Original Research

Prevalence & Positivity Of Cbnaat (Cartridge Based Nucleic Acid Amplification Test) In Extra-Pulmonary Tuberculosis In Correlation With Smear Microscopy At Chirayu Medical College & Hospital, Bhopal

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ABSTRACT

Background: Tuberculosis is a major global health concern, particularly in India, where it accounts for 10-15% of total TB cases. Extrapulmonary tuberculosis (EPTB) accounts for 20% and are often undetected due to its diverse clinical presentation and lack of diagnostic means. Mycobacterium tuberculosis complex organisms (MTBC) cause TB primarily in the lungs but can affect other organs, causing EPTB. Diagnosis of EPTB is challenging due to low numbers of MTB bacilli and difficulty in obtaining clinical specimens. Early detection of tuberculosis and multidrug resistance is crucial to reduce complications and mortality. Conventional microscopy and culture are essential for early diagnosis, but they are expensive, time-consuming, and require a long time to detect bacilli. The GeneXpert MTB/RIF assay, an automated real-time PCR system, has high sensitivity and specificity for the diagnosis of MTB as compared to ZN smear microscopy that can detect M. tuberculosis and rifampicin resistance with less turnaround time (two hours),which has proven itself as an effective tool in initiation of early treatment of suspected extra-pulmonary tuberculosis.In this study we compare the CBNAAT positive results with ZN staining to understand the sensitivity percentage. **Aim:** This study, at Microbiology department of Chirayu Medical College & Hospital, India, aimed to understand CBNAAT positivity prevalence in extra-pulmonary tuberculosis cases, and correlate it with smear microscopy from February 2023 to July 2023. Data was collected, analysed, and a correlation was established between CBNAAT and ZN microscopy. **Method:** This study involved 565 samples of suspected extra-pulmonary tuberculosis cases. The samples received from IPD and OPD were divided into two parts;one for ZN staining and the other for GeneXpert. The GeneXpert MTB/RIF assay runs within 2 hours and results are interpreted as the presence of MTB with RIF resistance detection. For ZN staining, fluid specimens are concentrated by centrifugation and the sediment was used directly. Smears are prepared and stained with Ziehl-Neelsen staining method according to NTEP guidelines. The statistical data collected were analysed for sensitivity and specificity for GeneXpert assay and ZN staining. The project analysis focused on positive cases obtained from GeneXpert MTB/RIF results. **Results:** In this study of six-month period, 565 patient samples found 105 (18.6%) cases of CBNAAT as positive reports, while 460 (81.4%) as negative reports. The majority of positive cases were from the IPD, with a gender distribution of 41 males and 64 females. The age distribution was 0-20 years, with 21 patients aged 0-20 years and 50 patients aged 31-60 years. The study found that only 2.9% of Ziehl-Neelsen staining cases showed positivity, indicating that the CBNAAT was able to detect Mycobacterium tuberculosis (MTB) at high and medium detection levels. The CBNAAT was able to achieve very low detection levels, with 97% of RIF susceptibility and only 5% resistance. The CBNAAT is an important development in rapid molecular TB diagnostics, endorsed by the World Health Organization (WHO) as an effective molecular technique for early diagnosis, treatment, and control of TB. It detects DNA specific for Mycobacterium tuberculosis by polymerase chain reaction. **Conclusion:** The GeneXpert MTB/RIF assay is a reliable method for detecting EPTB with high sensitivity and specificity, making it suitable for resource-constrained settings. Its fast turnaround time and minimal infrastructure requirement make it ideal for areas with high risk of MDR-TB or HIV-associated TB. CBNAAT, a more sensitive technique, has a higher positivity rate than AFB smear, making it a better choice for rapid disease diagnosis.

Keywords: Mycobacterium tuberculosis (MTB), Extrapulmonary tuberculosis (EPTB), Cartridge based nucleic acid amplification test (CBNAAT), GeneXpert MTB/RIF, Ziehl-Neelsen staining (ZN).

