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GENE XPERT BASED ONE YEAR ANALYSIS OF PULMONARY KOCH'S FROM NORTH MAHARASHTRA REGION

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ABSTRACT

Introduction: Tuberculosis is a major public health problem in the world, especially in the developing countries like India. Also MDR-TB and HIV: TB co-infection are major hurdles to achieve the aim and objectives of our national tuberculosis programme.

Objectives:

1. To diagnose Mycobacterium tuberculosis (MTB) infection in clinically suspected cases of pulmonary tuberculosis using Gene Xpert.
2. To find out HIV: TB co-infection rate.
3. To find out prevalence of Rifampicin sensitive and resistant cases in diagnosed tuberculosis patients.
4. To study factors responsible for Rifampicin resistance (MDR-TB)

Material & Methods: This retrospective study included 278 sputum samples from Jan 2015-Dec 2015 from registered RNTCP patients. The samples were subjected to Xpert MTB/RIF Assay for use with the Cepheid Gene Xpert. The Ziehl - Neelsen smear finding was provided by the RNTCP –DOTS regional centres.

Results: A total number of 278 sputum samples were subjected to Gene Xpert analysis in the year 2015. MTB could be detected in 137 (49.28%) cases. In the rest 141 cases where MTB was not detected 14 samples reported error on Gene Xpert. Out of 278 cases, 209 (75.18%) were HIV negative, and 69 (24.82%) were HIV positive. In 69 clinically suspected HIV: TB co-infection cases, MTB could be detected in 22 (31.88%) cases. Out of these 22 cases, 4 (18.18%) were smear positive. Out of 137 MTB detected samples, 117 (85.4%) were rifampicin sensitive, 2 (1.46%) were rifampicin indeterminate resistant and 18 (13.14%) were rifampicin resistant. According to RNTCP programme criteria for suspected MDR –TB, out of 18 MDR-TB cases, 12 (66.67%) cases were - smear positive at diagnosis , retreatment case; 3 (16.67%) cases were - any follow up smear positive, 2 (11.11%) - had contact of known MDR -TB case and 1(5.56%) - was HIV: TB case.

Conclusion: Gene Xpert was a useful tool in detection of HIV-TB co-infection. In our study out of 69 clinically suspects HIV: TB co-infection cases, one third cases were confirmed by Gene Xpert. Thus, 44 patients were not put on unnecessary AKT and were kept on follow-up. Even though the association of MDR-TB and HIV co-infection was not very significant in this study, it would not be too long before witnessing a rapid increase of MDR-TB among HIV patients if adequate and immediate measures are not taken. In the present study, MDR-TB detection rate was high among re-treatment cases. Emphasis has to be given for completion of primary treatment on time and taking proper nutrition. Patients usually stop treatment once they feel better within 2 month of starting the treatment. Special counselling and education is needed at this juncture. In our study, two cases of primary MDR-TB were from household contact. Hence, health workers must generate awareness and educate patients and family members about the risk of acquiring Primary MDR-TB to prevent its spread.

Key Words: Gene Xpert, Pulmonary tuberculosis, MDR-TB, HIV: TB co-infection

INTRODUCTION

India has the world's maximum burden of TB (approximately 3.4 million cases), which accounts for one fifth of global incident cases, and ranks first among the 22 TB high-burden countries.[1] Drug-resistant TB (DR-TB) is commonly seen in India; although its existence has been recognized since anti-tuberculosis drugs were first introduced for the cure

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of TB, adequate information regarding the prevalence of drug-resistant tuberculosis (TB) has not been reported from India.[2] According to WHO Reports on global TB control, Multidrug-resistant tuberculosis (MDR-TB) in India has been reported between 2.5–2.8% amongst new TB cases and 14–17% in retreated patients.[3-7] MDR-TB is a growing worldwide threat, most cases arising from a combination of physician error and patient non-compliance during treatment of susceptible TB. The amount of burden of MDR-TB varies significantly from nation to nation and region to region.[8]

