22%) and axillary lymphadenopathy (16%). Tissue sample was sent from 25 patients and fine needle aspirate from 45 patients and pus sent from 30 patients. Tuberculosis was evident in 19 cases (76%) either in the form of granuloma or necrosis or both. AFB culture was sent in all patients. Phenotypic detection through Liquid Culture (MGIT 960 BACTEC) was possible in lymph node samples from 60 patients (60%).

CONCLUSIONS

GeneXpert MTB/RIF when compared to traditional conventional methods to diagnose tubercular lymphadenitis, showed high sensitivity, specificity, positive predictive value and negative predictive value. In TB endemic settings, its implementation could significantly improve the rapid diagnosis of TB Lymphadenopathy and rule out MOTT.

KEYWORDS

Tuberculosis, Lymphadenitis, CBNAAT, MGIT 960 BACTEC

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BACKGROUND

Tuberculosis is a global health burden especially in the developing world. India accounts for one fourth of the global TB burden, 2.2 million out of 9.6 million new cases annually. In India more than 40% population is infected with mycobacterium tuberculosis.[1] Extrapulmonary tuberculosis (EPTB) refers to TB in parts of the body other than lungs and is known to affect virtually every part, with the lymph nodes and pleura being the most common site.[2] Lymphadenitis is the most common extra-pulmonary manifestation of TB that represents 35% of EPTB and 15-20% of all cases of TB.[3]

Over the past decades, the efficacy of the fine needle aspiration cytology (FNAC) and conventional smear microscopy were validated as a diagnostic tool for lymph node TB because of their simplicity, rapidity and performance friendly nature. But FNAC has the low specificity because of the presence of similar cytological indicators in lesions, other than those associated with TB.[4] Though histopathology is most reliable method for diagnosis of TB lymphadenitis, its feasibility is limited due to its non-acceptability, as it is invasive procedure and associated complications like sinus tract and fistula formation.[5] Bacteriological confirmation by the use of solid culture (8 weeks). Liquid culture (automated BACTEC MGIT 960 system, 4 weeks with DST) considerably improves the isolation and decreases the time required to detect mycobacteria from biological specimens.[6]

The main shortcoming of these parameters was that none of these were able to diagnose tuberculosis, perform speciation of the genus mycobacteria and identifying drug resistance in a short period, thus causing misery to the clinician to initiate therapy. Thus there is a need for a rapid and cost effective technique for reliable diagnosis of TB lymph node particularly in low-resource settings. GeneXpert MTB/RIF has the potential in the diagnosis of active TB in non-respiratory specimens (lymph node, pleura, CSF etc) from patients suspected having extra-pulmonary tuberculosis and multidrug resistant. CBNAAT, an automated instrument which works on the principle i.e. sample processing, nucleic acid amplification and detection of the target sequences in simple or complex samples using real time PCR and reverse transcriptase PCR. Results become available in less than 2 hours. The rapid detection of *Mycobacterium tuberculosis* and its resistance and indirect exclusion of MOTT (mycobacterium Other Than Tuberculosis) allows the physician to make critical decisions in the management.[7]

METHODS

This study was conducted in selected 100 patients of clinically suspected tubercular lymphadenitis admitted to the TB ward of the department of pulmonary medicine, SCB Medical College, Cuttack. A detailed clinical history, local and general clinical examination done. All routine blood test, sputum for AFB, chest x-ray PA and lateral view, BT, CT, PT-INR, ultrasound of neck, abdomen and pelvis, CT thorax if needed, pus/aspirate/tissue from lymph node for CBNAAT and liquid culture by MGIT, tissue from lymph node for Histopathology were done. CBNAAT was used as a diagnostic tool as well to detect rifampicin resistance in 2 hours. This study was conducted in a period from July 2016- to Jan 2018. The study population includes patients of both sexes. Ethical clearance from institutional ethical committee was taken.

Inclusion Criteria

Patients having single or multiple palpable lymph nodes with relevant constitutional symptoms of tuberculosis. No previous history of tuberculosis or anti tubercular treatment.

Exclusion Criteria

- Patient not giving consent.
- Patient with previous history of tuberculosis or who has taken anti tubercular treatment previously.
- Contraindications to biopsy.

