Prevalence and detection of drug-resistant tuberculosis in Hazara Division, Pakistan

Accepted 14th May, 2018

ABSTRACT

Tuberculosis (TB) is ranked as the second most infectious disease worldwide after acquired immune deficiency syndrome. However, the exact frequency of drug resistant TB in developing countries is not yet known. This study was conducted at the Programmatic Management of Drug resistant TB (PMDT) site of Ayub Teaching Hospital Abbottabad. A total of 635 sputum samples were collected from clinically suspected drug resistant TB cases and examined by GeneXpert MTB/Rif assay. Of the 635 samples, 468 patients with history of Cat-I were tested and 27 (5.76%) were found to be rifampicin resistant. Similarly, out of 137 Cat-II samples, 9 (6.56%) cases were rifampicin resistant. Moreover, 30 close contacts of MDR-TB were tested through GeneXpert MTB/Rif assay and 4 (13.3%) were found to be rifampicin resistant. The prevalence of drug resistant TB in Hazara Division of Pakistan is studied for the first time, using rifampicin resistance as a marker. A total of 40 MDR-TB suspects were detected and confirmed as rifampicin resistant which showed emergence of drug resistant TB in Hazara region. The findings of the current study may help in designing and executing TB treatments in the developing countries in future.

Key words: Contagious, drug resistance, GeneXpert, rifampicin, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is one of the most widespread and fatal disease in developing countries. The general factors that speed up the process of infection include poverty, overcrowding, and addiction to alcohol, drug abuse, and immunodeficiency (Padmanesan et al., 2013; Juan-Pablo et al., 2013). It is a considerable challenge for health due to its serious infectious and communicable nature, morbidity, and mortality in adults (World Health Organization, 2013). Tuberculosis is ranked second across the globe after acquired immune deficiency syndrome, as one of the most prevalent causes of death from infectious diseases (World Health Organization, 2016). TB infects an estimated eight million people and out of which two million people are dying every year (World Health Organization, 2013). According to WHO estimates, 9.6 million TB cases occurred globally in 2014, with 133 cases per 100,000 population (World Health Organization, 2016). Pakistan ranks fifth among the 22 High Burdon Countries and shares about 65% of the total TB burden in the Eastern Mediterranean Region. Treating TB with antibiotics was a significant achievement to win the battle in between humans and Mycobacterium tuberculosis, but the use of antibiotics has also been connected with the development of drug resistance. The causes of drug resistance include inaccurate usage of anti-TB drugs, wrong administration of medicines, low patient willingness or substandard quality of drugs. Drug resistant TB is an emerging challenge and poses a continuous threat to the global control efforts of TB (Migliori et al., 2007; Raviglione and Smith, 2007).

Assessment of drug resistant TB prevalence is the fundamental part of TB control (Pablos-Méndez et al., 1998). Many studies have reported the prevalence of DR-TB. According to WHO global report 2016, 480,00 people developed multidrug resistant TB annually (World Health
Organization, 2016). Pakistan stands at fourth position among 27 MDR high burden countries in the world (World Health Organization, 2016). Although the existence of drug resistance in Pakistan has been common, but no detailed report is yet to be documented due to limited centers of AFB culture and drug sensitivity testing throughout the country (Ejaz et al., 2010).

Exact and early diagnosis is crucial to control the disease. Drug sensitivity testing (DST) on cultured samples is the predictable technique used to identify resistance to first- and second-line TB drugs. The foremost disadvantage of culture is that it is time consuming as it takes weeks to get results (Al-Zamel, 2009). Development of GeneXpert MTB/RIF Assay has ended the need of a rapid test for detection of drug resistance. In 2010, the first rapid molecular test, GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), was introduced for detection of MTB and rifampicin resistance simultaneously (Steingart et al., 2013). Rifampicin is most powerful antibiotic and important part of anti-tuberculosis therapy (Blumberg et al., 2003). It interferes with bacterial DNA dependent RNA polymerase and inhibit transcription (Sulochana et al., 2014; Floss and Yu, 2005; Ho et al., 2009). The rpoB encodes for an active site enzyme. rpoB of gene contain β subunit of bacterial RNA polymerase which is sensitive to rifampicin. Any type of mutation, such as point mutation, insertion and deletion, in rpoB gene could results in rifampicin resistance (Campbell et al., 2001; Soniya and Molly, 2014). GeneXpert MTB/RIF assay detects MTBC DNA and mutation in RNA polymerase β-subunit gene (rpoB gene) in a single reaction (Helb, 2010; Van Rie et al., 2010).

