Diagnosis of Tubercular Lymphadenitis by Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and its Correlation with Ziehl-Neelsen Stain on Fine Needle Aspiration Cytology at KIMS Koppal

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Abstract

Background: According to WHO 2020 India (27%) is one amongst three countries which share the largest global TB burden. The diagnosis of lymph node tuberculosis is challenging by conventional microbiological tools like ZN stain due to its pauci-bacillary nature in lymph node aspirates. Recently, WHO recommends application of CBNAAT to be used as the initial diagnostic tool in all presumptive cases of tubercular lymphadenitis. CBNAAT is a fully-automated diagnostic molecular test which simultaneously detects Mycobacterium tuberculosis (TB) and rifampicin (RIF) drug resistance. The purpose of this study, To know the utility of CBNAAT test in presumptive tubercular lymphadenitis, To know the rifampicin resistance in Tubercular lymphadenitis, Comparing the results of CBNAAT test with routine cytology and ZN stain for AFB in lymph node aspirates. **Material and Methods:** This study is a retrospective study of 3 yrs (January 2018 to December 2020) duration at the cytology section of department of Pathology Koppal Institute of Medical Sciences, koppal (KIMS). Lymph node aspirations were processed simultaneously for routine cytological stains, ZN stain along with CBNAAT. All presumptive cases of TB lymphadenitis of all age groups were included in the study.

Resuts: 360 samples which were of presumptive tubercular lymphadenitis were subjected to FNAC, ZN stain and CBNAAT. Out of 360 cases, 180 cases (50%) were showing granulomatous inflammation cytologically and are suggestive of tuberculosis, amongst 180 cases 102 cases (56.66%) were AFB positive on ZN-stained smears and in 159 (88.33%) were confirmed M. TB by CBNAAT. Majority of the cases were in the 11-20 years age.

Conclusion: This study enightens widespread use of CBNAAT in early diagnosis of tuberculosis within 2 hours and accurately. CBNAAT detects pulmonary TB in people living with HIV with greater efficacy and it also detects rifampicin resistance also complement usual methods of conventional microscopy, culture, cytology and histopathology.
Keywords: FNAC, Lymphadenopathy, CBNAAT, Tuberculosis, Zn Stain.

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Introduction

According to the Global Tuberculosis report of 2014 of World Health Organization (WHO), Tuberculosis (TB) remains one of the world's deadliest communicable diseases which is caused by the Bacterium Mycobacterium tuberculosis (MTB).[1] Tuberculosis is a leading cause of morbidity and mortality in India with an incidence of 2.74 million including 0.13 million drug resistant cases. There were 0.41 deaths in India in 2017.[1]

The most common extrapulmonary sites are lymph nodes followed by pleural effusions and other sites.[1] Because of paucibacillary nature of lymph node aspirations, sensitivity rate of

ZN stain preparation for AFB is low.[2] According to the 2019 global report, a total of 4,77, 461 TB cases among people living with HIV were reported and TB is the leading cause of death among people living with HIV.[3] Standard sputum-based methods to detect pulmonary tuberculosis include sputum microscopy and culture. Because of the very long incubation period of M TB of four to eight weeks and also because of lack of caseous necrosis in sputum, it reduces sensitivity and specificity of sputum microscopy as a diagnostic tool. To overcome all these hurdles attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity, CBNAAT has been shown to be rapid, with a result for TB and RIF drug resistance under 2 h.[4] It was introduced in INDIA by the Revised National Tuberculosis Control Programme (RNTCP) in 2012 as a pilot project in Maharashtra.[5] In 2014, WHO also recommended its use in non-respiratory specimens from patients with extra pulmonary tuberculosis.[6]

Material and Methods
Study Design: Retrospective study.
Study participants: All presumptive cases of tubercular lymphadenitis.
Age group: This study includes all age groups.
Study Duration: This retrospective study was conducted for 36 months from January 2018 to December 2020 at the Cytology section of the Department of Pathology, at KOPPAL, Institute of Medical Sciences, Koppal Karnataka.
Sample size: 360 cases.
Procedure: FNA was done with the consent of the patient/guardian in children under aseptic precautions and material sent to CBNAAT.
Sample Collection: Samples collected from FNAC of presumptive tubercular lymphadenitis at cytology section of dept of pathology and processed for routine cyto stains, ZN staining and for CBNAAT.

