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ORIGINAL RESEARCH

Comparison Of Fine Needle Aspiration Cytology Sensitivity To RTPCR For The Diagnosis Of Tubercular Lymphadenitis In Tertiary Care Centre, Jaipur, Rajasthan

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ABSTRACT

Introduction: Tuberculosis is an infection caused by a slender shaped, non-spore-forming aerobic acid fast bacilli named -*Mycobacterium tuberculosis.* Lymph node tuberculosis constitutes 20-40% of extrapulmonary tuberculosis. It is more common in developing and underdeveloped countries.

Materials & Methods: A total of 76 patients were enrolled in this study who were having a non-malignant lymphadenopathy more than 1cm above 10 years of age were included in this study. The material obtained from FNAC was divided into three parts. One part was used to prepare a dry fix smear for Giemsa staining for cytology examination. Second part was used to prepare ZN staining and remaining part was used for molecular detection of mycobacterium tuberculosis by RTPCR.

Results: A total of 76 patients were enrolled in this study. Out of 76 cases 52 (68.42%) were male while 24 (31.58%) were females and mean age group was 31-40 yrs was found. On FNAC staining tubercular bacilli was found only in 27 (35.5%) cases while on RT-PCR 48 (63.15%) cases were found positive for *M. tuberculosis*. RT-PCR showed sensitivity of 100%, specificity of 96.55%. Positive predictive value of 97.92%, negative predictive value of 100% and an accuracy of 98.68%.

Conclusion: For extra pulmonary lymphadenopathy cases molecular methods are more reliable and sensitive method in a comparison of cytological microscopic methods. Molecular detection required extensive lab setup but it would be a great tool along with a combination of cytological method for the accurate diagnosis of tubercular lymphadenitis.

Key words: Tubercular Lymphadenitis, FNAC, RTPCR.

INTRODUCTION

Tuberculosis is an infection caused by a slender shaped, non-spore-forming, aerobic, acid fast bacilli named -*Mycobacterium tuberculosis*. Extrapulmonary tuberculosis (EPTB) is reported to be increasing over the last several years. Organs affected in EPTB include lymph nodes, pleura, central nervous system, eyes, musculoskeletal system, genitourinary tract, and gastrointestinal tract. Symptoms and clinical presentations of EPTB are variable and depend on the organ involved.[1]Lymph node tuberculosis constitutes 20-40% of extrapulmonary tuberculosis. It is more common in developing and underdeveloped countries.[2]

Granulomatous lymphadenopathy has an exhaustive list of differential diagnosis comprising of tuberculosis, sarcoidosis, fungal infections and other inflammatory conditions. An added complexity is a high clinical suspicion of extra pulmonary tuberculosis but lack of bacteriological proof on conventional microscopy. The standard protocol for tubercular lymphadenitis on cytology includes identification of epithelioid granulomas with giant cells, necrosis and a positive Acid-Fast Bacilli detected with ZN stain. This method is time taking & showed poor sensitivity of ZN stain is the probable cause of delaying diagnosis of the disease.[3]

MATERIALS& METHODS

A descriptive type of observational study was carried out in the Departments of Microbiology and Pathology, Jaipur National University Institute for Medical Sciences & Research Centre from Dec, 2020 to Nov, 2022. A total of 76 patients were enrolled in this study who were having a non-malignant lymphadenopathy more than 1cm and above 10 years of age were included in this study.

A dedicated clinical examination was conducted on patients with non-malignant lymphadenopathy who were having clinical symptoms such as fever, loss of weight, cough with expectoration or malaise. FNAC was performed under aseptic precautions using a 23-gauge needle and 10mL disposable syringe. Multiple passes were performed. The material obtained was divided into three parts. One part was used to prepare a dry fix smear for Giemsa staining for cytologyexamination.[4] Second part was used to prepare ZN staining and remaining part was used for molecular detection of mycobacterium tuberculosis by RTPCR. Cytological interpretation was done on Giemsa and ZN-stained slides. Interpretation was done on the basis of cellularity, epithelioid cell granulomas, Langhans giant cells and on caseation necrosis. DNA extraction was done on Qiagen DNA mini kit and RTPCR was done by TRU PCR MTBC detection kit as per standard protocol.

RESULTS

A total of 76 patients were enrolled in this study who were having non-malignant lymphadenopathy. Fine needle aspiration was done at Department of Pathology, Jaipur National University Institute for Medical Sciences & Research Centre. Out of 76 cases 52 (68.42%) were male while 24 (31.58%) were females and mean age group was 31-40 yrs was found. (Fig.1) Most fine needle aspiration was collected from cervical & axillary region.

