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Original Research Article

Comparison of CBNAAT and AFB screening using Ziehl Neelsen stain and fluorescent stain on FNAC sample for rapid diagnosis of tubercular lymphadenitis

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ABSTRACT

Background: Tuberculous lymphadenitis is the most common etiology of cervical lymphadenopathy in endemic countries. Fine needle aspiration of palpable lymph nodes is done for rapid diagnosis of tuberculous lymphadenitis This study presents a comparative evaluation of fine needle aspiration cytology (FNAC) with acid fast bacilli screening using Ziehl Neelsen stain, Fluorescent stain with Cartridge based nucleic acid amplification test (CBNAAT) for the rapid diagnosis of tubercular lymphadenitis.

Materials and Methods: An observational cross sectional study was done over a period of 15 months from January 2020 to March 2021. All newly diagnosed cases of tubercular lymphadenitis irrespective of age and sex were included. Fine needle aspiration was performed from the palpable lymph node. Smears were prepared using Giemsa, Papanicolaou, Ziehl Neelsen and Auramine O stain.Rest of the sample was used for Mycobacterial growth indicator test (MGIT) and CBNAAT. Statistical Analysis was performed using McNemar test. For gold standard, MGIT as well as a composite reference standard on parameters that included MGIT, radiological findings of tuberculosis, Positive Mantoux test and Positive response to ATT seen in the form of complete resolution of clinical and radiological findings.

Results: The diagnostic value of CBNAAT differed with respect to the chosen gold standard. With MGIT as gold standard, CBNAAT had the highest sensitivity, specificity, positive predictive value and negative predictive value. The diagnostic accuracy of CBNAAT was also the highest. Using CRS (Composite Reference standard) as gold standard, CBNAAT showed the highest specificity and positive predictive value.

Conclusion: With CBNAAT showing statistically significant data of a higher diagnostic value in our study as well as showing rapid result, being automated and not subjected to observer interpretation, we conclude that CBNAAT is more efficient in the diagnosis of tubercular lymphadenitis as compared to AFB screening methods. The only limitation of CBNAAT in our study was its ability to show positive results for three atypical mycobacteria due to possible cross contamination.

1. Introduction

Tuberculous lymphadenitis is the most common etiology of cervical lymphadenopathy in endemic countries and constitutes approximately 30% to 80% of extrapulmonary tuberculosis. The causes of cervical lymphadenopathy, however, range from nonspecific inflammation to life-threatening malignancies in both endemic countries and the western world.[1] Extrapulmonary tuberculosis most commonly affects the lymphatic, genitourinary, bone and joint, and central nervous system, followed by peritoneal and other abdominal organ involvement.[2]

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Fine needle aspiration of palpable lymph nodes is an easy procedure for rapid confirmation of tuberculous lymphadenitis using cytomorphology and direct mycobacterial visualization.[3] Investigations like Ziehl Neelsen and Fluorescent staining of FNAC smear obtained are done to confirm tuberculosis. However due to the paucibacillary nature of the FNAC smear obtained other ancillary investigations like CBNAAT are required.

According to WHO recent guidelines on tuberculosis infection prevention and control 2019, the global number of deaths related to tuberculosis have declined by 42% between 2000 and 2017, and the annual decline in the global TB incidence rate is currently 1.5%. Tuberculosis is still prevelant in India, despite radical measures to eradicate it. As per the Global TB Report 2021, the estimated incidence of all forms of TB in India for the year 2020 was 188 per 100,000 population (129-257 per 100,000 population)

Hence, Rapid diagnostic methods to detect Tuberculosis are required for faster rates of detection and elimination of this disease.

This is a comparative study of FNAC with AFB screening using Ziehl Neelsen stain, fluorescent stain with CB NAAT for the rapid diagnosis of tubercular lymphadenitis.

2. Materials and Methods

This study was done over a period of 15 months from January 2020 to March 2021. All newly suspected cases with clinical symptoms such as cough, fever and enlarged lymph nodes irrespective of age and sex were included. Patients already diagnosed with tuberculosis on Anti tubercular treatment were excluded. Patients with known malignancies with metastatic carcinoma in the lymph node were also excluded. FNAC procedure was performed. An adequate sample was taken. 3 to 5 smears were prepared on glass slides. Rest of the aspirate were collected in sterile containers for CBNAAT and MGIT. These smears were subsequently stained with Papanicolaou stain and Giemsa stain. Heat fixed smears were stained with Ziehl Neelsen stain and Auramine O stain.

Results of various diagnostic tests were analysed using liquid culture MGIT and a composite reference standard (CRS) as gold standard.

Using CRS, patients were classified as Definite TB or Probable TB.

1. Definite TB - TB cases showing growth on MGIT culture
2. Probable TB - Cases suspected to be Tubercular due to any of the two reasons:
   (a) Clinical / Radiological features suggesting tuberculosis.
   (b) Positive Mantoux test.
   (c) Positive response to ATT seen in the form of complete resolution of clinical and radiological findings.

