Role of fine needle aspiration cytology as a diagnostic tool in lymphadenopathy with utility of CBNAAT in tuberculous lymphadenopathy

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Abstract

Introduction: Lymphadenopathy is a common, nonspecific clinical sign. Fine Needle Aspiration Cytology (FNAC) is the first line of investigation in the evaluation of lymphadenopathy. FNAC material can be used for cytological evaluation and other ancillary tests in Tuberculous Lymphadenopathy.

Aims and Objectives

1. To evaluate the role of FNAC as a diagnostic tool in lymphadenopathy.
2. To assess the role of Cartridge based nucleic acid amplification test (CBNAAT) in suspected cases of tuberculous lymphadenopathy.

Materials and methods: The study conducted at department of Pathology in MVJMC &RH, Hoskote, Bangalore from January 2017 to June 2018. A total of 104 cases of lymphadenopathy were subjected to FNAC. In each case at least 3 smears for H & E, Giemsa stain and Special stain like AFB done for tuberculous lymphadenopathy. FNAC material sent for CBNAAT in suspected cases of Tuberculosis.

Results: The age ranged from 1-90years, Male:Female of ratio1.26:1. Most common site was cervical and cytological pattern was granulomatous -41(39.4%) followed by reactive -38(36.5%).Majority of them presented with solitary node (77%) and followed by bilateral/multiple (23%). One case of reactive (11%), two cases of suppurative (50%) and ten cases of granulomatous (37%) were found to be positive for Tuberculosis by CBNAAT.

Conclusion: FNAC is simple, cost effective diagnostic tool with high degree of accuracy.FNAC coupled with AFB staining is the first line of investigation for Tuberculous lymphadenopathy. According to New Revised National TB Control Programme (RNTCP) guidelines CBNAAT sputum is a must for paediatric cases. The present study highlights the utility of CBNAAT from FNAC material as one of the rapid and adjuvant diagnostic tool in tuberculous lymphadenopathy.

Keywords: FNAC, Tuberculous lymphadenopathy, CBNAAT.

Introduction

Fine needle aspiration cytology (FNAC) is widely used as a diagnostic test for the assessment of lymphadenopathy and it provides a high level of diagnostic accuracy.[1] Common causes are granulomatous inflammation, reactive, lymphoproliferative and metastatic deposits.In India, the incidence of tuberculosis (TB) is high and it is the leading cause of lymphadenopathy.[2] With the advent of Human Immunodeficiency virus (HIV), there is a global upsurge of mycobacterial infection and TB has become a major cause of morbidity and mortality.

Incidence of TB is 2.2 million and prevalence is 2.5 million. There were 5.8 lakhs estimated new cases of multi-drug resistant TB(MDR-TB) and Rifampicin resistant (RR-TB). India, one of the countries with high burden of TB, has an estimated 79,000 MDR-TB cases among notified pulmonary TB cases. The estimated incidence of MDR-TB is 2% among new cases and 15% among re-treatment cases.

The conventional methods of diagnosis like sputum examination and chest X-ray are accurate in detecting the pulmonary disease. However they are not helpful in diagnosis of extrapulmonary tuberculosis and not able to detect Rifampicin resistant cases.

Superficial lymhadenopathy is the most common extra-pulmonary manifestation of TB and tissue diagnosis is the main stay in the management of these cases. Ancillary tests like Acid fast bacili (AFB) stain and Auromine & Rhodamine stain from FNAC material aid in definite diagnosis. Newer molecular methods like Cartridge based nucleic acid amplification test (CBNAAT) can be done from FNAC material which act as an adjuvant for the diagnosis and can also detect Rifampicin resistant cases, hence this study was undertaken.

Aims and Objectives

1. To evaluate the role of FNAC as a diagnostic tool in lymphadenopathy.
2. To assess the role of CBNAAT in suspected cases of tuberculous lymphadenopathy.

Materials and Methods

A descriptive study conducted at department of Pathology in MVJMC &RH, Hoskote, Bangalore from January 2017 to June 2018.This study was conducted after approval from the

Institutional Ethics Committee. FNAC was done using 22 gauge needle, under strict aseptic precautions. In each case, part of the aspirate was used for preparing 3 smears at least, one each for H & E stain, Giemsa stain and AFB stain. Remaining aspirate material in suspected cases of tuberculosis were collected in a sterile cartridge container and sent for CBNAAT.

Inclusion criteria

1. Patients having clinical feature of extra-pulmonary tuberculosis.

2. Patients giving consent for the study.

Exclusion criteria

1. Patients with features suggestive of pyogenic pus.
2. Patients on antitubercular treatment for more than 1 month.
3. Patients not giving consent for the study.

