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Original article

EARLY MONITORING, EARLY TREATMENT CAN END TB- RAPID MOLECULAR DETECTION OF MDR-TB AND XDR-TB IN ADILABAD DISTRICT, TELANGANA STATE.

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ABSTRACT

BACKGROUND: *Tuberculosis is one of the deadliest bacterial communicable diseases in the world. Early detection and monitoring of active Tuberculosis cases can promote prompt recovery and reduce transmission. This study aims to demonstrate the molecular diagnostic methods' superiority over the conventional phenotypic methods in the diagnosis of MDR-TB and XDT-TB especially in endemic area of Adilabad district.*

METHODOLOGY: *A cross-sectional analysis of 1529 patients who attended RIMS General Hospital, Adilabad was done. The period of data collection was from Jan to Dec 2023.A simple sampling technique was employed and all patients underwent fluorescent staining, TrueNat MTB/MTB plus, MTB RIFDx, GeneXpert CBNAAT(Cepheid) and MGIT.*

RESULTS: *Of the total 1529 samples examined using three different platforms, fluorescent microscopy (FM) revealed 73(4.7%) positive results for tuberculosis, GeneXpert CBNAAT showed 793(51.8%) positive results for tuberculosis and TrueNat (Molbio) showed 119(7.7%) positive for tuberculosis. Follow up of 80 TB positive cases after receiving treatment done with BACTECT MGIT, 44 cases (55%) were detected to be MDR-TB cases. According to this study results, GeneXpert CBNAAT showed higher specificity and sensitivity compared to TrueNat and FM.*

CONCLUSIONS: *In TB endemic areas like Adilabad, Early monitoring by rapid molecular detection of MDR-TB and XDR-TB led to early treatment which can fasten the elimination and minimize the community transmission to finally END TB.*

Keywords*: Tuberculosis, Rapid molecular diagnosis, MDR and XDR-TB*

Introduction:

Tuberculosis (TB), caused by the mycobacterium tuberculosis complex, is one of the oldest diseases known to humanity and ranks as the leading cause of infectious mortality globally, excluding COVID-19. TB is a communicable disease that spreads through the air. Approximately 85-90% of cases manifest as pulmonary TB (PTB), which affects the lungs, while extrapulmonary TB (EPTB) accounts for about 15-20% of cases, impacting areas such as the pleura, lymphatic system, central nervous system, bones and joints, and the genitourinary system. This disease poses a significant health challenge in developing nations, with around 10 million individuals diagnosed with TB annually, representing one-third of the global population. The rise in TB cases in underdeveloped regions can be attributed to inadequate sanitation, overcrowding, and a higher prevalence of HIV infection. India bears a substantial share of the global TB burden, accounting for one-fourth of all cases. According to the WHO's 2018 report, India had the highest number of TB patients worldwide. By 2019, the country also reported the largest incidence of drug-resistant TB. The number of notified TB cases rose to 2.15 million in 2019, compared to 1.83 million in 2018, largely due to advancements in early diagnosis methods such as the rapid cartridge-based nucleic acid amplification test (CBNAAT) and the TrueNat chip-based nucleic acid amplification technique[2].The Xpert MTB/RIF assay utilizes five unique molecular beacons, which are nucleic acid probes, each tagged with a distinctively colored fluorophore that targets a specific nucleic acid sequence within the rpoB gene of Mycobacterium tuberculosis. This assay is capable of identifying tuberculosis and rifampicin resistance in under two hours, directly from untreated sputum samples[7]. With prompt detection and appropriate treatment, the majority of tuberculosis cases can be effectively cured, thereby minimizing the risk of further transmission[2]. The diagnosis of tuberculosis has progressed into a phase of molecular detection, offering quicker and more economical approaches to diagnose and confirm drug resistance in tuberculosis cases. In contrast, traditional culture methods, while regarded as the gold standard, necessitate several weeks for results. Recent advancements in molecular detection techniques, such as the expedited and