The Institutional Ethics Committee approved the protocol according to the Good Clinical practices. (GCP) – Central Drugs standard control organization (CDSCO)/Indian Council of Medical Research (ICMR)/Schedule Y guidelines /International Comimitee on Harmonizaion (ICH – GCP)) In this study, we enrolled 65 cases of suspected tubercular lymphadenitis in the study taking into account the inclusion and exclusion criterias. Cytomorphological features of tubercular lymphadenitis were classified into five patterns. These smears were then evaluated with AFB screening using ZN staining and fluorescent staining.

The data analysis was done with the use of Statistical Package for Social Sciences (SPSS) software. The diagnostic value of CBNAAT with respect to Zn stain and fluorescent stain for predicting Tuberculosis was calculated using McNamer test using both MGIT and composite reference standard as the gold standard.

3. Results

The age of the patients varied from 06 to 71 years, the predominant population was in the age group of 21-30 years. Female preponderance was seen in this study with 40 females and 25 males. The male:female ratio was 0.62:1. The most common complaint among the patients were fever (n=18/65), followed by pain in swelling (n=13/65). Loss of weight was seen in 7 patients (10.8%), cough in 6 patients (9.2%), cough with expectoration in 3 (4.6%), and loss of appetite in 4 patients (6.2%). One patient each had complaint of hemoptysis (1.5%) and itching (1.5%). No complaints were observed in 12 (18.5%) patients.

Majority of patients presented with lymphadenopathy of cervical region (n=45/65) followed by supraclavicular region (n=18/65). Least number of patients presented with axillary swelling (n=2/65). The Size of the Lymph nodes involved ranged from <1 cm to 4.5 cms. Maximum number of patients presented with single lymph node enlargement (n=48/65) followed by patients with multiple matted lymph nodes (n=15/65). Least number of patients presented with multiple discrete lymph nodes (n=2/65). Maximum number of patients revealed pus aspirate (n=47/65) on FNAC and 18 patients (n=18/65) aspirated bloody sample. Mantoux test was done in 12 patients only, out of which 11 (16.9%) patients showed positive result and only one patient showed negative result.

Smears prepared were classified into five patterns based on cytomorphology.

1. Pattern 1: Epithelioid granuloma with Langhan's giant cells and caseous necrosis.
2. Pattern 2: Epithelioid cells in a reactive lymphoid background.

3. Pattern 3: Caseous necrosis with epithelioid like cells.
4. Pattern 4: Caseous necrosis with lymphocytes and histiocytes. No epithelioid cells seen.
5. Pattern 5: Smears with predominantly neutrophils and degenerating epithelioid cells and necrotic material.

Maximum number of cases showed pattern 5 (n=40/65) followed by pattern 2 (n=9/65), Pattern 3(n=8/65) and pattern 1 (n=5/65). Least number of cases showed pattern 4 (n=3/65).

Cytology smears from all the patients were stained with Ziehl Neelsen stain and fluorescent staining methods and interpreted as per RNTCP guidelines as shown in Table 1.

Table 1: Showing AFB screening (ZN stain & Flourescent stain) on FNAC smears in suspected patients with tubercular lymphadenitis

AFB screening on FNAC	No. of patients	Percentage %
Zn stain		
Negative	31	47.7
Doubtful	12	18.5
1+	19	29.2
2+	2	3.1
3+	1	1.5
Fluorescent stain		
Negative	24	36.9
Doubtful	13	20.0
1+	21	32.3
2+	7	10.8

In routine ZN staining of cytology smears, AFB positivity was found to be 52.3% (n=34/65). As shown in Table 1, Maximum number of patients showed grade 1 positivity (n=19/65) followed by doubtful positivity (n=12/65). Grade 2 positivity was observed in 2 (3.1%) patients, Grade 3 positivity was seen in just one case (1.5%). 31 smears were negative for AFB on ZN staining (47.7%).

With fluorescent stain, AFB positivity was found to be 63.1% (n=41/65). Maximum number of patients showed grade 1 positivity (n=21/65) followed by doubtful positivity (n=13/65). Grade 2 positivity was found in 7 patients (10.8%). 24 patients (36.9%) were negative for AFB on fluorescent staining. Lymph node aspirates from all 65 cases were sent for CBNAAT testing to microbiology lab in a sterile tube. CBNAAT test results showed presence or absence of Tubercular bacilli, along with the presence or absence of its resistance to Rifampicin drug. CBNAAT test showed a positivity rate of 53.9% (n=35/65). Out of the 35 patients positive for Tubercular bacilli on CBNAAT testing as shown in Table 2, 24 (68.6%) cases were sensitive to Rifampicin treatment, 3 (8.6%) were resistant and 5 (14.3%) patients showed indeterminate results. Reaction to rifampicin was not detected in 3 (8.6%) patients. Rest of the aspirated sample was used for Mycobacterial growth indicator test (MGIT) culture. After 6 weeks of incubation, growth was seen in 21 patients (32.2%) as shown in Figure 1. Out of 65 cases, 17 (26.2%) patients showed Mycobacterium TB growth, whereas 4 (6.2%) patients showed growth of Atypical mycobacterium. Rest of the 44 (67.7%) patients failed to show any growth on MGIT culture.

