COMPARATIVE ANALYSIS OF CYTOMORPHOLOGY PATTERNS AND AFB POSITIVITY OF LYMPH NODE ASPIRATE VS CBNAAT FOR DETECTION OF TUBERCULAR LYMPHADENOPATHY

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ABSTRACT

Background: TB remains a major health concern world wide with extrapulmonary TB accounting for 20% cases in India. Tubercular lymphadenopathy is most common extrapulmonary manifestation in TB. **Aim:** To compare and analyse cytomorphology patterns, AFB positivity and CBNAAT for detection of tubercular lymphadenopathy **Methodology:** a total of 80 FNAC samples from lymph nodes were taken, one part was sent for CBNAAT and other part was used to prepare slides and stained with H&E, PAP, MGG, ZN stain for AFB. **Results:** Out of 80 cases, 52 (65%) were granulomatous, 14 (17.5%) each were of reactive and suppurative. Among these 52 cases reported as granulomatous 32 were positive for CBNAAT, 11 AFB positive. Only 1 case out of 14 reactive cases was positive for CBNAAT. Out of 14 suppurative cases, 4 cases were CBNAAT positive. **Conclusion:** CBNAAT is a rapid test, can detect even if bacilli is less in number but FNAC along with AFB is simple procedure in resource limited area.

KEYWORDS

CBNAAT, FNAC, AFB

INTRODUCTION

Lymphadenopathy often occurs in the head and neck or inguinal areas and usually noted in clinical practice. Enlarged lymph nodes could be due to infection, metastatic malignancy or lymphoma.¹ Tuberculous lymphadenitis is the most common Extrapulmonary (EP) manifestation of TB and the majority of cases have no active lung involvement.^{2,3}

Tuberculosis (TB) is an important health problem in low and middle income countries and remains a key challenge to public health and has an estimated global annual incidence of 9.6 million cases in India according to WHO annual report.^{4,5} Our ability to tackle this disease has been severely hampered by inadequate diagnostic assays⁴. No single diagnostic test is satisfying to all the demand of rapid, affordable and easy confirmatory test.

FNAC offers a feasible and safe option for specimen collection. Fine Needle Aspiration Cytology (FNAC) is an economical and reliable first line investigation in lymphadenopathy. FNAC is now considered as a valuable diagnostic aid as it gives early results, simple procedure, minimal trauma and complications.³

Culture can also be done but it is also time consuming. Histology is time-consuming and remains difficult to undertake and establish the diagnosis of TB with high specificity.⁶

The use of cytology together with the confirmation of acid fastness by Ziehl-Neelsen (ZN) staining and Papanicolaou stain-induced fluorescence microscopy as well as mycobacterial detection by culture offers excellent yields^{7,8} but remains limited by the absence of species confirmation, slow turnaround times, and/or lack of drug resistance guidance.

Several Nucleic Acid Amplification Technology (NAAT), tests have been developed that rapidly detect M. tuberculosis DNA in patient samples and look for DNA changes that make M. tuberculosis drug-resistant.⁹

Cartridge Based Nucleic Acid Amplification test (CB-NAAT), is an automated DNA test that detects M. tuberculosis and rifampicin resistance (an indicator of MDR-TB) within two hours for the investigation of patients who might have TB³.

In this study we compared the results of ZN stained smears obtained by FNAC of lymph nodes and CBNAAT for the diagnosis of tubercular lymphadenopathy.

MATERIAL & METHODS

Study design- prospective, observational study

Duration of study- 1 year (September 2018- September 2019)

Sample size- 80

Study area- patients with clinical suspicion of TB visiting OPD of various dept of Dr Bhim Rao Ambedkar hospital and Pt. J.N.M Medical college Raipur.

Inclusion criteria:

Enlarged and palpable lymph nodes with clinically presumptive TB sent for FNAC in dept of pathology, referred from any dept of Bhim Rao Ambedkar Memorial Hospital.

