Research article

Molecular Epidemiology of *Mycobacterium tuberculosis* in Di- vision Mirpur, Azad Jammu & Kashmir

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Abstract: The causative agent for TB is Mycobacterium tuberculosis a member of Mycobaterium Tuberculosis Complex. It is non-motile, slow growing, acid fast and rod in shape (bacilli). According to WHO in 2013, 9 million people contracted while 1.5 million died due to this disease, 550,000 children got TB while 80,000 were died who were HIV-negative. Furthermore, approximately 0.5 million people developed multidrug resistant TB (MDR-TB) and they needed comparatively longer and costly treatment. Pakistan has high incidence and prevalence of TB. The total 580 patients were examined during the study, out which of 332 were males while 248 were females. Only 17 percent were admitted (indoor patient department) in different hospitals of the division while 83 percent were from outdoor patient department of various settings. Pulmonary tuberculosis was detected in 97 patients while extra pulmonary tuberculosis was noticed in 5 individuals. All samples were examined by Direct Microscopy (88%) followed by Fluorescent Microscopy and found presence of Acid Fast Bacilli in 91 cases, Light microscopy missed 3 positive cases. Of the 109 specimens 68 grew during seven weeks of incubation while 20 grew in four weeks and 21 grew in second week of incubation. GeneXpert detected 102 samples as positive for Mycobacterium tuberculosis. Rifampicin (RIF) a first line treatment drug was detected as resistant in 2 (1.96%) patients thus, multi-drug resistant (MDR) was found in two cases, which was associated with Pro-E gene mutation.

Keywords: Tuberculosis (TB), GeneXpert, Fluorescent microscopy, Mirpur

Introduction

The causative agent for TB is Mycobacterium tuberculosis which is a member of Mycobacterium Tuberculosis Complex (MTC). Mycobacterium tuberculosis is a non-motile, with a very slow growth rate, acid fast and rod in shape (bacilli) belongs to the Mycobacterium genus and differs substantially from other bacteria due to the exceptionally thick cell wall and high genomic guanine-cytosine content. On DNA level species of MTC are alike to each other but different on the basis of phenotype, host tropism and disease causing ability (1,2,3). In 2013, 9 million people contracted while 1.5 million died due to this disease. According to World Health Organization (WHO), estimation 550,000 children got TB while 80,000 were died who were HIV-negative in the same year. Furthermore, approximately 0.5 million people developed multidrug resistant TB (MDR-TB) and they needed comparatively longer and costly treatment. With high occurrence of TB and perhaps getting high number of MDR and XDR. Pakistan is among the top five in the world (4), with overall rate of 85–100/100,000 but in Northern area of Pakistan, the rate is even higher where the rate has been reported to be 554/100,000.

For the first time, twenty diverse enzymes having cytochrome P450 or CYP were programmed for genome sequencing for H37Rv strain of M. tuberculosis (6). The important functions of these enzymes were described by huge number of P450s. The main function of P450 was recognized, to metabolize cho- lest-4-en-3-one (ie CYP125A1 and CYP142A1), chain lipids branched (ie CYP124A1), cyclic dipeptides oxidative couture (ie CYP121A1) sterol demethylation (ie CYP51B1) and menaquinone hydroxylation (ie CYP128A1) respectively in the host (7,8,9,10,11,12,13,14). In organisms that do not have true nucleus in it, M. tuberculosis P450 was the first categorized on the basis of structure and chemical as CYP51B1 and the principal associate of CYP51 gene (15). For the diagnosis of active TB after physician's thought radiological e.g; X-rays followed by different laboratory confirmation including culture initially smear microscopy of sputum is needed. Rapid diagnosis and related treatment is required to control the TB and drug resistance especially in resource constrains and prevalent entities but through these techniques it's impossible to assume the drug resistance in TB causing bacteria. Although the occurrence of TB and death rate reduced but MDR strains of TB bacteria are responsible for 480,000 cases of MDR in 2014 which is a great threat for patients as well as for TB control programs. Maximum frequency of MDR-TB is in European countries located on east.

