Original Article

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Pathology Section

Comparison of Fine Needle Aspiration Cytology, Ziehl-Neelsen Staining and GeneXpert Methods in Suspected Cases of Tubercular Lymphadenopathy

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ABSTRACT

Introduction: The Extrapulmonary Tuberculosis (EPTB) is challenging due to the pauci-bacillary nature of the disease and limited tests available for early diagnosis. It is the most common extrapulmonary manifestation of tuberculosis. The samples from extrapulmonary infection will have low bacterial count as compared with sputum specimens. It is also necessary to rule out other causes of granulomatous inflammation on Fine Needle Aspiration Cytology (FNAC) and confirmation by Ziehl Neelsen (ZN) stain and Cartridge Based Nucleic Acid Amplification Test (CBNAAT). In December 2010, World Health Organisation (WHO) recommended GeneXpert/CBNAAT to be used as the initial diagnostic test in suspected EPTB cases.

Aim: To compare FNAC and ZN stain with CBNAAT in the diagnosis of suspected tubercular lymphadenopathy in a tertiary care centre and to know the importance of CBNAAT in the modern era.

Materials and Methods: This was a hospital based prospective study carried out over a period of 12 months (July 2019 to June 2020) in the Department of Pathology and Microbiology, ESIC Medical College. All presumptive cases of tubercular lymphadenopathy and purulent aspirates from the lymph nodes of various sites were included in the study. Smears were made after FNA and stained with Haematoxylin and Eosin (H&E) stain and ZN stain and sample was also processed for CBNAAT in all cases of lymphadenopathy. Statistics were done using SPSS software version 20.00.

Results: The total number of cases with presumptive tubercular lymphadenitis was 119. Majority of the aspirates are from jugular lymph nodal and cervical swellings 77 (64.7%) out of 119. FNAC has detected tuberculosis in 20 (16.8%) cases and ZN stain detected Acid Fast Bacilli (AFB) in 6 (5%) cases. CBNAAT has detected 28 (23.5%) cases, among them 15 (12.6%) cases which were not detected by FNAC. Diagnostic Performance of CBNAAT versus FNAC 28 out of 119 showed sensitivity, specificity, negative predictive value and positive predictive value 80%, 85.8%, 65% and 45.5%.

Conclusion: The CBNAAT can be added with FNAC to get more specific results. CBNAAT is less sensitive for blood stained samples than purulent samples and hence FNAC still remains as the cheapest and first line test to diagnose in cases suspected of tubercular lymphadenoapathy.

Keywords: Extrapulmonary tuberculosis, Haemorrhagic aspirate, Necrotic, Sputum specimen

INTRODUCTION

Tuberculosis (TB) is a leading public health problem worldwide [1]. Majority of the cases will not show active lung involvement other than tuberculous lymphadenitis. It is the most common extrapulmonary manifestation of tuberculosis [2]. The samples from extrapulmonary infection will have low bacterial count as compared with sputum specimens [3]. In peripheral adenopathy assessment, FNAC supposed to have a major role and provide a better alternative to excisional biopsy as the precise diagnosis of these enlarged lymph nodes is often difficult by history, physical examination, and radiographic studies alone [1,4].

The diagnosis of TB by culture is gold standard but it takes 4-6 weeks, hence other modalities are needed for rapid diagnosis. FNAC remains the primary diagnostic tool and in which provisional diagnosis of TB lymphadenopathy is made by presence of epithelioid granulomas and caseous necrosis. It is also necessary to rule out other causes of granulomatous inflammation and confirmation of Mycobacteria by Acid Fast Bacilli (AFB) staining, CBNAAT and culture [5]. Being a fully automated rapid molecular assay, CBNAAT is based on Real-Time Polymerase Chain Reaction (RT-PCR) that detects *Mycobacterium tuberculosis (M. tuberculosis)* along with Rifampicin (RIF) resistance simultaneously [6]. Other than conventional tests (conventional microscopy, culture or histopathology), CBNAAT is recommended in patients suspected of having EPTB by WHO in 2014 [7-9]. Aims and objectives of this study were to know the importance of CBNAAT in this modern era along with other tests like ZN stain in the diagnosis of lymphadenopathy.

