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"Prevalence of Rifampicin resistance in newly diagnosed and previously treated cases of pulmonary tuberculosis by Xpert MTB / RIF assay"

Microbiology

Dr. Badrunnesa Khatun Medical officer - District Tuberculosis office (MO-DTC), RNTCP. Completed MD Microbiology from Seth GS Medical College and KEM Hospital, Mumbai - 2016.

Dr. Swapna Kanade* Associate Professor, Dept. of Microbiology, Seth GS Medical College and KEM Hospital, Mumbai. *Corresponding Author

Dr. Gita Nataraj Professor, Dept. of Microbiology, Seth GS Medical College and KEM Hospital, Mumbai.

Dr. Preeti Mehta Professor and Head, Dept. of Microbiology, Seth GS Medical College and KEM Hospital, Mumbai.

ABSTRACT

Objective:

- To determine the prevalence of Rifampicin resistance in new and previously treated PTB
- To compare Rifampicin resistance obtained by Xpert and proportion method
- To determine the prevalence of Isoniazid mono-resistance by proportion method

Design:

This cross-sectional study was carried over a period of one year (January - December 2015).

Results:

In 100 new TB suspects, smear positivity was 21% and culture positivity was 49%. Xpert assay detected MTB in 46% cases. In 100 previously treated cases, smear positivity was 40% and culture as well as Xpert MTB/RIF assay positivity was 47%. Xpert MTB/RIF assay has a sensitivity of 93.75% and specificity of 97.11% as compared to culture. Six (12.24%) Rifampicin resistant cases were observed in new TB cases & 19 (40.42%) in previously treated cases. All Rifampicin resistant cases were Isoniazid resistant also. INH mono-resistance was observed in nine cases.

Conclusion:

Rifampicin resistance, a surrogate marker of MDR-TB is more prevalent in previously treated cases. Xpert MTB/RIF assay provide the much-needed diagnostic capacity for effective identification of MDR TB cases in lesser time.

KEYWORDS:

Xpert MTB/RIF assay, newly diagnosed pulmonary tuberculosis, previously treated cases of pulmonary tuberculosis

INTRODUCTION

Tuberculosis (TB) caused by Mycobacterium tuberculosis complex (MTB) is still a leading cause of morbidity and mortality. [1] Multidrug resistant TB (MDRTB) is resistance to at least Isoniazid (INH) and Rifampicin (RIF). Globally, it is estimated that 3.3% of new cases and 20% of previously treated cases have MDRTB. [2] In India, MDRTB prevalence is estimated to be 2.2% among new cases and 15% among re-treatment cases. [2] MDRTB is a major public health problem because the treatment is complicated, cure rates are lower than drug susceptible tuberculosis and if treatment is not effective, an infected patient can spread the disease for months. The occurrence of MDRTB in new cases is either due to spontaneous mutations in susceptible strains or acquisition of resistant strain from close contacts. In previously treated cases, MDRTB is more commonly due to inadequate treatment and poor compliance. [3]

Early diagnosis and appropriate treatment are the main pillars of TB control program to reduce the burden of MDRTB. Under RNTCP, sputum microscopy is the initial screening test for the diagnosis of pulmonary TB. It has very low sensitivity (20-80%) and cannot comment on drug susceptibility of the organism.[4] Culture is considered as a gold standard but major limitations are lengthy turnaround time and need of advanced biosafety level.[5] Automated liquid culture systems provide results in 2-4 weeks but are costly and prone to more contamination.[5] Various molecular techniques such as Line probe assay and Real time PCR can predict drug resistance in clinical strains within one to two working days, but expense and infrastructure required for it is a barrier for implementation in resource constrained settings.[6]

Xpert MTB / RIF assay (Cepheid, Sunnyvale, CA) is a rapid molecular test with potential to improve TB diagnosis as it gives results within 2 hrs.[7] Xpert assay is based on single use sample processing cartridge system integrated multicolor real time PCR. It has the potential to greatly simplify nucleic acid amplification tests. This technology employs a novel six colour dye test to detect M. tuberculosis and identify Rifampicin resistance directly from the untreated sputum of patients in less than 2 hours. [8] The advantages of this system include hands free operation, on board sample processing and ultrasensitive hemi nested PCR with sensitivity equal to culture method, not prone to cross contamination, minimal biosafety facilities & high sensitivity in smear negative pulmonary TB. [9] This assay was endorsed by WHO in December 2010 for diagnosis of pulmonary tuberculosis.