The threat of development of active tuberculosis is high in HIV infected patients. Although antiretroviral therapy for HIV reduces this risk, TB still remains 5 times more common in HIV/AIDS persons.[9] The present study was undertaken with following primary objectives:

1. To diagnose Mycobacterium tuberculosis infection in clinically suspected cases of tuberculosis using Gene Xpert
2. To find out HIV: TB co-infection rate.
3. To find out prevalence of Rifampicin sensitive and resistant cases in diagnosed tuberculosis patients.
4. To study factors responsible for Rifampicin resistance(MDR-TB)

MATERIALS AND METHODS

Study Design: Retrospective study

Study period: From Jan 2015 to Dec 2015

The study was approved by Institutional Ethical Committee of SBHGMC, Dhule. Clinical data was obtained from the RNTCP filled requisition form sent along with the samples.

Collection of specimen: Sputum samples from registered RNTCP patients were subjected to Xpert MTB/RIF Assay for use with the Cepheid Gene Xpert system, a semi-quantitative, nested real-time PCR *in-vitro* diagnostic test. After proper instruction to the patient, two sputum samples were collected per patient. The Ziehl - Neelsen smear finding was provided by the RNTCP-DOTS regional centres. The specimen were stored and transported at 2 to 8°C prior to processing whenever needed. In the laboratory, specimens were examined for the presence of obvious food particles or other solid particulates and were rejected.

Processing of the samples:[10]

Each Xpert MTB/RIF cartridge was labelled with the sample ID. The lid of the sputum collection container was opened carefully and approximately 2 times the volume of the sample reagent (SR) to the sputum (2:1 dilution; SR: sputum) was poured in the container. The lid was replaced and shaked vigorously 10 to 20 times. The samples was incubated for 10 minutes at room temperature, and then shaked vigorously 10 to 20 times. The sample was incubated at room temperature for an additional 5 minutes. The test was started within 4 hours of adding the sample to the cartridge. The cartridge lid was opened and using the provided transfer pipette, the liquefied sample was aspirated to the line on the pipette. Care was taken so that no air bubble enters the pipette. The sample was transferred into the sample chamber of the Xpert MTB/RIF cartridge and was dispensed slowly to minimize the risk of aerosol formation. The cartridge lid was closed firmly and loaded as per the instruction given by the manufacturer.

RESULTS

A total number of 278 sputum samples were subjected to Gene Xpert analysis in the year 2015. MTB could be detected in 137 (49.28%) cases, while in 141(50.72%) cases MTB could not be detected.

In the rest 141 cases where MTB was not detected 14 samples reported error on Gene Xpert.

Out of 278 cases, 209 (75.18%) were HIV negative, and 69 (24.82%) were HIV positive.

In 69 clinically suspected HIV: TB co-infection cases, MTB could be detected only in 22 (31.88%). Out of these 22 HIV: TB cases, only 4 (18.18%) were smear positive (Table 1).

Out of 137 MTB detected samples, 117 (85.4%) were rifampicin sensitive, 2 (1.46%) were rifampicin indeterminate resistant and 18 (13.14%) were rifampicin resistant (Figure 1).

According to RNTCP programme criteria for suspected MDR –TB, out of 18 MDR-TB cases, 12 (66.67%) cases were - smear positive at diagnosis , retreatment case; 3 (16.67%) cases were - any follow up smear positive, 2 (11.11%) - had contact of known MDR -TB case and 1(5.56%) - was HIV: TB case. Out of the 22 HIV: TB co-infection cases, MDR-TB was detected only in 1 case.

DISCUSSION

The WHO South-East Asia Region (SEAR), having almost one fourth of the world's inhabitants, accounts for 38% morbidity and 39% mortality of the global burden of tuberculosis, with an approximate 4.5 million prevalent and 3.4 million incident cases and 440,000 deaths in 2013. India having a population of about 1252 million is the largest country in the Region, and is ranked first among the high-burden countries having contributed approximately 24% of the global incident TB cases and about 20% of global TB-related deaths in 2013. In 2013, the prevalence and incidence rates of all forms of tuberculosis were 211 and 171 respectively per

100,000 population.[11] Thus, India is the highest TB burden nation accounting for one fifth of the worldwide incidence.[12]

In the present study, a total number of 278 sputum samples were subjected to Gene Xpert analysis in the year 2015. All these samples were from RNTCP programme of Dhule district. MTB could be detected in 137 (49.28%) cases, while MTB was not detected in 141 (50.72%) cases.

