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RESEARCH ARTICLE

DIAGNOSIS OF TUBERCULAR LYMPHADENOPATHY BY CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST(CBNAAT)AND ITS CORRELATION WITH FINE NEEDLE ASPIRATION CYTOLOGY: AN INSTITUTION-BASED STUDY

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Abstract

Introduction: The highest burden of tuberculosis is seen in India, accounting for 21% incidence globally and a major cause of lymphadenopathy. Fine-needle aspiration cytology with Ziehl-Neelsen staining is routinely used as the diagnostic modalityfor testing Extra Pulmonary Tuberculosis. Although a presumptive diagnosis can be made easily, but due to low sensitivity, many a times definitive diagnosis is difficult. For overcoming these limitations a rapid and reliable Cartridge Based Nucleic Acid Amplification Test method by WHO (2010) has been endorsed as an initial diagnostic tool in laboratories.

Aims and Objectives: To assess the efficacy of CBNAAT in the diagnosis of tubercular lymphadenopathy and to compare it with cytological features on FNA and ZN stain.

Materials and methods: This is a one-year study done on 111 clinically suspected cases of EPTB with lymphadenopathy in the Department of Pathology. Fine needle aspirates of the lymphnodes were subjected to CBNAAT. Cytological features and ZN stain of the aspirate were compared with CBNAAT results.

Results: Of the 111 suspected cases of EPTB, 32(28.8%) were positive on ZN staining whereas 65 (58.5%) were positive by CBNAAT. Of these 65 CBNAAT positive cases, cytology revealed epithelioid granulomas with necrosis in 37 (56.9%), granulomas without necrosis in 22 (33.8%)and scattered epithelioid cells in 6 (9.3%) cases respectively.

Conclusion: Compared to FNAC and Z-N staining, CBNAAT is an effective, sensitive and rapid diagnostic tool for diagnosis of EPTB and hence suggested to be done in all suspected cases of EPTB. CBNAAT provides an extra edge in management of those undiagnosed cases which are of global concern.

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Introduction:-

India being one of the world's largest tuberculosis (TB) burdened country, which accounts for around 21% of the TB incidence globally.[1]Pulmonary involvement although is the most common presentation, but can potentially affect

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any organ or system of the body.[1]According to WHO classification criteria, Extra Pulmonary Tuberculosis (EPTB) is definedas an infection caused by M. tuberculosis which affects tissues and organs outside the pulmonary parenchyma e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints, bones and meninges.[2]. EPTB accounts for about 25% of TB cases.[3]

Diagnosis of EPTB is complex, as the number of Mycobacterium Tuberculosis Bacilli (MTB) present at the suspected clinical site is often low and also it is difficult to obtain clinical material from deep seated lymphnodes.[4] In India and other developing countries, tubercular lymphadenitis continues to be the most common form of EPTB and non-tuberculous mycobacteria causing lymphadenitis is seen rarely.[5]

Fine needle aspiration cytology (FNAC) has been available for nearly past two decades and serves as the first-line diagnostic technique even now in superficial tubercular lymphadenopathy, with sensitivity and specificity being 79% and 94% respectively.[4]FNAC and Z-N staining is an initial diagnostic tool in resource poor countries. It is a rapid diagnostic technique but has very low sensitivity due to paucicellular nature. Although a presumptive diagnosis can be made easily, but due to low sensitivity, many a times definitive diagnosis is not possible.[3]Mycobacterial culture and drug susceptibility testing has a turnaround time of four to eight weeks which is very high.[6]

To overcome all these limitations WHO has endorsed a rapid and reliable method Cartridge Based Nucleic Acid Amplification Test (CBNAAT)/GeneXpert MTB/RIF1 (Cepheid, USA).[6]CBNAAT is comparatively new, fully automated, real-time hemi-nested polymerase chain reaction (PCR) system that is a most sensitive, rapid and cost-effective test for the diagnosis of TB in paucibacillary samples, recommended by WHO.[3]It also helps in obtaining thediagnosis of TB within 2 hours.[6]

This study is intended for the rapid diagnosis of Mycobacterium tuberculosis in clinically suspected cases of lymph node tuberculosis by CBNAAT and comparing it with conventional methods like lymphnode cytology and ZN staining.

Materials and Methods:-

This study done was done in the Department of Pathology, Gulbarga Institute of Medical Sciences, Kalaburagi, from October 2022 to September 2023. Total of 111 cases of lymphadenopathy were studied.

