

Development of Drug Resistance among Comorbid and Non-Comorbid HIV-Negative Relapsed Cases of Tuberculosis in Lahore, Pakistan

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Research article

Keywords: Comorbid, Drug Resistant TB, MDR-TB, XDR-TB, Relapse

Posted Date: March 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-16991/v1>

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Abstract

Background: Mycobacterium tuberculosis sometimes become resistant to the drugs that are used to treat it. Drug resistant TB (DR-TB) is spread in the same way as drug susceptible TB. DR-TB is a public health crisis. This study aims to find the pattern of drug resistance and correlations between drug resistance and comorbid/non-comorbid conditions in patients with a relapse of TB.

Methodology: A cross-sectional study was conducted among 200 HIV-negative relapsed TB patients from 2016-2017 in Mayo Hospital Lahore. The patients' sputum samples were tested by Ziehl-Neelsen staining to observe acid-fast bacilli. The demographics and medical history of patients was recorded, who were positive for AFB in their sputum samples. Molecular procedure of Gene-Xpert assay was conducted to detect the presence of MTB and rifampicin resistance in the samples. Whereas, the drug susceptibility test (DST) was conducted on the LJ culture medium containing drugs.

Results: Out of 200 relapsed TB cases; 97 were comorbid, 99 were non-comorbid. The most prevalent comorbidities were hypertension (42 cases- 43.3%), diabetes (45 cases-46.4%) and hepatitis B (14 cases- 14.4%). Among 97 comorbid patients; 37 worked as laborers, 43 earned less than 20,000 PKR and 23 were found to have a history of imprisonment. Whereas in non-comorbid patients; 20 worked as laborers, 28 earned less than 20,000 PKR and 12 had been in prison before.

The Gene-Xpert test detected rifampicin resistance (RR) in 20 comorbid (20.6%) and 33 non-comorbid (33.3%) patients. Whereas, the drug susceptibility test (DST) showed that 22 comorbid (22.7%) and 33 non-comorbid (33.3%) patients were RR. A contrast was seen in the results of Gene-Xpert and DST; Gene-Xpert detected 3 cases of RR-negative whereas the same 3 cases were found to be RR-positive on DST. Only 1 case was RR-positive on Gene-Xpert but RR-negative on DST.

17 comorbid patients (17.5%) were diagnosed with MDR-TB and 5 (5.2%) with XDR-TB. Whereas, in non-comorbid patients, there were 26 cases of MDR-TB (26.3%) and 5 cases of XDR-TB (5.1%). There were 2 patients (2.1%) resistant to all drugs.

Conclusion: There was a deviation in the results of molecular Gene-Xpert assay compared to the conventional culture methods. Drug resistance was relatively higher in non-comorbid patients than comorbid patients, however, the difference between the two is not very significant.

Introduction

Tuberculosis, caused by *Mycobacterium Tuberculosis* (MTB), continues to be responsible for many deaths worldwide. In 2017, around 10 million people in the world developed this disease. Two thirds of these cases occurred in India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%)¹. Anti-TB drugs, aimed for treatment of TB, are taken for a time period of 6–9 months. The U.S. Food and Drug Administration (FDA) has approved the

usage of 10 drugs for the treatment of TB. Drug susceptible TB is treated by first line anti-TB agents including; isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamide (PZA) ².

Sometimes patients who have once received treatment and recovered from the initial episode of TB infection, can undergo a relapse of TB. This recurrence of TB can be due to the regrowth of MTB, which caused the initial infection, and may lead to the development of drug-resistant TB. This is because sometimes *Mycobacterium tuberculosis* becomes resistant to the drugs that are used to treat it. Drug resistant TB (DR-TB) is spread in the same way as drug susceptible TB. A patient could develop DR-TB directly from a DR-TB patient, by misuse or mismanagement of drugs used for treatment, in case of a relapse, or from an area where drug resistance is common. Three categories of drug resistance are used for global surveillance and treatment; Rifampicin Resistance (RR-TB), Multi Drug resistance (MDR-TB) and Extensive Drug Resistance (XDR-TB). RR-TB is resistant to rifampicin but susceptible to isoniazid. MDR-TB is the resistance to rifampicin and isoniazid, the two most potent anti-TB drugs. Whereas, XDR-TB includes; MDR, resistance to a fluoroquinolone (Levofloxacin, Moxifloxacin, Gatifloxacin, Ofloxacin) and at least one of the three second-line injectable drugs (Amikacin, Kanamycin, or Capreomycin) ^{1, 2, 3}. Other core second line anti-TB drugs include Cycloserine, Ethionamide and Linezolid, among others ³.