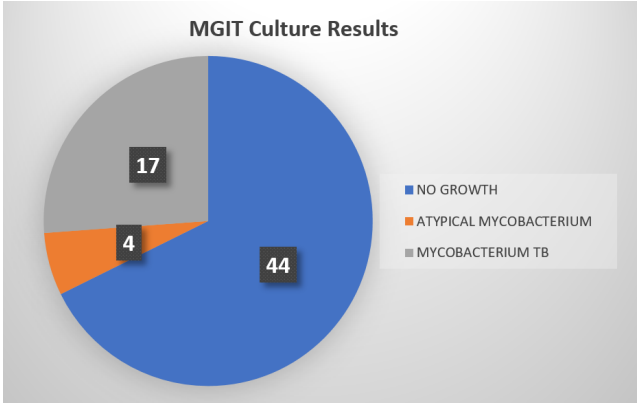

Fig. 1: Results of MGIT culture in suspected patients with tubercular lymphadenitis

Table 2: Showing reaction to Rifampicin treatment on CBNAAT positive patients

Reaction to Rifampicin	CBNAAT positive patients	Percentage %
Rifampicin sensitive	24	68.6
Rifampicin indeterminate	5	14.3
Rifampicin resistant	3	8.6
Rifampicin not detected	3	8.6
Total	35	100

On routine follow up of 65 patients, 49 patients (75.4%) who were detected positive for tuberculosis, had started Anti tubercular treatment (ATT) with significant resolution of symptoms. Enlarged, palpable swelling of the lymph node was the persistent symptom in patients with ongoing ATT, however other symptoms like fever, pain in swelling, cough and loss of appetite were completely resolved. Among the 49 patients, 6 (9.2%) patients had completed 6 months of treatment with complete resolution of the disease, while 43 patients (66.2%) were still undergoing ATT. Among the 43 patients still undergoing ATT, 3 patients who were tested negative on ZN staining, Fluorescent staining, CBNAAT and MGIT culture were tested positive via other ancillary investigations such as sputum culture etc. They had started ATT with significant resolution of symptoms during treatment. Out of the 65 patients with suspected tubercular lymphadenitis, radiology investigations were available in 37 patients only. This is depicted in Table 3 28 (43.08%)

patients had not undergone any radiological investigation. On Chest X-ray, 27 (41.54%) patients revealed pulmonary consolidation. One (1.54%) patient showed left lower zone infiltration with left costophrenic (CP) angle blunting. 6 (9.23%) patients showed a normal study. On CECT chest, one (1.54%) patient revealed ground glass opacities and consolidation in left upper lobe, one (1.54%) patient showed radio opacity in right hilar region and one patient (1.54%) showed soft tissue opacity in paratracheal region.

Table 3: Radiological findings in suspected patients of tubercular lymphadenitis

Radiological findings	No. of patients	Percentage %
Not done	28	43.1
X – Ray Chest		
Pulmonary Consolidation	27	41.5
Normal study	6	9.2
Left lower zone infiltration, left CP angle blunting	1	1.5
CECT Chest		
Ground glass opacities & consolidation in left upper lobe	1	1.5
Radio-opacity in rt. hilar region	1	1.5
Soft tissue opacity in paratracheal region	1	1.5
Total	65	100

In the present study, using the CRS (composite reference standard), patients were divided as Definite TB or Probable TB.

A total of 49 (75.4%) out of 65 patients were detected to have Tuberculosis using CRS as shown in Figure 2. 21 (32.3%) patients were diagnosed as Definite TB and 28 (43.1%) patients were diagnosed as Probable TB. On Comparison of CBNAAT, ZN & fluorescent staining taking CRS as gold standard using McNemar's test as shown in Table 4.

Using CRS as gold standard, fluorescent staining showed the highest sensitivity (77.6%), followed by CBNAAT (69.4%) and ZN stain (63.3%). CBNAAT showed the highest specificity (93.8%), followed by both Zn stain and fluorescent stain showing specificity of 81.3% each.

The diagnostic accuracy of fluorescent stain was the highest (78.5%) followed by CBNAAT (75.4%) and ZN stain (67.7%).

The positive predictive value of CBNAAT was the highest (97.1%), followed by fluorescent stain (92.7%) and ZN stain.(91.2%).

The negative predictive value of fluorescent stain was the highest (54.2%), followed by CBNAAT (50.0%) and ZN stain (41.9%).

On comparison of the p values of sensitivity and specificity of CBNAAT with respect to sensitivity and specificity of ZN and fluorescent stain respectively, no significant difference was found.