Inclusion criteria:
All presumptive cases of tubercular lymphadenitis.

Exclusion criteria:
Head and Neck cancers and secondaries.

Results
All 360 samples which were of presumptive tubercular lymphadenitis were subjected to FNAC, ZN stain and CBNAAT. Out of 360 cases, 180 cases (50%) were showing granulomatous inflammation cytologically and are suggestive of tuberculosis, amongst 180 cases 102 cases (56.66%) were AFB positive on ZN-stained smears and in 159 (88.33%) were confirmed M. TB by CBNAAT. Majority of the cases were in the 11-20 years age group followed by 0-10 years and 21-30 years (Table1), with male preponderance [Table 4] and in the present study Majority of the CBNAAT positive cases are also seen in between 11-30 years age group [Table 1].

Table 1: Age wise distribution of cases of TB lymphadenitis by cytologically.

S I No	Age	Number
1	0-10yrs	33(18.3%)
2	11-20yrs	51(28.3%)
3	21-30yrs	33(18.3%)
4	31-40yrs	18(08%)
5	41-50yrs	30(08%)

6	51-60yrs	15(08%)
7	>60 yrs	15(08%)
Total		180

Table 2: Sex distribution of TB lymphadenitis

	Number of cases	Percentage
Male	102/180	56.66%
Female	78/180	43.33%

Table 3: Microscopic diagnosis of lymphadenopathies

S I No	Diagnosis	
1	Caseating necrotising lymphadenopathy	90(25%)
3	Granulomatous lymphadenitis	60(16.67%)
4	Cold abscess	30(8.34%)
5	Others	180(50%)
	Total	360

Microscopic diagnosis amongst presumptive TB cases reveals 50% of cases diagnosed as TB by cytology.

Table 4: Comparison of cytomorphological diagnosis with CBNAAT (n=360).

Cytomorphological FNAC diagnosis	Total	CBNAAT + ve cases
Tuberculosis	180(50%)	159
Abscess	24(6.6%)	-
Others	156 (43.3%)	-
Total	360	159

[Table 4] Shows number of cytomorphological diagnosed tubercular cases which were then confirmed by CBNAAT amongst 360 presumptive TB cases.

Table 5: Group of Lymph nodes distribution

Site	Number of cases	CBNAAT +ve cases
Cervical	300	141/300
Submandibular	27	14/27
Axillary	26	7/26
Inguinal	7	0/7
Total	360	162

Amongst 360 presumptive TB cases, involvement of the cervical group of lymph nodes were highest followed by submandibular, axillary, inguinal group of lymph nodes.

Table 6: Distribution of FNA aspirates which showed TB +ve along with CBNAAT results (n=180)

Type of aspirate	Total number of CBNAAT cases	CBNAAT Positive cases
Purulent	140	124 (51.2%)
Thick gray(Cheesy)	40	35(26.7%)
Total	180	159

Table 7: Table revealing TB amongst People Living With HIV and their correlation with CBNAAT.

Total no of ART cases	CBNAAT +ve cases
48	30

Out of 180 cases of TB 48 were People Living With HIV, out of which 30 cases showed sensitivity to CBNAAT.

Table 8: Cycle threshold value for CBNAAT positive cases

S I No		CBNAAT "+ve"			
Ct values	Very low	Low	Medium	High	Error
No of cases	90	40	24	13	13

Ct values of CBNAAT cases, highest number Tb lymphadenitis 50% cases showed highest sensitivity to very low.

Table 9: Correlation of cases of lymph node aspiration for both CBNAAT and AFB.

S I no	Total number of cases	Percentage
Tuberculosis	180/360	50%
CBNAAT	159/180 (RS-130, RR-29)	88.34%
AFB	102/180(RS-90, RR-12)	56.68%

Out of 360 lymph node aspirations 180 cases showed TB, Out of 180 TB cases, 159 cases were CBNAAT positive and 102 cases were AFB positive.

This comparison illustrates that CBNAAT is a faster and good diagnostic tool with high sensitivity and specificity to diagnose tubercular lymphadenopathy than the conventional ZN staining and microscopy.

Although there is no previous study available, the present data points to an alarmingly high prevalence of TB lymph nodes at KOPPAL.