Granulomatous lymphadenitis 32 (42.10%) was the most common cytological diagnosis followed by tuberculous lymphadenitis 24 (31.57%), reactive lymphadenitis 15 (19.73%) and necrotizing lymphadenitis 5 (6.57%). (Table 1)

On ZN staining Acid fast bacilli was found only in 27 (35.5%) cases while on RT-PCR 48 (63.15%) cases were found positive for *M. tuberculosis*. (Fig.2) RTPCR was done at department of Microbiology, Jaipur National University Institute for Medical Sciences & Research Centre.

In the present study, RT-PCR showed sensitivity of 100%, specificity of 96.55%. Positive predictive value of 97.92%, negative predictive value of 100% and an accuracy of 98.68%.

(Table 2) While in fine needle aspirate cytology sensitivity of 56.52%, specificity of 96.67%. Positive predictive value of 96.30%, negative predictive value of 100% and an accuracy of 59.18%. (Table3)

Fig 1: Gender wise distribution of LNTB

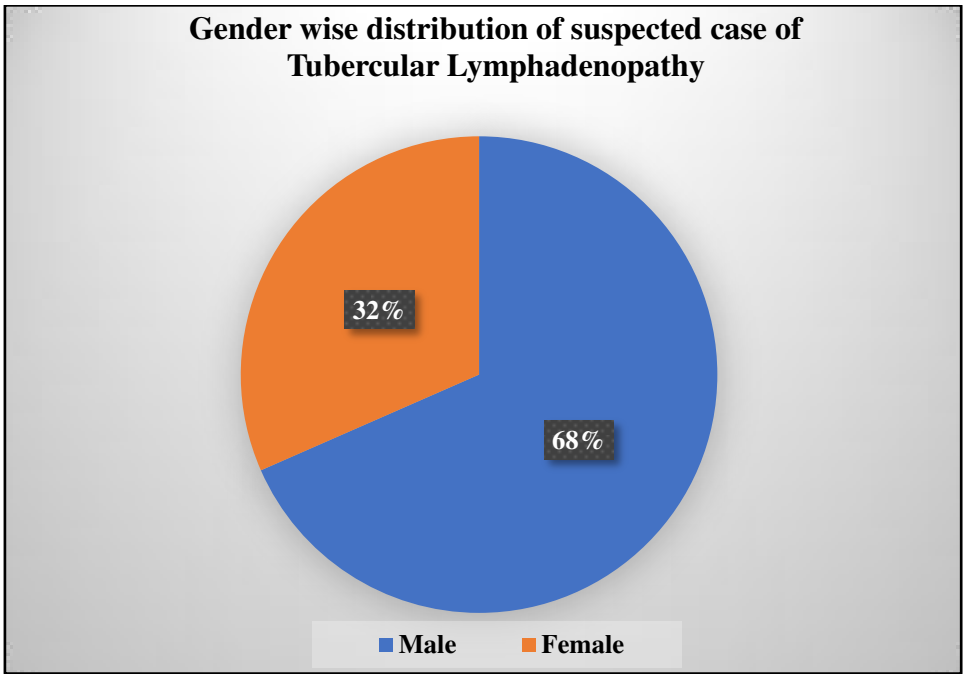

Fig 2: Comparison of FNAC& RT-PCR

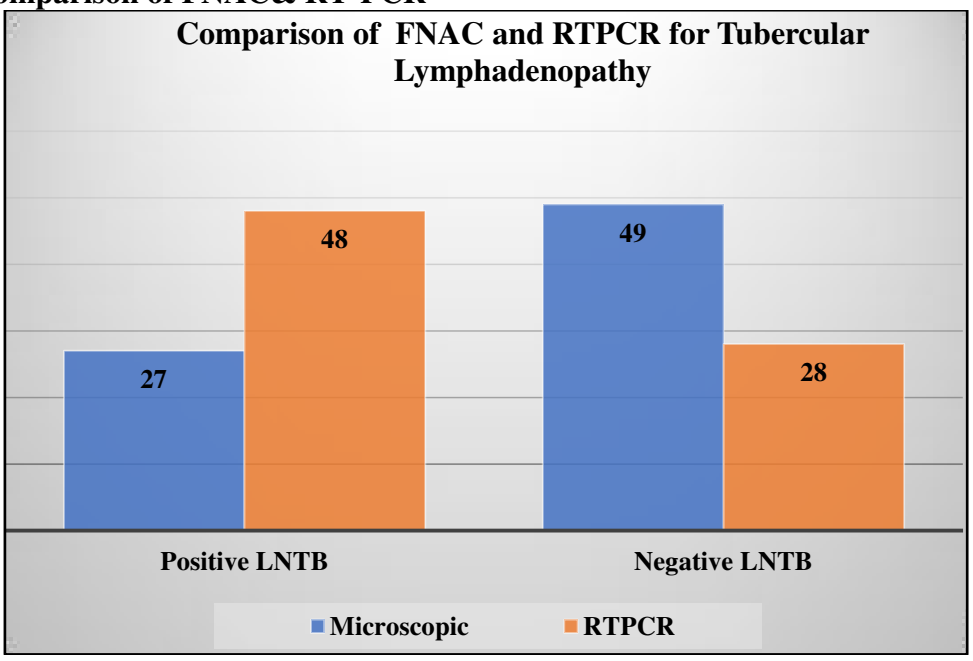

Table 1: Showing cytological findings in the present study.