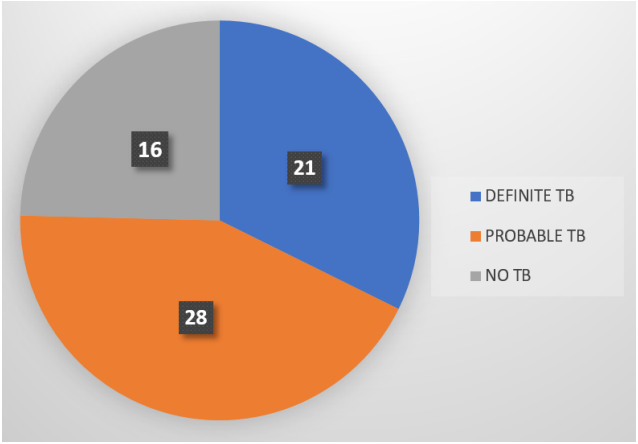

Fig. 2: Pie diagram showing distribution of tuberculosis according to Composite Reference Standards (CRS) of study subjects

On comparison of CBNAAT, ZN & fluorescent staining taking MGIT culture as gold standard as shown in Table 5, CBNAAT showed the highest sensitivity (81.0%), followed by fluorescent stain (71.4%) and ZN stain (47.6%). CBNAAT also showed the highest specificity (59.1%) followed by ZN stain (45.5%) and fluorescent stain (40.9%).

The diagnostic accuracy of CBNAAT was the highest (66.2%) followed by fluorescent stain(50.8%) and ZN stain (46.2%).

The positive predictive value for CBNAAT was the highest (48.6%) followed by fluorescent stain (36.6%) and ZN stain (29.5%).

The negative predictive value for CBNAAT was also the highest (86.7%) followed by fluorescent stain (75.0%) and ZN stain (64.5%).

No significant difference was seen in the sensitivity of CBNAAT as compared to fluorescent stain (p value=0.727) and in the specificity of CBNAAT as compared to Zn stain (p value=0.109).

Sensitivity of CBNAAT was significantly higher as compared to Zn stain (p value=0.039) and specificity of CBNAAT was significantly higher as compared to fluorescent stain (p value=0.021).

4. Discussion

Tuberculous lymphadenitis is one of the most common sites for extra pulmonary tuberculosis. Early diagnosis and optimal treatment will not only completely cure the patient but will also curb the transmission of infection and disease within the community. According to WHO guidelines for TB 2019, Ending the tuberculosis epidemic by 2030 is an achievable target by the United Nations Sustainable Development Goals (SDGs).

Due to the low volume bacterial load, it is difficult to find a definitive diagnostic method for diagnosis. Fine-

Table 4: Sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT, Zn stain and Fluoroscent stain for predicting TB taking CRS as gold standard

CRS	Sensitivity (%)	Specificity (%)	AUC	Positive Predictive value %	Negative predictive value %	Diagnostic accuracy (%)	Comparison of sensitivity with CBNAAT	Comparison of specificity with CBNAAT
CBNAAT	69.4	93.8	0.82	97.1	50.0	75.4	-	-
Zn stain	63.3	81.3	0.72	91.2	41.9	67.7	0.629*	0.5*
Fluorescent stain	77.6	81.3	0.79	92.7	54.2	78.5	0.454*	0.5*

Table 5: Sensitivity, specificity, positive predictive value and negative predictive value of CBNAAT, Zn stain and Fluoroscent stain for predicting TB taking MGIT as gold standard

	Sensitivity (%)	Specificity (%)	AUC	Positive predictive Value %	Negative predictive Value %	Diagnostic accuracy (%)	Comparison of sensitivity with CBNAAT	Comparison of specificity with CBNAAT
CBNAAT	81.0	60.0	0.7	48.6	86.7	66.2	-	-
Zn stain	47.6	45.5	0.47	29.4	65.0	46.2	0.039*	0.109*
Fluorescent stain	71.4	40.9	0.56	36.5	75.0	50.8	0.727*	0.021*

needle aspiration (FNA) is a commonly used diagnostic tool and is a rapid and cost-effective method of diagnosing tuberculous lymphadenitis. The cytomorphological features of tubercular lymphadenitis is epithelioid cell granulomas, multinucleated giant cells and caseous necrosis. However, granulomas and necrosis are also seen in conditions like sarcoidosis, fungal infections and other inflammatory conditions other than TB and hence, may not be very useful.[3]

The diagnosis of tuberculosis on FNAC is enhanced by AFB screening methods like Ziehl Neelsen staining and fluorescent staining. Although these techniques are specific and rapid; they also have low sensitivity in the detection of tubercle bacilli in various clinical samples.

The detection of tubercle bacilli by culture is required as definite evidence of mycobacterial infection. However, due to lack of laboratory equipment and safety procedures, this method is not practiced in resource-poor settings.[4]

Nucleic acid methods (CBNAAT) is being used for rapid detection of mycobacteria from both cultured and uncultured clinical samples. The diagnostic modalities must be done according to the needs of the population and the epidemiology of that region.

