RIFAMPICIN RESISTANCE IN PULMONARY TUBERCULOSIS BY GENE Xpert MTB/RIF ASSAY

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ABSTRACT

Objective: Pulmonary Tuberculosis a communicable disease is caused by *Mycobacterium tuberculosis* (MTB). *M.tuberculosis* is developing resistance to first line Anti-TB drugs, thus leading to drug resistant pulmonary tuberculosis (DR-PTB). Our study attempted to determine rifampcin resistance in mycobacterium tuberculosis isolated from patients with pulmonary tuberculosis.

Study Design: A cross sectional study.

Place and Duration: Department of Chest Medicine ward-12 JPMC Karachi from October 2015- September 2016.

Material and Methods: The study was conducted on 220 pulmonary TB suspects (fresh and retreated) on clinical and radiological grounds. A history of contact with MDR- TB patients and previous use of anti-tuberculosis therapy were documented. All sputum samples were analyzed by the Gene Xpert lab.

Results: Among 220 PTB cases 169 (76 %) were MTB positive. Out of 169, 37(21%) were RIF positive. Among RIF resistant cases male to female ratio was 6:1. Primary and secondary RIF resistance were found as 4.74%, 17.16% respectively. Among secondary resistance 11.2% and 5.91% were found defaulters and relapse patients consecutively.

Conclusion: Secondary rifampicin resistant PTB is continuously increasing in our community so early detection of RIF resistance in PTB cases by Gene Xpert RIF/assay is essential for further stoppage of emergence of MDR-PTB.

Key words: Rifampicin, Multidrug resistant tuberculosis, Mycobacterium TB, Pulmonary TB

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INTRODUCTION:

Tuberculosis can engage any site of the body, however Pulmonary tuberculosis is the commonest form accounting for almost 80% of cases[1].

Mycobacterium tuberculosis, is an obligate aerobe and facultative intracellular parasite to mankind[2]. It is also known as tubercle bacillus because it produces tubercles in the lungs[3].

M.tuberculosis (MTB) is a slow growing bacteria with a doubling time of 16-22 hours[4]. MTB is strongly acid fast, varies 1- 4×0.3 - 0.6 µm in length and width respectively[5].

Pulmonary tuberculosis in humans is absolutely transmitted by respiratory droplets[6]. Pulmonary tuberculosis (PTB) starts with capture of *Mycobacterium tuberculosis* into respiratory tract at the level of the alveoli[7].

Mycobacterium tuberculosis bacilli are becoming resistant to anti-tuberculous therapy (ATT) thus PTB may be drug sensitive or resistant[8]. Drug resistance is a serious threat for TB control and prevention programmes in underprivileged and developed countries[9].

Multidrug resistant pulmonary tuberculosis (MDR-PTB) is caused by *Mycobacterium*

*tuberculous*strains unresponsive to at least rifampicin and isoniazid[8,10].

Rifampicin is the most effective first line drug (FLD) used for intensive and continuation phases[11].

Primary drug resistance is now known as drug resistance among fresh cases who have never taken Anti-tuberculosis drugs[10].

Secondary drug resistance is not inborn and has been acquired as a result of ineffective management of pulmonary tuberculosis and which is currently called drug resistance among previously treated cases[12].

The risk factors associated with drug resistant TB (DR-TB) are the past history of PTB and its treatment (defaulters and relapsed), contact with PTB patients and those failing the therapy[13].

Rapid isolation of MTB and detection of rifampicin resistance is a key component of management of pulmonary tuberculosis[14].

The Gene Xpert MTB/RIF is specifically meant for testing sputum samples in patients with pulmonary tuberculosis. It simultaneously detects MTB and rifampicin resistance in sputum samples within two hours. It is a rapid reliable and an automated test with minimum chance of cross contamination which needs the least technical assistance, has the highest sensitivity (99.0%) for smear positive cases[15]. Current study was designed to detect primary or secondary rifampcin resistance in pulmonary tuberculosis suspects by Gene Xpert MTB/RIF assay.

MATERIALS AND METHODS:

This cross-sectional study, was carried out in collaboration with the Gene Xpert lab at Department of Chest Medicine (Ward-12) and Department of Microbiology, BMSI, JPMC Karachi. It comprised of sputum Samples from pulmonary tuberculosis suspects of adult age on the clinico- radiological and hematological grounds, was conducted during October 2015 to September 2016. Indoor or outdoor patients on anti-tuberculosis treatment were excluded.