Results

A total of 104 cases were studied. The age group range was from 1-90 yrs with M:F ratio- 1.26:1.Majority of them presented with solitary node (77%) and followed by bilateral/multiple (23%).Site wise distribution of cases, majority were from cervical (91%), followed by axillary (6%) and inguinal (3%).

The various cytological pattern seen in our study as shown in Table 1 composed of granulomatous pattern followed by reactive and metastatic. In the present study we further sub categorized granulomatous lymphadenopathy in to three category types as granuloma with necrosis, granuloma without necrosis and only necrosis as shown in Table 2 with AFB results. All clinical suspected tuberculosis cases (41) were sent for CBNAAT in which majority of cases showed granulomatous pattern on cytology with ten cases (37%) showed CBNAAT positive, one case of reactive (11%) showed CBNAAT positive, two cases of suppurative (50%) showed CBNAAT positive as shown in Table 3. In present study granulomatous without necrosis showed 19% AFB positive and 14% CBNAAT positive whereas in 14 cases of granulomatous with necrosis showed 07% AFB positive and 21.4% CBNAAT positive. In six cases of only necrosis showed 83% AFB positive and 66% CBNAAT positive. Smears showing predominantly necrosis has shown more AFB positivity (83%) than the other cases. All the cases sent for CBNAAT in our study were Rifampicin sensitive.

Table 1: Results of Cytological Pattern.

Pattern	Cases	Percentage
Granulomatous lymphadenopathy	41	39.4%
Reactive	38	36.5%
Metastatic deposits	15	14.4
Suppurative lymphadenitis	7	6.7%
Lymphoma and Lymphoproliferative	3	2.8%
Total	104	100%

Table 2: Subtypes of granulomatous lesion with AFB & CBNAAT results.

Pattern	Cases	AFB Positive	CBNAAT
Granuloma without necrosis	21(51.2%)	04(19%)	03(14%)
Granuloma with necrosis	14(34.2%)	01(7%)	03(21.4%)
Only Necrosis	06(14.6%)	05(83%)	04(66%)
Total	41	10	10

Table 3: CBNAAT results.

CBNAAT	Positive	Percentage	Negative	Percentage
Granulomatous-27	10	37%	17	63%
Reactive -09	01	11%	08	88%
Suppurative-04	02	50%	02	50%
Metastatic-02	00	00%	02	100%
Total	13	-	29	-

Discussion

FNAC is a first line investigation in the diagnosis of lymph node lesions. The mean age was 34 yrs and M:F ratio was 1.26:1, which was concordant with study done by Chand et al[3] and Devi et al.[4] The most common site of presentation in our study was cervical (91%), followed by axillary (6%) and inguinal (3%), which is concordant with Khajuria et al[2] and Chand et al[3] study.

For cytodiagnosis of tubercular lymphadenitis, we need to demonstrate granulomas composed of epithelioid cells, Langhans giant cells with or without caseous necrosis along with demonstration of AFB positivity. Studies by Gomes et al[5] and Das et al[6] also followed the same cytological parameters.

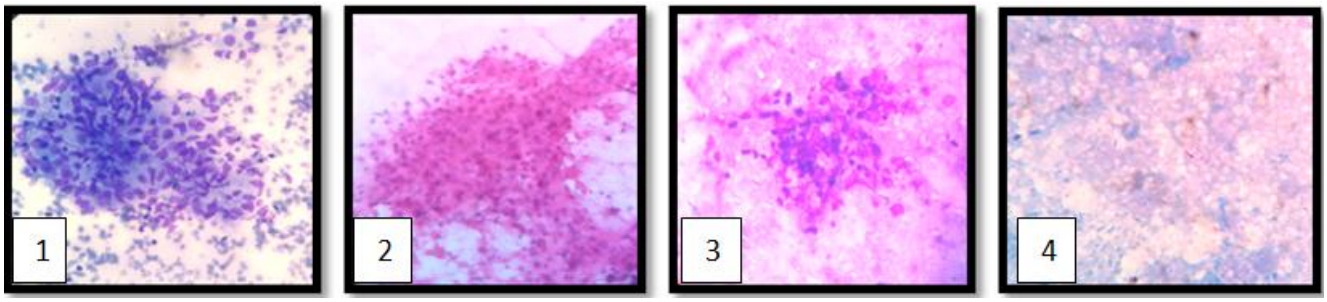

Fig. 1: Well formed granuloma without necrosis,Giemsa stain 400x, Fig. 2: Well formed granuloma without necrosis H & E stain 400x, Fig. 3: Granuloma with background showing necrosis, Giemsa stain 400x, Fig. 4: Only necrosis with Mycobacterium bacilli AFB stain 1000x

In granulomatous lesions the present study showed 51.2% granulomatous without necrosis (Fig.1 & 2),34.2% granulomatous with necrosis(Fig.3) and 14.6% only necrosis (Fig.4) whereas study done by Bhavani et al[7] showed 9.8% granulomatous without necrosis, 27.17% granulomatous with necrosis and 5.28% only necrosis.The various cytological pattern comparison with other studies as shown in Table.4