Materials and Methods

The study was conducted at the state TB reference laboratory situated within the premises of Divisional Head Quarter Teaching Hospital, Mirpur, Azad Jammu & Kashmir (AJ&K). The laboratory was established by the Ministry of National Health Services, Regulation & Coordination while functioning under the supervision of Health Department of government of AJ&K. The laboratory has separate reception, an office of the manager and separate room for every procedure. The said setting has state of the art equipment/instrument including PCR-based fully automatic GeneXpert, bio-safety cabinets (BSCs), autoclave, incubator, fume hood, conventional microscope, fluorescence microscope and all personnel protective equipments (PPEs). Research was conducted between september 2019 to march 2020. The patients referred by their physicians or coming directly to the centre of Mirpur division and present any of the sign and symptom enrolled; the presence of symptoms like persistent cough >7 days, low or high grade fever, gradually or suddenly loss of weight, night sweating and tiredness. Two sputum samples were taken from adult individuals while gastric wash was taken from infants and young children who were suspecting pulmonary tuberculosis. Patients who were suspecting for extra pulmonary tuberculosis, all specimens except blood e.g; biopsy, aspiration of other body fluid were obtained. The culture for mycobacterium tuberculosis was performed by using Lowenstein-Jensen culture medium and incubated at 37^0C for six weeks in specially designed incubator. Molecular Analysis of Detection of TB by GeneXpert GeneXpert which is most precise and accurate tool for the molecular detection of Mycobacterium tuberculosis was also done on all 580 samples. GeneXpert works on Polymerase Chain Reaction (PCR) principal basis detect Mycobacterium and it's resistant against anti-tuberculosis drugs.

All suspected patients with TB aged new born and above residing in division Mirpur, AJ&K during the research period and informed consent were included in the study while the patients disinclination to consent were excluded. The patients coming for follow-up were excluded and those with <1.5 ml sputum samples or salivary samples were also excluded from the study. The Study was approved by local Ethics committee.

Figure 1. Geographical map of Azad Jammu and Kashmir (AJ&K)

Results

A total of 580 patients were examined during the study out of which 332 were males while 248 were females (Figure 1). Only 17 percent were admitted in different hospitals of the division while 83% were from outdoor patient department of various settings. The age distribution of the patients is presented in Figure 2; where 9 patients were under one year of age while 105 patients between 1 to 20 years of age. Pulmonary and Extra Pulmonary Tuberculosis were detected in 97 and 5 patients reseptively (Figure 3). Out of 580 patients 89 were positive for AFB with diverse loud of AFBs which mean, in some samples the loud of AFBs was scanty while others shows 1+, 2+, 3+ and 4+ respectively. The males were predominant over female. In 69 males tuberculosis was detected while 33 female were also victim by this disease

Detection of TB by Fluorescent Microscopy

All 580 samples were than confirmed by Fluorescent Microscopy and found presence of Acid Fast Bacilli in 91 cases which means Light microscopy missed 3 positive cases (Figure 4). The quantity of Acid Fast Bacilli was variable, e.g; scanty, 1+, 2+, 3+ and 4+. 491 samples were negative for Acid Fast Bacilli after examining Z.N stained smear under Fluorescent microscope.

Detection of M. Tuberculosis by Culture Culture

It was done on all patients and 109 showed growth of Mycobacterium tuberculosis while 471 did not grow (Figure 5). Of the 109 specimens 68 grew during seven weeks of incubation while 20 grew in four week and 21 grew in second week of incubation. In Mycobacterium tuberculosis culture positive category the ratio of males 61% (n=67) was greater in comparison with females 39% (n=42).

GeneXpert

GeneXpert detected 102 samples as positive for Mycobacterium tuberculosis. Rifampicin (RIF) which is one of the drugs from first line treatment was detected as resistant in 2 (1.96%) patients so, multi-drug resistant (MDR) was found in two cases. The mutation was noticed in Pro-E gene which was responsible for Rifampicin resistance. There was no drug resistance seen in 99 patients who were positive for tuberculosis by GeneXpert. In one sample status was shown as In-determined (IND) as GeneXpert cut off value is > 131/ml of sample. The bacteria other than Mycobacterium tuberculosis from Mycobacterium Complex (MTC) were also found in 3 patients.