The detection of rifampicin resistance may act as an alternate marker for drug resistance (Amita and Geeta, 2015) because mono resistance to rifampicin is quite rare. It was also observed that 90% of rifampicin resistant cases also indicate resistance to isoniazid (Kheira et al., 2016). The GeneXpert MTB/RIF is an innovative completely automatic diagnostic molecular test with a diagnostic sensitivity of five genomic copies of purified DNA and 131 cfu/ml of M. tuberculosis in sputum. Furthermore, it is capable of identifying more than 99.5% mutations of rifampicin resistance and indicates DR-TB, in just two hours (Van Rie et al., 2010; Chang et al., 2014). The present study was aimed to determine the prevalence of drug resistant Tuberculosis in Hazara Division of Pakistan.

METHODS

This study was conducted at the Programmatic Management of Drug resistant TB (PMDT) site Ayub Teaching Hospital (ATH) Abbottabad from, April 2014 to March, 2015. A total of 635 sputum samples were collected from clinically suspected drug resistant tuberculosis cases and examined by GeneXpert MTB/RIF assay in the PMDT site, ATH Abbottabad. The samples were collected from the patients, which were referred to PMDT site ATH Abbottabad from all the health facilities of Hazara Division Health including District Headquarter Hospitals (DHQs), Tehsil Headquarter Hospitals (THQs), District TB control Centers (DTCs), Private Clinics and Basic Health Units (BHUs). The study was part of the ongoing treatment of the TB patients and therefore an ethical approval was not needed. Moreover, there is no active ethical committee both at the site of the research conducted nor in the author institution.

Data regarding past anti-tuberculosis treatment history, clinical findings, and laboratory reports were collected from the subjects and recorded in a pre-design Performa. In the current study, 2–4 ml early morning sputum samples were collected into sterile screw capped plastic bottles (graduated 50 ml). Then the samples were labeled bearing the name and identification number of the patient.

The samples were preceded for molecular detection of MTB and rifampicin resistance by using GeneXpert MTB/RIF assay. The GeneXpert system consists of GeneXpert Dx instrument and Xpert MTB/RIF cartridges. Cartridge contained all the components required for a PCR reaction. Sample reagents (Sodium Hydroxide and Isopropanol) were added in the ratio 2:1 (v/v) to sample and shaken vigorously (10-20 times) and incubated for 10 min at room temperature. The specimens were shaken again and incubated for 5 min. It was made sure that samples liquefied with no visible clumps of sputum. Thereafter, 2 ml sample was added into the cartridges using the sterile transfer pipette. Samples were dispensed slowly to minimize the risk of aerosol formation. Then cartridges were loaded into the GeneXpert Dx instrument and tests were initiated. The results were interpreted by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and displayed as “MTB detected (High, medium, Low, very Low) or “not detected” and “Rifampicin resistance detected” or “Rifampicin resistance not detected”.

RESULTS

District wise detection of MTB

In a the total of 635 samples collected, 297 were MTB positive, out of which 40 were found to be rifampicin resistant. Of the 40 rifampicin resistant cases, 18 (45%) belong to district Abbottabad, 17 (42.5%) to district Mansehra, and 5 (12.5%) to district Haripur, as shown in Figure 3. This shows that Abbottabad and the close areas are more prone to MTB rifampicin resistance than the other areas (Figure 1).

Male and female cases

In all referred specimens of 297 cases, 147 (49.5%) and
150 (50.5%) were found as MTB in males and females, respectively. While out of total 40 RIF resistant cases, 19 (47.5%) and 21 (52.5%) were detected as RIF resistant in males and females, respectively (Figure 2). This shows that resistant strains almost equally infected both genders.

