FREQUENCY OF RIFAMPICIN RESISTANCE AND SMEAR CONVERSION IN CATEGORY II TUBERCULOSIS PATIENTS USING GENEXPERT ASSAY IN QUETTA, PAKISTAN

Amir Muhammad¹, Kaleemullah *¹, Muhammad Shafee², Shireen Khan², Bibi Hajira¹, Mohammad Ashraf², Sajid Nabi¹, Bibi Tahira¹, Muhammad Umar²

¹ Department of Microbiology, University of Balochistan, Quetta, Pakistan.
² Fatima Jinnah TB Hospital, Quetta, Pakistan
³ Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan.

Received: 10 April 2017          Accepted: 30 April 2017

Abstract:
Background: Pulmonary tuberculosis is highly contagious disease commonly transmitted through air droplets and is a global public health concern. Almost one third of world population is infected tuberculosis and in last 2-3 decades the resurgence of drug resistance have posed serious threat over the globe. This study was aimed to evaluate RIF resistance in category II TB patients. Methods: A total of 100 sputum samples were collected from category ii (relapse, loss of follow up, failure, interrupted) TB patients visiting Fatima Jinnah Chest Hospital Brewery Road Quetta. All the samples were examined through GeneXpert MTB/RIF assay and other relevant information were collected from patients using predesigned questionnaire.

Results: About 23 patients were found RIF resistant with 7/23 (30 %) male and 16/23 (70%) female. While, 5 (21.7 %) patients from 15-34 year age group converted to smear negative in first month of treatment. Likewise, in second month 3/23(13%) patients in 15-54 years, followed by 5/23 (21.7%) in third month in 15-64 years and in fourth month 3/23 (13%) patients were seroconverted in 15-24 years age group. Similarly, in fifth month 4/23 (17.4%) patients were converted in the age 35-65 Years followed by seventh month 1/23 (4.3%) in, 15-24 years age group. While 2 (8.7%) in the age 25-34 were died.

Conclusion: Higher prevalence of RIF resistance in category II TB patients and sero-conversion was found in 15-34 years age group.

Keywords: Tuberculosis, geneXpert MTB/RIF, rpoB gene, Sputum smear conversion

Corresponding author:
Kaleemullah,
Department of Microbiology,
University of Balochistan,
Quetta, Pakistan.
E-mail: kaleemullah@um.uob.edu.pk

Please cite this article in press as Kaleemullah et al. Frequency of Rifampicin Resistance and Smear Conversion in Category II Tuberculosis Patients Using Genexpert Assay in Quetta, Pakistan, Indo Am. J. P. Sci, 2017; 4(04).
INTRODUCTION:
Tuberculosis (TB) is a chronic debilitating disease causing more than 1.8 deaths annually over the globe. The worldwide spread disease ‘Tuberculosis’ is the oldest chronic infectious disease which has been evolved with human for million years. In human, TB infections have been documented at least from last 9000 years [1]. *M. tuberculosis* infects about one-third of the human population. It damages the vital human organs such as lungs, central nervous system, lymphatic system and circulatory system [2]. For the diagnosis of TB, different specimens such as sputum, bronchial lavage, CSF, pleural fluid and urine, are proceeded for Ziehl-Nelson and oramine staining. Similarly, Culture for tubercle bacilli and drug susceptibility testing are also used for TB diagnosis. NAAT (nucleic acid amplification tests), Pyrosequencing (PSQ), Line-probe assay (LPA), GeneXpert MTB/RIF are also used for the diagnosis of TB [3]. Worldwide increase in drug-resistant tuberculosis poses a major public health threat. First-line anti-tuberculosis treatment often fails in patients with rifampin-nonresistant or multidrug-resistant tuberculosis. Inappropriate treatment can result in the development of resistance to additional antibiotics and increased mortality.

Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin (RIF), uses hemi nested real-time polymerase-chain-reaction (PCR) assay to amplify an MTB-specific sequence of the *rpoB* gene. It is probed with molecular beacons for mutations within the rifampicin-resistance determining region [4]. The Global Project analyze the resistance varying in different countries with different percentages. Multidrug-resistant tuberculosis with the highest rates have been reported in Gujarat, India with (33.8%) Nepal (48.0%), New York City, 30.1%, Korea (14.5%) and Bolivia (15.3%) respectively [5]. Early diagnosis may help to control tuberculosis through increased case detection rates and early diagnosis that may help in early treatment and hence reducing morbidity rate in the community [6]. In urban cities advanced diagnostic facilities like, culture and molecular based diagnosis may be available, but such facilities are still unavailable in most of the per urban health care centers in low income countries.