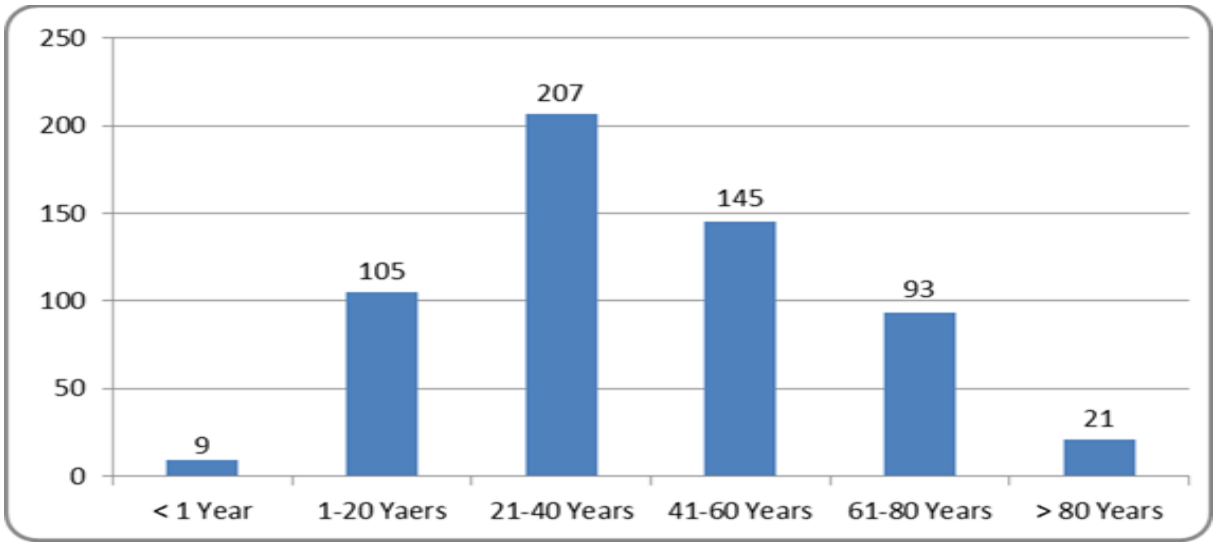

Figure 2: Age distribution of study population

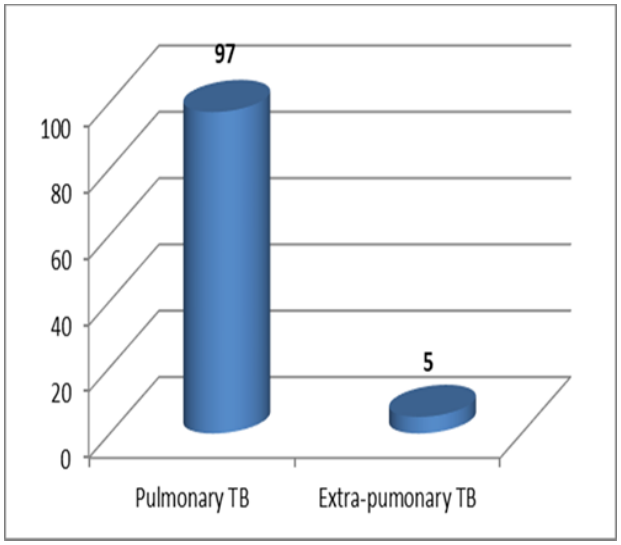

Figure 3: Pulmonary vs Extra-pulmonary TB breakout

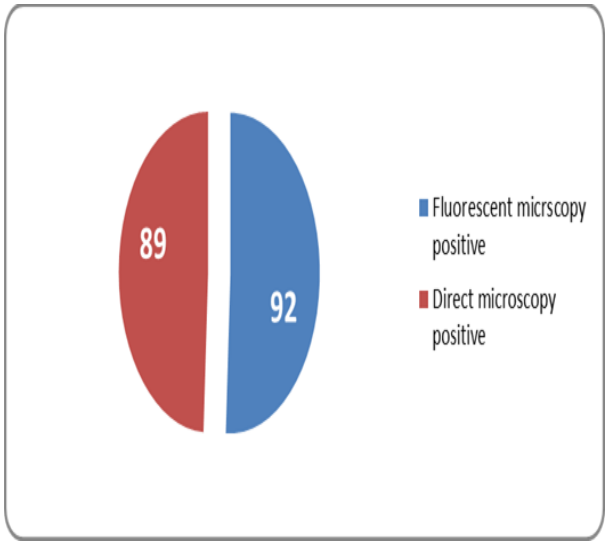

Figure 4: Light versus Fluorescent Microscopy in detection mycobacterium Tuberculosis

DISCUSSION:

The study presented, a detailed picture of TB, in the division Mirpur, Azad Jammu & Kashmir, Pakistan by combining PCR based GeneXpert, biochemical analysis and classical epidemiological methods. In the researches from around the globe and in our country the prevalence of drug-resistant tuberculosis was different. According to our study out of 580 clinical samples (both pulmonary and extra-pulmonary) 102 (17.58%) were positive for Mycobacterium tuberculosis and the prevalence of Rifampicin resistance to tuberculosis was only 1.96% from all pulmonary samples as well as extra-pulmonary samples.