MATERIALS AND METHODS

The hospital based prospective study was done in the Department of Pathology and Microbiology, ESIC Medical College, Kalaburgi, Karnataka, India, over a period of 12 months (July 2019 to June 2020) with a sample size of 119 cases. The study was done after the approval from Institutional Ethics Committee (IEC ref no. 13, ESICMCGLB/IEC/2018-19).

Inclusion criteria: The study included all clinically suspicious cases of EPTB, purulent aspirates on FNAC from the various sites between the age group of <1 year to 70 years

Exclusion criteria: Cases that were already diagnosed with CBNAAT before, recurrent, follow-up cases of EPTB were excluded from the study.

Study Procedure

Before doing FNAC, consent from all the patients and guardians in case of children were taken. FNAC specimens were collected from 119 patients by using a 23- or 25- gauge needle attached to a 5 mL syringe. Clinical features of the case and gross specimen appearance (caseous, purulent, and/or blood stained) were recorded at the time of specimen collection. Three smears were prepared

from each aspirate, two fixed with commercial cytology fixative for H&E staining and Papanicolou (PAP) staining were evaluated for adequacy and examined for the presence of epithelioid cells with or without necrosis, another slide was stained with ZN stain and observed under oil immersion to look for slender beaded bacilli. The aspirate was also tested for CBNAAT on Xpert-MTB/RIF (2010) [10]. The procedure was performed according to the manufacturer's instructions (CEPHEID, Sunnyvale, CA, USA).

According to standard operating procedure, two times the volume of sampling reagent (containing sodium hydroxide and isopropanol) was added to 1 part volume of sample and kept at room temperature for 15 minutes. After 15 minutes of incubation with intermittent shaking, 2 mL of this treated sample was filled in the cartridge and the cartridge was inserted in the module of CBNAAT machine. Polymerase chain reaction process with all the steps takes place inside the single chamber in real time with holding time of one hour and 50 minutes. The results were displayed on the monitor depending on the presence or absence of bacteria as *M. tuberculosis* is detected or not after the reaction is complete [11].

STATISTICAL ANALYSIS

Performance calculations, including test sensitivity, specificity, positive and negative predictive value done using SPSS software version 20.00.

RESULTS

In the present study, all 119 cases were subjected to FNAC and CBNAAT. Majority of the cases are in between 21-30 years age group with female preponderance [Table/Fig-1] and in the present study majority of the CBNAAT positive cases are also seen in between 21-30 years age group with female preponderance [Table/Fig-2].

In the present study, majority of the cases (73.9%) had fever as associated symptom along with lymphadenopathy followed by cough (54.6%) and weight loss (26%), respectively [Table/Fig-3].

Age group (years)	No. of cases	Male	Female
1-10	17	11	06
11-20	20	14	06
21-30	26	12	14
31-40	25	11	14
41-50	14	08	06
51-60	10	06	04
61-70	07	05	02
Total	119	67 (56.30%)	52 (43.69%)

[Table/Fig-1]: Age and sex distribution of total cases.

Age group (years)	No. of cases	Total	Male	Female
1-10	17	05	02	03
11-20	20	03	03	00
21-30	26	09	03	06
31-40	25	06	00	06
41-50	14	03	03	00
51-60	10	01	01	00
61-70	07	01	00	01
Total	119	28 (23.5%)	12 (42.85%)	16 (57.14%)

[Table/Fig-2]: Age and sex distribution of CBNAAT positive cases.
CBNAAT: Catridge based nucleic acid amplification test

Clinical features	No. of cases	Percentage
Swelling	119	100%
Fever	88	73.9%
Cough	65	54.6%
Weight loss	31	26.0%

[Table/Fig-3]: Clinical presentation of cases.