DR-TB is a public health crisis. According to the WHO Global TB Report 2018, around 558,000 people developed RR-TB, 82% of which were cases of MDR-TB. Almost half of the World's cases of MDR/RR-TB were from three countries; India (24%), China (13%) and Russia (10%). Around 8.5% of World's MDR-TB cases were extensively drug resistant (XDR-TB) ¹. Pakistan has the fourth highest prevalence of MDR-TB⁴.

Certain medical conditions are associated with the development of TB. Some underlying social determinants are also responsible for the spread of TB. HIV, diabetes, malnutrition, tobacco smoking and alcohol consumption are among some comorbidities and risk factors that increase the likelihood of developing TB disease. These conditions can worsen the clinical course of TB and are contributors of TB disease burden ⁵. This study aims to assess the development of drug resistance in comorbid and non-comorbid cases of relapsed TB.

Methods

A cross-sectional study was conducted in patients from Mayo Hospital Lahore, which is located centrally in Lahore, with patients coming in from all over Punjab. A total of 200 HIV-negative patients were included in the study, who had previously received a successful treatment for TB and had fallen into a relapse. These patients were asked to submit their sputum samples for the laboratory diagnosis of MTB. The laboratory diagnosis was performed by the microscopic examination of sputum (by Ziehl-Neelsen staining to observe acid-fast bacilli) and culturing. Upon positive confirmation of AFB in their sputum samples, the patients were interviewed through a semi-structured questionnaire to assess their demographic/social characteristics and medical history. For over 100 years, Ziehl-Neelsen (ZN) staining has been used as the primary diagnostic technique to stain smears using 1% Carbol Fuchsin, 25%

sulphuric Acid and 0.3% methylene blue. The results were reported according to WHO/International Union Against Tuberculosis and Lung Disease (IUATLD), according to which; no AFB observed per 100 high power fields is reported as negative, 1–9 AFB per 100 fields is reported as the exact count of bacilli per 100 fields, 10–99 AFB per 100 fields is reported as 1+, 1–10 AFB per field (at least 50 fields) is reported as 2+ and more than 10 AFB per field (at least 20 fields) is reported as 3+. All the sputum samples were processed for culture by Petroff's method. LJ medium, with pH 6.8, was used for culturing of MTB. The sputa samples were tested for drug susceptibility of 10 first/second-line anti-TB drugs namely; rifampicin, isoniazid, streptomycin, ethambutol, ofloxacin, kanamycin, amikacin, capriomycin, ethionamide and cycloserine. A modern molecular procedure, Gene-Xpert assay, was used to detect the presence of MTB and rifampicin resistance in the samples. Whereas, the drug susceptibility test (DST) was conducted on the LJ culture medium containing drugs with the following concentrations using standard proportion method: Rifampicin 40.0 µg/ml, Isoniazid 0.2 µg/ml, Streptomycin 4.0 µg/ml, Ethambutol 2.0 µg/ml and Pyrazinamide 100.0 µg/ml and the second line drugs was used in their respective concentrations ⁶.

Results

Out of 200 relapsed TB cases; 97 were comorbid, 99 were non-comorbid and the data of 4 patients regarding comorbidities was unavailable. Maximum number of comorbid patients (34 cases) were from the age group of 30–40. The demographics of the patients can be seen in table 1.

The most prevalent comorbidities were hypertension (42 cases- 43.3%), diabetes (45 cases–46.4%) and hepatitis B (14 cases–14.4%). The comorbid cases can be seen in figure 1.