simplified nucleic acid amplification test (NAAT) and whole-genome sequencing (WGS), have significantly reduced the time required for diagnosis, leading to swifter treatment for tuberculosis [3]. Tuberculosis (TB) is both preventable and treatable [4]. However, cases of TB that remain untreated or those that experience interruptions in therapy can lead to the development of multi-drug resistant (MDR) or extensively drug-resistant (XDR) strains, complicating treatment efforts. Advanced molecular diagnostic tools, such as nucleic acid amplification tests (NAAT) and the liquid culture system provided by MGIT BACTEC, are employed for the identification of mycobacteria. These methods enhance the sensitivity and specificity of drug resistance detection in various strains, while also offering a significantly reduced turnaround time, thereby enabling quicker and more accurate identification of Tuberculosis. Timely detection of TB infections and the immediate commencement of treatment are crucial in lowering both mortality rates and the incidence of drug resistance associated with Tuberculosis. The rise of drug-resistant TB, particularly MDR-TB—which is characterized by resistance to at least isoniazid and rifampicin—and XDR-TB—which includes MDR-TB plus resistance to any fluoroquinolone and either kanamycin, amikacin, or capreomycin—poses a significant challenge to global TB control efforts, primarily due to the complexities involved in diagnosis and treatment. Although the latest liquid culture diagnostics and molecular line probe assays offer advanced capabilities, their high costs and the requirement for biosafety measures and skilled personnel limit their application in resource-constrained environments. This study aims to demonstrate the advantages of new molecular techniques over traditional phenotypic methods in the diagnosis of MDR-TB and XDR-TB.

METHODS

The study was conducted for a period of one year from Jan 2023 to Dec 2023, from patients who attended District Microscopy Center, RIMS General Hospital, Adilabad. The patients with presumptive diagnosis of TB including pulmonary and extrapulmonary tuberculosis based on history, clinical presentation, radiological findings were included in the study. A total of 1592 samples collected from suspected patients were included in the present study. A simple sampling technique was employed and all patients underwent fluorescent staining, True Nat MTB/MTB plus, /MTB RIFDx, GeneXpert CBNAAT(Cepheid) and MGIT at TB, C&DST Laboratory. Rajiv Gandhi Institute of medical sciences, Adilabad, Telangana State. The corresponding specimens like sputum 5ml or 2 ml of fluid (CSF, pus, ascitic fluid, and pericardial fluid) and aspirate from lymph nodes were collected according to standard protocol. All samples were subjected to fluorescent staining, TrueNat MTB/MTB plus/MTB RIFDx, CBNAAT (GeneXpert). Follow up of 80 TB positive cases after receiving treatment were done with for MDR-TB cases.Fluorochrome stain is more sensitive than Ziehl-Neelsen stain because the smear spreading over an area approximately 1 x 2 cm. can be examined under a lower power objective, thus more fields can be read in the same amount of time, and the bacilli stand out brightly.

STATISTICAL ANALYSIS

The statistical analysis was done using statistical package for the social sciences (SPSS) version 20.0

Result:

A total of 1,529 individuals exhibiting clinical symptoms such as fever, cough, anorexia, weight loss, and various radiological findings were evaluated at the TB Testing Laboratory-RIMS in Adilabad from January 2023 to December 2023. This study encompassed all specimens related to both pulmonary and extrapulmonary tuberculosis. Each of the 1,529 specimens underwent testing using LED Fluorescent Microscopy, the Truenat Molbio chip-based nucleic acid amplification method, the GeneXpert CBNAAT-MTB/RIF assay, and the MGIT BACTECT liquid culture system. The most frequently affected age group was found to be under 20 years (1%), followed by those aged 21-40 years (16%), 41-60 years (56%), and over 61 years (15%). The majority of the patients were female (74%), while males constituted 26% (Figure 2). The samples collected included lymph node aspirates (50, 6.3%), sputum (1,149, 75%), cerebrospinal fluid (40, 2.6%), pus (250, 16%), ascitic fluid (20, 1.3%), and pericardial fluid (20, 1.3%). Testing across three different platforms revealed that fluorescent microscopy identified 73 positive cases (4.7%) for tuberculosis, True Nat (Molbio) detected 119 positive cases (7.7%), and GeneXpert CBNAAT identified 793 positive cases (51.8%). A follow-up of 80 TB-positive patients who received treatment via the BACTECT MGIT method indicated that 44 cases (55%) were identified as multidrug-resistant tuberculosis (MDR-TB). The findings of this study suggest that GeneXpert CBNAAT demonstrated superior specificity and sensitivity compared to True Nat and fluorescent microscopy (Figure 1). Furthermore, the fluorochrome stain proved to be more sensitive than the Ziehl-Neelsen stain, as the smear

covering an area of approximately 1 x 2 cm can be examined under a lower power objective, allowing for the review of more fields in the same timeframe, with the bacilli appearing distinctly.