Majority of the (64.7%) cases were aspirated from cervical (jugular) lymph node swellings followed by inguinal and axillary lymph nodes. Out of these, 17 (22%) of jugular lymph nodal swellings were positive for CBNAAT followed by supraclavicular and axillary [Table/Fig-4]. Majority of the cases 79 (66.38%) were blood mixed aspirates out of which 1 (1.26%) of cases were CBNAAT positive [Table/Fig-5].

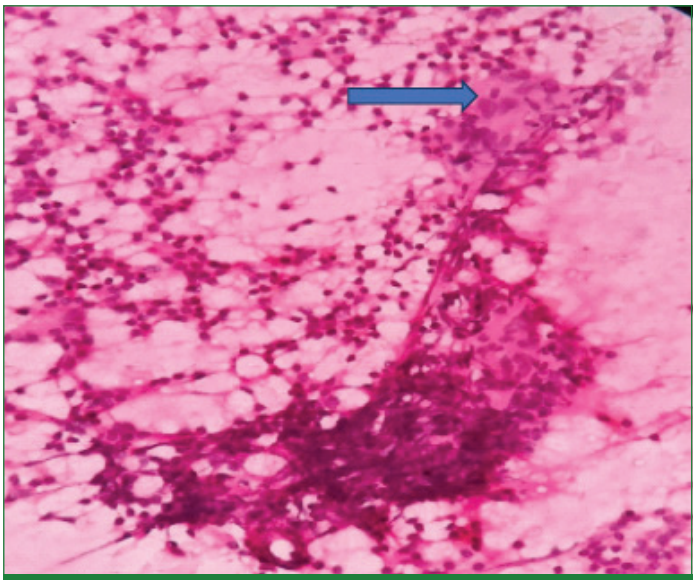
Site	Total	Percentage (%)	CBNAAT positive
Submandibular	07	5.9	02
Supraclavicular	07	5.9	04
Postauricular	05	4.2	00
Submental	02	1.6	00
Jugular	77	64.7	17
Axillary	08	6.7	04
Inguinal	13	10.9	01
Total	119	100	28

[Table/Fig-4]: Site wise distribution of total cases along with CBNAAT positivity (n=119).

Type of aspirate	Total	CBNAAT Positive	CBNAAT Negative
Purulent	22 (18.48%)	15 (68.18%)	07
Cheesy	18 (15.12%)	12 (66.66%)	06
Blood mixed	79 (66.38%)	1 (1.26%)	78
Total	119	28 (23.52%)	91

[Table/Fig-5]: Distribution of type of FNA aspirates along with CBNAAT results (n=119).
FNA: Fine needle aspiration

Out of 119 cases, cytomorphological features (FNAC) consistent with reactive lymphadenitis was noted in 58 (48.74%) of cases, granulomatous lymphadenitis were 20 (16.80%) [Table/Fig-6] suppurative lymphadenitis and necrotising lymphadenitis accounted for 14 (11.76%) [Table/Fig-7] cases each.

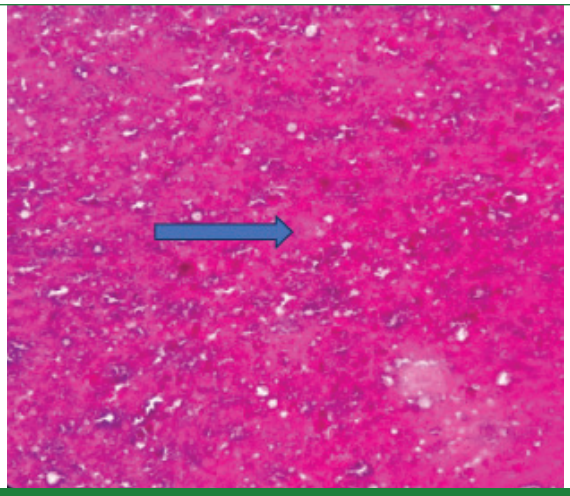


[Table/Fig-6]: Granuloma showing epithelioid giant cells and lymphocytes (H&E stain, 100X).

