International Journal of Community Medicine and Public Health
Patil SB et al. Int J Community Med Public Health. 2020 Apr;7(4):1511-1515
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: http://dx.doi.org/10.18203/2394-6040.ijcmph20201465

Original Research Article

Cartridge based nucleic acid amplification test: a sensitive diagnostic tool for tuberculosis on fine needle aspirates samples

Suwarna B. Patil[1], Shweta M. Dhage[1]*, Pradeep S. Umap[1], S. V. Ghorpade[2], Shyamkant Patharwat[1]

[1]Department of Pathology, [2]Department of Respiratory Medicine, Govt Medical College, Akola, Maharashtra, India

Received: 01 February 2020
Revised: 06 March 2020
Accepted: 07 March 2020

***Correspondence:**
Dr. Shweta M. Dhage,
E-mail: dhageshwetagmc@gmail.com

ABSTRACT

Background: The extrapulmonary tuberculosis (EPTB) is challenging to diagnose due to its pauci-bacillary nature. According to recent research, WHO recommends cartridge based nucleic acid amplification test (CBNAAT) to be used as initial diagnostic test in suspected cases of extrapulmonary tuberculosis. Aim of the present study is to assess the role of CBNAAT in comparison with cytomorphological features upon fine needle aspiration cytology (FNAC) and Ziehl-Neelson (ZN) stain in clinically suspected cases of EPTB.
Methods: Present pilot study is descriptive cross-sectional study of 439 cases of clinically suspected EPTB over a period of 12 months (January 2019 to December 2019). After procedure of fine needle aspirates, smears were stained with routine H&E, papanicolaou stain and ZN stain. In the same setting, aspirate was also sent for CBNAAT. Results were obtained after detailed study.
Results: Out of 439 cases, presumptive tuberculosis was diagnosed in 192 cases showing either epithelioid cell granulomas or caseous necrosis or both upon morphology, while overall 94 cases were positive on ZN stain and 146 cases were CBNAAT positive with the sensitivity of 84.04% and specificity of 80.57%.
Conclusions: FNAC is the cheapest and simplest method to diagnose extrapulmonary tuberculosis, however those smears where tuberculosis cannot be diagnosed on FNAC like suppurative lesions, reactive lymphadenitis and low cellularity, CBNAAT plays a key role for the correct diagnosis thereby significantly reducing the morbidity and mortality.

Keywords: Extrapulmonary tuberculosis, FNAC, CBNAAT, ZN

INTRODUCTION

Tuberculosis (TB) is one of the world's deadliest communicable diseases and worldwide around 10 million people fall ill with TB each year according to global tuberculosis report 2019 of World Health Organisation.[1] The causative agent is *Mycobacterium tuberculosis*. India stands first with the highest burden of TB.[1] Inspite of common involvement of lungs, TB can affect any organ or system of the body. Extra pulmonary tuberculosis according to WHO classification criteria is an infection by *M. tuberculosis* affecting tissues and organs outside the pulmonary parenchyma.[2,3] In India, 10 to 15% of total TB cases are of extra pulmonary tuberculosis which commonly involves the pleura, lymph nodes, gastrointestinal tract and other organs with a significant mortality rate (25 to 50%).[4] Cytology and conventional smear microscopy have been used as the initial diagnostic tools for tuberculous lymphadenitis in resource poor settings.[5] Fine needle aspiration cytology (FNAC) is a

simple and rapid diagnostic technique, but with low specificity due to the presence of similar cytomorphological features in lesions other than those associated with TB.[6,7] Conventional smear microscopy lacks sensitivity due to the pauci-bacillary nature of fine needle aspirates (FNA).[5] Mycobacterial culture is a gold standard for diagnosis of TB and because drug susceptibility testing are not always available in resource poor settings, their results may take 4 to 8 weeks or even longer.[2] Considering these limitations, more rapid and reliable methods are needed.

In December 2010, WHO endorsed CBNAAT or Gene Xpert MTB/RIF1 for use in TB laboratories and in India it was adopted by RNTCP in 2012. It first started as a pilot project in Maharashtra state, India.[8] The CBNAAT assay consists of a closed system that is based on real-time polymerase chain reaction (RT-PCR). It requires minimal technical expertise in the diagnosis of TB and rifampicin resistance within 2 hours.[9] The GeneXpert utilizes a DNA-PCR technique for simultaneous detection of *M. tuberculosis* and rifampicin resistance-related mutations. It is fully automated benchtop cartridge-based nucleic acid amplification (CB-NAAT) assay for TB detection 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.[10,11] Patients with a high risk of tuberculosis-like HIV-associated TB patients and extrapulmonary cases in whom Ziehl-Neelson (ZN) stain smear examination is usually negative are the most likely to be benefited from GeneXpert.[11] WHO strongly recommends widespread use of CBNAAT for these groups of patients.[2]

With this aim, the present study was carried out and the results of FNA smear cytology were compared with ZN stain and CBNAAT findings.