4.1. Site of lymphadenopathy

In the study conducted by Andrea Polesky et al[5] (2003), maximum patients (57%) had enlarged cervical nodes followed by supraclavicular nodes in 26% patients, submandibular lymphadenopathy in 13% and axillary adenopathy in 12% of patients.

In a study conducted by Annam et al[6] (2009) out of the 102 lymph nodes evaluated, maximum number of aspirates were from cervical lymph nodes (76/102) followed by inguinal nodes (11/102) and axillary nodes (15/102)

Dnyaneshwar et al[7] (2019) revealed the most common site of involvement was cervical region with 27 out of 52 cases (51.92%), followed by supraclavicular region (17.31%) and axillary region (11.53%).

In the present study, majority of patients presented with lymph node enlargement of cervical region (n=45/65) followed by supraclavicular region (n =18/65). Least number of patients presented with axillary lymphadenopathy (n=2/65).

The findings were similar to the above studies.

4.2. Clinical presentation

In a study conducted by Brijesh et al[8] (2013) with 145 cases of suspected tubercular lymphadenitis, Mantoux test was positive in 64.4% (58/90) cases. Chest X-ray showed signs of pulmonary tuberculosis in 32.2% (29/90) cases.

90% of tuberculous lymphadenitis mainly appears in the cervical lymph nodes more often than other lymph nodes.[9]

In a study conducted by Hitender Gautam et al[10] (2018), 140 cases of cervical lymph node enlargement were studied. Lymph node size of 3cm–6 cm in diameter was seen in 74 (52.85%) cases, followed by >6-cm diameter in 42 (30%), and <3 cm diameter in 24 cases (17.14%). Associated lung lesions were found in 18 by X-ray (12.86%).

The systemic features seen in these patients were fever (75%), followed by weight loss in 83 (59.28%), and night

sweats in 81 (57.85%) patients.

ESR was raised in 67 (47.85%) patients, and the Mantoux test was positive in 44 (31.42%) cases.

In the present study, maximum number of patients presented with single lymph nodes. (n= 48/65) followed by patients with multiple matted lymph nodes (n=15/65).

Lymph nodes of 1-2 cm size were seen in majority of patients (44.6%) followed by patients with lymph node of size 2-3 cm (35.38%). Lymph nodes of size 3-4 cm were found in (9.23%) and lymph nodes of 4-5 cm size in 3.08% of patients.

Out of 65 patients, 47 patients (72.31%) revealed pus aspirate on FNAC and rest 18 (27.69%) revealed bloody aspirate on FNAC.

Most common systemic symptom in patients was fever (27.69%), followed by pain in swelling (20.00%). Other complaints included weight loss (10.77%) and cough (9.2%). One patient presented with hemoptysis (1.54%).

Radiological investigations were available in only 37 (56.92%) while 28 patients (43.08%) had not done any radiological investigation.

Among the 65 patients,34 patients had done Xray. Of which, 27 patients (41.54%) revealed pulmonary consolidation on chest Xray. One patient (1.54%) showed left lower zone infiltration with left costophrenic (CP) angle blunting. 6 patients showed normal study in Xray. (9.23%)

Among the 3 patients with CECT chest findings, one patient (1.54%) showed ground glass opacities and consolidation in left upper lobe and one patient (1.54%) had radio opacity in right hilar region. One patient (1.54%) also showed soft tissue opacity in paratracheal region.

Mantoux test was positive in 16.92% (n=11/65) patients.

4.3. Response to treatment

In a study conducted by Andrea Polesky[5] (2003) with 106 patients of peripheral lymphadenitis, 101 patients were diagnosed as peripheral tuberculous lymphadenitis. 82% received their entire therapy under regular observation, and response to antitubercular therapy was uniformly successful.

In the study conducted by Hitender Gautam et al[10] (2018), on 140 patients, Category I treatment was started in 80 (57.14%) patients as per RNTCP guidelines and 75% of these patients responded within 6 months of treatment.13.75% of patients were cured within 9 months, and 11.25% needed 1 year of treatment.

In the present study, on routine follow up of 65 patients,49 patients (75.38%) who were detected positive for tuberculosis had started anti tubercular treatment with significant resolution of symptoms. Lymph node enlargement was a persistent symptom in patients with ongoing ATT, however other symptoms like fever, pain in swelling, cough and loss of appetite were completely resolved.

Among the 49 positive patients, 6 (9.23%) had completed 6 months of treatment with complete resolution of the disease and 43 patients (66.15%) were still continuing with ATT.

In the present study, the results of various diagnostic tests were first compared using liquid culture MGIT as gold standard. The results were also evaluated using a composite reference standard (CRS) for a final diagnosis of TB.

A series of meta-analyses has shown that nucleic acid amplification tests (NAATs) have high specificity and positive predictive value with highly variable sensitivity, commonly in cases of extra pulmonary TB. In those studies, NAAT has usually been compared to culture, which is known to be a very suboptimal reference standard for EPTB.[11] Hence, we have also used CRS score to evaluate the true diagnostic potential of these various diagnostic tests included in our study.