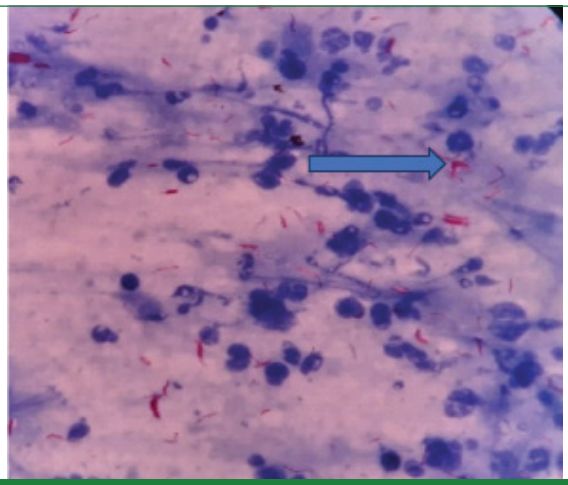
Positivity for AFB on ZN staining [Table/Fig-8] was seen in 04 (20%) cases out of 20 granulomatous lymphadenitis cases and 02 (14%) cases of 14 necrotising lymphadenitis cases [Table/Fig-9].

In 16.8% cases of granulomatous lymphadenitis on cytology, 65% of cases were CBNAAT positive. CBNAAT result of total seven cases was not correlated with FNAC. A 64.28% of suppurative lymphadenitis cases and 42.85% of cases were positive for CBNAAT [Table/Fig-9].

Out of seven cases which were FNAC+CBNAAT- six cases were blood mixed, whereas aspirates of majority of CBNAAT+ FNAC- cases were purulent [Table/Fig-10].



[Table/Fig-7]: Granular caseous material (PAP stain, 100X).



[Table/Fig-8]: (Oil immersion) ZN stain showing slender beaded bacilli.

Cytomorphological (FNA) diagnosis	Total	CBNAAT+	Not correlated with FNAC CBNAAT+/-	ZN stain for AFB
Granulomatous lymphadenitis	20 (16.80%)	13 (65%)	07	04
Suppurative lymphadenitis	14 (11.76%)	09 (64.28%)	05	00
Necrotising lymphadenitis	14 (11.76%)	06 (42.85%)	08	02
Reactive lymphadenitis	58 (48.74%)	--	--	--
Metastatic deposits	09 (7.56%)	--	--	--
Lymphoid hyperplasia	04 (3.36%)	--	--	--
Total	119	28	20	06

[Table/Fig-9]: Comparison of cytomorphological diagnosis with CBNAAT (n=119).

FNAC+ CBNAAT-	Purulent	Blood stained	Caseous
07	01	06	00
FNAC- CBNAAT+			
15	10	00	05

[Table/Fig-10]: Gross appearance of aspirates which are FNAC+ and CBNAAT- and vise versa.
*FNAC+ CBNAAT: Those cases which showed features of TB on FNAC but CBNAAT was negative for *M.tuberculosis*

CBNAAT showed 80% sensitivity when compared to FNAC and specificity of 86%. Diagnostic performance of the CBNAAT versus FNAC (28/119) showed sensitivity, specificity, positive predictive value and negative predictive value of 80%, 85.84%, 65% and 45.5%, respectively.

DISCUSSION

The present study is a hospital based prospective study in the diagnosis of suspected tubercular lymphadenopathy by CBNAAT in comparison to FNAC. In this new diagnostic era, FNAC plays invaluable role in diagnosis of diseases. FNAC being an outpatient department procedure, inexpensive and minimally invasive, it is routinely used now a days for diagnosing extrapulmonary TB [1]. This study used FNAC as primary investigation and compared it with CBNAAT to know the role of CBNAAT and also to get more rapid results.