Exclusion Criteria:

Cases already taking treatment for TB, active or suspected malignancy, reactive lymphadenitis, terminal disease with poor prognosis, non-cooperative patients, patients not willing to give consent for study

The skin of the patient, over the suspected lymph node to be aspirated was cleaned and disinfected using spirit swabs. The lymph node was then fixed following this, an 18 gauge needle attached to 10ml syringe was introduced into the lymph node and adequate material was aspirated. One part of this aspirated material was put in a sterile container and sent for CB-NAAT and the other part was smeared on slides. One smear slide was fixed in 95% ethyl alcohol for staining with Haematoxylin-Eosin and Papanicolaou stain, the other was air dried for Giemsa staining and few slides were kept aside for special stains i.e ZN stain for AFB for TB. The slides were examined under the microscope for visualization of granulomas and AFB.

RESULT

Table-1: Gross appearance in FNAC-

Gross appearance	No. of cases
Purulent	30 (37.5%)
Bloody stained	33 (41.2%)
Blood with pus	17 (21.3%)

Table 2: Results of Cytological pattern

Cytology Pattern	Cases	Percentage
Granulomatous	52	65%
Reactive	14	17.5%
Suppurative	14	17.5%
Total	80	100%

Table 3: Subtype of Granulomatous lesion with AFB & CBNAAT results

Type	No. of cases	AFB Positive	CBNAAT Positive
Granuloma without necrosis	4 (7.8%)	00	0
Granuloma with necrosis	33 (63.4%)	7 (21.2%)	19 (57.5%)
Only necrosis	15 (28.8%)	4 (26.6%)	13 (86.6%)
Total	52 (100%)	11	32

Table 4: CBNAAT results

CBNAAT	Positive	Percentage	Negative	Percentage
Granulomatous (52)	32	61.5%	20	38.5%
Reactive (14)	1	7%	13	93%
Suppurative (14)	4	28.5%	10	71.5%
Total	37	-	43	-

Present study included 80 subjects with cervical lymphadenopathy. FNAC done of that lymphnode was compared with ZN staining and CBNAAT findings. The age group ranges from 7-85 yrs with maximum number of cases between the age group of 21-40 years i.e 45 cases. Male female ratio was 1:1.2.

Gross appearance of aspirated material of FNAC is shown in table 1. 41.2%(33 cases) were bloody, purulent in 37.5% cases and 21.3% cases had a mixed aspirate of blood with pus.

The various cytological pattern seen in our study as shown in table 2 composed of predominantly granulomatous lymph node (65%), reactive and suppurative LN being 17.5% each. In the present study we further sub-categorized granulomatous lymphadenopathy into 3 categories i.e. Granuloma with necrosis (33cases), Granuloma without necrosis (4 cases) and necrosis only (15 cases) as shown in table 3. AFB and CBNAAT was performed and it was negative in granulomatous without necrosis. AFB was positive in 7 cases while CBNAAT was positive in 19 cases of granuloma with necrosis. Smears that showed only necrosis had 4 cases AFB positive while 13 cases showed CBNAAT positive.

Overall CBNAAT results are shown in table 4. Granulomatous lesion showed 61.5% positive result, reactive lymph node 7% and suppurative lesion showed 28.5% positivity.

DISCUSSION

The annual global incidence of extra pulmonary TB has been increasing in the last decade due to changing TB control practices, spread of HIV and population growth. The diagnosis of EPTB poses particular challenge for clinicians because of atypical presentations and lacking health facilities.¹⁰

The peak age group with granulomatous lymphadenitis was 21-40 yrs. The similar age distribution was found by Mulualet al and Louis et al^{11,13}

Out of 80 cases 46.25% cases were positive for CBNAAT which was comparable with study of Gour et al¹² and Srwar et al¹⁵ whereas higher percentage of CBNAAT positivity was found in studies of Moure et al¹⁴, Gupta et al¹⁵ and Ligthelm et al¹⁶

Of the 80 patients included in this study, cytomorphological features associated with TB were 52 patients with majority being females. Out of 52 patients 32 (61.5%) showed CBNAAT positivity while ZN was positive in 11 (21.1%) cases.