4.4. Cytomorphological patterns

In a study conducted by Chandrashekar et al[12] (2012) Cytomorphological features of all the cases were analyzed based on the similar pattern as our study. Pattern 2 was observed in 24%, pattern 3 in 7%, pattern 4 in 15% and pattern 5 in 22%. The classic pattern of tuberculosis (Pattern 1) of epithelioid cell granuloma, langhan giant cells and caseous necrosis was seen in 37.5% cases.

In a study conducted by A Hemalatha[13] (2014) with 150 cases, Cytomorphological patterns were categorized into four patterns.29 cases showed epithelioid granuloma without necrosis, 84 casesshowed epithelioid granuloma with necrosis, 4 cases)showed necrosis without epithelioid granuloma with neutrophilic infiltrate. 3 cases showed numerous macrophages.

In a study conducted by Jamsheed et al[14](2020) Among 193 cases of necrotizing granulomatous lymphadenitis, pattern A (Epithelioid cells with caseous necrosis) was observed in 33.7% patients, followed by pattern B (caseous necrosis with few scattered epithelioid histiocytes and lymphocytes) in 31.1%. Pattern C (caseous necrosis with suppurative inflammation) was seen in 30.6%, Pattern D (Caseous necrosis only) in 3.6% and pattern E (non necrotising epithelioid granuloma with positive acid fast bacilli) in 1.03%.

In the present study, the microscopic features of lymph node aspirates were categorized into 5 patterns according to the presence or absence of epithelioid cells, epithelioid cell granulomas, caseous necrosis, lymphocytes, histiocytes and neutrophils.

The maximum number of cases were from Pattern 5(61.54%) followed by pattern 2 (13.85%). Pattern 3 was found in 12.31% and Pattern 1 in 7.69%. Least number of cases were from Pattern 4(4.62%).

In the present study, TB positivity using MGIT as gold standard (Definite TB) was observed in 32.31%

(21/65) patients. Using CRS as gold standard, TB positivity (Definite TB + Probable TB) was observed in 75.39% (49/65) patients.

Comparison of AFB positivity by routine ZN staining and fluorescent stain method.

Variable AFB positivity by ZN staining has been observed by different authors. The interpretation is entirely subjective to the observers interpretation. Also, the severe dilution of mycobacteria in these smears makes it difficult. [15]

In a study conducted by Annam et al[6] in 2009, out of the 102 lymph node aspirates, the smear positivity for AFB on conventional ZN method was 44.11% (45/102) while the positivity increased to 81.37% (83/102) on the modified fluorescent method.

In a study conducted by P Joshi[16] (2013) with 80 cases, ZN staining was positive in 37.5% (30/80) of cases, while autofluorescence was positive in 57.5% (46/80) of patients and was found to have 81.8% specificity, 95% sensitivity, and a positive predictive value of 82.6%.

In a study conducted by Brijesh Thakur et al[8] (2013) smear positivity for Mycobacteria by Ziehl-Neelsen method was 26.67% (24/90), while positivity increased to 34.44% (31/90) by auramine-rhodamine and 42.22% (38/90) on autofluorescence. Culture was positive in 27.78% (25/90) aspirates.

In a study conducted by Olifan Zewdie et al[4] (2017) with 132 patients, 56.1% (74/132) were positive for M. tuberculosis on culture. The detection rate of direct Ziehl Neelsen smear microscopy and the concentration method were 29.5% and 65.2% respectively.

The benefit of Auramine techniques is that slides can be screened at a lower magnification and allows the examination of much larger area per unit of time. In fluorescence microscopy the same area that needs examination for 10 minutes with a light microscope can be examined in 2 minutes.

In the study conducted by N Gizaw et al[17] (2020), the sensitivity of concentrated Auramine-O (AO) and direct Auramine-O staining were extremely high as compared to conventional ZN staining (71.8% vs. 44.5% and 62.7% vs. 44.5% respectively). This sputum with staining of Auramine O had a high rate (18.6%) of detecting smears graded as doubtful, as compared to conventional ZN method.

In our study, ZN staining showed AFB positivity of 52.30% (n=34/65) whereas Fluorescent stain showed a AFB positivity rate of 63.07% (n=41/65). Majority of the studies proved screening with fluorescent stain to be easier i.e bright rod shaped bacteria against a dark background.

Using CRS as gold standard in our current study, ZN stain and fluorescent stain had sensitivity of 63.27% and 77.55% respectively. Specificity of both ZN stain and fluorescent stain was 81.25% each respectively.

Using MGIT culture as gold standard, sensitivity of fluorescent stain (71.43%) was higher than ZN stain.(47.62%). However, specificity of ZN and fluorescent stain were 45.45% and 40.91% respectively.

Sensitivity of fluorescent stain was higher than ZN stain in our study and this was consistent with literature.

4.5. Diagnostic value of GeneXpert

Molecular method (CBNAAT) is a rapid and excellent diagnostic tool for both smear negative lymph node aspirated sample as well as for positive patients clinically suspected with TB.