INTRODUCTION

Tuberculosis is a major health concern worldwide and it is a great public health problem in India, leading to a large number of deaths, and hence is a great health hazard**.**[1] In India EPTB constitutes 10-15% of total TB cases which primarily involve the pleura, lymph nodes, gastrointestinal tract and other organs with a significant case mortality rate (25-50%). Extra-pulmonary tuberculosis accounts for 20% cases which remain undetected due to its diverse clinical presentation and lack of diagnostic means.[2]Mycobacterium tuberculosis complex organism (MTBC) cause TB primarily in the lungs but can also affect other organs, causing extrapulmonary tuberculosis (EPTB). Extra pulmonary tuberculosis is defined according to WHO classification criteria as an infection by Mycobacterium tuberculosis which affects tissues and organs outside the pulmonary parenchyma.[3]EPTB can be either primary (at the site of initial infection) or secondary (disseminated), which usually occurs due to hematogenous or lymphatic spread of bacteria from the primary organ, reactivation of latent TB, ingestion of infected sputum, or spread locally from adjacent organs.[4]Diagnosis of extrapulmonary tuberculosis (EPTB) remains especially challenging since the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain.[5] In such situation, not only early diagnosis and treatment is very crucial but also may save many lives. To reduce all complications and mortality due to it, early detection of tuberculosis and multidrug resistance is a priority in diagnosis of tuberculosis. Conventional microscopy and culture are very important in the early diagnosis of this infection. Culture is the gold standard of diagnosis, being able to diagnose both viable and drug-sensitive/ resistant bacteria, but it is expensive, time consuming, and requires a long time (about 6–8 weeks) to detect the bacilli.[6] On the other hand, conventional staining by Ziehl–Neelsen stain is simple, easy, cheap, and fast, but it has low sensitivity.To overcome these limitations, CBNAAT or GeneXpert MTB/RIF assay, a fully automated real-time semi-nested polymerase chain (PCR) system give results within 2 hours, and detects RIF resistant gene. The high performance of GeneXpert MTB/RIF in TB samples is well established, and the same principle was applied to extrapulmonary cases where the diagnosis is difficult because of the paucibacillary nature of the infection and the presence of a variety of clinical features. All clinically suspected cases of EPTB samples from OPD and IPD patients for the study period of 6 months from February 2023 to July 2023, were received at Department of Microbiology at Chirayu Medical College & Hospital, Bhopal, India. The Ethics Committee approved the research study (CMCH/RC/2023/159) with research project No. 2023/192. All cases with clinical suspicion of EPTB were enrolled regardless of gender and age. Details of patients including clinical history, demographic profile, socio-economic status, and contact numbers were also recorded. The exclusion criteria included patients already diagnosed with EPTB and on anti-tubercular treatment along with patients of suspected cases for pulmonary TB. The samples received for GeneXpert MTB/RIF assay were also subjected to Ziehl-Neelsen staining. The data were recorded in a master chart using a Microsoft excel and correlation was analysed. The sensitivity and specificity of CBNAAT (GeneXpert) was compared with the diagnostic accuracy of ZN staining microscopy Aim of this study was to comprehend CBNAAT (Cartridge Based Nucleic Acid Amplification Test) positivity prevalence for extra-pulmonary tuberculosis cases and its correlation with smear microscopy at Chirayu Medical College & Hospital in Bhopal, IndiaObjective for the study were to identify samples of extrapulmonary tuberculosis suspected patients that were obtained at the microbiology lab for GeneXpert MTB/RIF from the IPD and OPD. To confirm Mycobacterium tuberculosis and rifampicin resistance using GeneXpert in comparison to ZN smear microscopy. To collect data, provide statistical analysis, and establish a correlation between CBNAAT and ZN microscopy. The need of the study was to help in diagnosis of extra-pulmonary tuberculosis (EPTB) even in the paucibacillary nature if disease and mutations that confer rifampicin resistance, to a high degree of specificity. This study will assess the value of GeneXpert in diagnosis of Mycobacterium tuberculosis complex in comparison with Ziehl-Neelsen smear microscopy and to observe the additional diagnostic value of the technique. This study will help for an early and accurate diagnosis and drug susceptibility testing which is essential to initiate the correct treatment regimen without delay.

MATERIALS AND METHODS

This study was conducted at Chirayu Medical College & Hospital in Bhopal, India for the period February 2023 to July 2023. During this time period, a total of 565 samples of suspected extra-pulmonary tuberculosis cases were received at the microbiology lab for Gene Xpert MTB/RIF. The suspected cases of EPTB samples obtained were from patients, regardless of gender and age, visiting OPD and admitted to IPD. The patients exempted for this study were those who were already diagnosed with EPTB

