Original Research Article

TB and HIV coinfection- GeneXpert a comparable tool to Line probe assay for diagnosis of MDR TB

Tadvi Khan Saleha[1*], More S.R[2], Sinha Rohit[3], Vimal Rathod[4]

[1]Assistant Professor, RMC Loni, Ahmednagar, Maharashtra, India
[2]Professor and Head, Department of Microbiology, DRSCGMC, Nanded, Maharashtra, India
[3]Assistant Professor, Department of Microbiology, DRSCGMC, Nanded, Maharashtra, India
[4]Assistant Professor, Department of Microbiology, DRSCGMC, Nanded, Maharashtra, India

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Abstract

Introduction: Tuberculosis (TB) and HIV duo forms the deadly synergy- the patients with these diseases more often will have unfavourable outcomes. It is important to see HIV TB correlation ad drug resistance in case of tuberculosis. **Aims and objectives:**

1. To detect HIV seropositivity in newly diagnosed sputum smear positive pulmonary tuberculosis patients.
2. To detect rifampicin resistance by CBNAAT.
3. To detect drug resistance of first and second line antitubercular drugs by conventional methods.
4. Comparison of drug resistance of Mycobacterium tuberculosis isolates in patients with and without HIV.

Material and methods: Prospective, cross sectional study was conducted in department of microbiology for one and half year. 200 newly diagnosed sputum positive samples were collected. They were subjected to Zeil Nelson(ZN) staining, culture, culture on Loweinstein Jensen(LJ)media, GeneXpert and Line Probe Assay(LPA). HIV test was done on blood sample. **Results:** Out of 200 patients, 123(61.5%) were males and 77(38.5%) were females. Male: Female ratio was 1.7:1. HIV positive were 12.5% and HIV negative were 87.5%. 65% showed growth on LJ media. 85% were positive by CBNAAT. Out of 25 HIV positive cases 48% were detected by CBNAAT. 2.35% were resistant to rifampicin. On LPA, all drugs that were resistant to rifampicin, were also resistant to isoniazid but sensitive to other second line drugs'. **Conclusion:** HIV testing of patients with TB and susceptibility testing of M. tuberculosis isolates from HIV-infected patients should be routine in settings where outbreaks or endemic transmission of MDR-TB is occurring in HIV-infected patients.

Keywords: CBNAAT, HIV, Line probe assay, rifampicin, sputum microscopy.

Introduction

Tuberculosis (TB) is the first infectious disease declared by WHO as a global health emergency in 1993. HIV and TB convergence have worsened and complicated the situation. One-third of the TB-infected patients are among 34 million people living with HIV[1]. Resistance of M. tuberculosis to anti-tubercular drugs is the result of a spontaneous genetic event and "manmade amplification of the natural phenomenon[2]." Under RNTCP, sputum microscopy is the mainstay in the diagnosis of pulmonary TB. It has very low sensitivity (20-80%), poor positive predictive value and cannot comment on drug susceptibility of the organism[3]. Culture is considered as gold standard for its higher sensitivity in detecting TB and takes 4-8 weeks for growth to be detected. Also drug susceptibility testing requires 4-6 weeks more[4]. Various molecular techniques have been developed in recent years such as Line probe assay and Real time PCR platforms to name a few.

Aims and objectives

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*Correspondence
Dr. Tadvi Khan Saleha
Assistant Professor, RMC Loni, Ahmednagar, Maharashtra, India
E-mail: khansalehambbs@gmail.com

Material and methods

Study design

Prospective, Cross-Sectional study

Duration of study

One and half year

Inclusion criteria

1. All newly diagnosed Patients having pulmonary tuberculosis by clinical findings and chest X-ray.
2. Smear positive sputum by using Ziehl Neelsen's acid fast staining.

Exclusion criteria

1. All clinically diagnosed extra pulmonary cases
2. Patient not willing for testing
3. Smear negative patient.

The present study was approved by Institutional Ethics committee**.**

Sample size

First 200 newly diagnosed smear positive patients of pulmonary tuberculosis attending TB chest OPD and wards.

Sample collected

Sputum sample

Procedure

Decontamination and concentration of sputum sample was done using Sodium hydroxide and N-acetyl cysteine. Smear was prepared and was stained using Zeil and Nelson staining. Culture was done on Loweinstein Jensen media. CBNAAT was performed as per manufacturer's guidelines. All MDR-MTB isolates obtained on CBNAAT were referred to reference centre of our institutes for drug

susceptibility by line probe assay. HIV test was carried out according to strategy 2B of HIV testing.