An ethical approval was obtained from the ethical committee of BMSI, JPMC . Informed consent was taken from patients included in the study.

A total of 220 samples were collected and sample size calculated by Open Epi software version 3.03 in reference of the study "Rapid diagnosis of tuberculosis using Xpert-MTB/RIF assay-Report from a developing country[16]."

One day protocol for collection of sputm was adopted. An early morning sputum from each suspect was taken and analyzed by Gene Xpert lab (ward -12) JPMC.

The main variables included age, gender, marital status, literacy, smoking, alcoholism, past history of Pulmonary TB treatment and history of contact with MDR-TB case. Data was entered, and analyzed using SPSS version 16.0. Quantitative and nominal data was presented as means with standard deviations and frequencies. Characteristics of TB patients with or without RIF resistance were compared using the Chi-square test. A difference was considered significant if p-value was ≤ 0.05.

RESULTS: The study involved analysis of 220 sputum samples from pulmonary tuberculosis suspects. Out of 220 suspects 169 (76%) were Gene Xpert assay MTB positive with 78(46.15%) being 31-40 years old and mean age was 36.02 years.

Males and females were were 97 (57.39%), 72 (42.59%) respectively. Married and unmarried were found at 100(59.18%) 69(40.82%) respectively. The literacy rate was 63(37.27%) while illiterate were 106 (62.72%). Nonsmokers 104 (61.54%) exceeded than smokers 65 (38.46%). Alcoholics were only a few 6(3.5%). History of TB treatment and contact with MDR-TB cases were found at 37(21.89%) and 45(26.62%). Out of 169 patients, 37 were found RIF resistant. The primary RIF resistance was lower 8 (4.73) than secondary resistance 29(17.15%). Among

the secondary RIF resistance 19(11.24%) and 10 (5.91%) were defaulters and relapse cases respectively. The smoking is considered a major risk factor for TB relapse and in this study was found in 21 (56%) RIF- resistant cases with strong statistical significance (p 0.021)

TABLE 1.GENDER DISTRIBUTION OF PULMONARY TUBERCULOSIS SUSPECTED PATIENTS (n=220)

Gender	Contamination n (%)	Negative n (%)	Positive n (%)	Total n (%)
Male	3	25	97	125 (56.81)
Female	2	21	72	95 (43.19)
Total	5	46	169	220 (100)

TABLE 2.POSITIVE PULMONARY TUBERCULOSIS CASES ACCORDING TO GENDER AND RIFAMPICIN RESISTANCE (n=169)

Gender	RIF sensitive n (%)	RIF resistant n (%)	No. of patients n (%)
Male	75 (44.37)	22 (13.01)	97 (57.38)
Female	57 (33.72)	15 (8.87)	72 (42.59)
Total	132 (78.10)	37 (21.89)	169 (100)

TABLE 3.FREQUENCY OF PRIMARY AND SECONDARY RIFAMPICIN RESISTANCE (n=169)

Type of resistance	Rifampicin Sensitive 132 (78.10)	Rifampicin Resistant 37 (21.90)	Total 169 (100)	p-value
Primary resistance	161(95.26)	8 (4.73)	169 (100)	0.000
Secondary resistance	140 (82.84)	29 (17.15)	169 (100)	

TABLE 4.FREQUENCY OF DEFAULTERS AND RELAPSE CASES IN SECONDARY RIFAMPICIN RESISTANT TUBERCULOSIS (n=169)

Type of a case	No. of patients	Percentage (%)
Defaulters	19	11.24
Relapse cases	10	5.91
Total	29	17.15

TABLE 5.DISTRIBUTION OF AGE OFPTB PATIENTS WITHAND WITHOUT RIFAMPICIN RESISTANCE(n=169)

Variables (years)	Patients without RIF resistance 132(78.10)	Patients with RIF Resistance 37(21.90)	p-value
>16-20	7 (5.30)	3 (8.12)	0.251
21-30	35 (26.70)	9 (24.32)	
31-40	66 (50.4)	12 (32.43)	
41-50	13 (9.9)	6 (16.21)	
51-60	6 (4.5)	5 (13.51)	
> 60	5 (3.78)	2 (5.41)	

