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Role of cartridge-based nucleic acid amplification test in detection of pulmonary tuberculosis in people living with human immunodeficiency virus

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Abstract:

INTRODUCTION: Tuberculosis (TB) in human immunodeficiency virus (HIV)-infected people remains a major global public health challenge. Cartridge-based nucleic acid amplification test (CBNAAT) has been proved to have a key role in diagnosing TB in patients having HIV infection.

METHOD: In this study, we intend to present a retrospective data of HIV-TB co-infected patients during our routine diagnostic algorithm under Revised National TB Control Program (RNTCP).

RESULT: A total of 59/207 (28.8%) HIV patients were diagnosed to be positive for TB by CBNAAT in the period January to December 2015.

Keywords:

Cartridge-based nucleic acid amplification test, People living with human immunodeficiency virus, Revised National Tuberculosis Control Program, Tuberculosis

Introduction

As per the global tuberculosis (TB) report (2014), 9 million people developed TB; out of whom 1.1 million cases were human immunodeficiency virus (HIV) positive. The global burden of multidrug-resistance-TB (MDR-TB) was estimated at 480,000 and 21,000 deaths have been reported in 2014.[1] As per the annual TB report; one-fourth of the global TB incident cases occur in India annually.[2] Of the total notified TB cases, nearly 25% are extrapulmonary and incidence is even higher in children and in immunocompromised people.[2,3] Prompt and accurate TB diagnosis is a prerequisite for early and effective treatment. However, there are certain technical limitations in the available diagnostic tools. Smear microscopy shows variable sensitivity, particularly in cases of HIV infection and extrapulmonary infection, due to paucibacillary nature of specimen,[4-6] liquid culture diagnostics and molecular line probe assays need expensive laboratory infrastructure and trained staff.[4] Biosafety requirements are stringent, especially in conventional diagnosis. Besides technical expertise and biosafety concerns, TB culture (e.g., using Lowenstein–Jensen medium) method, 'the gold standard test', takes several weeks to produce result causing delayed onset of treatment.[6] In December 2010, the WHO recommended use of CB-NAAT (GeneXpert) in detection of pulmonary and extrapulmonary TB[1] along with Rifampicin resistance. The test can be completed in about two h, directly from various clinical samples.[3,7] Under Revised National TB Control Programme (RNTCP), we are currently using Xpert MTB/RIF (Cepheid, USA) to diagnose pulmonary TB, paediatric TB, extrapulmonary TB and Rifampicin resistance in high-risk

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population such as HIV-positive cases, as recommended by the WHO under 2013 policy recommendations.[1,3,7]

Materials and Methods

This study was conducted at TB culture and drug susceptibility testing (DST) laboratory at Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University under RNTCP. This is a retrospective analysis of the data generated during January to December 2015.

The sputum specimens of multidrug-resistant TB (MDR-TB) suspects were received from 12 linked districts of eastern Uttar Pradesh. As per programmatic management of DR TB (PMDT) guidelines,[8] all the specimens were collected in pre-sterilised Falcon tubes with three-layer packing system, after thorough rinsing of the oral cavity with clean water. Samples along with prescribed requisition containing details of patients such as name, address, age, sex, HIV status and name of the referring facility was received in the TB culture and DST laboratory. The smears of all the specimens were prepared, Ziehl–Neelsen staining and microscopy was performed. A total of 207 sputum specimens received from HIV-positive patients were processed for cartridge-based nucleic acid amplification test (CBNAAT) as per the manufacturer's instructions.[9] In brief, 1–2 ml of sputum was collected in the conical tube. Sample reagent was added in 1:2 proportion and shaken 10–20 times vigorously. After 5 min of incubation the mixture was again shaken for 10–20 min and kept for 10 min. Two ml of sample was loaded in the cartridges, placed in the GeneXpert instrument and the programme was launched. The patients carrying Rifampicin-sensitive mycobacteria were treated under standardised treatment of either Category I that is six months of Isoniazid, Rifampicin, Pyrazinamide and Ethambutol (HRZE) or eight months of HRZE and Streptomycin (Category II) antitubercular treatment (ATT). The patients who were diagnosed as having Rifampicin-resistant TB were treated with MDR (Category IV) treatment. To know the status of MDR-TB treatment; patients were followed-up at 3, 4, 5, 6, 7, 9, 12, 15, 18, 21 and 24 months from the date of initiation of treatment. During culture follow-up, isolates of those found positive at six months of treatment, were tested for sensitivity to second-line drugs (Kanamycin [Km] and Ofloxacin [Ofx] drugs). In the case an MDR or XDR, TB patient on treatment decided to migrate and informs the healthcare worker, the patient can transferred out to the district where he/she wishes to migrate, provided that district is implementing PMDT services. The transfer out was brought to the notice of the DR-TB centre by the concerned District TB Officer.