Inclusion Criteria:

All clinically suspected cases of tubercular lymphadenopathy during study period.

Exclusion Criteria:

1. Patients on Anti Tubercular Therapy (ATT) for more than one month are excluded as they were already diagnosed, and due to low bacterial count probably CBNAAT will give false negative results.
2. Patients who had refused to give consent for both tests.

Methodology:-

Consent was obtained from the patients and demographic details and site of lymphadenopathy were documented. Fine needle aspiration was done with the help of 22 to 23 G needle attached to a 10 ml syringe. Air dried smears were subjected to Giemsa stain for conventional microscopy and one smear was used for Z-N staining for Acid Fast Bacilli (AFB). Rest of the sample was subjected to CBNAAT using Xpert MTB/RIF (Cepheid, Dx System Version 5.1c). The sample was processed as per the recommendations of operator's manual provided. The sample was mixed with sample reagent (Sodium hydroxide and Isopropranol) in 1:2 ratio in a pre-sterilized container and incubated at room temperature for 30min. Using a Pasteur pipette,two ml of this reagent sample mixture was then transferred to an Xpert cartridge and the cartridge was loaded onto Gene Xpert machine.

Descriptive fine needle aspiration cytological features on Giemsa-stained smears were recorded. ZN stain of the smear was reported as negative or positive as per RNTCP guidelines published by Central TB Division, DGHS, MoHFW, New Delhi. CBNAAT results were interpreted as negative or positive. Positive results provide a semi quantitative estimate of the concentration of bacilli defined by the cycle threshold (Ct) range and are graded as (high, <16; medium, 16-22; low, 22-28; very low, >28).

The results of FNAC, AFB and CBNAAT were compared and correlated. Sensitivity, specificity, positive predictive value and negative predictive values of FNAC, CBNAAT and AFB positivity were calculated.

Results:-

A total of 111 clinically suspected cases of EPTB were included in the study. 52(46.9%) were males and 59(53.1%) were female.

Table 1:- Age distribution in suspected EPTB patients.

Age group (in years)	Suspected EPTB	Percentage
0-10	16	14.4%
11-20	30	27%
21-30	30	27%
31-40	13	11.7%
41-50	13	11.7%
51-60	05	0.04%
61-70	02	0.01%
71-80	02	0.01%
Total	111	100%

Majority of the patients were in the age group of 11-20 yrs and 21-30 yrs which included 30(27%) each, where as those between 0-10 yrs are 16(14.4%) and in between 31-40 and 41-50 yrs are 13(11.7%) each, between 51-60 yrs were 5(0.04%),61-70 and 71-80 were 2(0.01%) each.

Table 2:- Sites of Fine-Needle Aspiration.

SITE	NO (%)
Right cervical	41(36.9%)
Left cervical	33(29.7%)
Right supraclavicular	10(9%)
Right posterior triangle	7(6.3%)
Left supraclavicular	5(4.5%)
Right submandibular	4(3.6%)
Left axillary	4(3.6%)
Left posterior triangle	3(2.7%)
Left submandibular	2(1.8%)
Right axillary	2(1.8%)
TOTAL	111(100%)

Right cervical region lymphadenopathy was the most common site of aspiration accounting for 41(36.9%), followed by left cervical 33(29.7%). Only 2 cases (1.8%) each of left submandibular and right axillary lymphnodes were seen.

Table 3:- Distribution of the total cases and their cytology findings.

Cytology findings	No of cases (%)
Granulomas with necrosis	45(40.6%)
Granulomas without necrosis	34(30.6%)
Reactive lymphadenitis	20(18%)
Scattered epithelioid cells	06(5.4%)
Acute suppurative lesion (Abscess)	04(3.6%)
Atypical lymphoid proliferation	01(0.9%)
Metastasis	01(0.9%)
Total	111 (100%)

On cytology, granulomas with necrosis were seen in 45 (40.6%) cases, followed by granulomas without necrosis in 34 (30.6%), Reactive lymphadenitis in 20(18%), Scattered epithelioid cells 06(5.4%) and Acute suppurative lesion (Abscess) in 04(3.6%) cases. Atypical lymphoid proliferation and metastasis was noted in 01 (0.9%) case each.