The study conducted by Louis Lighthelm et al[18] (2011) revealed the excellent diagnostic accuracy of the Xpert MTB/RIF test in patients with tuberculous lymphadenitis. Xpert MTB/RIF correctly identified 29 out of 30 TB cases (96.6% sensitivity). The test sensitivity and specificity were 96.7% and 88.9% respectively. The Xpert MTB/RIF test was positive in all 6 smear-negative culture-positive cases and correctly identified 1 of the 2 rifampin-resistant cases.

In a study conducted by Laura Maynard Smith et al[19](2014), in 6,026 non respiratory samples, Xpert had high specificity and detected vast majority of non respiratory samples including cases with smear-positive and approximately two-thirds of smear-negative samples.

According to a metanalaysis conducted by Steingart et al[20] (2014), although culture is considered the best reference standard for TB, it may lead to misclassification of some cases of extrapulmonary TB as ‘Not TB’ owing to the paucibacillary nature of the disease. Thus, culture may have low sensitivity for Extrapulmonary TB overall. This misclassification by culture may lead to overestimation or underestimation of the diagnostic accuracy of Xpert.

Culture results was negative due to inefficient sample collection, differing bacterial load, and contamination. [20] Contamination of cultures is a problem which is particularly more associated with liquid media. [21]

The low sensitivity of culture means that false negatives may be misclassified as true negatives when MGIT culture is used as the reference standard.

Therefore, when Xpert is evaluated against culture, the number of false negatives (classified as positive by the index test and negative by the reference test) may decrease and Xpert sensitivity overestimated. Hence, this could be the reason why the sensitivity of CBNAAT using MGIT culture as gold standard was relatively higher as compared to CRS.

Similarly, the low sensitivity of culture means also that index test true positives may be misclassified as false positives when culture is used as the reference standard. Therefore, when Xpert is evaluated against culture, the number of false positives (classified as positive by the index test and negative by the reference test) may be increased and Xpert specificity may be underestimated.

In our study, sensitivity and specificity of CBNAAT using CRS was 69.39% and 93.75% respectively. Sensitivity and specificity of CBNAAT using MGIT as gold standard was 80.95% and 59.09% respectively. The sensitivity of CBNAAT was overestimated when MGIT was taken as gold standard as compared to CRS. The specificity of Gene Xpert calculated using MGIT as gold standard was underestimated as compared to the specificity of Gene Xpert using CRS culture as gold standard. Hence, this was consistent with literature.

In the study conducted by Sarmeen Sarfaraz[22] 2018) in 297 patients, tubercular lymphadenitis was diagnosed on histopathology in 89.6% of cases, followed by GeneXpert in 32.6%, mycobacterial culture in 26.6%, and AFB smear positivity in 12.5% cases. The majority of tubercular lymphadenitis patients (88.7%) responded favorably to ATT.

In the present study, CBNAAT showed AFB positivity of 53.85% (n=35/65) with Rifampicin resistance in only 3 cases (4.6%). Among the 35 cases detected positive by CBNAAT, 17 cases showed growth in MGIT culture.

In a study conducted by Hitender Gautam et al[10] (2018), positive detection rates of tuberculosis by Xpert MTB/RIF and MGIT 960 culture were 25.71% and 17.85% respectively.

In a metaanalysis study conducted by Guocan Yu et al[23] (2019) showed that sensitivity of Xpert MTB/RIF, performed on FNA samples was 80% using CRS as gold standard and 90% using MGIT culture as gold standard. The pooled specificity was 96% using CRS as gold standard and 89% using MGIT as gold standard. Sensitivity of Xpert using CRS as gold standard was overestimated and specificity of Xpert was underestimated in this study. This was also consistent with our study.

4.6. Detection of atypical mycobacterium by CBNAAT

Out of 4 atypical mycobacterium species grown in culture, 3 cases were detected positive (nonspecific) by CBNAAT. The possible causes of false-positivity/ cross reactivity could be specimen contamination at the time of sample preparation.[24]

Pang et al[25] (2013) conducted a study in which out of 12 nontuberculous mycobacteria (NTM) species, 5 were identified as Mycobacterium tuberculosis (MTB) by GeneXpert at a bacterial load of 106. This false detection of tuberculosis is a problem by Xpert assay. They hypothesized that a high burden of organisms might cause fluorescence cross-talk in the detection channels, which could lead to false-positive results.

It is important to distinguish between tuberculous lymphadenitis and Non Tuberculous Mycobacteria lymphadenitis as the treatment modalities are different.[2]

In a study conducted by Huh H J[24] (2019), five Non tuberculous mycobacteria were used (M. abscessus, M. marinum, M. smegmatis, M. phlei, and M. aurum) to test the diagnostic accuracy of CBNAAT. This study showed only one specimen out of 180 specimens (0.6%) exhibited a false-positive result. They tested the cross-reactivity between MTB and five NTM species at high bacterial load (1×107 CFU/mL equivalents) and did not observe any false-positive reactions. They concluded that the possible causes of false-positivity could be specimen contamination at the time of sample preparation.