**Distribution of MTB and RIF resistance in different age groups**

All the patients were distributed into five groups based on age: ≤ 15, 16-30, 31-45, 46-60, 60-75 and 76-90 years age
Figure 3: Determination of the MTB and rifampicin resistant cases on the basis of age groups.

groups. The distribution of patients by age showed that most of the MTB and rifampicin resistance detected cases (63.9% MTB and 10.6% rifampicin resistance) belong to the age group 16 to 30 years (Figure 3). This means that young stage is more prone to MTB.

Incidence of MTB and RIF resistance in CATI and CATII

Retreatment cases of CAT-I and CAT-II are more vulnerable to drug resistance, therefore suspects from different registration groups of both categories (CAT-I & CAT-II) were tested. Out of 468 samples of CAT-I retreated patients, MTB was detected in 235 (50.2%) suspects and rifampicin resistance was detected in 27 (5.8%) suspects. Most of the rifampicin resistance detected cases, that is, 10 and 8 belong to groups relapse and failure, respectively. Moreover, 45 (29.2%) MTB and 4 (2.6%) Rifampicin resistant detected cases were observed from registration group cured (Figure 4).

While determining the MTB and rifampicin resistance cases in CAT-II retreatment cases, the results of GeneXpert showed that out of 137 cases, a total of 53 (38.7%) were MTB detected. Of these 53 cases, 8 (20.5%), 18 (64.3%), 14 (66.7%) 1 (25%), 5 (16.7%) MTB cases were detected in cured, relapse, failure, default and treatment completed patients, respectively (Figure 5). While in the 137 tested cases, a total of 9 (6.6%) were rifampicin resistant. Out of these 9 (6.6%) were observed 0, 5 (17.9%), 3 (14.3%), 1 (25%), and 0, in cured, relapse, failure, default and treatment completed patients, respectively (Figure 5).

MDR-contact

Close family contacts of drug resistant TB patients were also tested for detection of rifampicin resistance. For this purpose, 30 MDR-TB family contacts were tested, out of which 4 (13.3%) were found positive (Figure 6).

Comparison of GeneXpert and AFB

Of the 281 total smear positive cases, 252 (89.7%) were found as MTD and though 354 cases were found to be smear negative while using AFB, but 45 (12.7%) of these smear negative cases were also found to be MTD using GeneXpert (Figure 7).

DISCUSSION

DR-TB poses a continuous threat to the global control efforts of TB (National Tb Control Program, 2013; Gandhi et al., 2010). Estimation of drug resistance TB is very much important for its epidemiology and to control its transmission. Drug resistant TB existence has been identified in Pakistan and it is frequently occurring, but there is no detail report because diagnostic centers are not enough in the country. There are few laboratories that perform AFB culture and drug sensitivity testing; therefore, most of the drug resistant TB cases are not properly diagnosed. Secondly, the diagnostic laboratories are limited to the urban areas while majority of these TB patients are
residing in remote areas and as such, cannot access to diagnostic facilities to carry out the required tests. The literacy rate is also extremely low in these areas so people do not understand the severeness of the disease.

Finally, TB is generally accepted as a mark of disgrace, so people are hesitating to be diagnosed. Past anti-tuberculosis treatments known to be a major risk factor for acquiring drug resistant TB. There are evidences that show

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**Figure 4:** Distribution of MTB and rifampicin resistant cases from different registration groups of CAT-I patients diagnosed by GeneXpert/Rif Assay.

**Figure 5:** Distribution of MTB and rifampicin resistant cases from different registration groups of CAT-II patients diagnosed by GeneXpert/Rif Assay.
more chances of DR-TB in Cat-I and Cat-II retreatment cases (Hahzad et al., 2013; Haji et al., 2009). Previous worldwide studies have focused on the need for drug sensitivity testing to prevent DR-TB prior to the start of Cat-II treatment after receiving Cat-I treatment. But the main disadvantage of the Drug Sensitivity Testing is, the more time it takes, the delay in the start of the Cat-II medicines which may be dangerous for patient itself and for the
community as well. Early diagnosis and early treatment are very much crucial to counter the transmission of TB. Therefore, there is a need for fast and valid test that can detect drug resistance rapidly. Recently, WHO recommended GeneXpert Rif/Assay as a preliminary diagnostic test for MTB and rifampicin resistance detection. In 1999, a study carried out in Siberian prison showed that the rate of treatment failure of Cat-II was 35% in Cat-I patients who failed to respond to anti-tubercular treatment ATT (Kimelring et al., 1999). Another study from GSVM Medical College India presented that the CAT-II failure is more when the pretreatment sputum bacterial burden is higher (Sabudh et al., 2008), whereas in the present study, a total of 468 patients with history of Cat-I were analyzed through GeneXpert MTB/Rif Assay, the results of which showed that 27 (5.76%) were found to be rifampicin resistant. Similarly, patients with Cat-II history were also analyzed. In this case, 137 samples were processed through GeneXpert MTB/Rif Assay, the results showed that 9 (6.56%) cases were rifampicin resistant as shown in Figures 4 and 5.