and on anti-tubercular treatment and patients with suspected cases of pulmonary TB. All the 565 samples were subjected to Ziehl-Neelsen (ZN) staining along with CBNAAT (Gene Xpert MTB/RIF). The samples received were of CSF, ascitic fluid, pleural fluid, synovial fluid, drain fluid, tissue/biopsies,gastric/lymphnode aspirates, endometrial tissue and pus. The samples received were divided into two parts; one for the ZN staining and other to be used for Gene Xpert MTB/RIF.In case of Gene Xpert MTB/RIF, the cartridges used were of version 5.3 according to the manufactures' recommendations. To inactivate mycobacteria and liquefy the samples, a 2:1 mixture of isopropyl alcohol and NaOH was added to the clinical specimen. Biopsy specimens were chopped into extremely small pieces using a sterile blade in a sterile petri dish, a 2:1 volume of sample reagent (SR) buffer was added for tissue and biopsy specimen. With the exception of CSF, which is often less than 1 ml, all fluids were treated immediately by adding a 2:1 amount of SR buffer. The sample-SR mixture was shaken vigorously and incubated for 10 minutes before being shaken again and kept at room temperature for another ten minutes. 2 ml of the digested material were added to the cartridge. After that, the cartridge was put into the GeneXpert device, where the rest of the process happened automatically. If there was enough material available, the sample was processed again and restarted in the event that the results were indicated as invalid, error, or no result. The Gene Xpert MTB/RIF assay run completes within 2 hours and the results are interpreted as presence of MTB; High, Medium, Low and Very low with RIF susceptible/resistant with respective MTB detected loads, and no result. In case of ZN staining, fluids specimens were concentrated by centrifugation and the sediment was used directly for ZN staining, and for tissue specimens, they were torn apart with the help of sterile needles placed in a petri dish with sterile water (2-4ml). Smears were prepared and stained with Ziehl-Neelsen staining method as per the guidelines of NTEP. The results are positive if Acid Fast Bacilli (AFB) are seen and negative if not present. The statistical data collected were analysed for sensitivity and specificity for Gene Xpert assay and ZN staining. The quantitative data was collected in tabulation form using Microsoft Excel and were correlated, calculated and visually presented through charts (graph, bar & pie). The project analysis was focused on the positive cases obtained from Gene Xpert MTB/RIF results.

RESULTS

A total of 565 patient samples were received during the 6 months study period and they were all subjected to CBNAAT analysis and ZN staining. From the total samples received, the focus of the study was on the positive cases of CBNAAT and its correlation with ZN staining. Of the total samples received,CBNAAT analysis shows 105(18.6%) cases aspositive results and 460 (81.4%) cases asnegative results as shown in Table 1.

Month	Total Test	Total Positive cases	Total Negative cases
February	84	28	56
March	102	15	87
April	130	24	106
May	97	17	80
June	74	10	64
July	78	11	67
Total	565	105	460
Percentage	18.6%	81.4%	

Table :1 shows total positive and negatives cases with their respective percentage in CBNAAT analysis.

Month	Male	Female
February	9	19
March	5	10
April	11	13
May	6	11
June	4	6
July	6	5
Total	41	64
Percentage	39%	61%

In the total CBNAAT positive results, only 4 cases were from the OPD and the rest 101 cases were from the IPD. In terms of gender distribution of CBNAAT positive results, 41 patients were males while 64 were females contributing to 39% and 61% respectively as seen in Table 2.

Age Group	No. of patients
0-20	21

21-30	25
31-60	50
>60	9
Total	**105**

Table :2 (left)& 3 (right) shows gender and age distribution of CBNAAT positive cases respectively.

In case of the age distribution in CBNAAT positive results, 21 patients fall into the age bracket of 0-20 years, 25 patients in the age group of 21-30 years, while 31-60 age group showed 50 patients and only 9 patients were found to be above 60 years as shown in Table 3.Of the total 105 positive CBNAAT cases, 7 samples were received from CSF, 36 samples were body fluid comprising of Ascitic, Pleural, Synovial and Drain constituting to 14, 18, 3 and 1 respectively, 5 samples were tissue and biopsy, 15 samples were of other aspirates (gastric and lymph node), while 8 and 34 were from pus and endometrial tissue respectively as shown in the Figure 1 and Table 4.