Investigations

- FNAC: FNAC procedures were carried out in the Department of pathology SCB Medical College, Cuttack as per standard protocol and aspirate was expressed on the slides for cytology study and another aspirate was sent in normal saline for CBNAAT and liquid culture.
- Histopathological Study: An open biopsy consists of surgically removing all or part of a node. The sample is divided into to two parts, one part is sent to pathology in formalin for HP study and another in normal saline for CBNAAT and liquid culture.
- CBNAAT was done in IRL (intermediate Reference Laboratory) from pus, aspirate and tissue biopsy specimen in the standardized operating procedure followed. Rifampicin resistance results were reported as susceptible, resistant, or indeterminate.
- Liquid AFB culture- automated fluorometric BACTEC MGIT 960 system was used for detection of mycobacteria from the biological samples used in our study in the Intermediate reference laboratory of the institute.

RESULTS

Age Group (in Years)	No. of Patients	No. of Male Patients	No. of Female Patients	(%)
0-15	8	4	4	8
16-30	48	22	26	48
31-45	33	14	19	33
46-60	6	2	4	6
61 and Above	5	3	2	5

Table 1. Age Distribution

The commonest age group in our study was 16-30 years (48 patients, 48%) followed by 31-45 years (33 patients, 33%). There were 55 females (55%) and 45 males (45%) with male to female ratio of 1:1.27.

Symptoms	Number of Patients	Percentage
Fever	47	47%
Loss of Weight	25	25%
Loss of Appetite	24	24%
Cough	15	15%
GI Symptoms	13	13%
Chest Pain	12	12%
Dyspnoea	10	10%
Discharging Sinus	5	5%
Generalised Weakness	4	4%

Table 2. Symptomatology in 100 Patients of Tuberculous Lymphadenitis

Among the symptoms**,** fever was the commonest symptom that was seen in 47 patients (47%) followed by loss of weight in 25 patients (25%), loss of appetite in 24 patients (24%), cough in 15 patients (15%).

Sites	No. of Cases	Single	Multiple	Fluctuant	Sinus
Cervical	52 (52%)	32 (61.53%)	20 (38.4%)	11 (21.1%)	2 (3.84%)
Supraclavicular	22 (25%)	17 (77.2%)	5 (22.7%)	4 (18.18%)	0
Axillary	16 (16%)	10 (62.5%)	6 (37.5%)	4 (25%)	2 (7.5%)
Cervical+axillary	1 (1%)	0	1 (100%)	0	0
Inguinal	3 (3%)	3 (100%)	0	1 (33.3%)	1 (33.3%)
Submandibular	3 (3%)	3 (100%)	0	0	0
Axillary+ submandibular	1 (1%)	0	1 (100%)	0	0
Submental	1 (1%)	1 (100%)	0	1 (100%)	0
Supraclavicular+ submandibular	1 (1%)	0	1 (100%)	0	0
Total	100	66 (66%)	34 (34%)	21 (21%)	5 (5%)

Table 3. Lymph Node Involvement Pattern on Local Examination

Cervical lymph node was the most common site detected in our study (52%) followed by supraclavicular lymph nodes involvement (22%) and axillary lymphadenopathy (16%). 66% of the patients presented with single discreet lymph node involvement while 34 cases presented with multiple lymph node involvement. Out of 34 cases 29 cases presented with matting 85%). In 21 patients (21%) lymph nodes were fluctuant and in 5 cases (5%) there was discharging sinus. On chest X-ray PA view, 22 cases (22%) had some form of intra-thoracic involvement. But 20 patients (20%) showed chest lesions suggestive of tuberculosis. In these 20 patients, hilar enlargement was present in 2 cases (10%), pleural effusion was present in 11 patients (55%), infiltration in 5 patients (25%) and cavitation in 2 patients (10%).

Out of 100 patients (image 1), biopsy of lymph node were carried out in 25 patients. Among them tuberculosis was evident in 19 cases (76%) either in the form of granuloma or necrosis or both. 6 cases had reactive hyperplasia. Other causes of granuloma were ruled out. AFB culture was found positive more commonly in necrosis pattern (n=8, 50%) followed by both granuloma and necrosis (n=7, 43.75%), and granuloma only (n=1, 6.25%) pattern out of 16 culture positive cases.