Pakistan stands 5th among high burden countries, while have been placed 4th among high MDR countries. The increasing trend in MDR tuberculosis poses causes great challenge in disease control throughout the world [7]. The acquisition of resistance by the bacterium is a random event, and in a given mycobacterial population, 1 in $10^8$ bacteria mutates to develop isoniazid resistance, while 1 in $10^9$ mutates to develop rifampin resistance [8]. Several rapid diagnostic techniques have been introduced for the diagnosis of MDR-TB and MTB cases more accurately and in less time. In recent few years WHO has introduced molecular beacon technology using PCR for the direct detection of MTB in clinical specimens. Similarly, WHO recommended to use GeneXpert (MTB/RIF) assay for all smear negative, MDR and HIV-TB cases. This study have been designed to estimate the frequency of rifampicin resistance and smear conversion in relation to treatment time in category I1 tuberculosis patients using gene expert assay in Quetta, Pakistan.

METHODS:
Study area & study design
This study was carried out in Fatima Jinnah TB Hospital, Quetta. The city is provincial capital with pleasant weather during hot summer months. General People migrate from far long areas of the province and even from adjoining Sindh province during summer season. It is the 6th largest city of the country which have also accommodated a notable number of afghan asylum seekers [9]. A total of 100 (male=44: female=56) patients of tuberculosis (category II pulmonary tuberculosis) were evaluated for rifampicin resistance in Relapse, Default (loss of follow up), Interrupted and failure category II pulmonary tuberculosis patients.

Study inclusive criteria
Patients who have been treated previously at least more than one month were included in this study. The data were collected using pre structured questionnaire.

Sample preparation and Gene Expert assay
The sputum samples were collected from patients in sterile disposable pre labeled container. All the samples were subjected for acid fast staining and also screened through Gene Expert for RIF resistance cases detection. The content were mixed with sample dilution buffer followed by vigorous shaking and incubation for 15 minutes at room temperature. The sample contents were then aspirated into cartridge and cartridge barcode was scanned and module was run automatically for 2 hrs following manufacturer’s instructions.

RESULTS:
A total of 100 TB patients in category II were evaluated for the assessment of prevalence of MDR cases and smear conversion with reference to time. On age basis no positive cases was found in male patients , while only 2 female patients were found positive 05-14 years age group followed by 10 male
and 11 female patients were found in 15-24 age group. Similarly, 9, 5, 3 male and 16, 5 and 6 female patients were recorded in 25-34, 35-44 and 45-54 years age group. While 6, 11 Male and 10, 11 female patients were found positive in 55-65 and 65+ age group correspondingly.

Out of 100 cases 23 were detected as RIF resistance comprising of 07 (6%) male and 16 (13%) female patients (Figure 3). Similarly, 25-34 years, the more productive age group were more susceptible 7/23 (30 %) to RIF resistance using expert assay.

In the sputum smear conversion intensive phase of relapse cases two patients expired in 25-34 age group. While one patient have shown smear conversion in the 1st month of treatment in the age group of 15-24 years. Similarly, other patients of the same age group have shown smear conversion in 3rd and 4th month of their treatment. Similarly, The total Three (3) RIF resistance loss of follow-up cases sputum smear conversion was hereby analyzed where the results illustrates that patients ranging from 35-44 converted in the 03 month of treatment, whereas the patient recover at the earliest in the 1st month at the age ranging from 25-34. In addition, the patient converted at the age ranging from 35-44 at the 2nd month. The smear conversion of thirteen (13) failure cases have shown conversion in different months in different age groups. Out of thirteen 3,4 and 2 patients were seroconverted in 15-24, 25-34 and 35-44 year group and 2, 1 and 1 patients from 45-54, 55-64 and 65 + age group, respectively. In interrupted cases one of two patients were found converted in the 1st month belonging from 15-24 whereas, the second patient was converted at the 7th month of the same age group. The comparative results regarding RIF resistance sputum conversion illustrates that 5 cases were converted in 1st month of treatment from 15-34 age group, followed by 3 patients with smear conversion in 2nd month each belonging to different age group (15-24, 25-34 and 35-44 years). However, 5 cases were also converted in the 3rd month (Figure. 1).