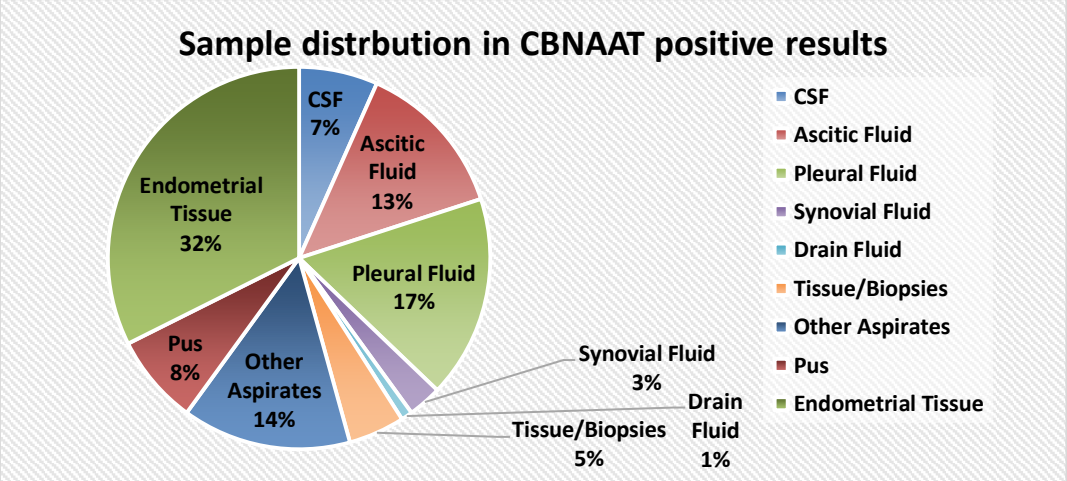

Figure 1: Distribution of extrapulmonary samples

Samples	No. of CBNAAT positive results	% contribution
CSF	7	6.7%
Ascitic Fluid	14	13.3%
Pleural Fluid	18	17.1%
Synovial Fluid	3	2.9%
Drain Fluid	1	1.0%
Tissue/Biopsies	5	4.8%
Other Aspirates	15	14.3%
Pus	8	7.6%
Endometrial Tissue	34	32.4%
Total	105	100%

Table 4 shows number of various extrapulmonary samples received.

Out of the total 105 positive cases of CBNAAT, only 2 (1.9%) cases of Ziehl-Neelsen (ZN) staining showed positivity. In this study result, these two positive cases of ZN staining correlated, only with the High and Medium detection level of Mycobacterium tuberculosis (MTB) with CBNAAT analysis, and of sample type pleural fluid and pus respectively, where RIF was found to be susceptible.

Samples	CBNAAT							ZN staining	
	MTB Detected					RIF Resistant		Positive	Negative
	High	Medium	Low	Very Low	Trace	Detected	Not detected		
CSF	--	--	3	--	--	--	3	--	3
CSF	--	--	--	3	--	--	3	--	3
CSF	--	--	--	--	1	--	1	--	1
Ascitic Fluid	--	--	1	--	--	--	1	--	1

Ascitic Fluid	--	--	--	6	--	--	6	--	6
Ascitic Fluid	--	--	--	--	7	--	7	--	7
Pleural Fluid	1	--	--	--	--	--	1	1	--
Pleural Fluid	--	--	3	--	--	--	3	--	3
Pleural Fluid	--	--	--	3	--	--	3	--	3
Pleural Fluid	--	--	--	--	11	--	11	--	11
Synovial Fluid	--	--	1	--	--	--	1	--	1
Synovial Fluid	--	--	--	--	2	--	2	--	2
Drain Fluid	--	--	--	1	--	--	1	--	1
Tissue/Biopsies	--	--	2	--	--	--	2	--	2
Tissue/Biopsies	--	--	--	1	--	--	1	--	1
Tissue/Biopsies	--	--	--	--	2	--	2	--	2
Gastric Aspirate	--	--	1	--	--	--	1	--	1
Gastric Aspirate	--	--	--	6	--	--	6	--	6
Gastric Aspirate	--	--	--	--	8	--	8	--	8
Pus	--	1	--	--	--	--	1	1	--
Pus	--	--	7	--	--	--	7	--	7
Endometrial tissue	--	--	4	--	--	1	3	--	4
Endometrial tissue	--	--	--	10	--	1	9	--	10
Endometrial tissue	--	--	--	--	20	--	20	--	20
Total	**1**	**1**	**22**	**30**	**51**	**2**	**103**	**2**	**103**

Table 5 shows result data for CBNAAT and ZN staining.

CBNAAT was able to perform low detection levels, with 21 (95%) cases of RIF susceptible and only 1 (5%) case of resistance. The CBNAAT was also able to achieve very low levels of detection with 29 (97%) cases of RIF susceptibility and only 1 (3%) case of resistance as shown in table 5. The two cases of resistance seen in both levels of detection, i.e. low and very low, were observed in the endometrial tissue sample received from gynaecology department.