While HIV/AIDS and tuberculosis can independently be the foremost causes for apprehension as stand-alone public health threats, the blend of the two has proven to have a far greater impact on the epidemiologic progression and subsequently on the impact it has on the global health prospect. The twin infection has been termed "accursed duet".[13] Research shows that of the opportunistic infections distressing HIV-infected patients, TB is found to be the most frequent with soaring risk for mortality.[14,15]

Worldwide, about 14.8% of patients with TB are co-infected with HIV.[16] There is extensive inconsistency in HIV seropositivity amongst TB patients in India, ranging from 9.4% in New Delhi to 30% in Mumbai.[17]

Out of 278 cases, 209 (75.18%) were HIV negative, and 69 (24.82%) were HIV positive.

Gene Xpert was very helpful in diagnosis of HIV: TB co-infection cases as HIV positive TB cases are more likely to be smear negative. In 69 clinically suspected HIV: TB co-infection cases, MTB could be detected in 22 (31.88%) cases. Out of these 22 cases, only 4 (18.18%) were smear positive.

Sethi *et al*[18] has reported the prevalence of HIV-TB as 20.1%, while Ghiya *et al* [19]and Noeske *et al* [20] have reported the prevalence of HIV:TB as 49.2% and 32% respectively.

MDR–TB is a most important challenge for TB control globally and is also linked with increased mortality and development of XDR-TB. Surveillance studies for the estimation of resistance rates and detection of MDR-TB are thus vital so as to optimize empiric drug treatment and to avoid the spreading of resistant strains in the community.[21]

Out of 137 MTB detected samples, 117 were rifampicin sensitive, 2 were rifampicin indeterminate resistant and 18 were rifampicin resistant i.e. rifampicin was sensitive in 85.4% of our diagnosed cases of tuberculosis and was resistant in 13.14% cases.

Worldwide, 5% of TB cases were estimated to have had MDR-TB in 2014.[22] While rates of MDR-TB infections are comparatively low in North America and Western Europe; they are progressively new grave dilemma worldwide, especially in areas of the Russian Federation, the former Soviet Union and other parts of Asia.[23]

In 2008, after China (100,000 cases), India had second highest total number of estimated MDR TB cases (99,000).[24]

According to WHO Reports on global TB control, MDR-TB in India continues to be reported between 2.5–2.8% and 14–17% amongst new TB and retreated patients respectively.[3-7]

According to RNTCP programme criteria for suspected MDR-TB, out of 18 MDR-TB cases, 12 (66.67%) cases were - smear positive at diagnosis , retreatment case; 3 (16.67%) cases were - any follow up smear positive, 2 (11.11%) - had contact of known MDR -TB case and 1(5.56%) - was HIV: TB case.

HIV may not constantly be a risk factor of MDR-TB, depending on the people investigated. In the present study, out of 22 HIV: TB confection patients only 1 case was MDR-TB. The hastening and magnifying sway of HIV infection and postponement in identification and diagnosis of tuberculosis were found to add to the outbreaks of MDR-TB among HIV infected patients in USA[25] and European countries.[26] However, a Thailand study [27] showed that the percentage of MDR-TB among HIV seropositives was the same as in the HIV negative group.