Table 4: Cytological pattern comparison with other studies

Pattern	Cases	Present Study	Bhavani et al[7]	Ligthelm et al[8]	Devi et al[4]	Naesreen et al[9]
Granulomatous	41	39.4%	42.26%	66.7%	31.2%	17.9%
Reactive	38	36.5%	35.47%	20.8%	55.2%	12.7%
Metastatic	15	14.4	11.32%	8.3%	6.25%	19.7%
Suppurative	7	6.7%	9.8%	2.1%	-	-
Lymphoma and Lymphoproliferative	3	2.8%	1.13%	-	6%	49.7%

In cases of extra-pulmonary tuberculosis clinicians have low level of suspicion due to varying symptoms and presentation. In Tuberculous lymphadenitis diagnosis conventional methods have low sensitivity with a range of 0-40%. Also it is limited by the inability to still remains challenging, as the Mycobacteria tuberculi bacilli load is low and the routine detect drug resistance. The culture methods yield vary from 30-80%. Drug susceptibility testing is complex, time consuming usually takes 2-8 weeks which makes delay in treatment decision.[10] While patients await diagnosis, they are likely to receive inappropriate or in effective treatment and consequently disease may progress. This results in an increased chance of morbidity from tuberculosis. They continue to transmit drug-resistant TB to others; especially family members and the resistance might have amplified. All these factors lead to delay in definitive diagnosis.[11]

Cytological diagnosis of tuberculosis coupled with AFB stain is the most preferred method for diagnosis of extra pulmonary tuberculosis. But this procedure has several limitations and pitfalls as explained above. To address this issue there was a need for a simple and rapid diagnostic tool and a new diagnostic test, CBNAAT was developed.

CBNAAT purifies, concentrates, amplifies and identifies the targeted rpoB nucleic acid sequences, and delivers the results in about 120 minutes. For CBNAAT examination the sample reagent were added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15 minute period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge. No cross reaction with other bacterial species was noted as it is a highly specific test which uses 3 specific primers and 5 unique molecular probes to target rpoB gene for MTB.[12] It is a rapid, fully automated test and based on PCR technique that detects DNA directly from the clinical specimens along with Rifampicin resistance detection.

Table 3 In the present study out of 21 cases of granulomatous without necrosis showed 19% AFB positive and 14% CBNAAT positive whereas in 14 cases of granulomatous with necrosis showed 07% AFB positive and 21.4% CBNAAT positive. In six cases of only necrosis showed 83% AFB positive and 66% CBNAAT positive. The overall AFB showed 24.4% positivity in suspected cases of tuberculosis which is higher than the study done by Aggarwal et al[13] and lower than the study done by Nidhi et al.[14]

The CBNAAT results in the present study showed 37% which was in concordance with the study done by Shakeel et al.[15] Various studies showed 36%-96% CBNAAT Positivity as shown in the Table 5. Low CBNAAT positivity may be due to various technical reasons like the second or third pass of FNAC sent for CBNAAT, amount of material sent. Also saline was used as diluent in our study, while in many other studies N acetyl cysteine was used as the diluent. The main negative point in our study was not able to compare the culture report which is considered as gold standard.

Table 5: CBNAAT Results comparison with other studies

Shakeel et al[15]	36.3%
Gour et al[16]	40%
Srwar et al[17]	51.7%
Moure et al[18]	58.3%
Gupta et al[10]	59.8%
Anmol et al[19]	62.7%
Ligthelm et al[8]	96%
Present Study	**37%**

In our study CBNAAT test showed Sensitivity of 37%, Specificity 80%, Positive predictive value 76.92%, Negative predictive value 41.38%, Accuracy 52.38%, Positive likelihood ratio 1.85 and Negative predictive ratio 0.79.There were no Rifampicin resistance cases in the present study whereas the studies conducted by Anmol et al,[19] Gupta et al,[10] Shakeel et al[15] showed 4.6%, 5% and 6.4% of Rifampicin resistant cases respectively.

Conclusion

FNAC is a reliable and economical investigating modality in lymphadenopathy. This provides high degree of accuracy in diagnosing and hence reducing morbidity and mortality of the disease.In a developing country like India with high prevalence rate of tuberculosis.

FNAC coupled with AFB staining should be the 1st line of investigation in cases with lymphadenopathy. According to New RNTCP guidelines CBNAAT sputum is a must for paediatric cases.The WHO 2012 [20] has also recommended the CBNAAT sputum for pulmonary tuberculosis and specially in pediatrics cases. The present study highlights the utility of CBNAAT from FNAC material as an adjuvant in the diagnosis of Tuberculosis lymphadenopathy.

