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ORIGINAL ARTICLE

Frequency of Rifampicin Resistance in Primary Pulmonary Tuberculosis at Sheikh Zayed Hospital Rahim Yar Khan

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ABSTRACT

Background: A World Health organization estimated show that almost 10 million people effect from TB and almost 1.5 million people died from this disease in 2018. Pakistan is ranked 5th in overall TB burden and ranked 4th in drug resistance development of developing countries. About 510,000 case of TB arise every year in Pakistan and 15000 patients develop drug resistance each year. Drug resistance cause huge mortality rate. It should confirm by using drug susceptibility test before treating the patient for drug resistance and for cure.

Objective: To find out the frequency of rifampicin resistance in primary pulmonary tuberculosis patient by using drug susceptibility test.

Methodology: This cross sectional study was conducted among all patient who visited department of pulmonology of Sheikh Zayed Hospital Rahim Yar Khan from 2016-2018. A total of 1184 smear positive TB patients having no history of ATT intake patient who visited pulmonology department were included in study. Patients sputum was further sent for PCR based Gene Xpert assay for detection of Mycobacterium tuberculosis and rifampicin resistance.

Results: A total 1184 TB patients were included in study carried out from 2016 to 2018 at pulmonology Department Sheikh Zayed hospital Rahim Yar Khan. Based on residential locality, 771 (65.11%) belonged to Rahim Yar Khan, 123 (10.38%) to Khanpur, 208 (17.56%) Sadiqabad, 82 (6.92%) Liaqatpur.Out of these 1184 TB cases Rifampicin resistance was detected in 65 (5.5%) cases. In current study rifampicin resistancewas detected in males and females40 (61.5%) and 25 (38.5%) respectively .

Conclusion: This study shows that rifampicin resistance among patient is directly affected with socioeconomic status of patient. TB is curable but when a TB patient is resistance to one or multiple drugs especially rifampicin, chance of cure will be less and patient will acquire more resistance to other drugs.

Keywords: Tuberculosis, rifampicin, Gene Xpert assay, drug susceptibility.

INTRODUCTION

Tuberculosis is most communicable and infectious disease globally.[1]In 2018 World Health organization estimated that about 10 million people suffer from TB in which 5.7, 3.2 and 1.1 million patients were men, women and children respectively, and almost 1.5 million died from the disease.World health organization (WHO) survey reported 6.5 million new cases in 2017 and 7 million new all over the world in 2018.[2] According to WHO report, cases noticed in 2018 is higher than 2017. It was seen that there was a huge growth in TB patient cases present during 2009-2012. In WHO survey report of 2018, it was observed that most of TB cases were present among areas of South-East-Asia, Africa and the Western pacific (44%), (24%) and (18%) likewise. Some other countries like India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh and south Africa have TB burden (27%), (9%), (8%), (6%), (6%), (4%), (4%) and (3%) likewise. These 8 countries carry 2/3 of TB burden globally.[3, 4] Pakistan ranked 5th in overall in TB burden countries and Ranked 4th in drug resistance. Pakistan is endemic for TB and about 510,000 TB cases arise every year and an estimated shows that almost 15000 patient developing drug resistance TB each year.[2]

Anti-tuberculosis drug resistance is a major public health problem worldwide. Drug resistance arises due to improper use of anti-tuberculosis chemotherapy like prescription of improper regimens, spurious drugs and failure to take complete course of treatment. Essentially, drug resistance arises in areas with weak tuberculosis control programmes.[5]

Rifampicin, a bactericidal drug from the rifampicin group is an essential drug for short course anti-tuberculosis therapy. Without rifampicin, treatment duration gets prolonged and is more likely to fail.[6] It acts by inhibiting the mycobacterial transcription by targeting DNA-dependent RNA polymerase. Resistance to rifampicin is developed due to mutations in rpoB gene.[7]In global tuberculosis report 2018, reported resistance to rifampicin in Pakistan was 4.2% in isolates from new cases (primary resistance) and 16% in retreatment cases.[8] A study done in Quetta Pakistan, primary resistance to rifampicin was 5.2%.[9]One study done at Lahore, Pakistan showed 4.9% of primary resistance to rifampicin.[10] Another study from same area showed 15.44% primary rifampicin resistance.[11]Drug resistance is a major determinant of treatment outcome.

