Section: Microbiology

Original Research Article

COMPARISON OF CBNAAT AND ZIEHL NEELSEN STAINED SMEAR MICROSCOPY IN DETECTION OF MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Background: Mycobacterium tuberculosis remains to be one of the most significant causes of death from an infectious agent. Smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive. Smear microscopy is the cornerstone for the diagnosis of TB in resource-limited settings but it has only modest sensitivity (35-80%). Rapid diagnosis of tuberculosis and detection of Rifampicin (RIF) resistance are essential for effective disease management. CBNAAT (Cartridge Based Nucliec Acid Amplification Test) also known as Gene Xpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens. **Aims Ans Objectives:** (1)To assess diagnostic usefulness of Gene Xpert MTB/RIF assay technique in management of mycobacterium tuberculosis. (2)To compare the rapidity and effectiveness of CBNAAT and ZN Stain smeared microscopy for mycobacterium tuberculosis. (3)To analyse epidemiology of CBNAAT positive cases of M. tuberculosis.

Materials And Methods: This prospective study is carried out using 750 sputum samples of suspected pulmonary TB patients from the time period of march 2021 to September 2021 (6 months). All the samples were subjected to Ziehl- Neelsen (ZN) stain and cartridge based nucleic acid amplification test (CBNAAT). They were compared for quantitative results. RESULTS: Out of 750 samples, MTB detected by CBNAAT were 116 while MTB detected by smear microscopy were 69. MTB detected cases with rifampicin sensitive were 105 (90.52%) and rifampicin resistant were 11 (9.48%). Most common age group was 18-60 yrs (78.44%), followed by below 18 yrs (12.93%), and above 60 yrs (8.62%). Males were more (64.66%) affected than females (35.34%).

Conclusion: Microscopy should not be the soul criteria to diagnose MTB as it can miss out many cases. Microscopy should be combined with CBNAAT owing to its high sensitivity and less turn over time.

Keywords: CBNAAT- Cartridge based nucleic acid amplification test, ZN stain- Ziehl Neelsen stain, MTB- Mycobacterium tuberculosis, RIF- rifampicin, TB- tuberculosis, RNTCP- Revised National Tuberculosis Control Programme, LJ medium- Lowenstein Jensen medium, PCR- Polymerase Chain Reaction, CT- Cycle Threshold, AFB- Acid Fast Bacilli.

INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality. significant global public health problem worldwide. According to the World Health Organization over the past two decades, tuberculosis remains a major global health problem among developing countries. Significant problem of the poor across the world - disease poverty trap.[1] Globally, India is a home for more than 25% of global Tuberculosis (TB) burden.[2] Out of the estimated global annual incidence of 9 million TB cases, India alone shares the incidence of 2.1 million cases/year (one fourth of global incidence).[2] RNTCP was launched in 1997 - most systemic and cost effective approach to revitalize the TB control programme in India.[3] Microscopic examination of sputum sample is the most commonly used screening test however requires a large no of bacteria (104 /ml of sputum).[4] Lowenstein Jensen (LJ) culture - Gold standard technique. Requires less no of bacteria 10-100 bacilli /ml for isolation. CBNAAT- early, affordable, easy diagnosis for TB.[5] Principle: real time PCR and reports can be given in 2 hrs.

CBNAAT test is routinely performed on the samples receives in tuberculosis laboratory in BJGMC. Hence this study was planned to see the concordance and efficacy of CBNAAT with smear microscopy for the diagnosis of TB at our set up along with future prospective of Tuberculosis Research.

Gene Xpert test is a semi-quantitative nested real-time PCR in-vitro diagnostic test with two uses:

1. The detection of Mycobacterium tuberculosis complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputum that are either acid-fast bacilli (AFB) smear positive or negative.
2. The detection of Rifampicin resistance associated mutations of the rpoB gene in samples from patients of Rifampicin resistance.[6]

Aim and Objectives

- To assess diagnostic usefulness of Gene Xpert MTB/RIF assay technique in management of mycobacterium tuberculosis.
- To compare the rapidity and effectiveness of CBNAAT and ZN Stain smeared microscopy for mycobacterium tuberculosis.
- Rapid detection of rifampicin resistance in smear-positive and smear-negative clinical specimens.
- To analyse Epidemiology of CBNAAT positive cases of M. tuberculosis.

MATERIALS AND METHODS

Study design

This is a prospective study. It is conducted at Department of Microbiology at B.J.G.M.C and SGH, Pune. Over a period of 6 months from march 2021 to september 2021

Sample size- Total sample size for this study is taken to be 750 as per the formula.

Sample size = z2 x (p) x (1-p)/c2 where z= 1.96, p is the prevalence in decimals which is taken to be 4 % (General population) and 25% in high risk group. C is the confidence level, expressed as decimal (0.05).

Inclusion Criteria

- Sputum samples of patients with clinical features suggestive of MTB
- Patients of all age groups of both sexes.