Out of these 100 patients (image 2), tissue samples from 25 patients, fine needle aspirate from 45 patients and lymph node pus from 30 patients were sent for liquid culture and CBNAAT. Phenotypic detection through Liquid Culture (MGIT 960 BACTEC) was possible in lymph node samples from 60 patients (60%) (n=100). Yielding of Mycobacterium in liquid culture was maximum from fine needle aspirate (68.89%) followed by tissue sample (64%) and pus (43.3%). Yielding of CBNAAT in lymph node aspirate is more (77.7%) than AFB culture, while yielding of both CBNAAT and culture was same (64%) in tissue biopsy sample and yielding of CBNAAT was more (46.6%) than culture in pus**.**

Out of 100 patients, lymph node aspirate was sent in 45 patients. Validity of Aspirate CBNAAT with Aspirate culture was studied, taking Culture as gold standard. In the study, it was found that Aspirate CBNAAT detected MTBC in 31 Culture positive cases and 4 in culture negative cases out of 45 cases.

Sensitivity = (True positive/True positive+ false negative) x 100
= (31/31+0) x 100=100%
Specificity = (True negative/True negative+ False positive) x 100
= (10/10+4) x 100=71.43%
Positive predictive value = (True positive /True positive + False positive) x 100
= (31/31+4) x 100=88.57%
Negative predictive value= (True negative/True negative + false negative)
= (10/10+0) x 100=100%

Out of 100 patients, lymph node Pus was sent in 30 patients. Validity of Pus CBNAAT with Pus culture was studied, taking Culture as gold standard. In the study, it was found that in Pus CBNAAT detected MTBC in 9 culture positive cases and detected MTBC in 5 Culture negative cases out of 30 cases.

Sensitivity = (True positive/True positive+ False negative) x 100
= (9/9+4) X 100= 69.23%
Specificity = (True negative/True negative+ False positive) x 100
= 12/12+5=70.58%
Positive predictive value= (True positive /True positive + False positive) x 100
= (9/9+5) x 100=64.28%
Negative predictive value= (True negative /True negative +
False negative) x 100= (12/12+4) x 100=75%

Out of 100 patients, lymph node Tissue was sent in 25 patients. Validity of Tissue CBNAAT with Tissue culture was studied, taking Culture as gold standard. In the study, it was found that, Tissue CBNAAT detected MTBC in 13 culture positive cases and 3 in Tissue Culture negative cases out of 25 cases.

Sensitivity = (True positive/True positive+ False negative) x 100
= (13/13+3) x 100=81.25%
Specificity = (True negative/True negative+ False positive) x 100
= (6/6+3) x 100=66.66%
Positive predictive value= (True positive/True positive|+False positive) X 100
= (13/13+3) x 100=81.25%
Negative predictive value= (True negative/True negative+ False negative) X 100
= (6/6+3) x 100=66.66%

Positivity of CBNAAT		Culture Positivity	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
PUS	CBNAAT (n=14)	9	69.23%	70.58%	64.28%	75%
Tissue	CBNAAT (n=16)	13	81.25%	66.66%	81.25%	66.66%
ASPIRATE	CBNAAT (n=35)	31	100%	71.43%	88.57%	100%

Table 4. Validity of Different Diagnostic Methods of Lymph Node Samples as Compared to Culture in 100 Patients

Out of total 100 cases of lymph node samples, culture was taken as gold standard. The sensitivity of CBNAAT was

highest in fine needle aspirate samples (100%) followed by tissues (81.25%) and pus (69.23%). The specificity of CBNAAT in all the three types of samples was nearly equal i.e. 71.43%, 70.58% and 66.66% in aspirate, pus and tissue respectively. AFB culture and CBNAAT were sent in all patients. AFB culture was found positive more commonly in necrosis pattern (n=8, 50%) followed by both granuloma and necrosis (n=7, 43.75%), and granuloma only (n=1, 6.25%) pattern. CBNAAT positivity was found highest in aspirate sample in 77.7%, pus (46.66%), tissue (64%), compared to culture positivity in aspirate (68.8%), pus (43.3%), and tissue (64%). The sensitivity of CBNAAT was highest in fine needle aspirate samples (100%) followed by tissues (81.25%) and pus (69.23%).

Out of 65 CBNAAT positive cases, 2 cases (3.17%) were found to have Rifampicin resistant and declared to have MDR TB and put on Cat-IV DOTS Regimen in DRTB Ward.