Out of the 14 reactive lesions and 14 suppurative lesion in FNAC; CBNAAT was positive in 1 (7%) and 4 (28.5%) cases respectively. Thus the lesions that were not identified as of tubercular lesion in FNAC were detected by CBNAAT, thereby showing the superiority of CBNAAT over FNAC.

CBNAAT was able to detect TB in 6.25% (1 reactive+4 suppurative=5/80)of cases which were not diagnosed by FNA, similarly Sunil et al studied 289 cases out of which 19 cases were false negative in FNAC, thus CBNAAT detected 6.5% of cases which were negative in FNAC.

ZN stain for AFB was positive in 21.1% cases of the granulomatous lymphadenitis. Overall out of the 80 cases 18% cases were ZN positive

which was comparable with the study of Subhan Ali et al, Brijesh thakur et al and anshu et al¹⁷ while in study of Majid momin et al it was positive in 62% of cases.

FNAC is a simple procedure which can be performed in an outpatient setting and is ideal in resource limited settings¹⁸. Specimen collection is simple, economical, easy to do and safe procedure¹⁹. But in a tertiary level hospital we cannot depend solely on FNAC for the diagnosis of tuberculosis.

During the last decade, a number of Nucleic acid amplification (NAA) methods have been developed for rapid detection and identification of MTB²⁰. newly administered Gene-xpert MTB/RIF assay take two hours to provide the final results, in addition distinguishes MTB and mycobacteria other than tuberculosis and also provide rifampicin resistance simultaneously¹⁰. Polymerase chain reaction (PCR) is based on NAA methods and is widely used for rapid diagnosis of TB²¹. This study supports the routine use of CBNAAT for the diagnosis of Extra pulmonary tuberculosis as time factor and drug resistance are the two important factors for the diagnosis and treatment of tuberculosis.

Though CBNAAT has several disadvantages there are few limitations like high cost, inability to differentiate MDR-TB and XDR-TB, not suitable for monitoring response to treatment and suggestive of conventional microscopy and culture are required for monitoring MDR-TB and few technical problems like requirement of stable electricity supply, limited temperature range and maintenance.

Culture being gold standard but takes 4-6 weeks for diagnosis. Newer faster radiometric (BACTEC) and Non- radiometric methods like fluorescent labelled mycobacterium growth indicator test (MGIT) methods being used and provide results in 7-10 days. It is time consuming and equipment expenses are much high, more skilled personals, continuous monitoring for several days and further confirmation of positive cultures by making ZN smears are required.²²

CONCLUSION

To conclude a high clinical suspicion and special diagnostic procedures are required for diagnosis of extrapulmonary tuberculosis. FNAC and ZN microscopy is the first line investigation and is also simple and safe procedure especially in resource limited area. Culture is gold standard but is time consuming. CBNAAT being the latest technique is less time consuming, can detect TB even if bacilli are less in number and also provides information about rifampicin resistance. But CBNAAT has a disadvantage of being costly, maintenance is high. To conclude combined techniques proved itself as an effective tool in OPD basis diagnosis and early initiation of treatment to prevent morbidity, mortality and economic loss.