METHODS

Present pilot study was the descriptive cross-sectional study conducted in the Department of Pathology, Government Medical College, Akola between January 2019 and December 2019. All those clinically suspected cases of EPTB which were sent for CBNAAT during the above-mentioned period were included in this study. So, in total, we had included 439 cases. While those already diagnosed, recurrent and follow up cases of EPTB were excluded.

Procedure

After obtaining detailed history and examining the patients, FNAC specimens were collected from 439 cases by performing 2-3 passes of 23 to 24 gauge needle attached to 5 ml syringe. Three smears were prepared from each aspirated material. Two were fixed with isopropyl alcohol each for H & E and papanicolaou staining. These smears were evaluated for adequacy and for the presence of epithelioid cell granulomas with or without caseous necrosis or only caseous necrosis. Third smear was for ZN staining. ZN stained smears were examined for bright pink beaded curved bacilli on bluish background and were reported as positive or negative for acid fast bacilli. The remaining aspirated material was sent in presterilized Falcon tube to the Microbiology Department, GMC Akola for CBNAAT where the final result was reported as positive or negative.

For calculating sensitivity and specificity following formulae were used.

Sensitivity=True positive

True positive+false negative

Specificity=True negative

True negative+false positive.

RESULTS

Four hundred thirty nine cases with clinical suspicion of tuberculosis subjected to FNAC, ZN stain and CBNAAT were studied. Most of the cases (29.3%) were found in the age group of 21 to 30 years with female preponderance (Table 1). The youngest patient was 1 year old child and 80 years old male was the oldest patient. Overall, females (59.2%) showed predominance over males (40.7%) as shown in Table 1.

Table 1: Age and sex wise distribution of total cases.

Age (years)	Number of patients	Males	Females
	N (%)	N (%)	N (%)
0-10	48 (10.9)	28 (6.3)	20 (4.5)
11-20	107 (24.3)	42 (9.5)	65 (14.8)
21-30	129 (29.3)	42 (9.5)	87 (19.8)
31-40	82 (18.3)	26 (5.9)	56 (12.7)
41-50	38 (8.6)	21 (4.7)	17 (3.8)
51-60	17 (3.8)	7 (1.5)	10 (2.2)
61-70	13 (2.9)	8 (1.8)	5 (1.1)
71-80	5 (1.1)	5 (1.1)	0
Total	439 (100.0)	179 (40.7)	260 (59.2)

Majority of CBNAAT positive cases (51 out of 146) were also in age group of 21 to 30 years with female preponderance and the least (3 out of 146) were seen in the age group of 71 to 80 years as shown in Figure 1.

The most common site to be involved was cervical neck swelling (67.1%) followed by axillary (8.6%), breast (6.3%) and submandibular swelling (5.2%) as shown Figure 2.

Out of 439 clinically suspected cases, reactive lymphadenitis comprised 38.4%, followed by suppurative inflammation (17.9%). Among FNAC diagnosed cases of

tuberculosis, cases of granulomatous lymphadenitis showing ill to well defined granulomas (Figure 3 A) were 15.9%, cases of tuberculous lymphadenitis showing both granulomas and caseous necrosis were 15.7%, necrotizing lymphadenitis showing only caseous necrosis (Figure 3C) were 9.5%. Breast was involved in 28 cases as tuberculous mastitis (6) and granulomatous mastitis (8) and remaining were given as suppurative inflammation showing plenty of polymophs and histiocytes. One case turned out tobe lymphoid malignancy as shown in Table 2.

