THE EMERGENCE OF PRE-EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS AMONG NEWLY DIAGNOSED MDR-TB PATIENTS IN THE LARKANO REGION, SINDH

Marvi Muzaffar [1], Sasui Wadho[1], Sindhu Zahid[2], Suhail Sarki[1], Ali Akbar[1], Almas Rahim Balouch[3], Muhammad Parial Shahani[4]

1. *Provincial TB Control Program Sindh*
2. *Isra University Karachi*
3. *Baqai Medical University, Karachi*
4. Shaheed Mohtarma Benzair Bhutto Medical University, Larkana

ABSTRACT

Background: The emergence of pre-extensively drug-resistant tuberculosis (Pre-XDR-TB) is the major hurdle for TB prevention and care programs especially in developing countries like Pakistan. This trend of drug-resistant mycobacterium increasing day by day, so we have discussed about emergence of pre-extensively drug resistant tuberculosis in this study, which help the peoples about awareness and through this, clinicians can reduce the emergence of drug resistant in future. Tuberculosis (TB) still remain a challenge to health authorities throughout the world. It is most dangerous in the form of drug resistant either multi-drug resistant or pre-extensively drug resistant.

Objective: We have conducted this study in order to determine pattern of pre-extensively drug resistance tuberculosis in MDR-TB patients Zero follow-up reports, pre XDR,

Materials and Methods: Study was conducted at the PMDT site Chandika Medical hospital, Larkano, Pakistan from Dec 2017 to 2020.All Zero month pulmonary samples of MDR and Pre-XDR DST patients of both genders having different ages were included in this research. Five to 10 ml of sputum samples were collected into a sterile 50 ml falcon tube at baseline and all specimens were stored at -20˚C until transported to Biosafety Level 03 laboratory Chandka Medical Hospital Larkano

The study protocol was approved by Additional Director of Tuberculosis program Sindh.
Results: Amongst 64 patients, 23 were females (35.94%) and rest 41 (64.06%) were males. Among 64 patients 15 (23.44%) were negative for microscopy and other 49 (76.56%) were positive. and 59.38% were between 10–40 years age group, rest of the participants ranged from 41 to 70 years. Mean age was 34.3±4.2. Moreover, 14 patients (21.88%) were pre-XDR, 45 patients (70.31%) were MDR and 5 sample (7.81%) were contaminated in DST.

Conclusion: Patients with resistance to isoniazid, rifampicin, Levofloxacin amikacin, and Moxifloxacin is called as (Pre-XDR TB) were 22%, however, 70% had multi drug resistance. Isoniazid and Rifampicin were the most resistant drugs in the study.

Keywords: Tuberculosis, Pre XDR-TB, MDR-TB, Larkano, Pakistan

Introduction

Tuberculosis (TB), a major infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), accounts for 1**.**8 million deaths and 10**.**4 million new cases annually Resistance to anti-TB drugs is an increasing global health threat [1]. Pakistan currently ranks third amongst countries with highest burden of tuberculosis globally. In an estimated 570,000 new cases in 2019 and 43,900 deaths attributable to the disease [2]. M. tuberculosis is an air-born infectious agent, and a patient with active TB will disseminate the bacterium within sputum droplets and droplet nuclei. When these droplets are inhaled by a healthy individual, M. tuberculosis reaches the respiratory alveoli, where it establishes a focus of infection [3]. Failure to eradicate the pathogen is largely due to the absence of a highly effective vaccine. The Bacille de Calmette et Guérin (BCG) vaccine is widely used throughout the world but it only partially prevents M. tuberculosis infection and mainly inhibits disease development in children; in adults it is less effective, thus allowing continued transmission of TB [4]. Wild-type M. tuberculosis is generally susceptible to all anti-tuberculosis drugs. However, spontaneous mutations in the bacterial genome during duplication may confer drug resistance [5]. Patients with active pulmonary TB, especially those with cavity lesions, may have > 109TB bacilli in the lung [6]. The estimated percentage of new TB cases with MDR-TB was reported to be 3.5%, however, the prevalence of XDR-TB is 9.5% worldwide [7]. Treatment of tuberculosis involves first and second-line anti-tuberculosis drugs for drug susceptible and drug resistant cases respectively. The first-line drugs consist of Isoniazid (H),Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) and Streptomycin (S) which are highlyeffective with a cure rate of approximately 98% inthe new cases [8]. Due to resistance to backbone drugs in the first-line TB regimen, the treatment of MDR-TB requires more expensive and more toxic second-line drugs, while the clinical outcome of MDR-TB is generally unsatisfactory [9-10]. The second line drugs are includes Fluoroquinolone (FQ), three injectable second-line drugs Amikacin, Kanamycin, and Capreomycin (AM) [11].