Different techniques are being practiced to detect Mycobacterium tuberculosis and drug resistance. Unfortunately, current methods of detection of resistance to anti-tuberculosis drugs are not ideal. Sputum for culture and sensitivity is considered gold standard, but it takes about two-three months. Other methods are automated liquid culture-based system and molecular methods of detection. Automated liquid culture based system includes Mycobacterial Growth Indicator Tube (MGIT) and Versatrek.[12] These methods take about two weeks for results, are very expensive and require trained technical personnel.

Recently introduced Gene Xpert MTB/RIF is a real time PCR that detects the presence of Mycobacterium tuberculosis complex and its susceptibility to rifampicin within two hours.[13]It is found in various studies that 90% of Rifampicin resistant isolates also exhibit resistance to isoniazid.[14] Therefore, the detection of rifampicin resistance may serve as a surrogate marker for multidrug resistant tuberculosis. According to available studies Gene Xpert achieved a pooled sensitivity of 94% and pooled specificity of 98%.[15]

For successful treatment of tuberculosis, a combination of drugs to which tuberculosis bacilli are susceptible is essential. If TB bacilli are resistant to one or more drugs especially to rifampicin, chances of cure will be less and bugs will more acquire resistance to other drugs. So, it is vital to know the susceptibility of TB bacilli to these drugs before start of treatment. Standard tests (culture and sensitivity on solid and liquid media) take longer time (6-12 weeks). If resistance present in a case, this delay will result

in progression of disease in that individual as well as risk of spread to others and may induce further resistance to other drugs. Gene Xpert MTB/RIF assay confirms presence of mycobacterium TB and detects resistance to rifampicin within 2 hours. Therefore, it is recommended by WHO as a rapid diagnostic test for tuberculosis and resistance to rifampicin to avoid above mentioned unfavorable outcome.[12] Prevalence of rifampicin resistance may differ in various countries and even various parts of same country. It is recommended to conduct a study to know the local figures to guide treatment. To best of my knowledge so far there is no such study has been done in this area.

MATERIALS AND METHODS

Setting: Rahim Yar Khan is a city in Punjab province of Pakistan. It is the 17th largest city of Pakistan by population.It is the capital of Rahim Yar Khan District and Rahim Yar Khan Tehsil.Sheikh Zayed Medical College, established in March 2003, is a public sector Medical College in Rahim Yar Khan. It is named in honor of His Royal Highness ShaikhZayed bin Sultan Al Nahyan. The Medical College is affiliated with ShaikhZayed Hospital which has 1700 beds, which is the biggest hospital of Rahim Yar Khan District. This Cross sectional study was done in the Department of Pulmonology Sheikh Zayed Hospital, Rahim Yar Khan during 01 January 2016 to 31 December 2018.

Ethical consideration: Ethical approval was taken from ethical committee of Sheikh Zayed Medical College / Hospital, Rahim Yar Khan.

Clinical samples: This research work was carried out on sputum specimens from 1184TB suspects patients. These selected patients were belonged to different localities of South Punjab including Rahim Yar Khan,Khanpur, Sadiqabad, Liaqatpur. These patients were initially screenedfor TB through AFB sputum smear microscopy. All suspected patientswith persistent clinical symptoms of TB werereferred to TB lab forfurther confirmation of MTB/DR by GeneXpertMTB/RIF assay. The experimental work for AFB smear microscopy andGeneXpert MTB/RIF assay was carried out at Shiekhzayed hospital Rahim Yar Khan. Before specimen collection, the patients wereinformed about the study and written consent wasobtained. The patients were asked to collect thesputum specimens following the standard sputumcollection technique using the recommended sterile plastic (50-mL Tarson) containers. One half ofthe collected sputum specimen was used for AFBsputum smear microscopy, whilethe remaining portion was used for GeneXpertMTB/RIF assay. The research study was conductedfollowing the approved guidelines framed by theresearch committee of ShiekhZayed Medical College Rahim Yar Khan.

Inclusion Criteria and Exclusion Criteria: Sputum smear positive newly diagnosed pulmonary tuberculosis cases of either sex having age more than 12 years were included in study. While, all cases of extra-pulmonary tuberculosis and sputum smear negative pulmonary tuberculosis were excluded from study.

Smear microscopy: Direct smears were prepared for microscopicexamination of AFB by applying one drop of sputum samples on a clean glass slide which waslabeled with the ID number. The smear was airdried followed by heat fixation and staining usingZN staining methods. The stained smears wereexamined microscopically using 100× objectivelens. Control slides (known positive and knownnegative) were included as procedural control with each run of ZN staining[16].