Exclusion Criteria

- Samples other than sputum sample.

Sample collection

Sputum samples were collected from 750 patients who were clinically suspected cases of M. TB and having symptoms of tuberculosis. Detailed history (Preformed questionnaire), findings of physical examination were recorded.

Early morning, deep coughed sputum specimens in sterile containers were included in the study.

Specimens were stored at 2-8°C in freezer till further processing. . However, the specimen can be safely stored at 35°C for three days.

Tests performed

After collection, Ziehl-Neelsen (ZN) staining was done on all samples in department of Microbiology and each sample was run on Gene Xpert.

RESULTS

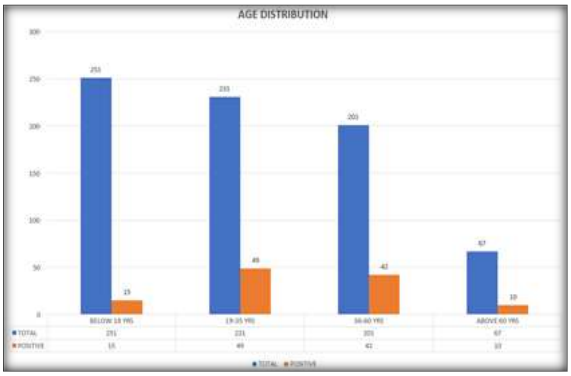

Figure 1: Age Distribution

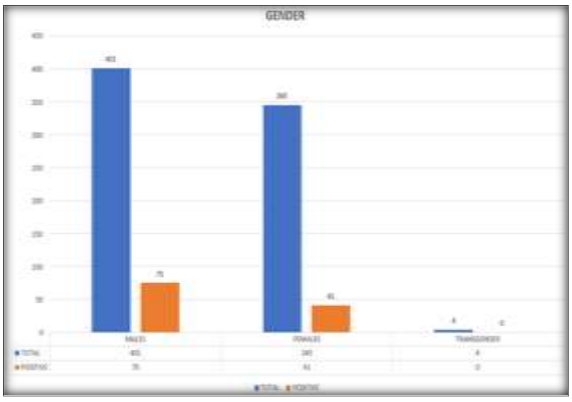

Figure 2: Gender Distribution

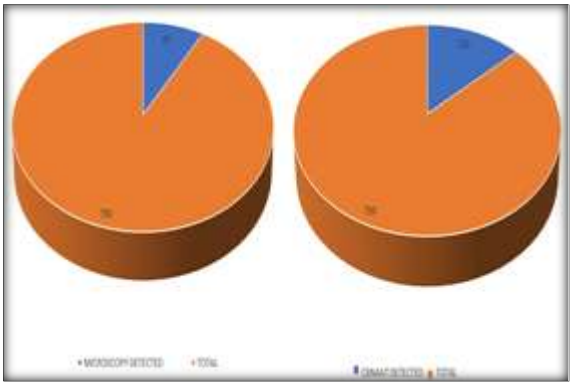

Figure 3:?

Figure 5:?

Figure 4:?

Figure 6:?

Table 1: ?

CBNAAT	MICROSCOPY	
POSITIVE	POSITIVE	69
NEGATIVE	NEGATIVE	634
POSITIVE	NEGATIVE	47
NEGATIVE	POSITIVE	00

The results of Gene Xpert and ZN staining are compared in our study. It is evident from the table that Gene Xpert MTB/RIF is more useful than ZN staining. As compared to ZN staining it can detect MTB even in 1ml of sputum.

CBNAAT also detects Rifampicin (RIF) resistance and helps us to diagnose multi-drug resistance tuberculosis (MDR TB).

Table 2: ?

RIFAMPICIN	MTB +VE	MTB -VE	TOTAL
SENSITIVE	105	634	739
RESISTANT	11	00	11
TOTAL SAMPLES	116	634	

Out of 47 samples which were smear negative but CBNAAT positive, about 25 samples were inoculated on LJ medium for culture and its was observed that the organism was grown in all the 25 samples which were CBNAAT positive but smear negative.

DISCUSSION

In this study, the performance of the CBNAAT with sputum specimens obtained during the clinical routine was compared with microscopy. In our study, the CBNAAT detected the agent in 15.46% (116/750) specimens, whereas sputum for AFB was able to detect only 9.2% (69/750) specimens. Out of 69 sputum smear positive cases, tuberculosis was detected in all 69 (100%) cases by CBNAAT and out of 681 sputum smear negative cases 47 (6.9%) cases were detected as positive by CBNAAT. In a study by Das & et al, smear positivity was found to be 17.6 % and positivity by CBNAAT was found to be 25.5% respectively. Slightly lower percentage in our study compared to the study by Das & et al is probably due to the large sample size in our study.[2] Similar finding was seen in the study by Salinita et al where MTB cases detected by CBNAAT were 29% where as MTB detected by microscopy were 19%.[11] Likewise similar findings in Sunil kumar et

al study where MTB detected by CBNAAT was in 19.4% cases and by microscopy was in 7.4% cases.