![Fig.1: Sputum conversion of RIF resistance cases of different age groups and months in category II TB patients](image-url)
In the first month out of 23 RIF resistant 5 patients in the age of 15-34 (21.7%) were converted. Likewise, in second month 3 Patients in the age of 15-54 (13%), followed by 5 patients in 3rd month with age range 15-64 and 3 (13%) patients were converted in fourth month in the age group of 15-24. In the fifth month 4 (17.4%) patients were converted in the age of 35-65+, while, in seventh month 1 (4.3 %) patient in the 15-24 age group and 2 (8.7%) patients have been died in 25-34 year age.

**DISCUSSION:**
Tuberculosis in Pakistan is on increasing trend and is placed on 5th position among high burden and 4th among high MDR countries throughout the world. MDR patients are resistant to at least first line drugs (Isoniazid and rifampicin). This high incidence and prevalence of tuberculosis may be attributed to many factors like, poverty, illiteracy, less access to health care facilities etc.

In this study, 23/100 (23 %) category II TB patients have been shown to be resistant to rifampicin. In Pakistan MDR cases varies from 2.3 % to 17.9 % in previously treated TB patients. Our findings are close in agreements with Taimor and Umair, 2009, who also reported that individuals with previous history of interrupted or discontinue treatment are at high risk of MDR TB [10]. Similar opinion have been reported from other researchers from Karachi, Pakistan [11]. This relatively higher MDR cases in our study may also be attributed to Afghan patients. Who may not complete their treatment due to infrequent border crossing and for follow up in such a lengthy course of treatment. Our these observation have also been supported by Ibrahim et al., 2002, who is also of the view that higher MDR cases in Pak afghan region may be due to visits of afghan patients [12].

In this study, 7/23 male and 16/23 female patients were found with MDR TB. Our these findings corroborate with Bawri et al., 2013, who reported more MDR cases in male than female patients while [13], these findings contradict with Ganguly et al., 2015, who reported 84/328 cases from male and 14/328 from female patients [14]. This higher number of RIF resistance cases in this study could be attributed to local culture, in which female patients can visit freely even to health care centers. These factors may help in delay of follow up leading to serious consequences.

In our study, most of the patients became sputum smear negative in 1st 4-5 months of treatment. Out of 23 patients, 5 were converted to sputum smear negative after 1st month treatment followed by 3 in second month 5 by 3rd month and 3 patients were converted in fourth month of their treatment. In the fifth month 4 patients and in seventh month only one patient were found sputum smear negative (Fig.2). Our findings are similar to Bawri et al., 2008, who studied 100 cases of sputum smear positive conversion into smear negative category-l pulmonary tuberculosis and reported 92 % conversion in 1st three months of treatment [15]. This quick smear conversion may be ascribed to the fact that healthy and more productive age of the patient may have good provoked immune system to combat the disease.

In this study, high rate of MDR 19/23 (83%) was observed in age 15-44 years in category 11 TB patients. Similarly, higher smear conversion 17/23 (74 %) was also observed in more productive age group, 15-44 years (Fig.1). This may possibly be due the fact that it is the more active age group that may
become more vulnerable to get infection and also due to strong immune system can encounter infection more effectively.

CONCLUSION:
In conclusion, Xpert assay is a valuable addition in the field of diagnostic mycobacteriology. It has greatly shortened the detection time for up to 2 hrs. Higher MDR TB cases were detected in category II patients with loss of follow up, relapse and interrupted treatment. Similarly, more productive age (15-44 Years) was more vulnerable to quicker smear conversion. Greater attention may be required while managing tuberculosis campaign in areas with afghan asylum seekers for continuous and proper follow up.

REFERENCES:
15. Bawri, S., Ali, S., Phukan, C., Tayal, B., & Baruwa, P. A study of sputum conversion in new smear positive pulmonary tuberculosis cases at the monthly intervals of 1, 2 & 3 month under directly observed treatment, short course (dots) regimen. Lung India. 2008; 25(3), 118-123. doi:10.4103/0970-2113.44122.