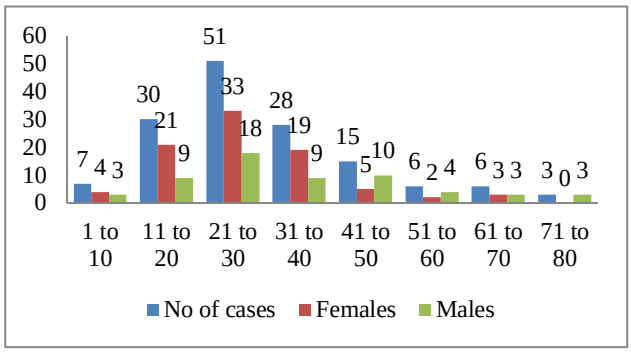

Figure 1: Age and sex wise distribution of CBNAAT positive cases.

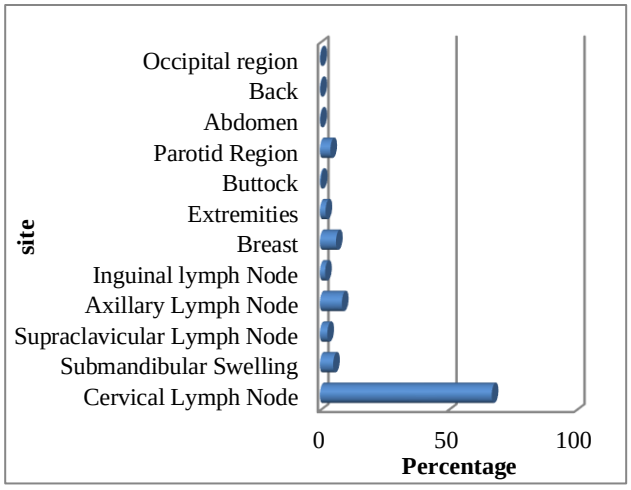

Figure 2: Site wise distribution of total cases.

Table 2: FNAC diagnosis

Diagnosis	Number of cases	%
Tuberculosis		
Chronic granulomatous lymphadenitis	70	15.9
Necrotizing lymphadenitis	39	8.8
Tuberculous lymphadenitis	69	15.7
Tuberculous mastitis	06	1.3
Chronic granulomatous mastitis	08	1.8
Reactive lymphadenitis	167	38.4
Suppurative inflammation	79	17.9
Lymphoid malignancy	01	0.2

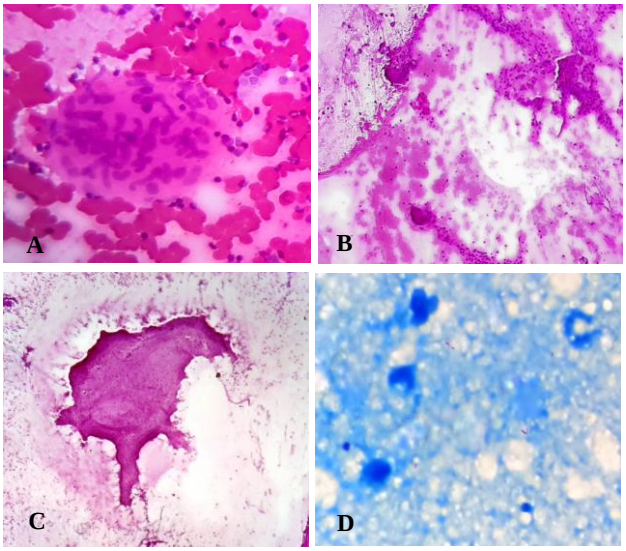

Figure 3: Different cytomorphological features of tuberculosis, (A) Granuloma consisting of epithelioid cells (H & E, 400X); (B) epithelioid cell granuloma and giant cells (H & E, 100X); (C) caseous necrosis (H & E, 200X); (D) ZN stain show positive acid fast bacilli (1000X-arrow and arrow head).

Table 3: Comparison of FNAC diagnosis with ZN stain and CBNAAT.

Diagnosis	Total no. of patients diagnosed on FNAC	Positive cases on ZN stain	Positive cases on CBNAAT
		N (%)	N (%)
Tuberculosis	192	89 (46.3)	106 (55.2)
Chronic granulomatous lymphadenitis	70	2 (2.8)	223(1.42)
Necrotizing lymphadenitis	39	20 (51.2)	22 (56.4)
Tuberculous lymphadenitis	69	62 (89.85)	53 (76.8)
Tuberculous mastitis	06	4 (66.6)	4 (66.6)
Chronic granulomatous mastits	08	1 (12.5)	5 (62.5)
Reactive lymphadenitis	167	1 (0.5)	8 (4.7)
Suppurative inflammation	79	4 (5.0)	32 (40.5)
Lymphoid malignancy	1	0	0
Total	439	94 (21.4)	146 (33.2)

Out of 439 cases, 21.4% cases were positive for acid fast bacilli while 33.2% cases were positive for CBNAAT. Among 192 cases of tuberculosis diagnosed on FNA, 46.3% cases were positive for acid fast bacilli and 55.2%

cases were positive for CBNAAT. Among 167 cases of reactive lymphadenitis, only one case was ZN positive and eight cases were CBNAAT positive while out of 79 cases of suppurative inflammation, four cases were ZN stain positive and thirty two cases were CBNAAT positive as shown in Table 3.