We had included total 200 pulmonary positive tuberculosis cases. Out of 200 cases, 123(61.5%) were males and 77(38.5%) were females. Male to female ratio was found to be 1.7:1 in the present study.

Results

Out of 200 cases, HIV status of patients is as follows-

Table 1: HIV status of the study population (n=200)

HIV status	Sputum smear positive (n=200)
HIV positive	25 (12.5 %)
HIV negative	175 (87.5 %)

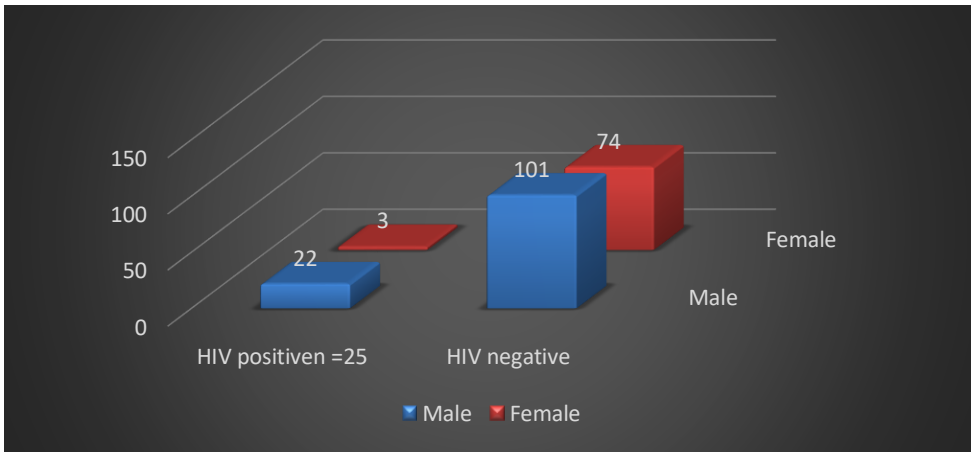

Fig. 1: Gender distribution of HIV positive and HIV negative patients

(Chi square value = 7.2432 with 1 degrees of freedom, p value is less than 0.007117).

Table 2: The growth of MTB on LJ media and detection of MTB by CBNAAT (n=200)

Result	Growth on LJ	CBNAAT
Positive	130	170
Negative	70	30
Total	200	200

Table 3: Shows MTB detected by CBNAAT in HIV positive and HIV negative patients

CBNAAT result	HIV Positive	HIV Negative	Total
MTB detected	12	158	170
MTB not detected	13	17	30
Total	25	175	200

(Chi square value = 30.6779 with 1 degrees of freedom, p value is less than 0.00001).

Amongst 200 Tuberculosis positive cases, 2.35 % population was resistant to Rifampicin (MDR-TB).

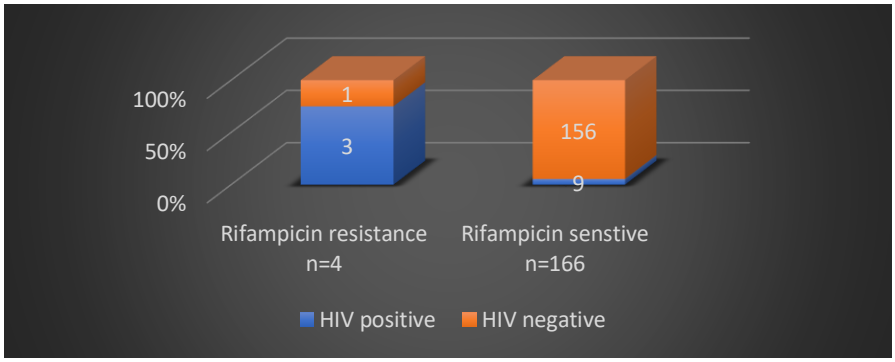

Fig. 2: Rifampicin resistance in HIV positive and HIV negative patients by CBNAAT (n=170)

Table 4: DST by Line Probe Assay

Drugs	Sensitivity	Total (N= 4)
Isoniazid	Resistant	4
Second Line Injectable Drugs	Sensitive	4
Quinolones	Sensitive	4

Above table & figure indicate that, all rifampicin resistant cases were isoniazid resistant and were sensitive to other drugs like quinolones and second line injectable drugs.