DISCUSSION

Tuberculosis (TB) remains a key challenge in the face of global public health and inadequate diagnostic assays have hampered our chances to tackle this disease effectively. Extra pulmonary tuberculosis accounts for 20% of total burden of tuberculosis globally it is estimated that approximately 70 million people will die from tuberculosis within the next 20 years and it is because of inadequate measures for TB control. As the number of bacilli are very less in extrapulmonary samples and because of difficulty in obtaining tissues from deep seated organs; diagnosis is delays in most cases. The CBNAAT marks an important development in the field of rapid molecular TB diagnostics. This assay was endorsed by WHO as an effective molecular technique in the early diagnosis, treatment and control of TB globally. This assay was a clear replacement, earlier for sputum smear microscopy, particularly in setting with high rates of HIVassociated TB and multidrug resistant TB developed for testing sputum samples, followed by its use in extrapulmonary samples where the assay has shown results with high sensitivity and specificity. This multifunctional diagnostic platform is an automated, closed system that perform real time PCR and can be used by operator with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hours.[7] This test detects DNA specific for Mycobacterium tuberculosis by polymerase chain reaction. Extra-pulmonary TB is far more complex because of thediversity of clinical sample types, difficulties in obtaining adequate tissue for analysis and in the extraction of M. tuberculosis DNA from the samples.

Culture technique being comparatively slow and complex, requires specialised laboratories and skilled staff. The *Mycobacterium* bacilli take days to grow in liquid media and 4-8 weeks in solid media.[8]Smear microscopy's primary flaws are to differentiate

between MTB complex and NTM (Nontuberculous mycobacterium) and also its inability to identify rifampicin resistance. Misdiagnosis of smears could be the reason for the false positive cases. Technical mistakes such as insufficient slide preparations, poor staining technique, observational inaccuracy, etc. could be to blame for the false negative cases. A repeat sample is advised or CBNAAT should be performed to confirm the diagnosis in the event of a false positive or suspected case in order to avoid needless medical intervention, pharmacological side-effects, and stress on the patient and their family.[9]Molecular method (CBNAAT) is a rapid diagnostic tool for both smear negative or positive patients clinically suspected with TB and it also gives information about rifampicin resistance. This study findings suggest that CBNAAT has higher sensitivity and specificity for detection of extrapulmonary tuberculosis cases compared to ZN staining. In our study the age distribution in CBNAAT positive results, 21 patients fall into the age bracket of 0-20 years, 25 patients in the age group of 21-30 years, while 31-60 age group showed 50 patients and only 9 patients were found to be above 60 yearsIn case of the age distribution in CBNAAT positive results, 21 patients fall into the age bracket of 0-20 years, 25 patients in the age group of 21-30 years, while 31-60 age group showed 50 patients and only 9 patients were found to be above 60 years. Arora & Gupta (2006)[10], conducted a study on trends of EPTB and found higher detection of EPTB cases in younger age group.

Our study findings suggest that CBNAAT has higher sensitivity for detection of pulmonary and extrapulmonary tuberculosis cases. The WHO 2012 has also recommended the CBNAAT for routine use under programmatic conditions.[11]In this study CBNAAT clearly shows results of two cases with high and medium levels of Mycobacterium tuberculosis which were also seen positive in ZN staining due to the presence of more than 10^4 - 10^5 per ml of Acid-fast bacilli in sample. But in paucibacillary cases, ZN staining showed all negatives results while the CBNAAT was able detect 131 bacilli per ml of specimen, being categorised in low and very low detection levels as can be seen in table 5. Therefore, as per the CBNAAT positive reports, the overall sensitivity of CBNAAT is 100% compared to ZN staining which was at 1.9%.

CONCLUSION

GeneXpert assay has proved to be a reliable method for the detection of EPTB and has high sensitivity and specificity compared to ZN-smear techniques.It is recommended even in resource-constrained settings due to its feasibility, fast turnaround time, and minimal infrastructure requirement. It is especially helpful in areas with a high risk of MDR-TB or HIV-associated TB. CBNAAT will help in the timely initiation of effective treatment in extra-pulmonary TB patients and rifampicin-resistant TB cases. AFB smear technique, though cost-effective, has low sensitivity as compared to CBNAAT in the diagnosis of EPTB. This present study revealed that more positivity rate by CBNAAT in comparison to ZN staining indicates that it is a more sensitive technique as compared to conventional methods.To sum up, CBNAAT or Gene Xpert assay has the advantages of less turn-around time (two hours) for detecting TB with high sensitivity of EPTB detection with simultaneous assessment of rifampicin resistance. Although the role of culture remains central in the microbiological diagnosis of EPTB, the sensitivity of Gene Xpert in rapid diagnose of disease makes it a much better choice compared to smear microscopy. The ability to rule out the disease still remains suboptimal, therefore fast diagnosis is crucial and thus has potential to replace the gold standard culture method.

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CONFLICT OF INTEREST

None declared

ETHICAL APPROVAL

The study was approved by the Research Committee at Chirayu Medical College & Hospital in Bhopal, India.

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