REFERENCES

- MukulPatar et al(2014). A clinicopathological evaluation of cervical lymphadenopathy in children (0-14 Years) By fine needle aspiration cytology and histopathological examination - A hospital based study. National Journal of Otorhinolaryngology and Head & Neck Surgery.2014;2(11):12-14.
- Ghartimagar D et al(2011). Utility of fine needle aspiration cytology in metastatic lymph nodes. Journal of Pathology of Nepal.2011;1(2):92-95
- Louis J.LigthelmXpert MTB/RIF for Rapid Diagnosis of Tuberculous Lymphadenitis from Fine-Needle-Aspiration Biopsy Specimens. Journal of clinical microbiology, Nov. 2011, p. 3967–3970 Vol. 49, No. 110095-1137. doi:10.1128/JCM.01310-11.
- Ardizzoni E et al(2015). Implementing the Xpert® MTB/RIF Diagnostic Test for Tuberculosis and Rifampicin Resistance: Outcomes and Lessons Learned in 18 Countries. PLoS One. 2015 Dec 15;10(12):e0144656. doi: 10.1371/ journal.pone.0144656. eCollection 2015. [PubMed]
- Catherina CB, Rapid molecular detection of TB and Rifampicin resistance, NEJM, 2010, Sep; 363:11. Doi: 10.1056/NEJMoa0907847.
- Lee JY.(2015) Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis (Seoul). 2015 Apr;78(2):47-55. doi: 10.4046/trd.2015.78.2.47. Epub 2015 Apr 2. [PubMed]
- Wright C. A., et al. (2008). Diagnosing mycobacterial lymphadenitis in children using fine-needle aspiration biopsy: cytomorphology, ZN staining and autofluorescence—making more of less. Diagn. Cytopathol. 36:245–251 [PubMed] [Google Scholar]
- Wright C. A et al.(2004). Mycobacterial autofluorescence in Papanicolaou-stained lymph node aspirates: a glimmer in the dark? Diagn. Cytopathol. 30:257–260 [PubMed] [Google Scholar]
- Singh KG et al (2017) Role of CB-NAAT in diagnosing Mycobacterial tuberculosis and rifampicin resistance in tubercular peripheral lymphadenopathy. Int J Med Res Rev 2017;5(03):242-246.doi:10.17511/ijmrr.2017.i03.05.
- majid momin et al (2017) comparative analysis of gene expert assay in addition to ZN microscopy for detection of extrapulmonary TB in fine needle aspiration samples. Sch.J.App.Med.Sci.,Jul2017;5(7D):2803-2808.
- Mulualet al. Genexpert MTB/RIF Assay for the diagnosis of tuberculous lymphadenitis on concentrated fine needle aspirates in high tuberculosis burden settings
- Gour sanjay et al(2017). Genotypic diagnosis of extrapulmonary tuberculosis-CBNAAT a novel tool. Medpulse Int J Med 2017;4(2):79-82.
- Srwar A et al (2014)Rapid detection of Mycobacterium tuberculosis and rifampicin resistance in extrapulmonary samples using Genexpert MTB/RIF assay. IOSR. J Dental

- Med Sci 2014;13(11):50-53.
14. Moure R et al(2012). Effectiveness of an integrated real time PCR method for detection of mycobacterium tuberculosis complex in smear negative extrapulmonary samples in an area of low tuberculosis prevalence. J Clin microbiol 2012;50(2):513-515
 15. Gupta S et al(2018). Role of gene Xpert Mtb/Rif in diagnosis of tuberculosis pus. Int J Curr res Med Sci 2018;4(2):81-85.
 16. Ligthelm L et al (2011). Xpert MTB/RIF for the rapid diagnosis of tuberculous lymphadenitis from fine needle aspiration biopsy specimens. J Clin Microbiol 2011;49(11):3967-3970.
 17. Kumar anshu et al (2018). A study on role of cartridge based nucleic acid amplification test (CBNAAT) for diagnosing pediatric tuberculosis in a tertiary care hospital in eastern India. J of pediatrics & neonatology ISSN 2474-7521
 18. Wright C et al (2009). Fine needle aspiration biopsy: an undervalued diagnostic modality in paediatric mycobacterial disease. Int. J. Tuberc. Lung Dis. 13:1467-1475
 19. Gupta PP et al. (2017) Efficacy of cartridge based nucleic acid amplification test to diagnose tubercular pleural effusion. Int J Res Med Sci. 2017 Aug;5(8):3637-3643
 20. Piersimoni C et al (2002). performance assessment of two commercial amplification assays for direct detection of mycobacterium tuberculosis complex from respiratory and extrapulmonary specimens. J. Of Clin microbiol. 2002 Nov 1;40(11):4138-42
 21. automated real time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance : xpert MTB/RIF system: policy statement; WHO 2011
 22. Theron G et al (2012). Do adjunct tuberculosis tests, when combined with xpert MTB/RIF, improve accuracy and cost of diagnosis in a resource poor setting ?. European respiratory journal. 2012 Jul 1;40(1):161-8.