Understanding the prevalence of MDRTB is important for management and from programmatic point of view. Hence this study was conducted to (i) determine the prevalence of Rifampicin resistance in new and previously treated pulmonary tubercular cases, (ii) compare RIF resistance obtained by Xpert and proportion method of drug susceptibility testing (DST) using solid culture and (iii) to determine the prevalence of INH monoresistance by proportion method using solid culture.

MATERIALS AND METHODS

This cross sectional study was carried over a period of one year (January 2015 - December 2015) in a tertiary care teaching hospital after obtaining institutional ethics committee permission [ethics no.EC/190/2013]. Adult patients of both genders, referred by clinicians for diagnosis of suspected PTB and willing to give written informed consent were included in the study. Patients already on anti-tuberculosis treatment were excluded. Two groups of 100 patients each were formed. One group included new suspected PTB patients without any history of TB and another group included

suspected PTB cases giving history of taking anti TB treatment in past.

STUDY PROCEDURE:

All processing was carried out in Bio Safety Cabinet (BSC) class 2 and level 2 bio safety practices were followed. Early morning sputum specimen was used for this study. Direct smear was stained by Ziehl Neelsen staining and screened for the presence of acid fast bacilli. All positive smears were graded as per RNTCP guidelines. [10] NALC (N-Acetyl-L-Cysteine) -NaOH method was used to decontaminate and digest the sputum specimen. Pellet obtained after centrifugation was inoculated on Lowenstein Jensen (LJ) medium and incubated aerobically at 37oC. Any growth observed on LJ medium was identified as MTB or mycobacterial other than tuberculosis [MOTT] using phenotypic characteristics. [11] Any acid fast isolate which was a slow grower, did not grow on LJ containing PNBA, formed buff coloured colony and MPT64 positive was characterized as MTB.

All MTB isolates obtained on LJ medium were tested for drug susceptibility by proportion method for RIF and INH as described by Canetti et al [11]. The recommended drug concentrations were 0.2µg/ml for Isoniazid and 40µg/ml for Rifampicin. Xpert MTB /RIF assay was performed on direct sputum specimen as per manufacturer's instructions. [12]

RESULTS AND OBSERVATIONS

Majority of patients in which MTB was detected by any of the test were in the age group of 21 to 40 years in both new TB suspects (54.34%) and in previously treated cases (65.95%). Male to female ratio was 1:0.72 in new case and 1:0.75 in previously treated cases. MTB detection rate was more in male but female predominance was noted in rifampicin resistance for both the groups.

In 100 new TB suspects, smear positivity was 21% and culture positivity was 49%. Xpert assay detected MTB in 46% cases. In 100 previously treated cases, smear positivity was 40% and culture as well as Xpert MTB/RIF assay positivity was 47%. As compared to microscopy, Xpert assay detected extra 25 and 7 cases in new and previously treated cases respectively within the similar time i.e. 2 hours. [Table 1&2]

Table 1: Relation between smear, culture & Xpert assay results in new cases

Culture	Microscopy (ZN staining)		Xpert assay		Total
	Positive	Negative	MTB Detected	MTB not Detected	
Positive	21	28	45	4	49
Negative	0	51	1	50	51
Total	21	79	46	54	100

Table 2: Relation between smear, culture & Xpert assay results in previously treated cases

Culture	Microscopy (ZN staining)		Xpert assay		Total
	Positive	Negative	MTB Detected	MTB not Detected	
Positive	40	7	45	2	47
Negative	0	53	2	51	53
Total	40	60	47	53	100

When 200 cases were considered together, Xpert MTB/RIF assay has a sensitivity of 93.75% and specificity of 97.11% as compared to culture. Discordance between culture &Xpert MTB/RIF assay was observed in total nine cases. Xpert MTB / RIF assay was positive in three culture negative cases (one in new cases & two in previously treated cases). Xpert MTB /RIF assay was negative in six culture positive cases (four in new cases & two in previously treated cases).

Six (12.24%) Rifampicin resistant cases were observed in new TB cases & 19 (40.42%) in previously treated cases. All Rifampicin resistant cases were isoniazid resistant also. INH monoresistance was observed in nine cases (four in new TB cases & five in previously treated cases). [Table 3 & 4]

Table 3: Results of DST (1% proportion method) in new and previously treated cases

Group	Total no of Strain for DST	RIF- S INH- S	RIF- S INH -R	RIF-R INH -S	RIF- R INH -R
New TB suspects	49	39	4	0	6
Previously Treated Suspects	47	23	5	0	19

DST=drug susceptibility Test, RIF=rifampicin, INH=isoniazid, S=Sensitive, R= Resistant.