MDR-TB is defined as TB strains resistant to at least two first line drugs, rifampin (RIF) and isoniazid (INH), pre-extensively drug resistant TB (Pre-XDR TB) is defined as MDR-TB strain that is resistant to either fluoro-quinolones (FQ) or second line injectable drug but not both. While XDR-TB is defined as MDR-TB strainthat is resistant to any FQs and one of the second line injectable drugs (capreomycin, kanamycin or amikacin) [12]. Treatment of XDR-TB is complicated, as it requires the use of second-line drugs that are less effective and more toxic, thus demanding longer treatment duration.

Detection of pre-XDR-TB cases among MDR-TB patients is an important step in the prevention of treatment failure of MDR-TB and in addition, it helps to take appropriate measures to halt the progression towards XDR-TB [13].

Drug-resistant strains of *Mycobacterium tuberculosis* arise due to spontaneous chromosomal mutations at a low frequency, but one study revealed that selection pressure that is caused by inappropriate utility of anti-TB drugs results in the emergence of drug resistant TB [14]. Resistance to first and second line anti-TB drugs has been linked to mutations of genes: *Kat*G and *inh*A for isoniazid resistance, *rpo*B for rifampicin resistance; *gyr*A and less frequent *gyr*B for FQ resistance; *rrs*and *eis*promoter region for aminoglycosides (amikacin/kanamycin); *rrs*and *tly*A for capreomycin resistance [15-16].

A person can get drug resistant TB through primary direct transmission and secondary due to inadequate TB treatment for

extended duration of time. Pre-XDR and XDR-TB patients are usually treated with regimens that include group V drugs (clofazimine, linezolid, high dose INH, bedaquiline, delamanid) as they are usually resistant to most of the effective drugs and treatment outcome in these patients is poor [17].

Drug-susceptibility testing (DST) is essential for detection and management of drug resistant TB**.** Although phenotypic DST using the proportional method is regarded as the gold standard [27]. The unavailable DST results make it difficult to design personalized treatment regimens for these patients, and the standardized second-line regimen has been used following the guidelines endorsed by WHO [18].

METHODOLOGY

Study site

Study was conducted at the PMDT site Chandika Medical hospital, Larkano, Pakistan from Dec 2017 to 2020.All Zero month pulmonary samples of MDR and Pre-XDR DST patients of both genders having different ages were included in this research.

Specimen collection

Five to 10 ml of sputum samples were collected into a sterile 50 ml falcon tube at baseline and all specimens were stored at -20˚C until transported to Biosafety Level 03 laboratory Chandika Medical Hospital Larkano

Auramine Staining

Concentrated smears of each sputum specimen were performed using Auramine staining for acid fast bacilli (AFB)as defined with small changes [19].

Specimen processing, analysis and Drug sensitivity

Sputum samples were decontaminated using N-Acetyl L-cysteine- Sodium Hydroxide (NALC-NaOH) solution and neutralizing by phosphate buffer, and inoculated onto Lowenstein– Jensen (L–J) medium as previously reported [25]. Bacterial colonies were analyzed four to eight weeks later for conventional DST and identification, MIGT media was used for DST, all work has been done in BSL3 lab with full safety precaution as followed by guidelines from WHO [20,26].

HIV Testing

HIV testing of all samples was done on whole blood using rapid HIV test. The test was done at the Provider Initiative Counseling and Testing (PICT) services of the respective health institutions.

Ethical Approval

The study protocol was approved by Additional Director of Tuberculosis program Sindh.

RESULTS

Demographic and Clinical Characteristics

Sixty four samples of zero month (Newly diagnosed), MDR and pre-XDR were included during time period of Dec 2017 to 2020 while others were excluded from this study, all were checked for HIV , 05 patients tested positive. Among 64 patients 23 were females (35.94%) and rest 41 (64.06%) were males. Among 64 patients 15 (23.44%) were negative for microscopy and other 49 (76.56%) were positive. and 59.38% were between 10–40 years age group, rest of the participants ranged from 41 to 70 years. Mean age was 34.3±4.2. Moreover, 14 patients (21.88%) were pre-XDR, 45 patients (70.31%) were MDR and 5 sample (7.81%) were contaminated in DST.