Cytological findings	No. of Cases
Granulomatous lymphadenitis	32(42.10%)
Tuberculous lymphadenitis	24(31.57%)
Reactive lymphadenitis	15(19.73%)
Necrotizing lymphadenitis	5(6.57%)

Fig 3: Granulomatous lymphadenitis in MGG

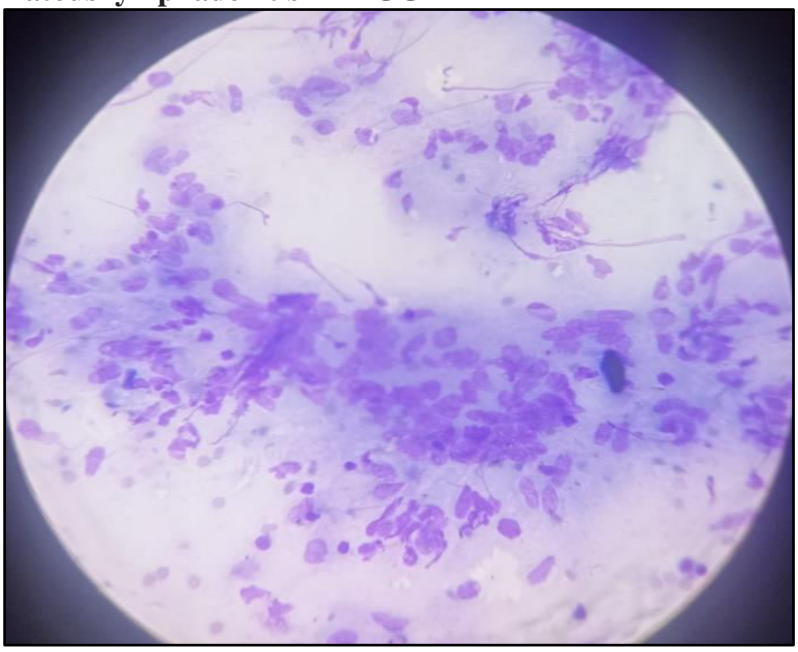

Fig 4: MTB detection in RTPCR

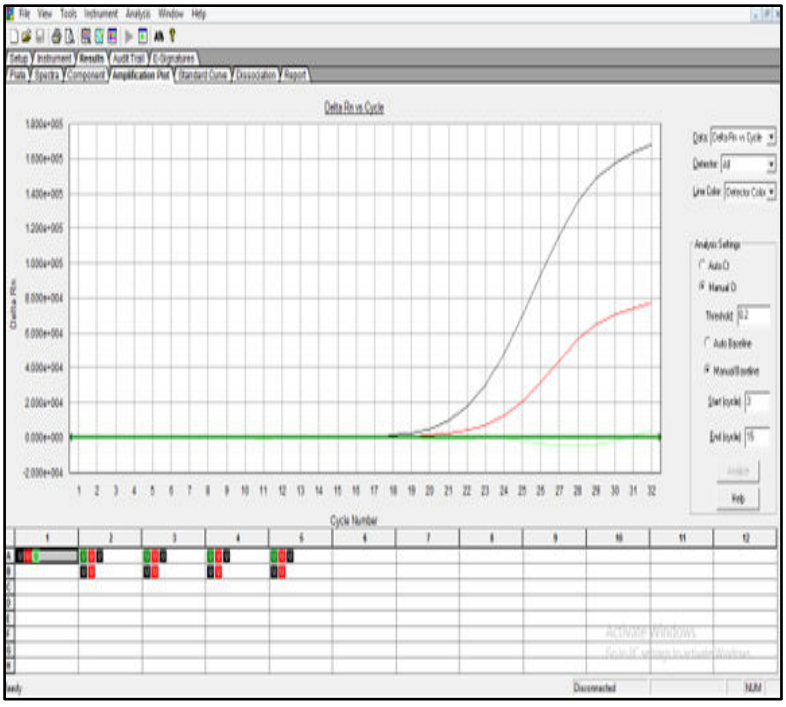

Table 2: Sensitivity & Specificity of FNAC

Statistic for Cytological Method	Value	95% CI
Sensitivity	56.52%	41.11% to 71.07%
Specificity	96.67%	82.78% to 99.92%
Positive Likelihood Ratio	16.96	2.43 to 118.43
Negative Likelihood Ratio	0.45	0.32 to 0.63
Disease prevalence (*)	60.53%	48.65% to 71.56%
Positive Predictive Value (*)	96.30%	78.83% to 99.45%
Negative Predictive Value (*)	59.18%	50.89% to 66.99%

Accuracy (*)	72.37%	60.91% to 82.01%

Table 3: Sensitivity & Specificity of RT-PCR.