DISCUSSION

India accounts for an estimated one fifth (21%) of all TB cases Worldwide as per Global TB control report-2010. Extrapulmonary tuberculosis constitutes 15-20% of all cases of tuberculosis in immune competent patients but this figure up to 50% as described by some authors (Sharma et al) in some tertiary care centres.[2] Tuberculosis lymphadenopathy accounted for up to two-third of all cases of lymphadenopathy. The clinical presentation of tuberculous lymphadenitis may mimic other non-tubercular causes like malignancy. The histopathological findings may be confused with granulomatous inflammation due to other microbial infections. Bacteriological examination by demonstration of acid fast bacilli in smear microscopy lacks sensitivity due to the paucibacillary nature of fine needle aspirates and culture is gold standard for TB diagnosis and to distinguish from other granulomatous lesions but it is time consuming, requires biosafety measures and needs trained laboratory personnel and reduced sensitivity in paucibacillary disease. In line with these limitations more rapid and reliable methods are needed. GeneXpert MTB/RIF which detected *M. tuberculosis* complex and Rifampicin resistant within 2 hrs. and is more beneficial for diagnosis and treatment of patients regarding therapy during the same visit.

This study was undertaken to assess the correlation between bacteriological and molecular diagnosis (gene Xpert MTB/RIF) of patients of suspected tubercular lymphadenitis and finally utility of these tests in arriving at a precise diagnosis of tubercular lymphadenitis. In present study, among 100 cases of tubercular lymphadenitis the commonest age group affected was young adults of age group 16-30 years (48 patients, 48%), followed by the age group of 31-45 years (33 patients, 33%). B C Jha et al., (2001) in a study of 56 cases of tuberculous cervical lymphadenopathy, observed that the commonest age group affected was 11-20 years (23 patients) followed by 21-30 years (20 patients).[8] As in agreement with most of the studies,[9,10] our study too found that tubercular lymphadenitis mostly affect the young. Male to female ratio in our study was 1:1.27. The explanation for female preponderance can be the poorer living condition, and because young females generally notice differences in their appearance earlier than males and they report to the health care facility earlier. This is similar to that found by B C Jha et al,[8] Dandapat et al (1: 1.2),[11] Subhrahmanyam (1:1.3).[9] Cervical lymph nodes were most common site of involvement (n=52, 52%) followed by supraclavicular nodes (n =22, 22%), axillary nodes (n = 16, 16%) in our study which is in accordance with most of previous literatures (Verma et al). In 11 cases (11%) of patients lymph node was fluctuant at the time of presentation and in 5 cases (5%) there was discharging sinus. This figure is similar to the study by B C Jha et al, Bayazit et al (2004), Dipti Gothi et al (2009).[8,12,13]

In the present study, when lymph nodes were clinically examined out of 100 patients, there was single discrete node in 66 patients (66%), multiple in 34 patients (34%). Multiple matted nodes were observed in 29 patients (29%) and multiple discrete nodes were seen in 5 patients (5%). B C Jha et al (2001) had observed multiple matted nodes forming the largest group (47%).[8] Desa et al (1981) had suggested multiplicity, matting and caseation are the three features that help to establish the diagnosis.[14] But Cheung et al (1988) had reported that 22% of their patients had an abscess or sinus at the time of presentation.[15] In contrast to most of the other studies the increase number of discrete nodes (66%) may be a representation of changing pattern of tubercular lymphadenitis and this could be due to increased awareness leading to early appreciation and detection. In our study fever was observed in 47 patients (47%), loss of appetite in 24 patients (24%) and loss of weight in 25 patients (25%). Cough was the complaint in 15 patients (15%). Review of previous literatures have shown significant variation in clinical presentations.[8,11]

In present study, chest X-ray PA view showed lesions in 22 patients, but 20 patients (20%) out of 100 cases of tubercular lymphadenitis showed chest lesions suggestive of tubercular origin. Out of these 20 patients, 11 patients had pleural effusion (55%), 5 patients had parenchyma infiltrates (25%), 2 patients had parenchyma cavitation (10%) and 2 patients had hilar enlargement (10%). Other similar studies have shown presence of chest x-ray lesion in 5% to 48% of cases.[8,11,16] Out of 100 patients, biopsy of lymph node was carried out in 25 patients. Diagnostic features suggestive of tuberculosis were found in 19 patients (76%). It was observed that epithelioid cell granuloma with caseous necrosis was predominant pattern that was present in 10 patients (40%). Only necrosis was observed in 8 patients (32%) and only epithelioid cell granuloma were found in 1 patients (4%). Predominant cells that were observed in tissue sections suggestive of tuberculosis were epithelioid cells, giant cells and lymphocytes. Our study can be compared with the study by Vemulapalli et al (2016)[16] in this regard.