In India 9% were Rifampicin (single drug-resistant) as reported in different studies. Surajit Lahiri et al. reported in 2011– 2012, Rifampicin mono-resistance in 4.69% while M. Giridhar Kumar et al. shows 0% Rifampicin resistance in Mycobacterium tuberculosis in 2010–2012 chiefly from South India. High prevalence of multi-drug resistant tuberculosis were reported in many studies from different region of India mainly in patients first-time treated with deterioration, treatment after evasion and treatment after failure. The prevalence of drug-resistance in our study is low as compared to studies mentioned above. 20 – 60 years of age male patients were mostly infected with Pulmonary Mycobacterium tuberculosis while in female the burden was found in 20 – 40 years of age group. Extra pulmonary tuberculosis was found in age group of 20 – 40 years in both male as well as in females. While in Rifampicin resistant, males were main victim within the age of 20 – 60 having pulmonary tuberculosis. And in case of Rifampicin resistance, majority of pulmonary cases found in males and females were also from the age group of 20 - 40 years.

The development of resistance to anti-TB drugs used to treat tuberculosis (TB), and chiefly multidrug-resistant TB (MDR-TB), has become a momentous public health threat in a number of nations and is a problem to actual TB control. Many researches for the surveillance of drug resistance conducted in India indicated relatively low rate of multi-drug resistance but, this interprets into a huge total number of drug resistant TB cases and as hitherto the management of patients with MDR-TB is insufficient. Within the Revised National Tuberculosis Control Program (RNTCP) specific measures are being taken to address the multi-drug resistant-TB problem through suitable management of individuals and policies to preclude the spread and dissemination of multi-drug resistant-TB. Antimicrobial drug resistance in TB has clinical, microbial and programmatic reasons. In case of microbiological perspective, the drug resistant is caused by the genetic alteration which stops the drug to effects the organism and show ineffectiveness against the altered bacilli. The second major cause of drug-resistance is the administration of insufficient and poorly treatment regimen which makes strain dominant in patient infected with tuberculosis. Therefore, the poor treatment, poor antimicrobial therapy is totally man-made phenomenon in the development of multi-drug resistance tuberculosis.

CONCLUSION:

The specimen for the diagnosis of Tuberculosis should be tested by all techniques including; direct and fluorescent microscopy, culture and GeneXpert technology. Microscopy by both technique has low sensitivity and specificity. GeneXpert technology is much better in terms of sensitivity and specify when compare with microscopy while till date culture is most predominant in the account of sensitivity but need longer time due to slow growth of Mycobacterium tuberculosis.

REFERENCES:

1. *Brosch, R., Gordon, S. V, Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier, T., Gutierrez, C., Hewinson, G., Kremer, K., Parsons, L. M., Pym, A. S., Samper, S., van Soolingen, D., & Cole, S. T. (2002). A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proceedings of the National Academy of Sciences of the United States of America, 99(6), 3684– 3689. https://doi.org/10.1073/pnas.052548299.*

2. *Huard, R. C., Lazzarini, L. C. de O., Butler, W. R., van Soolingen, D., & Ho, J. L. (2003). PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. Journal of Clinical Microbiology, 41(4), 1637–1650. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12682155.*

3. *Smith, N. H., Gordon, S. V., de la Rua-Domenech, R., Clifton-Hadley, R. S., & Hewinson, R. G. (2006). Bottlenecks and broomsticks: the molecular evolution of Mycobacterium bovis. Nature Reviews Microbiology, 4(9), 670–681. https://doi.org/10.1038/nrmicro1472.*

4. *Cyan, M., Price, M., & Rider, M. (2017). A Health Literacy RCT toward Improvement of Programmatic Outcomes of Tuberculosis Control in the Tribal Areas of Pakistan Governance Support Program Post-Crisis. International Center for Public Policy Working Paper Series, at AYSPS, GSU. Retrieved from https://ideas.repec.org/p/ays/ispwps/paper1711.html.*