GeneXpert MTB/RIF assay: Two mL of sample reagent, 1 mL of sputum specimen was added and the sputum specimen wasallowed to liquefy for 15 min. Using a sterile dropper provided with the kit, 2 mL of mixture wastransferred into the GeneXpert cartridge. This cartridge contained the required reagents for nucleicacid amplification and RIF drug resistance detection. The inoculated cartridge was loaded into theGeneXpert machine, as shown in Figure 1. Resultswere displayed by the GeneXpert system automatically within 2 h. The operator can read and print thetest results as “MTB detected; RIF resistance notdetected.”

Data analysis: Data was analyzed with the help of SPSS version 20. Qualitative variables was presented as frequency and percentages.

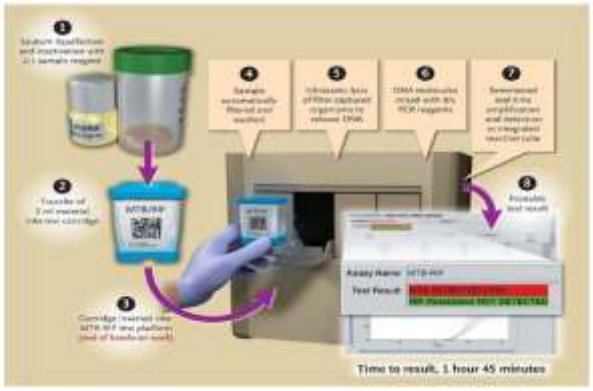

Figure 1: Different procedural steps in the Xpert MTB/RIF assay process.[17]

RESULTS

A total of 1184 TB patients were enrolled in current study. Our data showed that frequency of TB patients was 399, 410 and 375 in 2016, 2017 and 2018 respectively (Figure 2). Study population was comprised on564 (47.64%) females and 620 (52.36%) males (Table 1). The male-to-female ratio was 1.10:1. Based on residential locality, 771 (65.11%) belonged to Rahim Yar Khan,123 (10.38%) to Khanpur, 208 (17.56%) Sadiqabad, 82 (6.92%) Liaqatpur (Figure 3).The results showed that majority of the TB patients were belonged to Rahim Yar Khan city while least were form Liaqatpur City. The Socio-economic status of 97 (8.19%) TB patients was very good followed by mediocre417 (35.21%) and majority of TB patients 670 (56.57%) were belong to poor status. Out these 1184 TB cases, Rifampicin resistance was detected in 65 (5.5%) cases(Table 2).It is worth mentioning that among positive cases of rifampicin resistance, 40 (61.5%) were males and 25 (38.5%) were females (Table 1). Our collected data also showed that rifampicin resistance was greater 38 (58.46%) in poor socio economic status patients and least 7 (10.76) was recorded in good socio economic status TBpateints Table 2.

DISCUSSION

Drug resistance of mycobacterium tuberculosis is a huge problem now a days. Recently, several studies have been reported that emergent nations have increased number of drug resistant population and it was observed that drug resistance was at peak in most densely populated areas.[18-20]In 1967 Salman H. et al. reported in his study that in Pakistan the rate of antituberculosis drug resistance was 87% in newly treated patients whereas, 47% rate was found with no treatment background.[21] A. Aziz et al. stated that in the population of Lahore 5.1% rifampicin resistant patients was found and it was also seen that rifampicin was newly introduced drug of those times so the resistance rate was low.[22] Similarly, in 1976 Siddiqiet al. reported that rifampicin resistance rate was 2-4% in the patients of the same population due to its limited usage.[21] Furthermore, the rate of Rifampicin resistance was increased due to the increase amount of its usage and freely available. In earlier reports it was observed that rifampicin resistance rate was higher due to multiple drug treatment in which usage of rifampicin is not necessarily included.

The present study showed that the rate of antituberculosis drug resistance was highly increased among patient who had visited Sheikh Zayed Hospital Rahim Yar Khan. Additionally, numerous studies have been reported high primary drug resistance among young and adult population of different areas.[23-25]

It was observed that primary and acquired drug resistance are main cause of treatment failure. There is significantly high drug

resistance was expected among population. It is observed that rifampicin resistance patient rate of present study is normal. Rifampicin resistance rate is very low or absence observed by multiple other studies conducted in different region.[22, 26-28]In present study it was observed that the primary rate of rifampicin resistance in patients were very low.

