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Rapid Diagnosis of Tubercular Lymphadenopathy by Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and its Correlation with Ziehl-Neelsen Staining on Fine Needle Aspiration Cytology

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ABSTRACT

Introduction: 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. An early diagnosis is required for treatment and control of infection. Conventional microscopy and Ziehl-Neelsen (Z-N) staining has low sensitivity in cervical tuberculosis due to paucicellular nature of the disease. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) has a well established role in detection of pulmonary tuberculosis. Recently, WHO has recommended its use in extrapulmonary tuberculosis as an initial diagnostic tool.

Material and Methods: This is a prospective study performed in department of pathology and included 100 patients of suspected recurrence of tubercular lymphadenopathy. Fine needle aspiration was done and the material was sent for CBNAAT. The rest of the material was used for preparing giemsa stained and Z-N stained smears. Of these, 24 cases were positive for acid fast bacilli (AFB) by microscopy and Z-N stain while 41 cases were positive for tuberculosis by CBNAAT indicating a higher sensitivity of CBNAAT. This difference was statistically significant (p value <0.05)

Conclusion: CBNAAT is more sensitive for detection of recurrent cervical tuberculosis in comparison to conventional microscopy and ZN staining. This should be used as an initial diagnostic tool for recurrent tubercular cases in countries with high prevalence like India.

Keyword: recurrent, tubercular lymphadenopathy, CBNAAT, Ziehl-Neelsen stain, Fine needle aspiration.

INTRODUCTION

Tuberculosis is a global health problem with one third of world's population being infected with tuberculosis. The most common presentation is pulmonary tuberculosis. Extrapulmonary tuberculosis accounts for about 25% cases of tuberculosis. [1] WHO defines extrapulmonary tuberculosis as an infection by M. tuberculosis which affects tissues and organs outside the pulmonary parenchyma. Extrapulmonary tuberculosis is mostly underdiagnosed due to high rates of smear negativity in these cases. The most common site is lymph nodes followed by pleural fluid and other sites. [2] As the extrapulmonary sites are mostly smear negative, it is believed that these cases are less contagious and thus these are not a priority in the campaigns done by national TB control programs. [3,4] Early diagnosis and timely treatment is required in these cases to prevent further spread and reduce the mortality.

Fine needle aspiration cytology 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 its paucicellular nature. [5] Mycobacterial culture and drug susceptibility has a very long turnaround time of four to eight weeks. To overcome all these limitations a rapid and reliable method Cartridge Based Nucleic Acid Amplification Test (CBNAAT)/GeneXpert MTB/RIF1 (Cepheid, USA) was endorsed by WHO in 2010 for use in TB laboratories. It was introduced in INDIA by Revised National Tuberculosis Control Programme (RNTCP) in 2012 as a pilot project in Maharashtra. [6]

CBNAAT is a real time polymerase chain reaction based assay which provides diagnosis of TB and rifampicin resistance within 2hours. [7] It is a rapid and cost effective test. Thus, WHO recommends its use in multidrug resistant and HIV associated tubercular cases. In 2014, WHO has also recommended its use in non respiratory specimens from patients with extra pulmonary tuberculosis. [8] In the present study patients suspected to have tubercular lymphadenopathy were evaluated by conventional microscopy, Z-N stain and CBNAAT. Comparative analysis of the results of Z-N staining and CBNAAT were done.

MATERIAL AND METHODS

The study was conducted in Department of Pathology over a period of two months. It included 100 patients with cervical lymphadenopathy who were clinically suspected of having recurrence of tuberculosis those and who had previously completed antitubercular treatment (ATT) and now again presented with lymphadenopathy. Fine needle aspiration was done (1 to 2 passes) with the help of 24 G needle attached to a 5ml syringe and the material was used to prepare one giemsa stained smear for conventional microscopy, one for Z-N staining and rest of the aspirate was mixed with buffer in 1:2 ratio in a pre-sterilized container and incubated at room temperature for 30min. Two ml of this reagent sample mixture was then transferred to an Xpert cartridge using a Pasteur pipette and the cartridge was loaded onto Gene Xpertmachine (Cepheid, Dx System Version 4.0c). CBNAAT results were reported as negative or positive. It provides a semiquantitative estimate of the concentration of bacilli as defined by the cycle threshold (Ct) range (high, <16; medium, 16-22; low, 22-28; very low, >28). ZN stain was also reported as negative or positive as per RNTCP guidelines (Table 1 and 2.). Also, Rifampicin resistance results were reported as resistant or susceptible. Statistical analysis was done by SPSS software.