Among the high risk groups of MDR-TB, failure of retreatment regimen and contact of MDR-TB may be the highest prospect of MDR-TB and warrant special attention. Rapid testing of resistance to rifampicin might be essentially helpful in the confirmation of MDR-TB.[28]

CONCLUSION

Gene expert was a useful tool in detection of HIV: TB co-infection as HIV positive TB cases are more likely to be smear negative, thus early diagnosis of TB in HIV: TB patients helps in the management of these patients, reducing the morbidity and mortality. In our study out of 69 clinically suspects HIV:TB co-infection cases, one third cases were confirmed by Gene Xpert. Thus, 44 patients were not put on unnecessary AKT and were kept on follow-up. Even though the association of MDR-TB and HIV co-infection was not very significant in this study, it would not be too long before witnessing a rapid increase of MDR-TB among HIV patients if adequate and immediate measures are not taken. In the present study, MDR-TB detection rate was high among retreatment cases. Emphasis has to be given for completion of primary treatment on time and taking proper nutrition. Patients usually stop treatment once they feel better within 2 month of starting the treatment. Special counselling and education is needed at this juncture. In our study, two cases of primary MDR-TB were from household contact. Hence, health workers must generate awareness and educate patient and family members about the risk of acquiring Primary MDR-TB to prevent its spread.

Abbreviations:

MDR-TB: Multidrug resistance tuberculosis

MTB: Mycobacterium tuberculosis

TB: Tuberculosis

RNTCP: Revised National Tuberculosis Programme

DOTS: Directly Observed Therapy, Short-Course

AKT: Anti Koch's treatment

XDR- TB: Extensively drug resistant tuberculosis

WHO: World Health Organisation

HIV: Human Immunodeficiency Virus

AIDS: Acquired Immunodeficiency Syndrome

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Table 1. Shows the Gene Xpert based analysis of MTB cases

MTB detected cases	Frequency	Percentage(%)
Rifampicin sensitive	117	85.4
Rifampicin resistance (MDR-TB)	18	13.14
Rifampicin indeterminate	2	1.46
Total	137	100

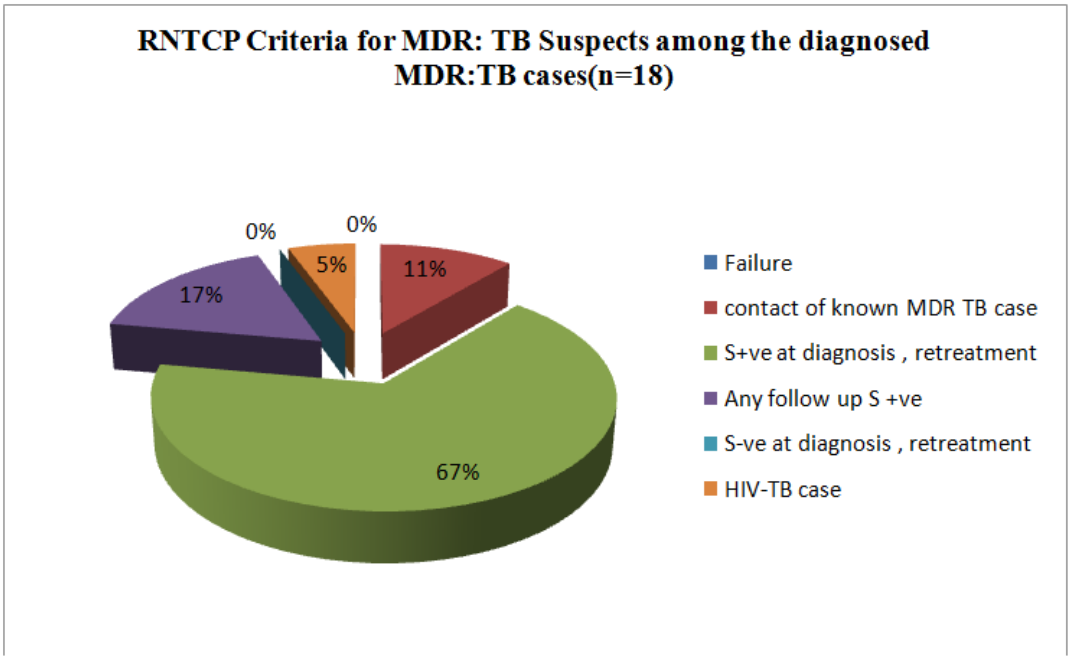

Figure 1: Shows RNTCP Criteria for MDR-TB Suspects among the diagnosed MDR:TB cases.