Table 4: Results of Xpert assay in new and previously treated suspects

Group	Total no of Strains tested	RIF sensitive	RIF resistant
New TB suspects	46	40	6
Previously Treated Suspects	47	28	19

DISCUSSION

Early detection of tuberculosis cases as well as understanding the burden of MDRTB is important for designing the protocol of diagnostic tests for tuberculosis and to decide measures for preventing transmission. As per previous RNTCP guidelines, MDR TB was first suspected if acid fast bacilli were demonstrated by microscopy done at the end of 2-3 months of anti-tuberculosis treatment [13]. Phenotypic culture and DST were performed to confirm MDR TB which required additional 2 to 3 months. So patient continued to spread MDRTB in community for 5-6 months, till proper treatment was started based on confirmed culture DST results. Xpert MTB/RIF assay was a milestone in TB diagnosis as it simultaneously detects MTB complex and susceptibility to Rifampicin directly from sputum specimen within 2 hours. WHO policy document (2013) continues to strongly advise use of Xpert MTB/RIF assay as the initial diagnostic test in people with HIV-associated TB, pediatric TB and cases of suspected extra pulmonary TB [14].

In the present study, MTB was detected mainly in the age group of 21 - 30 years (28.26 %) followed by 31 – 40 years (26.08 %) in new TB suspects and 38.30 % & 27.66 % in same age groups in previously treated cases. It coincide with the higher number of the patients enrolled from this age group. Similar finding has been observed in a study by Shittu O Rasaki et al. [15] MTB detection rate was more in male than female. Similar findings were observed in other studies also. [15, 16, 17] In present study, female predominance was noted in Rifampicin resistance for both the groups (1:5 in new TB suspects & 1:1.38 in previously treated cases). In the study by Joydeep et al., male to female ratio was 6:1 among Rifampicin resistant cases. [6] Same socioeconomic & cultural factors may contribute to continued compliances with therapy for women causing drug resistant TB in them. Fear of social isolation is a strong element in denial of disease in women in the male dominated society. [18]

When all 200 cases were considered together, comparison of bacteriological detection by the three methods revealed that culture detected more number of TB cases (48%) as compared to Xpert MTB/RIF assay (46.5%). Microscopy test detected the least number of cases (30.5%). Smear positivity was higher in previously treated suspects (40%) than new TB suspects (21%). (p value .003522). High smear positivity in previously treated cases may be due to low cure rates in them leading to reactivation of the disease. These patients become chronic TB patients & probably excrete TB bacilli in greater number over an extended period.[19] National smear positivity among total patients screened was 11.43 % in 2013 & in Maharashtra it was 8.9 %. [20] In present study, average smear positivity was 30.5 %

(61/ 200). It is far higher than the above mentioned national value; it may be due to the criteria of patient selection. Strongly suspected TB patients & patients with prior history of TB were included in this study and also concentrated sputum pellets were used for microscopy. Rasaki et al reported smear positivity of 34.3 % in MDRTB suspects [15].

The culture positivity in the present study was 49% in new TB suspects and 47 % in previously treated suspects. Culture is the gold standard for diagnosing active tuberculosis. It is highly specific and more sensitive because it can detect as few as 10 viable bacteria per ml. [8] but the limitation of solid culture is that it takes 2-8 weeks due to the slow replication rate. [21, 22]Median time to detect MTB in LJ medium in new suspects was 26 days and in previously treated was 24 days. The turnaround time was slightly higher in specimen having scanty bacilli. It is comparable to the study conducted from the same institution in 2007 where median time to detect MTB was 24 days. [23] The high culture positivity rate in the present study may also be attributed to patient selection.

Xpert assay detected MTB in 46 % of new suspects & 47% of previously treated suspects. The sensitivity, specificity, PPV and NPV of Xpert MTB /RIF assay for MTB detection was 91.84 %, 98.04%, 97.83% and 92.59% in new cases and 95.74 %, 96.23%, 95.74% and 96.23%in previously treated cases respectively. All smear positive cases were culture & Xpert positive in both new TB suspects & previously treated suspects. Xpert MTB/ RIF assay detected 25 more cases in new TB suspects & seven in previously treated as compared to microscopy. High / medium bacterial load was observed by Xpert assay in high grade (3+,2+)smear positive cases while low / very low bacterial load was observed in low grade (1+, scanty) smear positive & smear negative cases.