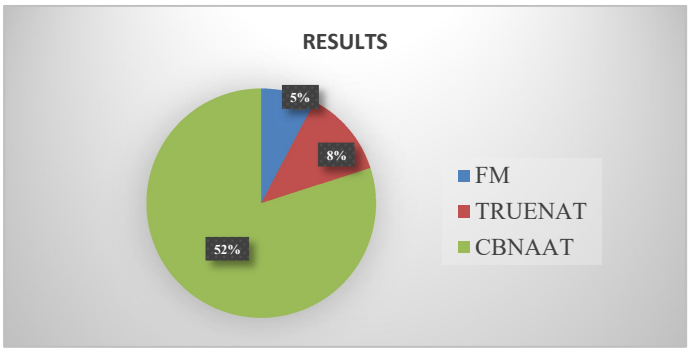

Figure 1: Molecular diagnostic tests for Tuberculosis

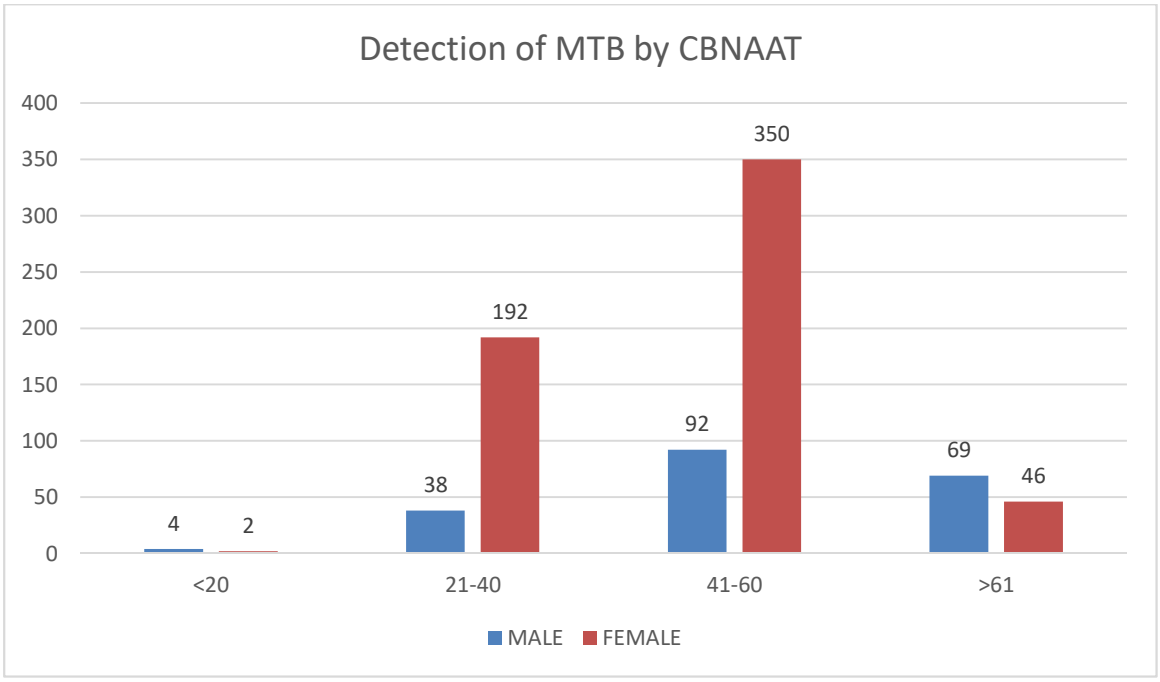

Figure2: Age and sex wise detection of MTB by GeneXpert CBNAAT

Discussion:

Tuberculosis is projected to account for approximately 10.6 million cases worldwide, with India reporting 2,537,235 cases in 2023. In the Adilabad district of Telangana, which has a population of 830,313, there were 1,529 confirmed cases within the year spanning from January to December 2023. The World Health Organization recommends the use of rapid molecular assays for the diagnosis of Tuberculosis, favoring them over smear microscopy due to their superior accuracy in early detection and treatment. Consequently, a significant number of undiagnosed TB cases may go unrecognized, posing a serious global health challenge. Tuberculosis affects individuals across all age groups. In India, the infection rate is 20% among those aged 15 to 24 years, while it is only 2% in the 0 to 14 age group. In contrast, in developed nations, the disease is more prevalent among older populations and is more common in males than females. In the current study, 1,529 samples were analyzed using three different diagnostic platforms. Fluorescent microscopy identified 73 (4.7%) positive cases of Tuberculosis. The age group most frequently suspected of having TB was 41 to 60 years (56%), followed by 21 to 40 years (16%) and those under 20 years (1%). The predominant demographic affected was female (74%), with males comprising 26%. These results align with previous studies, which reported 56 (43.41%) cases in males and 73 (56.59%) in females (Rashmi M et al., 2021), corroborating the