Discussion

Worldwide, Tuberculosis is one of the top ten causes of death and the leading cause from a single infectious agent. According to WHO As India (27%) is one amongst three countries which share largest global TB burden,[7] there are nearly 10 million new cases and 4 million deaths from tuberculosis globally in 2019,[7] TB caused an estimated 1.2 million deaths among HIV-negative people and there were an additional 208000 deaths in people living with HIV people.[7] Men (≥15 yrs) accounted for 56%, women 32%, children(aged< 15 yrs) for 12%. Among all those affected, 8.2% were living with HIV.[7] Tuberculosis can involve any organ system in the body.[8,9] Number of studies have demonstrated the utility of CBNAAT in diagnosis of extrapulmonary tuberculosis.[10-13]

In India there are only a few studies on the utility of CBNAAT in extrapulmonary Tb. A study done in 2011 in Hyderabad showed incremental case detection of 10.8% when CBNAAT was used to diagnose tuberculosis over and above fluorescent microscopy.[14] A multicentre assessment at five trial sites in Peru, Azerbaijan, South Africa, Durban and India by Boehme et al demonstrated sensitivity of nearly 100% by CBNAAT.[15]

Under National Tuberculosis Elimination Programme (NTEP) which was previously known as RNTCP, the impact of CBNAAT in diagnosis of pulmonary TB additional 2,493 patients were diagnosed and amongst 30,000 presumptive pulmonary TB detected when compared to sputum microscopy.[16,17]

In this retrospective study, a total of 360 patients with presumptive TB lymphadenitis were included, 180 cases were diagnosed as TB lymphadenitis with male: female ratio of 1.3:1.

Most (28.34%) of the patients having TB were in the second decade (51cases) followed by the 1st (33cases) and 3rd (33 cases) decades. Right cervical group of lymph nodes was the most common site of involvement followed by the left cervical group of lymph nodes. Most of the patients had only one site of involvement. Among all presenting constitutional symptoms, loss of weight 33 (75%) and loss of appetite 30 (68.2) were the common.[18]

Table 10: Comparison of results obtained in our study with various other similar studies.

	Present study		Age	Authors	
Common Age group at presentation	11-20 yrs		30-40yrs	R deewan et al,[5]	
			11-30yrs	Komanapalli sk et al,[19]	
			15-24yrs	Yaseen et al,[20]	
			15-24yrs	Arorvek et al,[21]	
			15-24yrs	Bryan et al,[22]	
			16-30yrs	Mulualem et al,[23]	
Male:Female ratio	Males	Females	Males	Females	Authors
	56.66%	43.33%	67%	33%	manju kumari et al,[24]
			23%	26.2%	Komanapalli et al,[19]
			46%	54%	Brain et al,[22]
			67%	76%	Mulualem et al,[23]
			31%	69%	Pooja Singh et al,[25]
Commonly affected group of lymph node	Cervical(83.34%)		Cervical (94.1%) by komanapalli sk et al,[19]		
People living with HIV	25%		2% young et al,[17] 10.66% Nikesh agarwal et al,[3]		
Rif resistance In TB lymphadenitis	18.23%		1)19.51% 2)13.55% 3)25% 4)53% 5) 6.38%	Manju kaumari et al,[24] D pragati rao et al,[26] R deewan et al,[5] R Tripathi et al,[27] Gour Sanjay et al,[25]	
Cycle threshold values of CBNAAT +ve cases	Very Low(90)>low(40) >medium(24)>high(13)		Very Low(24)>low(11)> Medium(4)>high(2)	Manju kumari et al,[24]	
			Very Low (7)>low(19)> Medium(17)>high(6)	Anish kumar P et al,[8]	
			Very Low(87)>low(40)> Medium(11)>high(4)	Komanapalli et al,[19]	

Conclusion

By doing this study we conclude that widespread use of CBNAAT enhances early diagnosis of tuberculosis within 2 hours and accurately, which aids in early treatment which helps in declining transmission rate and case fatality rates and increases survival rate. CBNAAT detects pulmonary TB in people living with HIV with greater efficacy and it also detects rifampicin resistance and can be used for screening of mdr-tb so that early therapy can be started, thus decreasing the incidence of mdr-tb. This rapid TB diagnostic test may complement usual methods of conventional microscopy, culture, cytology and histopathology.

As tuberculosis is endemic at Koppal district of north Karnataka, CBNAAT provides a robust and a promising role in early diagnosis of TB, its high specificity and less time-consuming procedure makes it an excellent tool for timely diagnosis of such cases.

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