Frequency of Rifampicin Resistance among Sputum Smear Negative Pulmonary Tuberculosis Cases By Real Time PCR

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MSM SA conceived idea, MSM UU AJ drafted the study, AJ FH collected data, MSM FH did statistical analysis and interpretation of data MSM SA UU critical review manuscript, All approved final version to be published.

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The authors declare that there is no conflict of interest.

Abstract

Background: Drug resistance pattern is changing among new cases of sputum smear negative Pulmonary Tuberculosis in our local population as indicated by gradual rise in multidrug resistance, but no local data is available. Real Time PCR (GeneXpert) is latest development which may help us achieve the pattern of drug resistance. Objective of the present study was to determine the frequency of rifampicin resistance among sputum smear negative Pulmonary Tuberculosis cases by Real Time PCR (GeneXpert).

Methodology: A cross-sectional survey study was conducted at department of pulmonology in Gulab Devi Chest Hospital/ Al-Aleem Medical College, Lahore from January 2018 to February 2019. After taking an informed consent of 210 patients having history and examination strongly suggestive of tuberculosis and sputum smear negative pulmonary tuberculosis were enrolled. Sputum was sent for GeneXpert analysis. Presence of rpo-B gene indicator of rifampicin resistance was noted.

Results: Mean age was 31.9±9.2 years and 109 (51.9%) were male while 101 (48.1%) were female. 17 patients (8.1%) had rifampicin resistance in the sampled population as determined by GeneXpert. There was no effect of gender, mean age, mean number of family members who previously had tuberculosis and mean duration of disease in both groups with and without resistance to rifampicin.

Conclusion: It is concluded that rifampicin resistance in sputum sever negative pulmonary tuberculosis is quite high i.e., 8.1% so its reasons need to be identified.

Key words: Sputum Smear Negative Pulmonary Tuberculosis; GeneXpert; Primary Drug Resistance in TB; Multidrug Resistance TB; Rifampicin Resistance

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Introduction

Rifampicin (RMP) and Isoniazid (INH) are the best medications for treating Mycobacterium Tuberculosis because they are the most efficacious, well tolerated, and cost-effective.[1] MDR-TB is described as tuberculosis caused by mycobacterium species resistant to the antibiotics INH and RMP. MDR-TB continues to threaten progress that Pakistan has made in controlling TB.[2] Pakistan ranks 5th among 27 high burden countries with high burden tuberculous countries.[3,4] As per WHO Global Tuberculosis report 2019, incidence of Tuberculosis in Pakistan is 265/100000. In the year 2018, out of 562000 a total of 3690610 cases were noted.

Drug-resistant tuberculosis (DR-TB) can develop in newly diagnosed or previously treated patients (including relapse, treatment after failure, treatment after loss to follow-up, other previously treated patients, and patients with unknown previous tuberculosis treatment history) or in any type of tuberculosis (pulmonary or extra-pulmonary, smear-positive or smear-negative).[1] In 2010, the percentage

of MDR-TB cases notified was 3.4 among new TB cases and 21 among retreated cases, according to WHO estimates.[2] A positive Mycobacterium tuberculosis culture and drug susceptibility testing are required for an appropriate diagnosis of MDR-TB.1 However, procedures that detect Rifampicin resistance with greater than 95% accuracy and are highly specific for MDR-TB are time demanding. Rifampicin is a key medication that is used to diagnose MDR-TB in more than 90% of cases.[2]

Various publications report an increased risk of MDR-TB in other circumstances, such as in patients treated in the private sector, patients from countries with a history of drug stock-outs or poor-quality drugs, patients with other co-morbidities facilitating malabsorption, etc. If resources are available, culture and DST against FLDs should be performed.[1] For the rapid detection of Rifampicin resistance among Tuberculosis patients, an automated integrated real – time system has recently been developed with the name of Xpert MTB / RIF assay, and evaluated.[6-8]

The new technique integrates genomic amplification (hemi nested PCR), DNA extraction, semi quantitative detection of Mycobacterium Tuberculosis Complex (MTC), and detection of Rifampicin (RIF) resistance with a single cartridge, thereby reducing cross contamination risk and labor time.9 Several patients with active Pulmonary TB have Sputum Smear results negative. They are diagnosed and treated according to history suggestive for Pulmonary TB however, their diagnosis can be confirmed by culture of Mycobacterium Tuberculosis.