Resistance to First Line Drugs

Among 64 patients 45 patients (70.31%) were MDR. Of 64 specimen 05 contaminated during DST, 11 (17.19%) were resistant to HRZ 45(70.31%) were resistant to Rifampicin, 52 (81.25%) were resistant to Isoniazid and 15 (23.44%) were resistant to Pyrazinamide.

Resistance to Second Line Drugs

Pre-XDRTB Definition: Resistance to isoniazid, rifampicin, Levofloxacin amikacin, and Moxifloxacin is called as Pre-XDR TB.

In this study, amongst 64 patients 14 patients (21.88%) were pre-XDR, and all were resistant to Levofloxacin amikacin, and Moxifloxacin.

Table 1: Baseline Characteristics of the Patients

Study Variable	n	%
Age Groups (Years)		
10 to 40	38	59.38
41 to 70	26	40.63
Mean±SD	34.3±4.2	
Gender		
Male	41	64.06
Females	23	35.94
Microscopy		
Negative	15	23.44
Positive	49	76.56
Drug Resistance		
MDR	45	70.31
Pre XDR	14	21.88
Contamination	5	7.81
Drug Resistance in Pre XDR (14)		
Levofloxacin	14	100.00
Moxifloxacin	14	100.00
Amikacin	14	100.00

Table II Resistance to First Line Drugs

Drug	Number	Percentage
HRZ	11	17.19
Rifampicin	45	70.31
Isoniazid	52	81.25
Pyrazinamide	15	23.44

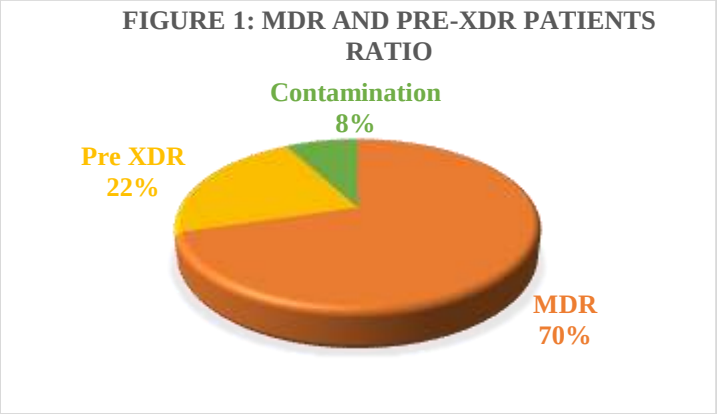

Figure 1: MDR and Pre-XDR patients ratio

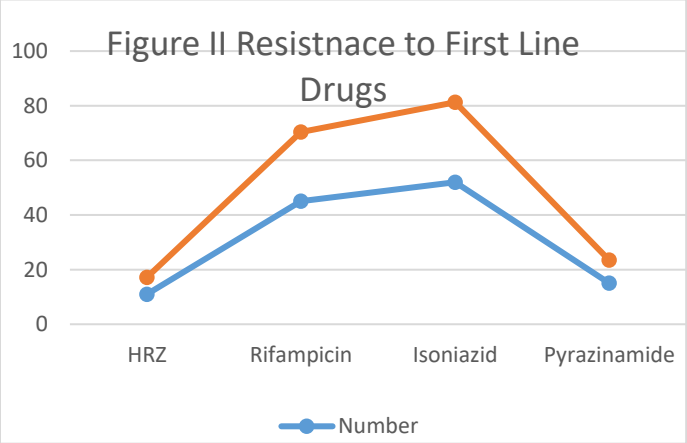

Figure 2: Resistance to First Line Drugs

DISCUSSION

Tuberculosis (TB) still remain a challenge to health authorities throughout the world. It is most dangerous in the form of drug resistant either multi-drug resistant or pre-extensively drug resistant. We have conducted this study in order to determine pattern of pre-extensively drug resistance tuberculosis in MDR-TB patients. As per WHO guideline samples were collected aseptically and analyzed for microscopy and cultured on L-J medium after NALC procedure and finally culture positive samples of 0 months were performed for DST.

Only 16 samples of 0 months were culture positive in time period of Dec 2017 to 2020

as per WHO and guidelines and mentioned in (W.H.O, 2021), among them 50% were females and 50% were male and 16 patients 03 (19%) were negative for microscopy and other 13 (81%) were positive as similarly done by [21]

Furthermore, all were performed for DST. Among 16 patients 12 patients (75%) were MDR. Of 16 specimen 01 was contaminated during DST, 11(69%) were resistant to Rifampicin, 14 (87.5%) were resistant to Isoniazid and 03 (18.75%) were resistant to Pyrazinamide as described in with small changes [22]. and 03 patients (18.75%) were resist to second line drug which were categorize into pre-XDR, and all were resistant to Levofloxacin and Moxifloxacin as previously analyzed by [23]. The all positive 0 months patients were checked for HIV by using kit method as used by [24].