Figure 3 shows the referral of suspects MDR from different district of Hazara division. This figure clearly shows that majority of these suspects (that is, 355 (56%) were referred from the district Abbottabad. This is because the diagnostic site is at the tertiary care hospital and most of these patients were visiting the said hospital for their routine checkup. Figure 1 also indicates that most of these detected cases were from district Abbottabad having 18 (2.8%) cases of Rifampicin resistant. Out of the rifampicin resistant detected cases, the ratio on the basis of gender, as shown in the Figure 4, was found to be higher in females 21 (52.5%) as compared with males 19 (47.5%). On the basis of age, detection of rifampicin resistance was minimal in age group less than 12 years. Figure 3 also shows that rifampicin resistance was high (22 out of 208 cases) among the people with age ranging from 16 to 30 years. It was also observed that out of the rifampicin resistant cases, most of these cases (55%) were between 16 and 30 years. Patients were categorized on district wise basis. The study of the district wise distribution showed that of the detected cases, more cases were diagnosed from district Abbottabad as compared with other district of the study. The Rifampicin resistance rate was 18 (45%), indicating its high prevalence in district Abbottabad. Out of the MTB detected cases, the highest proportion was found in females which was 150 (50.5%) as compared with males which was 147 (49.5%). Sex wise distribution of rifampicin resistance showed that female had more cases as compared with males, which was 21 (52.2%) and 19 (47.5%), respectively. Age wise, the patients were divided into five groups in which the highest proportion of MTB and rifampicin resistance (63.9 and 10.6% respectively) was observed in the age group range of 16-30 years. This study showed that females and age group 16-30 years are more susceptible to MTB and rifampicin resistance.

Another risk factor which has been an issue in many countries is extended person to person transmission of DR-TB. This study also documented person to person transmission of drug resistant TB. For this purpose, 30 close contacts of MDR-TB were tested through GeneXpert MTB/Rif assay and out of them, 4 (13.3 %) were found to be rifampicin resistant as shown in Figure 6. The comparison of the efficacy of the DST staining and MTB GeneXpert Rif assay is shown in Figure 7. It is obvious that MTB GeneXpert is a more sensitive technique and could be preferred over the DST staining for an accurate MTB detection and drug resistance.

As the samples were collected and processed in a single center, therefore these results may not depict the picture of the whole country. However, Ayub Teaching Hospital is very old and as the only tertiary care center in the Hazara region, people usually come from all the districts and as such, our results to some extent could reflect drug resistant TB burden in Hazara region. The current study documents the considerable drug resistant to rifampicin and the indication of MDR-TB, constituting a huge health concern in Hazara division of Pakistan. The present study clearly increases health concerns to reconsider the re-treatment regimens in order to overcome the rising outbreaks of DR-TB and its associated fatality.

**Conclusion**

This study reports for the first time the emergence of drug resistant TB in Hazara Division. It found that resistance was high among patients having previous history of antituberculosis drugs. The total MTB detected was 47% and rifampicin resistance was 6.2%. Moreover, it was found that GeneXpert MTB/Rif assay was much more sensitive as compared with AFB microscopy for the detection of MTB and its rapidly improved drug susceptibility testing facilities in the rural areas.

**ACKNOWLEDGEMENTS**

The authors are grateful to Dr. Shahid Wali coordinator and Naseer Khan Lab attendant PMDT site Ayub Teaching Hospital Abbottabad for providing the necessary facilities for this study. They would also like to thank Dr. Fazli Wahid for his critical review of the manuscript.

**REFERENCES**