Sputum smear negative cases of PTB make a significant part of the total burden of Tuberculosis, and according to the National TB Control Program of Pakistan, 23,999 cases were reported with sputum smear negative PTB in 2010 – 11. In a study (Moure et al) Sixty-four of 85 (75.3%) smear-negative respiratory (n=78) and non-respiratory (n=7) samples with positive cultures of Mycobacterium Tuberculosis Complex (MTC) were detected by the GeneXpert system using the Xpert MTB/RIF assay (GX). 6 samples contained rpo-B mutation reflective of rifampicin resistance. (6/64, 9.35%).[9]

The emergence of MDR is threatening the whole world, especially the developing countries like Pakistan where lack of financial resources poses a great hindrance in war against Tuberculosis. Conventional techniques to diagnose drug resistance are costly, while GeneXpert is a cost effective alternative.

It is generally believed that MDR only presents as sputum smear positive cases, but significant evidence is available regarding sputum smear negative cases of MDR.[6,7,8] Also, there is no local study available about the prevalence of Rifampicin resistance among sputum smear negative cases in Pakistan.

The aim of the present study is to evaluate the effectiveness of GeneXpert for assessment of Rifampicin resistance in smear-negative sputum samples, not done so far in our population. Estimation of Rifampicin resistance among sputum smear negative Pulmonary TB patients is the need of the hour for designing effective empirical regimens, to monitor functioning and progress of national level efforts being carried out against MDR TB. This study will help us to achieve above said goals.

Methodology:

A cross-sectional survey study was conducted at department of pulmonology in Gulab Devi Chest Hospital/ Al-Aleem Medical College, Lahore from January 2018 to February 2019. After taking an informed consent of 210 patients having history and examination strongly suggestive of tuberculosis and sputum smear negative pulmonary tuberculosis were enrolled. Sputum was sent for GeneXpert analysis. Presence of rpo-B gene indicator of rifampicin resistance was noted. For study purpose strict

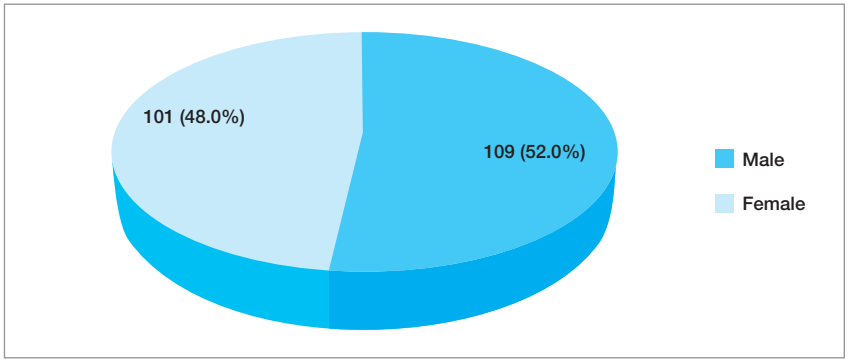

Figure 1: Gender distribution of study cases

Table 1. Common Associated Injuries in Traumatic Diaphragmatic Hernia

	Rifampicin Resistance Found	N	Mean	St. Deviation	St. Error	P-Value
Age in Years	No	193	31.93	9.357	0.674	0.923
	Yes	17	31.71	7.515	1.823	

inclusion and exclusion criteria were followed. Inclusion criteria include patients of either gender having age greater than 16 years of age with sputum smear negative TB, whereas exclusion criteria included Patients having history of relapse, treatment after failure, treatment after lost to follow up, other previously treated patient, patients with unknown previous tuberculosis treatment history, diabetic patients and any connective tissue disorder determined by history or physical examination.

Data Collection

After an informed consent, 210 patients were able to be recruited in the study after fulfilling the inclusion criteria. A questionnaire was used as research instrument, containing background information i.e., age, sex, and duration of symptoms and no of family member (living under one roof) having TB.