The performance of Xpert MTB/RIF assay has been compared with culture on Lowenstein Jensen medium. Overall nine discordant results were obtained. Six specimens were culture positive which can be explained by high sensitivity of culture (10–100 viable organisms per ml) than Xpert assay (131 bacilli per ml). [8] Another reason for Xpert negativity may be absence of the target sequence (IS6110) which is expected to be amplified by this method. [24] Such results require confirmation by another molecular assay which was not done in the present study. Three cases were culture negative & Xpert positive. Xpert positivity in culture negative cases may be either due to presence of dead bacilli in the specimen or presence of few viable bacilli in the specimen which may get killed during deconta mination. All three specimens positive by Xpert assay and negative by culture were smear negative indicating very low bacterial load which was missed by microscopy. These cases may get detected if liquid culture method was used instead of solid culture.

Very high Rifampicin resistance (40.42%) was observed in previously treated cased as opposed to 12.24% in newly diagnosed cases which is statistically significant (p-value is .00166). Various studies reported rifampicin resistance ranging from 3.64 % to 25.4 %in new TB cases [25, 26] and 27.55% to 50.94 %in MDR suspects [27, 28]. No discrepancy in DST result was observed between the phenotypic method and Xpert assay. All 25 rifampicin resistant strains were isoniazid resistant also indicating that rifampicin resistance is a good marker for predicting MDRTB.

Nine cases showed isoniazid mono resistance by proportion method. Isoniazid is one of the most effective first-line drugs in anti-TB therapy. Resistance to isoniazid is the most common form of mono-resistance with a prevalence of 10% among new tuberculosis (TB) cases and 28% among retreatment cases reported in 2009 globally. [29] History of previous treatment for tuberculosis is strongly associated with subsequent Isoniazid mono-resistance. [30] Rifampicin sensitive Isoniazid mono-resistant patients will be treated with first line drugs which may not be completely effective. It will result in development of manmade acquired resistance to Rifampicin. Rifampicin mono-resistant patients (though rare) will not be treated with isoniazid, which is a safe and useful drug for them. [31] Therefore, use of rapid tests to detect both Rifampicin resistance and Isoniazid resistance would have better outcomes than tests to detect Rifampicin resistance alone.

A person develops drug resistant TB as a result of either spontaneous mutation or transmission of resistant strain [20, 32]. Spontaneous occurrence of drug resistant mutants in wild strain of mycobacteria for rifampicin is 1 strain in 10^8 bacilli and for Isoniazid is 1 strain in 10^6 bacilli. So resistance to both RIF and INH simultaneously will occur in 1 strain in 10^{14} bacilli [31 IJSS 2015].A cavitatory lesion usually harbors 10^8 - 10^9 bacilli. It is likely to contain only 1 naturally rifampicin resistant and 100-1000 naturally isoniazid resistant strains. A very high bacterial load mainly due to inappropriate treatment with respect to drugs, doses and duration is required to develop acquired resistance. Hence previous treatment for TB is considered as strongest risk factor for development of acquired resistance. It is a measure of effectiveness of ongoing TB control program.

The emergence of drug-resistant TB in Mumbai is alarming as Mumbai has higher levels of MDR-TB than in other parts of India (24%–30% of new cases and 11%–67% of treated case).[33] National Average of MDR TB is 12%-17%, but Mumbai has reported ~25% of MDR TB cases even with a limited diagnostic facility. Extensively drug-resistant (XDR TB was detected in Mumbai in 2005. [33] Responding to the growing threat of MDRTB, Municipal Corporation of Greater Mumbai has rapidly scaled up the access to Gene xpert technology since 2012. [34] A very high rate of drug resistance in newly diagnosed TB cases (12%) in this study should alarm the TB control program to take stringent actions to prevent spread of TB at least in confirmed infectious smear positive DRTB cases.

To conclude, Rifampicin resistance which is a surrogate marker of MDR-TB is more prevalent in previously treated cases (40.42%) than in new TB cases (12.24 %). By providing same day result both for detection and Rifampicin resistance, Xpert MTB/RIF assay provide the much needed diagnostic capacity for effective identification of MDR TB cases.

Considering the high Rifampicin resistance observed in our study in previously treated cases and no additional cases detected by microscopy alone, it is advisable for RNTCP to incorporate Xpert MTB/RIF assay as the first screening test in previously treated TB suspects.

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