Out of the 111 cases 32 were positive for AFB and 79 were negative. Of the 32 AFB positive cases, 30 (93.75%) showed granulomas with necrosis and 02 (06.25%) cases showed only granulomas on cytology.

CBNAAT positivity was seen in 65 cases (58.5%). Majority of the positive CBNAAT cases showed granulomas with necrosis (37), followed by granulomas without necrosis (22) and 6 cases showed scattered epithelioid cells in the lymphnode aspirate.

Table 4:- Comparison of Cytology with ZN Staining and CBNAAT results.

Cytology findings	**No of cases (%)**	**AFB**		**CBNAAT**	
		+	-	+	-
Granulomas with necrosis	45(40.6%)	30	15	37	08
Granulomas without necrosis	34(30.6%)	02	32	22	12
Reactive lymphadenitis	20(18%)	-	20	-	20
Scattered epithelioid cells	06(5.4%)	-	06	06	-
Acute suppurative lesion (Abscess)	04(3.6%)	-	04	-	04
Atypical lymphoid proliferation	01(0.9%)	-	01	-	01
Metastasis	01(0.9%)	-	01	-	01
Total	111 (100%)	32	79	65	46

Table 5:- Comparison of ZN staining with CBNAAT findings.

ZN Stain	**CBNAAT**		**TOTAL**
	POSITIVE	**NEGATIVE**	
POSITIVE	27	05	32
NEGATIVE	38	41	79
TOTAL	65	46	111

Out of 111 cases from suspected EPTB patients, 32 showed AFB in ZN stain and 65 were positive in CBNAAT. In all, 46 samples of suspected EPTB patients were negative in CBNAAT, and in 05 cases AFB was detected in these samples.

The sensitivity of CBNAAT with ZN stain was only 41.5%, whereas specificity was 89%. Positive predictive value is 84.3%, Negative predictive value is 51.8%. Diagnostic accuracy of CBNAAT over ZN staining was 61.2% in the diagnosis of EPTB.

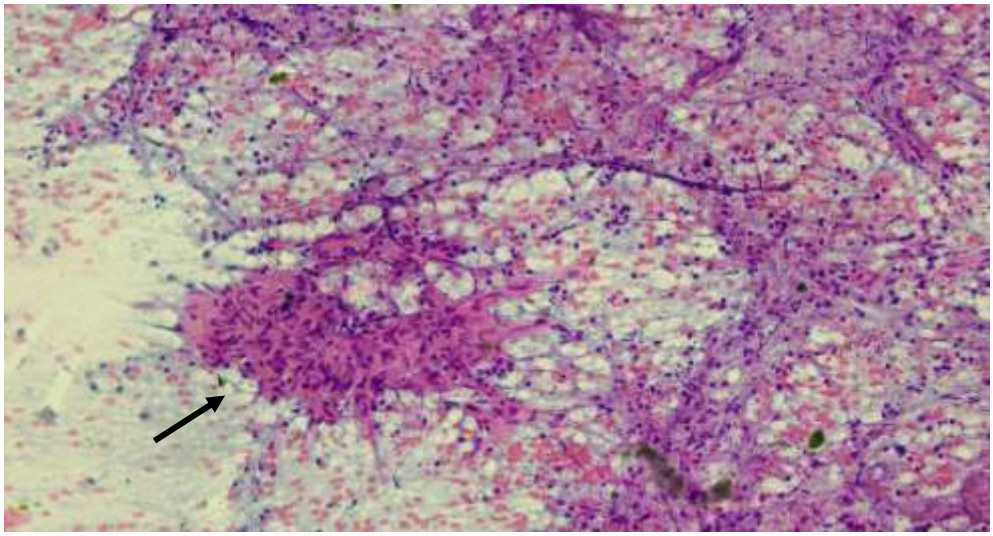

Figure 1:- Well formed Granuloma with epithelioid cells (arrow). Pap stain (20x).