Table 4: Diagnostic performance of CBNAAT versus FNAC.

CBNAAT	Positive	Negative	Total
Positive	106	40	146
Negative	86	207	293
Total	192	247	439

Sensitivity and Specificity of CBNAAT with FNA was 55.20% and 83.80% respectively (Table 4).

Table 5: Diagnostic performance of ZN stain versus FNA.

ZN stain	Positive	Negative	Total
Positive	89	5	94
Negative	103	242	345
Total	192	247	439

Sensitivity and specificity of ZN stain with FNA was 46.35% and 97.97% respectively (Table 5).

Table 6: Diagnostic performance of CBNAAT versus ZN stain.

CBNAAT	Positive	Negative	Total
Positive	79	67	94
Negative	15	278	293
Total	94	345	439

Sensitivity and Specificity of CBNAAT with ZN Stain was 84.04% and 80.57% respectively (Table 6).

DISCUSSION

The present study is retrospective study on the diagnosis of extrapulmonary tuberculosis by CBNAAT in comparison to FNAC and ZN stain in a tertiary care hospital.

In this study, we compared the age and sex wise distribution of total cases and CBNAAT positive cases with different studies. Out of 439 cases, majority was in the age group of 21 to 30 years with female predominance which was similar to study conducted by Konamapalli et al.[2] Most of the CBNAAT positive patients were in younger age group which was comparable to other studies as shown in Table 7. Similar to this study, female preponderance was noted in other studies (Table 8).

We also compared anatomical site distribution of cases. The most common site involved in this study was cervical lymph node which was also noted by the study done by Majed et al and Lavanya et al.[16,17]

Table 7: Comparison of age wise distribution of CBNAAT positive cases with other studies:

Study	Age group	% of cases
Present study	11-30	53.7
Konamapalli et al[2]	11-30	30.4
Yassin et al[12]	15-24	30.7
Arora et al[13]	15-24	38
Bryan et al[14]	15-24	43
Mulualem et al[15]	16-30	58

Table 8: Comparison of sex wise distribution of CBNAAT positive cases with other studies.

Study	Male (%)	Female (%)
Present study	40.4	59.5
Konamapalli et al[2]	23	26.2
Bryan et al[14]	46	54
Mulualem et al[15]	67	76

In present study, among 192 diagnosed cases of EPTB on FNAC, 46.35% cases were positive on ZN stain while 55.20% cases were positive for CBNAAT which was comparable to the study done by Lavanya et al.[17] Among suppurative inflammatory lesions, 40.50% cases were positive for CBNAAT and 5% were positive for ZN stain which indicates that there is a strong possibility of tuberculosis among suppurative lesions. This finding was also noted by Lavanya et al.[17]

In the present study, surprisingly, among reactive lymphadenitis 4.7% cases were positive for CBNAAT and one case was positive for acid fast bacilli indicating the importance of CBNAAT.

CBNAAT had an overall sensitivity of 55.20% which is superior to ZN stain smear microscopy with a sensitivity of 46.35% in the current study. So, in concordance with other studies, CBNAAT has higher sensitivity than ZN stain.[17-19]

CONCLUSION

As accepted worldwide, FNA is a safe, cost effective, non invasive and rapid method to diagnose tuberculosis based upon cytomorphological features and ZN stain. However, it becomes very difficult to diagnose TB in suppurative lesions, reactive lymphadenitis and low cellularity. In such context if in a single prick; cytology smears, ZN stain and CBNAAT procedures are carried out, rapid diagnosis of TB is possible reducing morbidity and mortality related to TB. So, coupling FNAC with ZN stain and CBNAAT increases diagnostic accuracy for tuberculosis. The use of CBNAAT greatly improves

diagnostic value specially in patients with low bacillary load those are likely to be missed on ZN stain.