The sputum smear microscopy and culture test results showed that out of all 200 patients, 186 patients were both smear and culture positive. 11 sputa samples were smear-negative and culture positive. However, 2 sputa samples were smear-positive but culture-negative. The results of Gene-Xpert test were 100% positive for MTB, which confirmed that these patients had a relapse of TB.

Table 2. shows rifampicin resistance found in patients through Gene-Xpert and Drug susceptibility Test (DST). A contrast was seen in the results of the two tests as DST showed two additional RR-positive cases which were shown to be RR-negative by Gene-Xpert test. However, the DST results of one patient were unavailable

The DST of first/second-line anti-TB drugs was conducted in all patients. Table 3. depicts the drug resistance of patients to first-line drugs, fluoroquinolones and second-line injectable drugs.

There were 2 patients (2.1%) resistant to all drugs. 67 out of 200 (33.5%) patients were found to be susceptible to all 10 drugs, out of which; 40 (59.7%) were comorbid and 27 (40.3%) were non-comorbid patients.

Discussion

The demographics of the patients were assessed. Out of 200 relapsed cases of TB, 97 were comorbid and 99 were non-comorbid patients. Most comorbid patients were substance abusers and belonged to the lower economic strata with a history of imprisonment as well. This was in contrast to the non-comorbid population which had a significantly lower proportion of substance abusers, people from lower economic class and criminal record holders. However, DR-TB cases were seen in the sample. There were more cases of MDR-TB in non-comorbid (26 cases, 26.3%) population as compared to the comorbid population (17 cases, 17.5%). The number of XDR-TB cases was the same in both populations (table 1.).

The conventional diagnostic methods include microscopy, culture and drug susceptibility testing (DST), but these techniques are time-consuming, taking around 8 weeks to give results. Recent developments in molecular diagnostics allow prompt and early diagnosis of TB. Gene-Xpert MTB/RIF assay is real-time PCR for the rapid detection of MTB and antibiotic sensitivity of the specimens within 2 hours. For laboratory diagnosis of the specimens, Gene-Xpert and DST were conducted. DST of MTB is determined by observing growth or inhibition of MTB in a culture medium consisting of anti-TB drugs^{7,8}. All specimens were positive for MTB on Gene-Xpert, as they were taken from relapsed patients of TB.

However, it was seen that the sensitivity of the two procedures was different. The Gene-Xpert test detected rifampicin resistance (RR) in 20 comorbid (20.6%) and 33 non-comorbid (33.3%) patients. Whereas, DST showed that 22 comorbid (22.7%) and 33 non-comorbid (33.3%) patients were rifampicin resistant. A contrast was seen in the results of Gene-Xpert and DST; Gene-Xpert detected 3 cases of RR-negative whereas the same 3 cases were found to be RR-positive on DST. Only 1 case was RR-positive on Gene-Xpert but RR-negative on DST (table 2.). So, the Gene-Xpert assay gave 3 false-negative and 1 false-positive results. Despite significant advancements in molecular testing, definitive testing for microbial identification and antimicrobial susceptibility can only be ensured by culture tests. This does not make molecular testing incompetent as it is adequately sensitive, specific and much rapid to be used in diagnostics. Although culture testing is considered to be a gold standard but it takes days to give results, which makes Gene-Xpert an effective and useful diagnostic tool.

The DST was conducted for 10 first- and second-line anti-TB drugs. The results showed that resistance to FLDs was relatively higher in non-comorbid patients, as compared to comorbid patients. In case of rifampicin and isoniazid resistance, a difference of around 11.3% and 14.5% was observed between comorbid and non-comorbid patients, respectively. Streptomycin resistance was relatively higher in comorbid patients than non-comorbid patients, with a difference of approximately 6.8% between the two. Ofloxacin resistance was relatively higher in comorbid patients, with a percentage difference of 4.5% as compared to non-comorbid patients. There was no significant difference in resistance to second-line injectable drugs between the two categories of patients. Moreover, other second line drugs ethionamide and cycloserine show relatively higher resistance in non-comorbid patients by a small percentage difference of around 2.6% and 2%, respectively (table 3.). In both comorbid and non-comorbid patients, MDR-TB was seen in greater prevalence relative to XDR-TB. Even though the number of XDR-TB cases was the same in the two categories of patients, MDR-TB cases were relatively higher in non-comorbid

cases. Although, the term 'resistant to all drugs' has not been classified by WHO, yet there were two patients who were resistant to all ten drugs.