RESULTS

The study includes 100 suspected patients with recurrence of extrapulmonary tuberculosis in cervical lymph node. All the patients had received complete ATT previously. The aspirate was purulent (53), cheesy (28) or blood mixed (19). The age of patients ranged from 16 years to 58 years, of which 67 were males and 33 were females. On routine microscopy61 were necrotizing lymphadenitis, 20 cases of granulomatous lymphadenitis, 19 were of acute lymphadenitis. Of these 41 patients were positive for Mycobacterium tuberculosis these included 25 males and 16 females. Whereas only 24 patients were smear positive on ZN staining which included 15 males and 9 females. The positivity for Mycobacterium tuberculosis was 41% by CBNAAT and 24% by conventional ZN staining method. 17% cases were detected with tuberculosis with the help of CBNAAT only and were reported as negative on ZN smears.

This difference was statistically significant with p value <0.05 (Z score -2.5665). Among these 41 cases which were detected by CBNAAT 33 were sensitive to rifampicin and 8 were resistant (Figure 2). This comparison illustrated that CBNAAT is a better diagnostic tool to diagnose recurrent tubercular lymphadenopathy than the conventional ZN staining and microscopy.

Table 1. Grading of acid fast bacilli as per RNTCP guidelines.

No. of bacilli	Result	Grading	No. of fields
>10/field	Positive	3+	20
1-10/field	Positive	2+	50
10-99/field	Positive	1+	100
1-9/field	Positive	Scanty	100
No bacilli	Negative	-	1000

Table 2. Cycle threshold of CBNAAT positive cases.

Cycle threshold	No. of CBNAAT positive cases
Very low	24
Low	11
Medium	04
High	02
Total	41

Table 3. Combined results of CBNAAT and AFB positivity with Rifampicin sensitivity

Sensitivity	CBNAAT+	AFB+
RS	33	17
RR	08	07
Total	41	24

RS=rifampicin sensitive, RR- Rifampicin resistance, CBNAAT=Cartridge-Based Nucleic Acid Amplification Test, AFB= Acid fast bacilli.

DISCUSSION

Extrapulmonary tuberculosis is a major burden of mortality and morbidity due to its paucicellular nature, subclinical presentation and difficulties in diagnosis. The conventional microscopy, ZN staining and culture have low sensitivity and are very time consuming resulting in delay in treatment of these cases. CBNAAT provides early diagnosis along with rifampicin sensitivity which is clinically very helpful for patient management. It provides the results within 2 hours and plays an important role in diagnosis of extra pulmonary tuberculosis.

In the present study out of 100 patients 41 cases were positive for tuberculosis by CBNAAT out of which only 24 were detected by FNAC and ZN staining. This shows that CBNAAT is superior to conventional FNAC and ZN staining for diagnosis of tubercular lymphadenopathy. The most common presentation of head and neck tuberculosis is lymphadenopathy. Out of 41 positive cases on CBNAAT 13 were showing granulomatous reaction while rest were showing mainly necrosis. Mycobacterium tuberculi is rarely found in granulomatous lymph nodes but it can be easily detected by CBNAAT in these cases. Also, previous drug intake leads to fragmentation of the bacilli along with reduction in bacterial load which further reduces the chances of getting AFB on ZN stained smears. CBNAAT is found to be of great significance in these scenarios.

Total sample positivity in the present study is 41% by CBNAAT and only 24% by Z-N staining. A sample positivity of 17.6% was found in a study carried out by Gour Sanjay M et al which is very less than that observed in the present study. [9] The reason could be that the present study has included only the suspected cases of recurrent tuberculosis. In a study done by Chinedum OK et al. CBNAAT was positive in 65.7% cases as compared to smear examination which was positive in 38.6% cases when used to diagnose TB. [10] Bajrami R et al could detect M. tuberculosis in 29.3% cases by CBNAAT as compared to Z-N staining alone which was positive in only 14.6% cases. [11] Similarly in an another study done by Mavenyengwa R et al, 32.20% samples were found to be positive for TB by CBNAAT assay, and only 24.05% were found to be positive by microscopy. [12] All these studies were having same findings as of our study.