In our study, amongst the positive patients, male preponderance was seen with 64.65 %. Similar findings were seen in a study by Ashwini BS et al wherein 55% of the affected population was of males.[1] Study by Das et al revealed similar findings. In this study, positive rate was found to be more in age group 19-35 yrs, that is 21.21% (49/231), followed by 36-60 yrs (20.89%) then above 60 yrs (14.92%) and below 18 yrs (5.97%). Similar findings were observed in the study of Das et al and Ashwini BS et al and also Avashia et at. The CBNAAT test is easy to perform and is less dependent on the user's skills. Routine staff with normal training can perform the test. Technicians can be trained in 1-2 days; Only 2 steps (addition of buffer and sputum sample) are manual and the rest of the steps are automated. The results are available within 90 minutes. Each table top-sized module can process 4 samples at one time (larger modules can run 16 tests at one time), and because it is a closed system, biosafety and contamination concerns are minimized. It has a short turn-around time and simultaneously detects M. tuberculosis and RIF resistance in less than 2 hours. Although the CBNAAT could be a useful tool for rapid identification of RIF-resistant M. tuberculosis, the test results must always be confirmed by culture and DST. It takes about 4-6 weeks for the organism to grow on LJ medium for culture, it was difficult to follow up all the samples that we received, also there are higher chances of the sample getting contaminated. In that case, in order to compare sensitivity and specificity of CBNAAT and smear microscopy with culture, we took 25 samples which were found to be positive by CBNAAT but negative by smear microscopy and grew them on culture. And the result we found was that organism was grown on all the 25 cultures which were positive by CBNAAT but negative by microscopy making CBNAAT more sensitive and specific compared to microscopy.

As all the samples that we receive in our laboratory for MTB detection, we perform microscopy of all those samples and concurrently we perform CBNAAT of all the samples. Now from all the study and results we observed that microscopy gives no additional benefit over CBNAAT, moreover there are higher chances that we could miss the detection of organism on microscopy, and in contrast CBNAAT gives more sensitive, rapid and accurate results, it is better to perform only CBNAAT of all the sample and skip performing microscopy. Also we don't give DOTS therapy only on the basis of microscopy, we confirm all the samples by CBNAAT, then what is the need of doing microscopy?

CONCLUSION

This study is to convince that all the samples to be tested for MTB should be detected by CBNAAT alone, and if we want to eliminate performing microscopy, this is the right time. Also, the centers where CBNAAT is not available, and the detection of MTB is done by microscopy, it is necessary to provide CBNAAT set up at those centers and replace microscopy by CBNAAT machines for better results.

REFERENCES

1. OBEAGU DE. Comparison of Microscopic Methods with CBNAAT in Suspected Pulmonary Tuberculosis Patients among HIV Seropositive
2. Das PK, Das NS, Debnath J, Saha A. Comparative assessment of Cartridge based Nucleic Acid amplification Technique with Microscopy among Pulmonary TB Patients in a Tertiary care Hospital of Tripura
3. Editoreal: Recent Changes in Technical and Operational Guidelines for Tuberculosis Control Programme in India – 2016: A Paradigm Shift in Tuberculosis Control. The Journal of Association of Chest Physicians, Jan-Jun 2017 , Volume 5, Issue 1
4. R Dewan, S Anuradha, A Khanna, S Garg, S Singla, P Ish, S Agarwal,A Narayana H, M Hanif, H Singh, S Uppal. Role of cartridge- based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. JIACM 2015; 16(2): 114-7
5. Monika Agrawal, Ashish Bajaj, Vinay Bhatia, Sarjana Dutt. Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis.JCDR2016;10(5):9-12
6. Sowjanya DS, Behera G, Ramana Reddy VV, Praveen JV. CBNAAT: a novel diagnostic tool for rapid and specific detection of mycobacterium tuberculosis in pulmonary samples. Int J Health Res Modern Integr Med Sci (IJHRMIMS), ISSN. 2014:2394-8612.
7. Boehme CC, Nicol MP, Nabeta P et al. Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study.
8. Helb D et al. 2010. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of ondemand, near- patient technology. J Clin Microbiol. 48: 229–237.
9. Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert (R) MTB/ RIF assay. J Clin Microbiol. 2010; 48:249–251.
10. Bodmer T, Ströhle A. Diagnosing Pulmonary Tuberculosis with the Xpert MTB/RIF Test. J Vis Exp. (62), e3547, doi: 10. 3791/3547
11. Salinita N, Supriya L, Usharani M, Singh HL. Detection of pulmonary tuberculosis using cartridge based nucleic acid amplification test (CBNAAT) and fluorescent microscopy. IJHRMLP. 2018 Jan 1;4:78-81.