In the present study, AFB culture was sent in all patients, tissue sample was sent from 25 patients and lymph node aspirate was sent from 45 patients and lymph node

pus was sent from 30 patients. Out of this 100 cases, mycobacterial growth was detected in lymph node samples from 60 patients (60%), which is comparable with the study by Patawardhan et al (2011).[17] Maximum positive yielding from culture was in lymph node aspirate (68.89%) followed by tissue sample (64%) and pus (43.33%) similar to the study by Fontanilla JM et al and Denkinger et al (2014)[18] In the present study tissue biopsy was done in 25 patients. Out of 25 patients, diagnostic features of tuberculosis in histopathological study were seen in 19 patients. Among histopathological pattern AFB culture was found positive more commonly in necrosis pattern (n=8, 50%) followed by both granuloma and necrosis (n=7, 43.75%), and granuloma only (n=1, 6.25%) pattern out of 16 culture positive cases which is similar to the study by Goswami and Parikh et al (2012).[10]

Out of 45 cases of lymph node aspirate of total 100 cases of lymph node samples, compared to culture as gold standard the sensitivity, specificity, positive predictive value, negative predictive value of aspirate CBNAAT were found 100%, 71.43%, 88.57%, 100% respectively. In their study by Asma Ghariani et al (2015), fine needle aspiration samples were collected from 174 patients, the sensitivity and specificity of the Xpert assay were 94.9% and 37.9% respectively, when compared with culture.[19] A systematic review and Meta-analysis conducted by Denkinger et al showed that Xpert test has a sensitivity ranging from 50% to 100% with pooled sensitivity of 83%.[18] The observations of our study are in agreement with above findings as described in other studies. Out of 100 patients, lymph node Tissue was sent in 25 patients, validity of tissue CBNAAT with tissue culture was studied taking Culture as gold standard. In the study, it was found that, tissue CBNAAT detected MTBC in 13 culture positive cases and 3 in tissue Culture negative cases out of 25 cases. The sensitivity, specificity, positive predictive value, negative predictive value of tissue CBNAAT were 81.25%, 66.66%, 81.25%, 66.66% respectively which is less in comparison to the other literatures,[20,21] may be due to inclusion of small sample size.

Out of 100 patients, lymph node Pus was sent in 30 patients. Validity of Pus CBNAAT with Pus culture was studied, taking Culture as gold standard. In the study, it was found that in Pus CBNAAT detected MTBC in 9 culture positive cases and detected MTBC in 5 Culture negative cases out of 30 cases. The sensitivity, specificity, positive predictive value, negative predictive values were 69.23%, 70.58%, 64.28%, 75% which coincides with the study by Tadasse M et al.[22] Out of total 100 cases of lymph node samples, culture was taken as gold standard. The sensitivity of CBNAAT was highest in fine needle aspirate samples (100%) followed by tissues (81.25%) and pus (69.23%). The specificity of CBNAAT in all the three types of samples was nearly equal i.e. 71.43%, 70.58% and 66.66% in aspirate, pus and tissue respectively. The observations of our study are in agreement with the findings as described in other studies (Ablanedo-Terrazas et al 2014).[23]

In those cases where rapid molecular test detects mycobacteria but there was no growth in liquid culture is assumed to be due to dead bacilli in the biological specimen due to prior exposure to antibiotics with anti-TB effect. Out of 65 CBNAAT positive cases, 2 cases (3.17%) were found to have Rifampicin resistant and declared to have MDR TB. Tadasse M et al (2015) in their study was found that rifampicin resistance was identified in 4.7% (4/86) of Xpert-positive cases.[22]

CONCLUSIONS

GeneXpert MTB/RIF when compared to traditional conventional methods to diagnose tubercular lymphadenitis showed high sensitivity, specificity, positive predictive value and negative predictive value. GeneXpert MTB/RIF has been endorsed by WHO in 2014 to be a promising tool to diagnose tuberculous lymphadenitis and rifampicin resistance within 2 hours even in peripheral health set ups and can replace all traditional conventional methods used previously. Fine needle aspirate is a simple OPD procedure, and samples subjected to GeneXpert MTB/RIF get results early and detects rifampicin resistance too.

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