TABLE 6.COMPARISON OF VARIABLES BETWEEN PTB PATIENTS WITH AND WITHOUT RIFAMPICIN RESISTANCE (n=169)

Variables	Patients without RIF resistance 132 (78.10)	Patient with RIF Resistance 37 (21.90)	p-value
Male	75 (56.81)	22 (59.45)	0.380
Female	57 (43.18)	15 (8.87)	
Married	85 (64.39)	15 (40.54)	0.015
Unmarried	47 (35.61)	22 (59.46)	
Illiterate	78 (59.09)	28 (75.67)	0.063
Literate	54 (40.91)	9 (24.33)	
Smoker	44 (33.33)	21 (56.75)	0.021
Nonsmoker	88 (66.67)	16 (43.25)	
Prior H/O TB treatment			
Yes	8 (6.0)	29 (78.37)	0.000
No	124 (94.0)	8 (21.63)	
Contact with MDR-TB case			
Yes	37 (28.03)	8 (21.62)	0.471
No	95 (71.93)	29 (77.80)	

DISCUSSION:

Various studies have evaluated the rifampicin resistance in pulmonary tuberculosis with different levels of depth.

The present study focused on identification of pulmonary tuberculosis patients with rifampicin resistance by Gene Xpert MTB/RIF assay. From the total of 220 MTB suspects, 76% (169) were Gene Xpert assay positive. In this study 57.39% (97/169) were predominantly male. This was consistent with an Indian study[17].

In this study Gene Xpert assay detected 21.89% (37/169) rifampicin resistant cases. This was in agreement with the study by Nazir et al[18]. (2009) from Lahore, Pakistan. Our study showed higher rifampicin resistance among male, 59.45% (22/37) in comparison to female 40.55% (15/37), the male predominance was supported by another study conducted by Gangly et al[19]. However the difference was statistically insignificant (p=0.380) when compared with or without rifampicin resistance. In this respect our study is supported by the study of Yazar et al[20]. Another study reflected male to female ratio (6:1) among rifampicin resistant cases. This issue might be attributable to behavioral factors for tuberculosis such as smoking and alcohol consumption.[19] Male preponderance for DR-TB is not universally accepted phenomenon, many studies reported no difference in the rate of drug resistance with respect to gender.[20]Most of the patients with rifampicin resistance were in the age group of 31-40 years (32.43%) followed by 21-30 years (24.32%), this was compatible with a study done by Kiran et al.,[13]. Khurram et al.,[21]. considered the smoking as a major risk factor for TB relapse. In our study smoking was found in 21 (56%) rifampicin resistant cases with a strong statistical significance (p 0.021) but Kiran et al[13] showed even higher frequency (66%) in rifampicin resistant cases.

Previous treatment for tuberculosis is a strong risk factor associated with rifampicin resistant tuberculosis (p<0.000). This finding is favored by Li et al.,[22] who inferred that retreated pulmonary TB patients are six times more likely to have rifampicin resistant tuberculosis (RR-TB) than newly diagnosed TB patients. Our research showed insignificant values for illiteracy and alcoholism. This mismatched with an Indian study.[23]

In our research the history of a close contact with MDR-TB cases was 21.62%, an almost similar finding was observed by Mulu.[24]

In this study primary and secondary rifampcin resistance was 4.74% (8/169) and 17.16% (29/169) respectively, and these findings correlated with a study by Adane et al[25]. In India and China primary rifampcin resistance was 2.8% and 5.7% respectively[26] . The result of this study showed that secondary rifampicin resistance was almost four times greater than primary resistance, this finding is supported by the previous research.[27]Secondary rifampicin resistance included 11.2% (19/169) and 5.91% (10/169) as defaulters and relapsed patients consecutively. However findings by Goswami.[28] showed higher level of relapsed cases (44%) when compared with our results.

CONCLUSION:

A speedy increase of rifampicin resistance in Pakistan and other striving countries is a serious threat for TB control programmes. Our study idtenfies the rifampicin resistance in pulmonary tuberculosis in urban community of Karachi.In our study primary rifampicin resistance (4.74%) was found much lower than secondary RIF resistance (17.16%). Defaulters possessed more RIF resistance (11.2%) than relapsed patients (5.91%). So we concluded that TB control programmes should be strengthened to avert discontinuity of ATT thus defaulters and relapses cases are reduced.

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