From the results it can be observed that there is a difference in the development of drug resistance between the two categories of patients, with more drug resistance being developed in non-comorbid patients. However, the difference is not significant enough to draw any clear conclusions. So, the development of drug resistance cannot entirely be attributed to the comorbid diseased condition of the patients.

Conclusion

There was a deviation in the results of molecular Gene-Xpert assay compared to the conventional culture methods. The conventional methods still stand authentic, however, the advanced molecular techniques may be used together with these methods, as both of these procedures have their pros and cons. There is also a need of emphasis on good laboratory practices to avoid cases of false-positives and false-negatives. Drug resistance was relatively higher in non-comorbid patients than comorbid patients, however, the difference between the two is not very significant. This shows that the development of drug resistance cannot be attributed solely to the development of comorbidities.

Declarations

Ethics approval and consent to participate:

The study was conducted with the approval of Pakistan Health Research Council ethical review committee. The data of the patients was collected with their written consent.

Consent for publication:

All the authors have consented for publication

Availability of data and material:

The data was collected from Mayo Hospital Lahore, with prior approval of competent authorities. All relevant data are within the paper and its supporting Information files.

Competing interests:

The authors have declared that no competing interests exist.

Funding:

This was a non-funded study.

Acknowledgements:

The authors are grateful to the teams at Pakistan Health Research Council for facilitating the study.

Abbreviations:

MTB- Mycobacterium Tuberculosis

TB- Tuberculosis

DR-TB- Drug Resistant Tuberculosis

RR-TB- Rifampicin Resistant Tuberculosis

MDR-TB- Multi-Drug Resistant Tuberculosis

XDR-TB- Extensive-Drug Resistant Tuberculosis

DST- Drug Susceptibility Testin

ZN- Ziehl-Neelsen

AFB- Acid Fast Bacilli

FLD- First Line Drugs

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Tables

Due to technical limitations, all tables are only available for download from the Supplementary Files section.

Figures

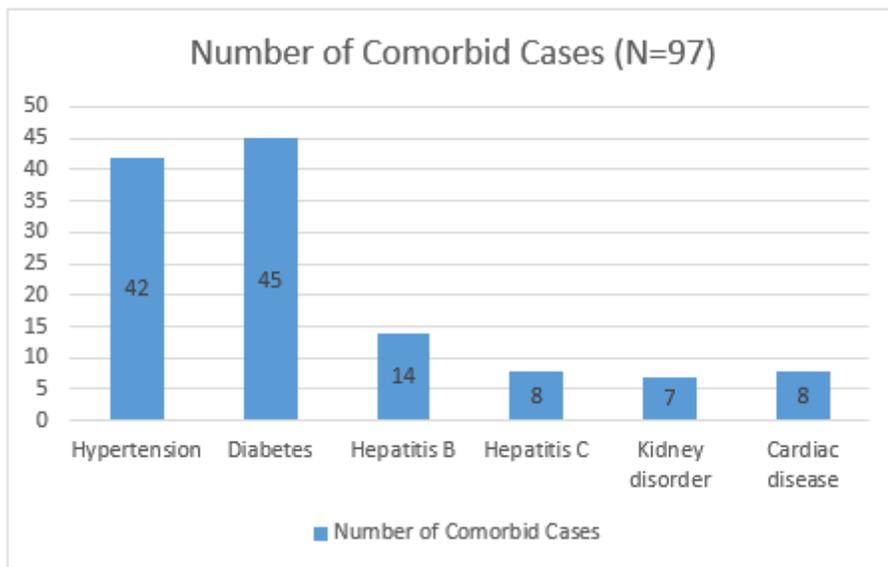


Figure 1

Comorbidities in the patients.

Supplementary Files

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- [table1.docx](#)