A study done Sharma et al, regarding to diagnosis of extra pulmonary tuberculosis, has shown an overall sensitivity of 71% and PPV ranging from 98 to 100%. [13] Also a meta-analysis done by Penz et al showed a sensitivity of 87% among lymph node specimen. [14]

Rifampicin resistance in the present study was 19.51% (8 cases out of 41). A study by D. Pragati Rao et al found 13.55% Rifampicin resistance and R Dewan et al found 25% of rifampicin resistance which was nearby similar to our findings. [15,16] Whereas R Tripathi et al found a very high (53%) rifampicin resistance in their study. [17] However, Gour Sanjay M et al found a very low rifampicin resistance of 6.38%. [9] This difference can be due to demographical variation of the populations included in the various studies. None of the case was CBNAAT negative and AFB positive in the present study.

Thus the present study highlights the impact of CBNAAT in diagnosis of recurrent tubercular lymphadenitis cases in resource poor countries like India, where tuberculosis is so prevalent. With early detection of these cases proper timely management could be done and further spread can be prevented.

CONCLUSION

CBNAAT is a sensitive, effective and rapid diagnostic tool for extrapulmonary tuberculosis as compared to FNAC and Z-N staining. This can diagnose more number of smear negative as well as multiple drug resistant tuberculosis very early. This provides an extra edge for tubercular management as these undiagnosed cases are of global concern. This should be used as an initial diagnostic tool for these cases.

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REFERENCES

1. World Health Organization. Global tuberculosis report 2012. Geneva, WHO, 2012. Available at: http://www.who.int/tb/publications/global_report/gtbr12_main.pdf.
2. Gupta S, Shenoy VP, Bairy I, Srinivasa H, Mukhopadhyay C. Diabetes mellitus and HIV as co-morbidities in tuberculosis patients of rural south India. J Infec Pub Heal. 2011 Aug 1;4(3):140-4.
3. World Health Organization. Tuberculosis programme: Framework for effective tuberculosis control. Geneva, Switzerland: WHO;1994:179.
4. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Ind J Med Res. 2004;120(4): 31653.
5. Annam V, Karigoudar MH, Yelikar BR. Improved microscopical detection of acid-fast bacilli by the modified bleach method in lymphnode aspirates. Indian J Pathol Microbiol. 2009;52:349-52.
6. Dewan R, Anuradha S, Khanna A, Garg S, Singla S, Ish P, Agarwal S. Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. JIACM. 2015;16(2):114-7.
7. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011;6(9):1067-82.
8. World Health Organization. Xpert MTB/RIF implementation manual: technical and operational 'how-to': practical considerations, 2014. Available at: http://www.who.int/tb/publications/xpert_implem_manual/en/.
9. Sanjay MG, Radha PM, Babu NP, Sushant M, Jitesh A. Genotypic diagnosis of extrapulmonary tuberculosis- CBNAAT a novel tool. Med Pulse Int J Med. 2017; 4(2);79-82
10. Chinedum OK, Emwiomwan A, Ifeanyi OE, Babayi A. Comparative Analysis of Ziehl-Neelsen and Genexpert Techniques for the Diagnosis of Tuberculosis in Human Immuno-Deficiency Virus Positive Patients in Benin City. Ann Clin Lab Res. 2017; 5(4):1–6.
11. Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L. Comparison of GeneXpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo. J Infect Dev Ctries. 2016;10(4):418–422.
12. Mavenyengwa R, Shaduka E, Maposa I. Evaluation of the Xpert MTB/RIF assay and microscopy for the diagnosis of Mycobacterium tuberculosis in Namibia. Infect Dis Poverty. 2017;13(6):1–5.
13. Sharma SK, Kohli M, Chaubey J, Yadav RN, Sharma R, Singh BK, et al. Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. EurRespir J. 2014 Oct;44(4):1090-3.
14. Penz E, Boffa J, Roberts DJ, Fisher D, Cooper R, Ronksley PE, James MT. Diagnostic accuracy of the Xpert MTB/RIF assay for extra-pulmonary tuberculosis: a meta-analysis. Int J Tuber Lung Dis. 2015 Mar;19(3):278-84.
15. Rao DP, Sowjanya KL. Role of CBNAAT in rapid detection of Mycobacterium Tuberculosis in PLHIV in a highly prevalent state. J Evid Based Med Healthc. 2012; 3(38):1896–1898.
16. Dewan R, Anuradha S, Khanna A, Garg S, Singla S, et al. Role of cartridge-based

nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. J Indian AcadClin Med. 2015;16(2):114–117.
17. Tripathi R, Sinha P, Kumari R, Chaubey P, Pandey A, et al. Detection of rifampicin resistance in tuberculosis by molecular methods: A report from Eastern Uttar Pradesh, India. Indian J Med Microbiol. 2016;34(1):92–94.

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