The study was done on Larkana patients, samples were collected at PMDT site Chandka Medical College Hospital and further process for microscopy, culture and DST in Biosafety level 03 lab Chandka Medical College Hospital.

CONCLUSION

Patients with resistance to isoniazid, rifampicin, Levofloxacin amikacin, and Moxifloxacin is called as (Pre-XDR TB) were 22%, however, 70% had multi drug resistance. Isoniazid and Rifampicin were the most resistant drugs in the study.

ACKNOWLEDGEMENT

The authors express their sincere appreciations to Provisional T.B control program and worthy Director General (DG Sindh).

REFERENCES

1. Demissie, T.A. and Belayneh, D., 2021. Magnitude of Mycobacterium tuberculosis Infection and Its Resistance to Rifampicin Using Xpert-MTB/RIF Assay Among Presumptive Tuberculosis Patients at Motta General Hospital, Northwest Ethiopia. *Infection and Drug Resistance*, *14*, p.1335.

2. Alba, S., Rood, E., Mec)atti, F., Ross, J.M., Dodd, P.J., Chang, S., Potgieter, M., Bertarelli, G., Henry, N.J., LeGrand, K.E. and Trouleau, W., 2022. TB Hackathon: Development and Comparison of Five Models to Predict Subnational Tuberculosis Prevalence in Pakistan. *Tropical medicine and infectious disease*, *7*(1), p.13.

3. Alzayer Z, Al Nasser Y. Primary Lung Tuberculosis. InStatPearls [Internet] 2022 Jan 5. StatPearls Publishing.

4. Seki, Mitsuko, Hongjo Choi, Kyungjong Kim, Jake Whang, Joohon Sung, and Satoshi Mitarai. "Tuberculosis: A persistent unpleasant neighbour of humans." *Journal of Infection and Public Health* 14, no. 4 (2021): 508-513.

5. Seki, Mitsuko, Hongjo Choi, Kyungjong Kim, Jake Whang, Joohon Sung, and Satoshi Mitarai. "Tuberculosis: A persistent unpleasant neighbour of humans." *Journal of Infection and Public Health* 14, no. 4 (2021): 508-513.

6. Sharma, A., 2019. A Study to Evaluate the Effectiveness of Structured Teaching Programme on Knowledge of Mothers Regarding Prevention of Selected Communicable Diseases Among Under Five Children in Selected Urban Areas of Fatehabad. *International Journal of Community Health Nursing*, *1*(1), pp.19-23.

7. Gallo JF, Pinhata JM, Simonsen V, Galesi VM, Ferrazoli L, Oliveira RS. Prevalence, associated factors, outcomes and transmission of extensively drug-resistant tuberculosis among multidrug-resistant tuberculosis patients in São Paulo, Brazil: a cross-sectional study. Clinical Microbiology and Infection. 2018 Aug 1;24(8):889-95.