Conflicts of Interest: None.

References

1. Steel BL, Schwartz MR, Ramzy I. Fine needle aspiration biopsy in the diagnosis of lymphadenopathy in 1,103 patients. Role, limitations and analysis of diagnostic pitfalls. *Acta Cytol* 1995;39(1):76-81.
2. Khajuria Ruchi, Goswami KC, Singh K, Dubey VK. Pattern of Lymphadenopathy on Fine Needle Aspiration Cytology In Jammu. *JK Science* 2006;8(3):158-160.
3. Chand P, Dogra R, Chauhan N, Gupta R, Khare P. Cytopathological Pattern of Tubercular Lymphadenopathy on FNAC: Analysis of 550 Consecutive Cases. *J Clin Diagn Res:JCDR* 2014;8(9):16-19.
4. Devi Thaker B,Devi A,Singh K: Pattern of Lymphadenopathy on Fine Needle Aspiration Cytology- A Retrospective Study. *JMSCR* 2017;5 (5):22088-22093
5. Gomes I,Trindade E,Vidal O, et al. Diagnosis of sputum smear–negative forms of pulmonary tuberculosis by transthoracic fine needle aspiration.*Tubercle* 1991:210- 213
6. Das DK, Pank JN, Chachra KL, et al. Tuberculosis lymphadenitis: correlation ofcellular components and necrosis in lymph node aspirate with AFB positivity andbacillary count. *Indian J Pathol Microbiol* 1990;33:1-10
7. Bhavani C, Neeraja M, Varalakshmi KPV, Ramana Babu PV, Chaitanya B, Sravani P. Role of FNAC in the Diagnosis of Cervical Lymphadenopathy. *Int J Med Res Rev* 2014;2(6):599-603.
8. Ligthelm L J, Nicol M P,Hock KG P, Jacobson R, Helden P D V, Marais B J et al.Xpert MTB/RIF for the rapid diagnosis of Tuberculous lymphadenitis from Fine needle aspiration biopsy specimens. *J Clin Microbiol* 2011;49(11):3967-3970.
9. Nesreen HH, Neveen ST, Reliability of FNAC as a diagnostic tool in cases of cervical lymphadenopathy. *J Egypt National Cancer inst* 2011;23:105-114
10. Gupta S, Kajal NC, Shukla AK,Singh A, Neki NS. Role of Gene Xpert Mtb/Rif in Diagnosis of Tuberculous Pus. *Int J Curr Res Med Sci* 2018;4(2):81-85.
11. 11. Kandi S,Reddy V,Nagaraja SB.Diagnosis of pulmonary and extrapulmonary tuberculosis: How best is CBNAAT when compared to conventinal methods of TB detection? *Pulm Res Respir Med Open J* 2017;4(2):38-41
12. Boehme CC, Nabeta P, Hillemann D,Nicol MP, Shenai S, Krapp F, Allen J,Tahirli R, Blakemore R, Rustomjee R,Milovic A. Rapid molecular detection of tuberculosis and rifampin resistance. *Eng J Med* 2010;363(11):1005-1015.
13. Aggarwal P, Wali JP, Singh S, Handa R, Wig N, Biswas A. A clinico-bacteriological study of peripheral tuberculosis lymphadenitis. *J Assoc Physicians India* 2001;49:808-812
14. Nidhi P, Sapna T,Shalini, Kumud G. FNAC In Tuberculosis Lymphadenitis: Experience From A Tertiary Level Referral Centre. *Indian J Tuberc* 2011;58:102-107
15. Shakeel K, Iram S, Akhar M et al Diagnostic validation of rapid molecular detection of Mycobacterium tuberculosis in pus samples by GeneXpert[R]. *J Pak Med Assoc* 2018;68(1):33-37
16. Gour Sanjay M,Radha PM, Babu NP,Sushant M,Jitesh.Genotypic diagnosis of extra pulmonary tuberculosis-CBNAAT a novel tool. *Medpulse Int J Med* 2017;4(2):79-82.
17. Srwar A, Akhtar R,Ahmed I et al.Rapid detection of Mycobacterium tuberculosis and Rifampicin Resistance in extra pulmonary samples using GeneXpert MTB/RIF assay.IOSR. *J Dental Med Sci* 2014;13(11):50-53
18. Moure R, Martin R, Alcaide F.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
19. Fuladi AB,Gupta PP.Challenges in the Diagnosis of Extrapulmonary Tuberculosis:Role of Gene Xpert Mycobacterium Tuberculosis/Rifampicin Assay. *Int J Sci Stud* 2017;5(7):75-79.
20. Xpert MTB/RIF implementation manual Technical and operational 'how-to': practical considerations [Internet].2014. Website. www.who.int/tb.Accessed January 12,2019.

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