findings of the present research. The majority of samples analyzed were derived from sputum, totaling 1,149 (75%), followed by pus with 250 (16%), lymph node aspirate with 50 (6.3%), cerebrospinal fluid (CSF) with 40 (2.6%), and ascitic and pericardial fluids, each accounting for 20 (1.3%). In contrast to our findings, another study reported 387 (96.0%) sputum samples and 7 (1.7%) bronchial wash samples, with extrapulmonary samples comprising 9 (2.3%) [6]. Clinicians predominantly concentrate on patients with positive Ziehl-Neelsen (ZN) smears, who are known to be highly infectious. However, it is important to note that ZN smear-negative patients contribute to approximately 17% of tuberculosis (TB) transmission, a factor that cannot be overlooked due to its significant implications for public health [9]. In the current study, a total of 1,529 samples were evaluated across three different diagnostic platforms. Fluorescent microscopy (FM) identified 73 (4.7%) positive cases for tuberculosis, while True Nat (Molbio) detected 119 (7.7%) positive cases, and GeneXpert CBNAAT revealed 793 (51.8%) positive results for tuberculosis. The cartridge-based nucleic acid amplification test (CBNAAT) has proven to be an essential diagnostic tool for the swift identification of Mycobacterium tuberculosis (MTB) in both pulmonary and extrapulmonary TB. Its capacity to deliver rapid and precise results significantly improves TB management, especially in instances where conventional methods may be inadequate. The subsequent sections will detail the critical aspects of CBNAAT's effectiveness in TB diagnosis. This study corroborates previous research indicating that CBNAAT exhibits high sensitivity and specificity, with sensitivity rates ranging from 86% to 91.5% and specificity rates between 83% and 89% (Vankayala et al., 2024) (Dash et al., 2023). The findings underscore the effectiveness of CBNAAT in diagnosing tuberculous sputum, demonstrating a sensitivity of 86% and a specificity of 83.33%, thereby highlighting its significance in the rapid detection of extrapulmonary TB cases. A recent investigation involving 593 samples from individuals suspected of having extrapulmonary tuberculosis (TB) revealed that the Cartridge-based Nucleic Acid Amplification Test (CBNAAT) identified 52 positive cases, surpassing the performance of smear microscopy. This study underscores the efficacy of CBNAAT in the rapid detection of Mycobacterium tuberculosis, particularly in cases of extrapulmonary tuberculosis (EPTB), and highlights its capability to uncover false negatives that may be overlooked by smear microscopy (Chattopadhyay et al., 2023). The test proves especially advantageous for diagnosing extrapulmonary TB, which frequently exhibits low bacillary loads, rendering conventional diagnostic methods less effective (Chattopadhyay et al., 2023). CBNAAT is adept at swiftly detecting Mycobacterium tuberculosis in both pulmonary and extrapulmonary forms of the disease, exhibiting high sensitivity (99.5%) and specificity (95%) in comparison to Ziehl-Neelsen staining, thus serving as a crucial diagnostic instrument for timely TB identification. Furthermore, CBNAAT can identify TB in smear-negative cases, which is essential for ensuring prompt treatment (Vankayala et al., 2024). The test delivers results within hours, enabling rapid clinical decision-making and the initiation of treatment, which is critical for controlling the spread of TB (Dash et al., 2023). Despite the significant advantages of CBNAAT, challenges persist, including the necessity for further optimization in resource-limited environments and the risk of false positives, which could complicate patient management (Calderon et al., 2023). The CBNAAT has proven effective for the rapid identification of Mycobacterium tuberculosis in both pulmonary and extrapulmonary TB, with the Xpert®MTB/RIF assay demonstrating notable effectiveness in diagnosing extrapulmonary cases alongside standard culture methods (Kannuri et al., 2022). The study emphasizes GeneXpert's superior performance in detecting Mycobacterium tuberculosis, reporting higher detection rates (15% and 12.6%) compared to AFB/ZN microscopy (8.17%, 5%, 3.39%). The detection of Mycobacterium tuberculosis (MTB) is notably enhanced, especially in instances of drug resistance, thereby improving the diagnosis of tuberculosis (TB) (Duke et al., 2022). This research emphasizes the application of the cartridge-based nucleic acid amplification test (CBNAAT) for the prompt identification of pulmonary tuberculosis in patients with negative sputum smear results, revealing increased rates of bacteriological confirmation; however, it does not consider extrapulmonary tuberculosis (Pankaj B et al., 2022).

CONCLUSION

The prompt identification of infections through highly sensitive rapid molecular techniques, such as GeneXpert CBNAAT detection and MGIT Bactec 960 drug susceptibility testing, facilitates the early diagnosis of tuberculosis. This timely detection enables the prompt initiation of anti-tuberculous therapy, which is essential for minimizing the transmission of TB. Additionally, these methods are crucial for the early identification of drug-resistant cases of multidrug-resistant tuberculosis (MDR-TB) in both pulmonary and extrapulmonary forms.

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