In the present study, cervical lymph node swellings (jugular lymph node) formed majority of cases. In this study, young adults are more commonly affected whereas in other studies where younger age groups were predominantly affected with TB [Table/Fig-11] [3,8,9] and female preponderance is seen in the present study which is correlated with other studies done by Komanapalli SK et al., Rock RB et al., and Tadesse M et al., [3,8,9].

This study also compared the distribution of type of FNAC aspirate along with CBNAAT result which is not correlated with Tadesse M et al., [9], study where caseous aspirates (69%) had more CBNAAT positivity compared to present study which has 93.75% cases of purulent aspirates with CBNAAT positivity which is in correlation with Komanapalli SK et al., [3].

In the present study, 13 cases out of 20 non correlated cases were CBNAAT positive. Among them, majority (eight) were caseous grossly which turned necrotising lymphadenitis on FNAC and in one case had blood mixed aspirate. Hence, the sensitivity for blood stained aspirate was 1 (7.7%) out of 13 with CBNAAT.

Therefore, in this study, the importance of CBNAAT lies in detecting the above mentioned 13 TB patients which were cytologically negative for TB, who were surely benefitted by the CBNAAT. Out of seven (FNAC+CBNAAT-cases), all cases were blood mixed. It is possible that in these cases representative sample might not be obtained as bacterial load may have been too low for the GeneXpert to detect the Deoxyribo-nucleic Acid (DNA) from *Mycobacterium Tuberculosis* (MTB)- complex [12].

On comparison of CBNAAT diagnostic performance of present study (sensitivity 80%, specificity 86%) (CRS) with Aruna L et al., (sensitivity-65%, specificity 92.45%), Komanapalli SK et al., (sensitivity 84.25%, specificity 86.71%) Patil SB et al., (sensitivity 55.5%, specificity 83.80%) and Kumar A et al., (sensitivity-92.7%, specificity 98.9%) showed less sensitivity and more specificity [Table/Fig-12] [2,3,13,14].

Therefore, FNAC should be included as first line investigation and it should be compared with CBNAAT to get more accurate results and to decrease the chances of missing cases by CBNAAT alone or FNAC alone.

Study	Year of study	Place of study	Age group (years)	Female	Male	Percentage of CBNAAT positivity
Komanapalli SK et al., [3]	April 2017-March 2018	Visakhapatnam, Andra Pradesh	11-30	23.8%	76.2%	58%
Rock RB et al., [8]	Jan1993-Dec 2003	Minnesota	15-24	46%	54%	43%
Tadesse M et al., [9]	May-Sept 2013	Jimma, Belgium	16-30	67%	76%	58%
Present study	July 2019-June 2020	Kalaburgi, Karnataka	21-30	42.85%	57.14%	21.84%

[Table/Fig-11]: Comparison of age and sex wise distribution of CBNAAT positive cases with other studies (n=119).

Study	Year of study	Place of study	Sensitivity (%)	Specificity (%)
Present study	July 2019-June 2020	Kalaburgi, Karnataka	80	86
Aruna L et al., [2]	July 2017-July 2018	Warangal, Telangala	65	92.45
Komanapalli SK et al., [3]	April 2017-March 2018	Visakapatnam, Andrapradesh	84.25	86.71
Patil SB et al., [13]	Jan 2019-Dec 2019	Akola, Maharastra	55.5	83.80
Kumar A et al., [14]	July 2016-July 2017	Kolkata	92.7	98.9

[Table/Fig-12]: Comparison of sensitivity and specificity with other studies.

Limitation(s)

Follow-up of patients could not be done in the present setting.

CONCLUSION(S)

CBNAAT detected 12.6% of cases which were not detected by FNAC. It was found less sensitive for blood stained samples than purulent samples. CBNAAT is more specific than FNAC for detection of extrapulmonary TB. FNAC is cost effective in the diagnosis of EPTB (suspected tubercular lymphadenopathy) but combining it with CBNAAT can add advantage of detection of FNAC missed cases and it is a rapid test available with high specificity.

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