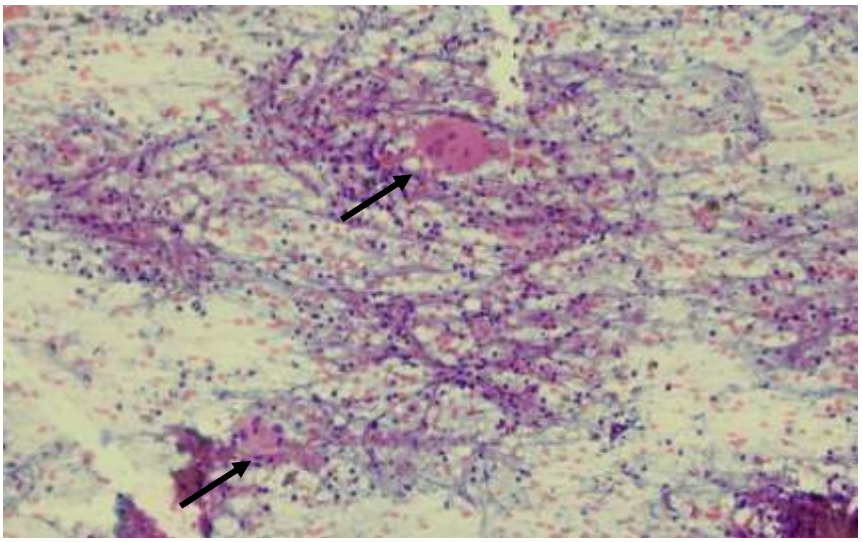

Figure 2:- Giant cells(arrow). Pap stain (20x).

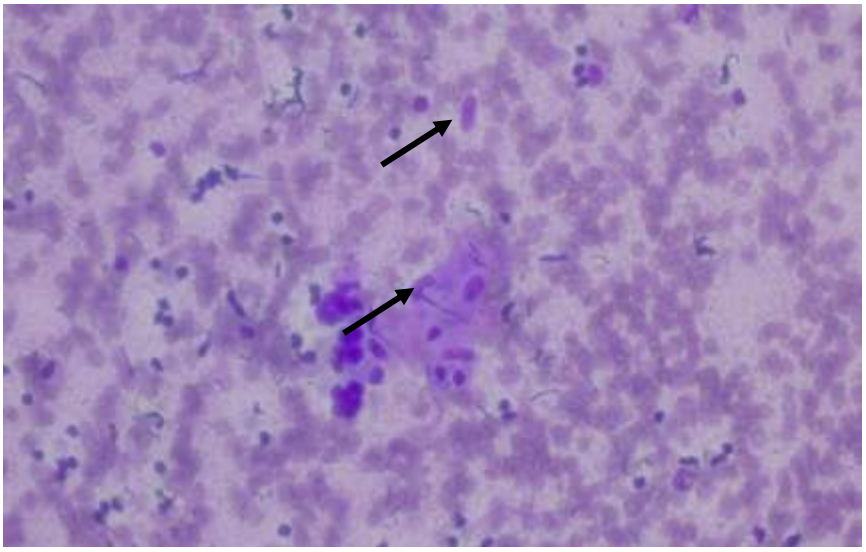

Figure 3:- Scattered epithelioid cells(arrow). Giemsa stain (20x).

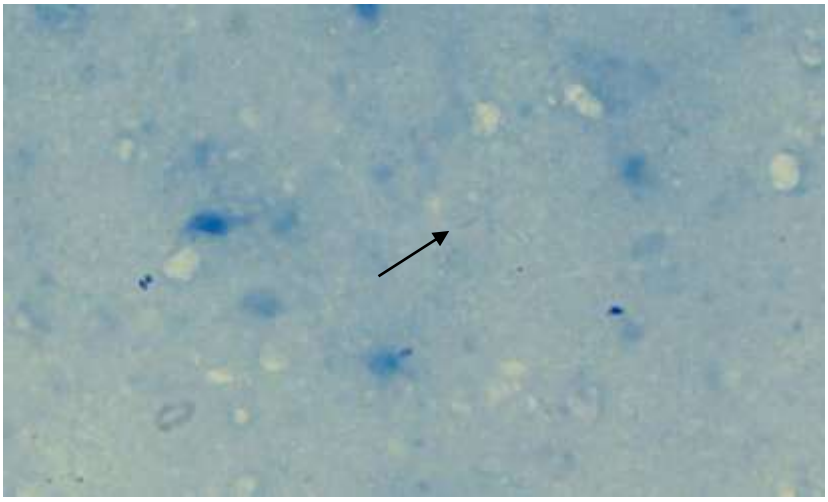

Figure 4:- Acid Fast Bacilli(arrow). ZN stain (100x).