Using purposive non-probability sampling, sputum sample was collected from all patients having history and examination suggestive of Pulmonary Tuberculosis as diagnosed and/or suspected by consultant pulmonologist, with initial sputum smear reports negative. These patients were enrolled through Pulmonology department (OPD and indoor) of Gulab Devi hospital Al-Aleem medical college Lahore.

Sample was collected in a sterile plastic container and transported to Pathology department within three hours for GeneXpert analysis. Presence of rpo B gene indicator of rifampicin resistance was noted. Duration of symptoms and no of family member having TB were used as effect modifier.

Data Analysis

Data collected was entered and analyzed in the SPSS version 20. Results were projected using descriptive statistics e.g., mean with standard deviation in case of continuous variables like age, duration of disease, family members with TB and percentages in case of categorical variables like gender and frequency of resistance. Data was stratified for age groups and gender. Post stratification proportions were compared using chi square or Fischer exact test. A p-value < 0.05 was taken as significant.

Results

A total of 210 patients with sputum smear negative pulmonary tuberculosis were included in the study according to inclusion criteria. 109 (51.9%) were male while 101 (48.1%) were female (Figure 1). Mean age of the sampled population was 31.9 ± 9.2 years ranging from 17 to 56 years. Mean number of family members with TB patients with previously diagnostics TB were 1.86 ± 1.4 ranging from 1 to 7. Mean duration of disease in the sampled population was 4.01 months ± 7.80.

Seventeen patients (8.1%) had rifampicin resistance in the sampled population as determined by GeneXpert (Figure 2). To determine whether rifampicin resistance is independent of gender or not we cross tabulated gender verses rifampicin resistance and when chi square test was applied p-value was 0.18 i.e., non-significant.

Similarly, independent sample t-test applied to determine the mean age distribution in with and without rifampicin resistance and we found that there is equal distribution among patients with and without rifampicin resistance (Table 1). Similarly, mean number of family members previous diagnosis of TB were equally distributed in patients with and without rifampicin resistance and mean disease duration has no effect on the rifampicin resistance (Table 2).

Discussion

More than 2 billion people (about one-third of the world population) are estimated to be infected with Mycobacterium tuberculosis. The global incidence of tuberculosis (TB) peaked around 2003 and appears to be declining slowly. Globally, TB incidence is falling at about 2% per year and between 2015 and 2019 the cumulative reduction was 9%. This was less than half-

Table 2. Mean disease duration distribution in patients with and without Rifampicin resistance

	Rifampicin Resistance Found	N	Mean	St. Deviation	St. Error	P-Value
Duration of Disease in months	No	193	3.97	1.836	0.132	0.82
	Yes	17	4.47	1.940	0.471	

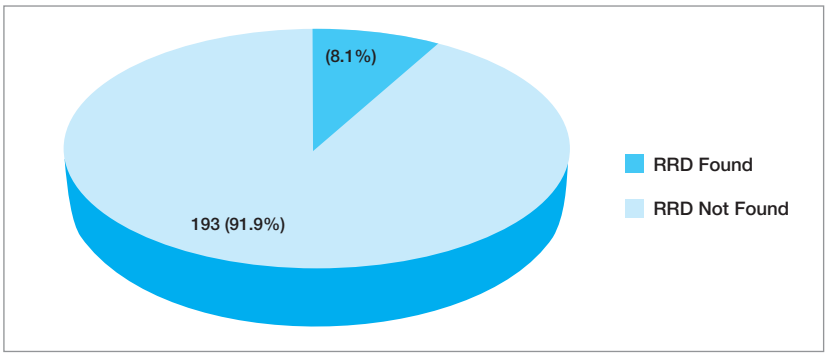

Figure 1: Gender distribution of study cases

way to the End TB Strategy milestone of 20% reduction between 2015 and 2020.

As per WHO, a total of 1.4 million people died from TB in 2019 (including 208 000 people with HIV). In 2019, an estimated 10 million people fell ill with TB worldwide. 5.6 million men, 3.2 million women and 1.2 million children. TB is present in all countries and age groups. But TB is curable and preventable. Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). In 2019, the 30 high TB burden countries accounted for 87% of new TB cases.