Discussion

In the present study, there was male preponderance, 123(61.5%) as against 77(38.5%) female this was also in agreement with the work of Taura et al[5] , and in concord with the work of Olusoji D[6], Imam TS[7], Otu A[8], Rasaki S[9] where male subjects had prevalence of 60% as against 40% of females.

From above observations it is seen that males are more susceptible for tuberculosis. This male predominance can be explained by the fact that male subjects are probably more exposed to risk factors of TB infection such as smoking, outdoor exposure, HIV-TB co infections, less compliance etc.

In India, the prevalence of HIV among TB patients was high in some areas, such as a 5 year (2005 to 2009) study by Menon S et al. Mumbai (2011) shows 47.54% strains were found to be MDR[10]. In the initial years (2005-65.5%, 2006-68.3%, 2007-46.5%) the percentage of MDR strains was quite high. However, in the year 2008 and 2009 the study noted 22.7% and 25.2% of MDR, respectively[10], Pune (28.75%) Tripathi S et al[11], com-pared with areas with a low prevalence, such as New Delhi (1.14%) Jain SK[12], Aurangabad (4.7%) Talib SH[13]. In the current study, covering 200 newly diagnosed sputum smear positive pulmonary tuberculosis patients reporting for the first time at tuberculosis clinics, the HIV infection found 12.5% was no different from the observation made by Magna Manjareeka et al[14].

The present study indicate significantly higher numbers of males 22/25 (88%) subjects with HIV-TB co-infection than affected females 3/25 i.e 12%. This study align with observation made by Magna Manjareeka et al[14], S.K Jain et al[12], Rasaki S et al[9].

The culture positivity in the present study was 65 % in new TB suspects. Xpert assay/CBNAAT detected MTB is 85 % of study population. The result of different studies shows that positivity for MTB detection by Xpert assay varies from 77.7 % to 95.7 % in PTB suspects, S. K Sharma et al, Nikam C et al[15,16]. Xpert negativity may also be due to the absence of the target (IS6110) which is expected to be amplified[17].

We found a low prevalence (2.35%) of MDR-TB among new cases of pulmonary TB. The reported prevalence of MDR-TB in new TB cases has varied from 0.14 to 5.3 % in previous studies from different parts of India and our findings are in consonance with such observations[18-20]. But there are a few studies which have reported a high prevalence of MDR-TB among new TB cases Jain A and D'souza DT et al[21,22]. Differences in methodology may account for such high prevalence of MDR-TB noted in these studies.

In present study MDR TB were higher 3/12 (25%) in HIV Positive patients as compare to HIV Negative patients. Recent meta-analyses have indicated that, on average, new HIV-positive tuberculosis (TB) patients are at increased risk of multidrug-resistant (MDR)-TB compared with HIV-negative patients, while this risk is less clear for previously treated TB patients Mesfin YM et al, Suchindran S et al[23,24].

Our findings carry some important implications. Firstly, the prevalence of MDR-TB has not risen over the years, which reflects the success of DOTS as effective treatment of drug-susceptible TB and preventing the emergence of MDR-TB. Secondly, since MDR-TB is rare in new TB cases, all new cases of pulmonary tuberculosis can be treated with empirical category I regimen composed of rifampicin (R), ethambutol (E), pyrazinamide (Z) and levofloxacin (Lfx)5, without the risk of treatment failures or aggravation of drug-resistance. The major limitation of the present study is the small sample size and therefore, it is not representative of the population at large. Nation-wise and State-wise representative data on the prevalence of MDR-TB are an urgent need of the hour to design effective empirical regimens, to monitor functioning and progress of the national TB control programme and for continued surveillance of MDR-TB among category I TB patients.

Conclusion

HIV testing of patients with TB and susceptibility testing of M. tuberculosis isolates from HIV-infected patients should be routine in settings where outbreaks or endemic transmission of MDR-TB is occurring in HIV-infected patients. In present study, Xpert MTB/ RIF assay has same result as compared to line probe assay done at reference center of our hospital. So Xpert assay is the right choice for detection of TB and MDR –TB. Since, Xpert assay can give result within 2 hours, the highly infectious patients can be enlisted under DOTS PLUS programme at earliest.

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