5. *Alvi, A. R., Hussain, S. F., Shah, M. A., Khalida, M., & Shamsudin, M. (1998). Prevalence of pulmonary tuberculosis on the roof of the world. The International Journal of Tuberculosis and Lung Disease, 2(11), 909–913. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9848612.*

6. *Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry, C. E., Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M. A., 64 | P a g e Rajandream, M.-A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J. E., Taylor, K., Whitehead, S., & Barrell, B. G. (1998). Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature, 393(6685), 537–544. https://doi.org/10.1038/31159.*

7. *Podust, L. M., Poulos, T. L., & Waterman, M. R. (2001). Crystal structure of cytochrome P450 14alpha -sterol demethylase (CYP51) from Mycobacterium tuberculosis in complex with azole inhibitors.*

Proceedings of the National Academy of Sciences of the United States of America, 98(6), 3068–3073. https://doi.org/10.1073/pnas.061562898.

8. Leys, D., Mowat, C. G., McLean, K. J., Richmond, A., Chapman, S. K., Walkinshaw, M. D., & Munro, A. W. (2003). Atomic structure of Mycobacterium tuberculosis CYP121 to 1.06 A reveals novel features of cytochrome P450. The Journal of Biological Chemistry, 278(7), 5141–5147. https://doi.org/10.1074/jbc.M209928200.

9. McLean, K. J., Lafite, P., Levy, C., Cheesman, M. R., Mast, N., Pikuleva, I. A., Leys, D., & Munro, A. W. (2009). The Structure of Mycobacterium tuberculosis CYP125. Journal of Biological Chemistry, 284(51), 35524–35533. https://doi.org/10.1074/jbc.M109.032706.

10. Holsclaw, C. M., Sogi, K. M., Gilmore, S. A., Schelle, M. W., Leavell, M. D., Bertozzi, C. R., & Leary, J. A. (2008). Structural Characterization of a Novel Sulfated Menaquinone produced by stf3 from Mycobacterium tuberculosis. ACS Chemical Biology, 3(10), 619–624. https://doi.org/10.1021/cb800145r.

11. Belin, P., Le Du, M. H., Fielding, A., Lequin, O., Jacquet, M., Charbonnier, J.-B., Lecoq, A., Thai, R., Courcon, M., Masson, C., Dugave, C., Genet, R., Pernodet, J.-L., & Gondry, M. (2009). Identification and structural basis of the reaction catalyzed by CYP121, an essential cytochrome P450 in Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences, 106(18), 7426– 7431. https://doi.org/10.1073/pnas.0812191106.

12. Capyk, J. K., Kalscheuer, R., Stewart, G. R., Liu, J., Kwon, H., Zhao, R., Okamoto, S., Jacobs, W. R., Eltis, L. D., & Mohn, W. W. (2009). Mycobacterial Cytochrome P450 125 (Cyp125) Catalyzes the Terminal Hydroxylation of C27 Steroids. Journal of Biological Chemistry, 284(51), 35534–35542. https://doi.org/10.1074/jbc.M109.072132.

13. Johnston, J. B., Kells, P. M., Podust, L. M., & Ortiz de Montellano, P. R. (2009). Biochemical and structural characterization of CYP124: A methyl-branched lipid -hydroxylase from Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences, 106(49), 20687–20692. https://doi.org/10.1073/pnas.0907398106.Driscoll, M. D., McLean, K. J., Levy, C., Mast, N., Pikuleva, I. A., Lafite, P., Rigby, S. E. J., Leys, D., & Munro, A. W. (2010). Structural and Biochemical Characterization of Mycobacterium tuberculosis CYP142. Journal of Biological Chemistry, 285(49), 38270–38282. https://doi.org/10.1074/jbc.M110.164293.

14. Hunter, R. L. (2016). Tuberculosis as a three-act play: A new paradigm for the pathogenesis of pulmonary tuberculosis. Tuberculosis, 97, 8–17. https://doi.org/10.1016/J.TUBE.2015.11.010.

15. Bellamine, A., Mangla, A. T., Nes, W. D., & Waterman, M. R. (1999). Characterization and catalytic properties of the sterol 14alpha-demethylase from Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences of the United States of America, 96(16), 8937–8942. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10430874.

16. WHO. (2015a). Global tuberculosis report 2015. World Health Organization. Access