Statistic for TB RT-PCR	Value	95% CI
Sensitivity	100.00%	92.45% to 100.00%
Specificity	96.55%	82.24% to 99.91%
Positive Likelihood Ratio	29.00	4.23 to 198.98
Negative Likelihood Ratio	0.00	
Disease prevalence (*)	61.84%	49.98% to 72.65%
Positive Predictive Value (*)	97.92%	87.26% to 99.69%
Negative Predictive Value (*)	**100.00%**	
Accuracy (*)	**98.68%**	**92.89% to 99.97%**

DISCUSSION

Diagnosis of TBLN in India mainly depends on FNAC as it is simple, sensitive, and in expensive but often times, it is based mainly on suggestive features of tuberculosis such as epithelioid granuloma and caseous necrosis rather than depending on the direct detection of bacteria which might occur due to factors other than Tuberculosis and may produces in conclusive results in some instances. In the present study, both cytological and molecular methods were used for the accurate diagnosis of tubercular lymphadenitis.

In the present study, RT-PCR showed sensitivity of 100%, specificity of 96.55%. Positive predictive value of 97.92%, negative predictive value of 100% and an accuracy of 98.68% which was in an agreement with the studies of Hilda Fernandes et al,2020[5], Patwardhan et al 2011[6] and Baek et al,2000,[7] where Patwardhan et al, 2011 reported PCR sensitivity 90.1% and specificity 100% for the diagnosis of tubercular lymphadenitis, CH Baek found PCR sensitivity was76.4% and specificity 100%. In the study of hilda fernandes et al, 2020 found PCR sensitivity was 100% and specificity was 92.59%.

PCR showed maximum positivity in cases which were cytomorphologically categorized as tubercular lymphadenitis with features of epithelioid granulomas and extensive caseation necrosis, similar other results of Gupta et al[8]and Pahwa et al[9]. Gupta et al, 2017 and Pahwa et al, 2005 were reported less sensitivity and specificity compared to present study. In the study of Gupta et al, 2017 sensitivity was 77.3% and specificity was 69.6% and Pahwa et al,2005 also reported PCR sensitivity of 89.5% and specificity of 86.1% in a comparison to present study.

CONCLUSION

For extra pulmonary lymphadenopathy cases molecular methods are more reliable and sensitive methods in a comparison of cytological microscopic methods. Molecular detection required extensive lab setup, but it would be a great tool along with a combination of cytological method for the accurate diagnosis of tubercular lymphadenitis.

REFERENCES

1. Purohit M, Mustafa T. Laboratory Diagnosis of Extra-pulmonary Tuberculosis (EPTB) in Resource-constrained Setting: State of the Art, Challenges and the Need. J Clin Diagn Res. 2015;9(4):EE01-6.
2. Gupta, P.R. dm*. Difficulties in managing lymph node tuberculosis. Lung india 2004; 21(4):p 50-53.
3. Alves F, Baptista A, Brito H, Mendonça I. Necrotising granulomatous lymphadenitis. BMJ Case Rep. 2011; 8; bcr.11.2010.3548.

4. Koo V, Lioe TF, Spence RA. Fine needle aspiration cytology (FNAC) in the diagnosis of granulomatous lymphadenitis. Ulster Med J. 2006 Jan;75(1):59-64.
5. Hilda F, Utility of PCR in the Diagnosis of Tubercular Lymphadenitis on Fine Needle Aspirates. APALM. 2022;9(5);3571
6. Patwardhan S, B hide V, Bhargava P, Kelkar D. A study of tubercular lymphadenitis: A comparison of various laboratory diagnostic modalities with a special reference to tubercular polymerase chain reaction. Indian Journal of Medical Microbiology. 2011;29(4):389.
7. Baek C, Kim S, Ko Y, Chu K. Polymerase Chain Reaction Detection of Mycobacterium tuberculosis From Fine-Needle Aspirate for the Diagnosis of Cervical Tuberculous Lymphadenitis. The Laryngoscope 2000; 110(1): 30-34.
8. Gupta V, Bhake A. Reactive Lymphoid Hyperplasia or Tubercular Lymphadenitis: Can Real-Time PCR on Fine-Needle Aspirates Help Physicians in Concluding the Diagnosis?. Acta Cytologica. 2018;62(3):204-208.
9. Pahwa R, Hedau S, Jain S, Jain N, Arora V, Kumar N et al. Assessment of possible tuberculous lymphadenopathy by PCR compared to non-molecular methods. Journal of Medical Microbiology. 2005;54(9):873-878.