Results

We received a total of 2551 patient samples for CBNAAT out of which 207 (8.1%) patients were HIV positive. They comprised of 145 (70.0%) males and 62 (30.0%) females. The mean age of the patients was 36.28. All smears were negative for acid–fast bacilli. A total of 59/207 (28.5%) HIV-positive cases were detected as positive for *Mycobacterium tuberculosis* by CBNAAT. Out of 59 HIV-TB co-infected cases, 45 were male and 14 were female. Rifampicin resistance was seen in 15/59 (25.4%) of TB confirmed cases and 44 (74.6%) were sensitive. Sex-wise distribution of resistance in HIV-TB co-infected patients is depicted in Figure 1. Treatment outcome of the patients are depicted in Table 1. In the course of ATT treatment, a total of 26 patients (65%) were declared cured or treatment completed, five patients were defaulters, nine were transferred out to nearby states and one patient died. Out of 15 Rifampicin-resistant cases, eight are still under MDR treatment. The consecutive follow-up cultures of four patients were negative; however, six-month follow-up of the four patients turned out positive. Hence, they were tested for second-line

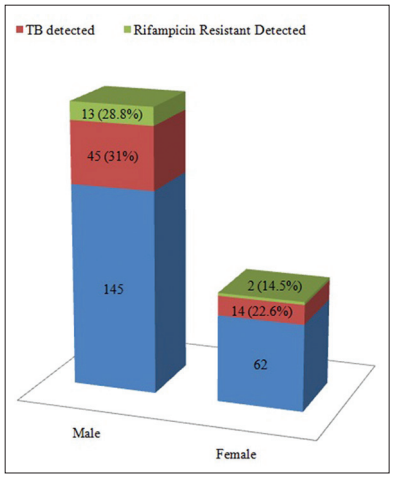

Figure 1: Status of tuberculosis Rifampicin-resistant detection in human immunodeficiency virus-positive male and female cases

Table 1: Treatment outcome in human immunodeficiency virus-tuberculosis co-infected cases

Treatment outcome	*n* (%)
Rifampicin-sensitive cases	
Cured or treatment completed	29/44 (65)
Treatment default	5/44 (11)
Died	1/44 (2)
Transfer out	9/44 (20)
Rifampicin-resistant cases	
On MDR treatment	8/15 (53)
MDR treatment default	1/15 (8)
Died	4/15 (26)
Transfer out	2/15 (13)

MDR: Multidrug resistance

DST. Two isolates were found sensitive to both the drugs, Km and Ofx, whereas one patient was diagnosed as sensitive to Km but resistant to Ofx. The result of the other patient is awaited. Four patients died, one was a defaulter and two were transferred out to other states.

Discussion

Globally, 48% of TB patients had a documented HIV test result in 2013.[1] India accounts for about 10% of the global burden of HIV-associated TB. The mortality in this group is very high and every year 42,000 people die among TB/HIV co-infected patients.[2] As per this study, all HIV-positive cases were smear negative and TB detection was low to very low in CBNAAT which further confirmed the low bacillary load in immunocompromised patients, thereby escaping detection by microscopy due to less cavitations. This study demonstrates overall 2.3% (59/2551) of TB-HIV co-infection among all the TB suspects tested, whereas in confirmed HIV-positive tested, the TB cases were 28.8% with 70% in males. Male predominance has been observed in other studies too.[10,11] Our results are in concordance with the study conducted in AIIMS-New Delhi, in which HIV-TB co-infection was diagnosed in 33.2% of patients of which 81.3% were male.[11] The migration of male person for job to other states may lead to risk behaviour. Furthermore, the mean age of 36 years points to the risk behaviour of younger age group, due to illiteracy and inaccessibility of healthcare facilities, the cases remain unreported, particularly in females.

In this report, 25.4% case of MDR-TB was detected among HIV-positive cases. Similarly, the study done by Sethi *et al*. at PGIMER, Chandigarh, reported significantly higher association of MDR-TB (27.3%) with HIV-seropositive patients as compared to HIV-seronegative patients (15.4%).[12] The study conducted by Arora *et al*. reported 15.7% of MDR-TB among HIV-positive cases.[13]

Reports from different parts of the world have also showed high sensitivity and specificity for CBNAAT. A study by Raizada *et al*. that covered a population of 8.8 million across 18 sub-district level TB units reported 28% bacteriologically TB confirmed cases, of which 27.6% TB cases were detected on CBNAAT against smear positivity rate of 12.9%. Positive predictive value (PPV) for Rifampicin resistance detection by CBNAAT was 97.7%.[14] In other study, Raizada *et al*. suggested on paediatric populations (0–14 year) that CBNAAT could detect TB in 98.4% in bacteriologically confirmed cases. Advantageously, it could detect TB in 54.9% smear-negative cases too.[15] Similarly, Singh *et al*. have conducted a study on HIV-positive adults referred to DOTS centre with pulmonary symptoms suggestive of TB and reported the contribution of CBNAAT in TB diagnosis.[16]

In the present report, treatment success in the co-infected cohort is similar to other TB patients in India.[1] However, the numbers of deaths in Rifampicin-resistant cases are higher than that of Rifampicin-sensitive cases. The reason may be the effectiveness of first-line drugs compared to drugs used in the MDR treatment. Our Institution is a tertiary care centre in Eastern Uttar Pradesh and patients from nearby states visit the hospital to utilise the facility for TB diagnosis, but for the treatment; they revert back to their own states leading to higher number of transfer out cases of TB.

Conclusion

CBNAAT plays a very important tool in detection of TB in HIV-positive cases where the bacillary load is below the sensitivity of microscopy. CBNAAT not only enabled diagnosis of TB but also helped in rapid detection of Rifampicin resistance and helps in the early initiation of treatment.

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Conflicts of interest
There are no conflicts of interest.

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