8. Khamouli, S., 2019. *Study of the structure, QSAR properties and molecular docking of some amino-pyrimidine derivatives as Novel Mycobacterium tuberculosis inhibitors for drug design* (Doctoral dissertation, UNIVERSITE MOHAMED KHIDER BISKRA).
9. Kibret, K.T., Moges, Y., Memiah, P. and Biadgilign, S., 2017. Treatment outcomes for multidrug-resistant tuberculosis under DOTS-Plus: a systematic review and meta-analysis of published studies. *Infectious diseases of poverty*, *6*(1), pp.1-8.
10. Gröschel, M.I., Walker, T.M., van der Werf, T.S., Lange, C., Niemann, S. and Merker, M., 2018. Pathogen-based precision medicine for drug-resistant tuberculosis. *PLoS pathogens*, *14*(10), p.e1007297.
11. Adwani, S., Desai, U.D. and Joshi, J.M., 2016. Prevalence of pre-extensively drug-resistant tuberculosis (Pre XDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) among pulmonary multidrug resistant tuberculosis (MDR-TB) at a tertiary care center in Mumbai. *Journal of Krishna Institute of Medical Sciences University*, *5*, pp.13-19.
12. Shibabaw, A., Gelaw, B., Gebreyes, W., Robinson, R., Wang, S.H. and Tessema, B., 2020. The burden of pre-extensively and extensively drug-resistant tuberculosis among MDR-TB patients in the Amhara region, Ethiopia. *PloS one*, *15*(2), p.e0229040.
13. Tasnim, T., Tarafder, S., Alam, F.M., Sattar, H. and Kamal, S.M., 2018. Pre-extensively drug resistant tuberculosis (Pre-XDR-TB) among pulmonary multidrug resistant tuberculosis (MDR-TB) patients in Bangladesh. *Journal of Tuberculosis Research*, *6*(3), pp.199-206.
14. Gandhi, N.R., Moll, A., Sturm, A.W., Pawinski, R., Govender, T., Lalloo, U., Zeller, K., Andrews, J. and Friedland, G., 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet*, *368*(9547), pp.1575-1580.
15. Nguyen, L., 2016. Antibiotic resistance mechanisms in M. tuberculosis: an update. *Archives of toxicology*, *90*(7), pp.1585-1604.
16. Zhang, Y. and Yew, W.W., 2015. Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. *The International Journal of Tuberculosis and Lung Disease*, *19*(11), pp.1276-1289.
17. Singh, A., Prasad, R., Balasubramanian, V. and Gupta, N., 2020. Drug-resistant tuberculosis and HIV infection: current perspectives. *HIV/AIDS (Auckland, NZ)*, *12*, p.9.
18. Falzon, D., Schünemann, H.J., Harausz, E., González-Angulo, L., Lienhardt, C., Jaramillo, E. and Weyer, K., 2017. World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update. *European Respiratory Journal*, *49*(3).
19. Qureshi, A.R., Irfan, M., Sajid, M., Mehmood, H., Ahmer, M., Khalid, A. and Bilal, H., 2021. Fluorescence Microscopy: A useful diagnostic

tool for Pulmonary Tuberculosis.(Study of 23,506 cases). *Asian Journal of Allied Health Sciences (AJAHS)*.

20. World Health Organization, 2021. *WHO consolidated guidelines on tuberculosis: module 2: screening: systematic screening for tuberculosis disease*. World Health Organization.
21. Sinshaw, W., Kebede, A., Bitew, A., Tesfaye, E., Tadesse, M., Mehamed, Z., Yenew, B., Amare, M., Dagne, B., Diriba, G. and Alemu, A., 2019. Prevalence of tuberculosis, multidrug resistant tuberculosis and associated risk factors among smear negative presumptive pulmonary tuberculosis patients in Addis Ababa, Ethiopia. *BMC infectious diseases*, *19*(1), pp.1-15.
22. Alemu, A., Bitew, Z.W. and Worku, T., 2020. Poor treatment outcome and its predictors among drug-resistant tuberculosis patients in Ethiopia: a systematic review and meta-analysis. *International Journal of Infectious Diseases*, *98*, pp.420-439.
23. Forsman, L.D., Niward, K., Kuhlin, J., Zheng, X., Zheng, R., Ke, R., Hong, C., Werngren, J., Paues, J., Simonsson, U.S. and Eliasson, E., 2021. Suboptimal moxifloxacin and levofloxacin drug exposure during treatment of patients with multidrug-resistant tuberculosis: results from a prospective study in China. *European Respiratory Journal*, *57*(3).
24. Meehan, S.A., Sloot, R., Draper, H.R., Naidoo, P., Burger, R. and Beyers, N., 2018. Factors associated with linkage to HIV care and TB treatment at community-based HIV testing services in Cape Town, South Africa. *PLoS One*, *13*(4), p.e0195208.
25. Liu, Z., Dong, H., Wu, B., Zhang, M., Zhu, Y., Pang, Y. and Wang, X., 2019. Is rifampin resistance a reliable predictive marker of multidrug-resistant tuberculosis in China: a meta-analysis of findings. *Journal of Infection*, *79*(4), pp.349-356
26. Li, Y., Pang, Y., Zhang, T., Xian, X., Yang, J., Wang, R., Wang, P., Zhang, M. and Chen, W., 2020. Genotypes of Mycobacterium tuberculosis isolates circulating in Shaanxi Province, China. *Plos one*, *15*(12), p.e0242971.
27. Pandey, P., Pant, N.D., Rijal, K.R., Shrestha, B., Kattel, S., Banjara, M.R., Maharjan, B. and Kc, R., 2017. Diagnostic accuracy of GeneXpert MTB/RIF assay in comparison to conventional drug susceptibility testing method for the diagnosis of multidrug-resistant tuberculosis. *PloS one*, *12*(1), p.e0169798.