In present study patients were divided in three major groups; high, mediocre and low socio-economic status. Firstly, it was observed that patient with primary pulmonary TB having high socio-economic status, has less rifampicin resistance frequency because they have enough money to bear the burden of the disease. Their socio-economic status fully cover their expenses. Second group is then divided in patient of mediocre socio-economic status with primary pulmonary TB has more frequency of rifampicin resistance than high socio-economic status. More frequency of mediocre patient is because they have less income to fulfill their household needs. They use self-medicine for treatment of disease and even they don't investigate the disease. Therefore, due to use of multiple unnecessary drugs can cause rifampicin resistance in primary pulmonary TB patients. Those patients with poor socio-economic status and having primary pulmonary TB are huge in number than mediocre and high socio-economic status. The reason behind this is, these patient does not have their home, they don't have enough money to buy good food, and they don't have money to buy medicine when they fall sick. Due to these reasons' rifampicin resistance frequency of these patient is higher than others. Other studies conducted among other regions also show same factor of higher and decrease in frequency of rifampicin resistance patient.

Male and female genders was also studied in this observation. In present study male has higher frequency 61% of rifampicin resistance than females 38%. A.Alemuet al. also observed higher frequency 55% of rifampicin resistance in males.[29] Frequency of male is because due to high rate of self-medicine among them. Patient with TB start eating medicine for cough and sickness. It cause the resistance to rifampicin among them.

Basic purpose of this study is to point out that problem of high frequency of drug resistance still exist in the country which is affecting the tuberculosis patient's treatment at large scale. Which the cause the mortality high among the population. It is observed that developing countries are more affected by drug resistance and frequency is high among these countries. It is needed to overcome the factors causing the drug resistance among tuberculosis patient. Tuberculosis program is in developing countries can overcome these factors by educating the patient about and medical and paramedical staff about accurate diagnosis and treatment. Accurate laboratory facilities should be provided to diagnose the drug susceptibility. There should be regular supply of primary and secondary drug for tuberculosis patient. In addition, tuberculosis drug should be recommended by a trained professional who examined the patient and he should be expert in his work.

Declaration of conflicting interests: The author(s) declared no potential conflicts of interestwith respect to the research, authorship, and/or publicationof this article.

Table 1: Frequency of Tuberculosis patient and Rifampicin resistance in study subjects.

Year	Cases	Male	Female
2016	399	200	199
2017	410	215	195
2018	375	205	170
Total	1184	620	564
Rifampicin resistance	65	40	25

Table 2: Socio-economic status of TB Patients

Socio-economic status	Total	Rifampicin resistance
Very good [i]	97	07
Mediocre [ii]	417	20
Poor [iii]	670	38

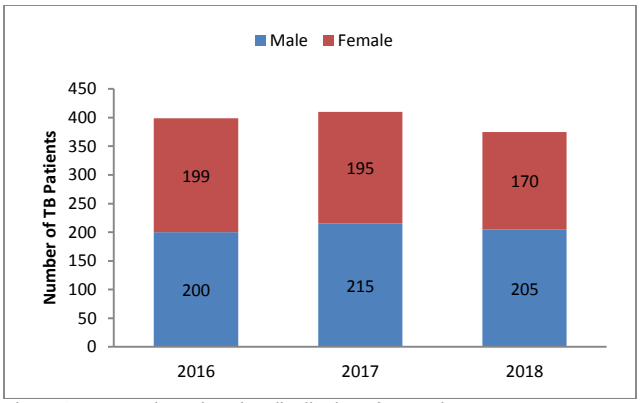

Figure 2: Year and gender wise distribution of TB patients.

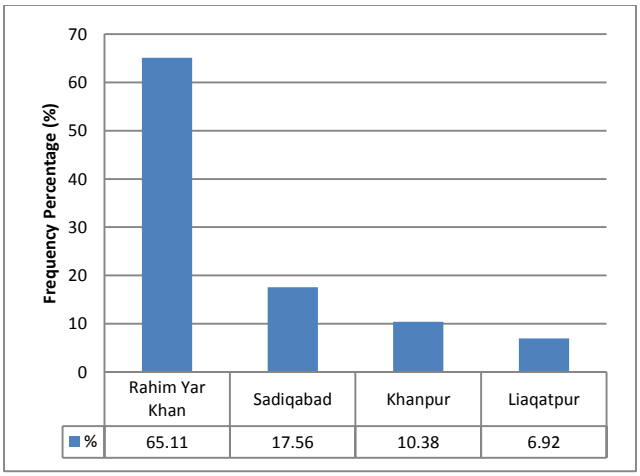

Figure 3: Column chart showing residential locality of study subjects.

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