Discussion:-

Extrapulmonary tuberculosis accounts for major burden in terms of mortality and morbidity due to its subclinical presentation, paucicellularity and difficulties in diagnosis.[6]Lymphadenopathy is the most common presentation of head and neck tuberculosis. FNAC and AFB positivity are the investigations of choice that are preferred for diagnosis. The criteria for diagnosis of tubercular lymphadenitis on cytology are the presence of granulomas with caseous necrosis and demonstration of AFB positivity on ZN staining.[7,8,9]However Mycobacterium tuberculi arefound rarely in granulomatous lymph nodes. The conventional microscopy, ZN staining have low sensitivity and culture is time consuming, resulting in treatment delay of these cases.To address these existing limitations, CBNAAT was developed. This innovative diagnostic tool identifies targeted rpoB (RNA polymerase β) nucleic acid sequences, providing results in approximately 2 hours. Key benefits of this are high specificity due to five unique molecular probes and three specific primers targeting the rpoB gene for MTB. No cross-reaction with other bacterial species.Fully automated PCR-based testing with directDNA detection and also simultaneous detection of rifampicin resistancefrom clinical specimens.[10]

CBNAAT provides an early diagnosis along with rifampicin sensitivity which is very helpful for patient management owing to its rapidity, sensitivity and specificity.[6]

In our study, the younger age group was affected more with female predominance, which correlated with other studies like Komanapalli SK et al.[1], Yassin et al.[11], Aroravk et al.[12], Brayn et al.[13] However few studies by Ali et al.[14] and Natrajet al.[15] showed higher number of male patients in their studies. The incidence of TB was found to be higher in females than males in our study. There may be many reasons for higher prevalence of TB in females. Firstly, females in rural areas are malnourished and have a low immunity, thus are vulnerable to infectious diseases like TB. Also, females remain restricted to indoors which are not properly ventilated and they are not exposed to fresh air, helping the TB bacilli to grow. Overcrowding, poor sanitation, and poor personal hygiene in females also owe to be the contributing factors. The present study showed cervical lymph node to be the most commonly involved (66.6%), which is similar to the study done by Adhikaryet al.[3],Vimal Set al.[16].

Majority of our cases 45(40.6%), 34(30.6%) showed granulomas with necrosis and granulomas without necrosis respectively which are similar to studies done by Adhikary et al.[3], Gouda et al.[17].

Out of 111 patients 65 cases were positive for tuberculosis by CBNAAT out of which only 27 detected AFB by ZN staining. This shows that CBNAAT is superior to conventional FNAC and ZN staining for diagnosis of tubercular lymphadenopathy easily detected by CBNAAT in these cases.
The present study showed 58.55% CBNAAT positivity which correlated with the studies done by Moure et al.[18](58.3%), Srwar et al.[19](51.7%), Anmol et al.[20](62.7%).

The sensitivity of CBNAAT with ZN stain was only 41.5%, whereas specificity was 89% in our study. But other studies, Lavanya et al. found CBNAAT has sensitivity of 83.3% and specificity of 84.8% in diagnosing EPTB over ZN staining.[21]Patil et al. compared diagnostic performance of CBNAAT versus ZN stain and found sensitivity 84.04% and specificity 80.57%[22]which have higher sensitivity and with nearly same specificity, which might be due to few reasons of false-negative results on CBNAAT, that decrease its sensitivity. Improper collection and transport of samples is one of the important causes of false negative result of CBNAAT.[23] Rarely, PCR inhibitors and other endogenous or exogenous host proteins may also interfere and lead to false negative results.[24] Another cause of false negative CBNAAT is the solid nature of cheesy material, which may have very low bacillary load compared to liquid caseous material with high bacillary load.[25] Therefore, CBNAAT should be repeated with fresh sample if there is high clinical suspicion of EPTB to exclude false negative results. Very rarely, contamination and presence of dead Mycobacterium tuberculosis bacilli in the samples can give false positive CBNAAT report.[24].Despite its limitations, CBNAAT emerges as a promising first-line diagnostic approach for lymphadenopathy cases, offering a valuable initial investigation tool.

Conclusion:-

CBNAAT is a highly effective, sensitive, and rapid diagnostic tool for extrapulmonary tuberculosis, outperforming FNACand Z-N staining. Its superior diagnostic capability enables early detection of smear-negative and multi-drug resistant tuberculosis cases, addressing a significant global health concern. Given its advantages, CBNAAT should be considered as the initial diagnostic tool for suspected extrapulmonary tuberculosis cases.

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