The limitation of the diagnostic value of CBNAAT in our study was its ability to show positive results for three atypical mycobacteria, which has an entirely different treatment regimen. However, the possible reason for this could be cross contamination as stated in a few other studies.

4.7. Comparison of CBNAAT with AFB screening (ZN and Fluorescent staining)

In a study conducted by Meenal Bagdia et al[26] (2018), positivity of ZN stain was 5.17%, of fluorescent microscopy was 10.34%, of culture was 12.06% and of Gene Xpert was 15.38%. In their study, sensitivity and specificity of LED-fluorescent microscopy was 82.6% and 98% respectively and sensitivity and specificity of Gene Xpert was 85.7% and 97.36% respectively. Specificity of LED fluorescent microscopy and ZN were highest in their study and sensitivity of Gene Xpert was the highest compared to other diagnostic tests in their study.

In a study conducted by Louis Lighthelm et al[18] (2018), sensitivity of ZN stain was 41.4%, Fluorescent microscopy was 75.9%. Gene Xpert had the highest sensitivity of 96.6%.

In the present study, AFB positivity of ZN stain was 52.30% (n=34/65). Fluorescent stain showed AFB positivity of 63.07% (n=41/65). MGIT culture growth positivity was seen in 21 patients (32.20%) out of which Mycobacterium TB were grown in 17 patients.(26.15%) and 4 showed growth of atypical mycobacteria. Positivity of Gene Xpert was 53.85% (n=35/65).

Using CRS as gold standard fluorescent staining showed the highest sensitivity (77.55%), followed by CBNAAT (69.39%) and ZN stain (63.27%). CBNAAT showed the highest specificity (93.75%), followed by both Zn stain and Fluorescent stain showing specificity of 81.25% each.

The diagnostic accuracy of fluorescent stain was the highest (78.46%) followed by CBNAAT (75.38%) and ZN stain.(67.69%).

The positive predictive value of CBNAAT was the highest (97.14%), followed by Fluorescent stain (92.68%) and ZN stain.(91.18%)

The negative predictive value of fluorescent stain was the highest (54.17%), followed by CBNAAT (50.00%) and ZN stain. (41.94%)

Using MGIT culture as gold standard, CBNAAT showed the highest sensitivity (80.95%), followed by fluorescent stain (71.43%) and ZN stain,(47.62%).

CBNAAT also showed highest specificity (59.09%), followed by Zn stain (45.45%) and fluorescent stain showing specificity of 40.91%.

The diagnostic accuracy of CBNAAT was also the highest (66.15%), followed by fluorescent stain (50.77%) and ZN stain (46.15%).

The positive predictive value for CBNAAT was the highest (48.57%), followed by Fluoroscent stain (36.59%) and ZN stain.(29.47%). The negative predictive value for CBNAAT was also the highest (86.67%), followed by Fluorescent stain(75%) and ZN stain.(64.52%).

CBNAAT showed highest specificity by using both MGIT and CRS as gold standard. The sensitivity of fluorescent stain was the highest using CRS as gold standard, and the sensitivity of CBNAAT was the highest using MGIT as gold standard.

On comparison of p values of CBNAAT with AFB screening (ZN stain and fluorescent stain) using CRS as gold standard. No significant difference was found.

Hence, on comparison of CBNAAT with AFB screening by using ZN staining and Fluorescent staining, the diagnostic value of CBNAAT differed with respect to the chosen gold standard. With MGIT as gold standard, CBNAAT had the highest senstivity, specificity, positive predictive value and negative predictive value. The diagnostic accuracy of CBNAAT was also the highest.

With CRS (Composite Reference standard) as gold standard, CBNAAT showed the highest specificity and positive predictive value. However, the sensitivity and diagnostic accuracy of CBNAAT was lower than fluorescent staining. This could be due to the overestimation of sensitivity of CBNAAT detected when MGIT was used as gold standard.

However, considering the tedious procedure involved in fluorescent staining as well as the subjective interpretation in the detection of AFB, it is better to opt for CBNAAT as the interpretation of AFB positivity is not subjected to interpretation by the observer and results are available within 2 hours and are less prone to bias.

5. Conclusion

CBNAAT showed the highest sensitivity, diagnostic accuracy, positive predictive value and negative predictive value followed by fluorescent stain and ZN stain using MGIT as gold standard.

Although, AFB screening using ZN stain and fluorescent stain uses less resources It is based on subjective interpretation by the observer. It requires experience and skill and is subjected to inter observer variability.

With CBNAAT showing statistically significant data of a higher diagnostic value in our study as well as showing rapid result, being automated and not subjected to observer interpretation, we conclude that CBNAAT is more efficient in the diagnosis of tubercular lymphadenitis as compared to AFB screening methods.

6. Source of Funding

None.

7. Conflict of Interest

None.

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