Eight countries account for two thirds of the total tuberculosis burden worldwide., With India leading the count, it is followed by Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa. Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. A global total of 206,030 people with multidrug- or rifampicin-resistant TB (MDR/RR-TB) were detected and notified in 2019. This is a 10% increase from 186,883 in 2018. Ending the TB epidemic by 2030 is among the health targets of the United Nations Sustainable Development Goals (SDGs). To achieve millennium development goals, thus drug resistance must be addressed.

One difficulty in achieving the goals described is that a dramatic TB resurgence occurred in some areas during the 1990s. In Africa, this resurgence was largely due to the HIV epidemic, but it was also compounded by poor access to health services. In Eastern Europe, this resurgence can be attributed to widespread economic decline, secondary declines in the overall quality of health services, poor living conditions, alcoholism, and the emergence of infection due to MDR strains of M. tuberculosis.[14]

The third MDG target (to reduce the incidence of TB to <1 case per million by 2050) appears to be unachievable currently. An annual decline of 15 percent in the worldwide incidence of TB is required to meet this goal. Even with complete implementation of all the control measures advocated by the World Health Agency, the third MDG target goal cannot be achieved by 2050.

As noted above, the two most important targets for TB control have been to reach at least 70 percent case detection and 85 percent treatment success.11 Case detection rates are highest in western Pacific, Americas, and Southeast Asia (68 to 70 percent) and lowest in Africa, Europe, and the eastern Mediterranean (46 to 52 percent). Asia and Africa account for most global cases of TB (55 and 31 percent, respectively). Two thirds of the "missing cases" occur in China, India, and Africa.[11]

Data on treatment success rates follow similar geographic patterns, although these rates reflect only the proportion of patients who received treatment. It is sometimes uncertain whether these patients completed an effective treatment course. The western Pacific, Americas, and Southeast Asia have had the greatest success (78 to 92 percent); Africa, Europe, and the eastern Mediterranean have fared less well (70 to 83 percent). Success is limited in part by the high prevalence of HIV infection in Africa and drug resistance in Eastern Europe.

Rifampicin is the most effective drug against tuberculosis. Situation seems regarding MDR-TB different in Pakistan, as poverty is on rise and with population exploding at exponential rate, our health system is at high burden.

Rifampicin resistance is rising as shown by our study. 17(8.1%) sputum smear negative TB patients had rifampicin resistance. This is quite high figure as reported by others which have shown almost 5% rifampicin resistance and usually these patients are neglected. In our sampled population male and female distribution was equal showing that tuberculosis is equally affecting both strata of our population. An infected woman will spread the disease to children and other household members if not detected early

rising trends of pulmonary tuberculosis in women may change the trends in epidemiology of tuberculosis. Younger teenagers and school children will be affected.

Mean age in our study was about 30 years which is most productive age in human life. It shows that tuberculosis is not only a chronic disease with many health implications but also a cause of loss of production and family support in part of the patients. Most of the patients included in our sample had one or more family members previously effected with TB. The problem here is the late presentation of the patients. Mean time lapse between start of symptoms and presentation to a treatment center was 4 months which is quite high. Although it ranged from 1 to 7 months but as much, treatment delay results in more complication and poor outcome. Rifampicin resistance is equally affecting both groups i.e., men and women in our sampled population showing that the change of mechanism rely other than the natural differences. Similarly mean age was also equally distributed showing no effect on rifampicin resistance in our sampled population. Mean number of family members had no effect and mean duration had no effect in producing the rifampicin resistance.

Limitation of current study is its small sample size and population selection from a tertiary care hospital which is not representative of our total population.

Conclusion

It is concluded that rifampicin resistance in sputum sever negative pulmonary tuberculosis is quite high i.e., 8.1% and patients with history of delayed presentation and other family member previously with TB should be diagnosed for rifampicin resistance by using GeneXpert. GeneXpert is a revolutionary invention regarding treatment and diagnoses of rifampicin resistance in our population. Strategies are needed to address the issue and get a real picture of isoniazid resistance in our population.

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