In developing country like India, such practices will definitely support WHO's goal of eradicating TB by 2025.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Global tuberculosis report 2019. Geneva: World Health Organisation; 2019. Licence: CCBY- NC-SA3.0IGO.
2. Komanapalli S K, Prasad U, Atla B, Vasundhara N, Yendluri D. Role of CB-NAAT in diagnosing extra pulmonary tuberculosis in correlation with FNA in a tertiary care center. Int J Res Med Sci. 2018;6(12):4039-45.
3. Ramirez-Lapausa M, Menendez-Saldana A, Noguerado-Asensio A. Extrapulmonary tuberculosis: an overview. Rev Esp Sanid Penit. 2015;17:3-11.
4. Takhar RP. NAAT: A new ray of hope in the early diagnosis of EPTB. Emerg Med (Los Angel). 2016;6:328.
5. Tadesse M, Abebe G, Abdissa K, Aragaw D, Abdella K, Bekele A, et al. GeneXpert MTB/ RIF Assay for the Diagnosis of Tuberculous Lymphadenitis on Concentrated Fine Needle Aspirates in High Tuberculosis Burden Settings. PLoS ONE. 2015;10(9):e0137471.
6. Ammari FF, Hani AHB, Ghariebeh KI. Tuberculosis of the lymph glands of the neck: a limited role for surgery. Otolaryngol Head Neck Surg. 2003;128:576-80.
7. Bekedam H, Boeree M, Kamenya A, Liomba G, Ngwira B, Subramanyam V, et al. Tuberculosis lymphadenitis, a diagnostic problem in areas of high prevalence of HIV and tuberculosis. Malawi Medical J. 2014;11:56-9.
8. Dewan R, Anuradha S, Khanna A, Garg S, Singla S, Ish P, et al. Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. JIACM. 2015;16(2):114-7.
9. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011;6(9):1067-82.
10. Piersimoni C, Scarparo C, Piccoli P, et al. Performance assessment of two commercial amplification assays for direct detection of Mycobacterium tuberculosis complex from respiratory and extrapulmonary specimens. J Clin Microbiol. 2002;40:4138-42.
11. Saglam L, Akgun M, Aktas E. Usefulness of induced sputum and fibreoptic bronchoscopy specimens in the diagnosis of pulmonary tuberculosis. J Int Med Res. 2005;33:260-5.
12. Yassin MA, Datiko DG, Shargie EB. Ten-year experiences of the tuberculosis control program in the southern region of Ethiopia. Int J Lung Dis. 2006;10(10):1166-71.
13. Arora VK, Gupta R. Trends of extrapulmonary tuberculosis under revised national tuberculosis control program: A study from South Delhi. Ind J Tuberc. 2006;53:77-83.
14. Rock RB, Sutherland WM, Baker C, Williams DN. Extrapulmonary tuberculosis among Somalis in Minnesota. Emerging infectious diseases. 2006;12(9):1434.
15. Tadesse M, Abebe G, Abdissa K, Aragaw D, Abdella K, Bekele A, et al. GeneXpert MTB/RIF assay for the diagnosis of tuberculous lymphadenitis on concentrated fine needle aspirates in high tuberculosis burden settings. PLoS ONE. 2015;10(9):1-9.
16. Majed M, Amitha G, Anamika A, Sandeep S.Comparative Analysis of Gene Expert Assay In Addition To Z-N Microscopy for Detection of Extra -Pulmonary TB in A Fine Needle Aspiration Samples. Sch J App Med Sci. 2017;5(7D):2803-8.
17. Lavanya G, Sujatha C, Faheem K, Anuradha B. Comparison of GeneXpert with ZN Stainin FNA samples of suspectedextrapulmonary tuberculosis. IOSR J Dent Med Sci. 2019;18(7):25-30.
18. Al-Ateah SM, Al-Dowaidi MM, El-Khizzi NA. Evaluation of direct detection of Mycobacterium tuberculosis complex in respiratory and non-respiratory clinical specimens using the Cepheid Gene Xpert® system. Saudi Med J. 2012;33(10):1100-5.
19. Ligthelm LJ, Nicol MP, Hoek KG, Jacobson R, van Helden PD, Marais BJ, et al. Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. J Clin Microbiol. 2011;49:3967-70.

Cite this article as: Patil SB, Dhage SM, Umap PS, Ghorpade SV, Patharwat S. Cartridge based nucleic acid amplification test: a sensitive diagnostic tool for tuberculosis on fine needle aspirates samples. Int J Community Med Public Health 2020;7:1511-5.