ORIGINAL ARTICLE

The Utility of Line Probe Assay in Early Diagnosis and Treatment of Multi Drug Resistant Tuberculosis in Baluchistan

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ABSTRACT

Objective: To determine the utility and role of the LPA for the early diagnosis of MDR-TB on sputum smear positive pulmonary tuberculosis patients in Baluchistan, Pakistan.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Internal Medicine Bolan Medical College Hospital Quetta from 1st January 2019 to 29th February 2020.
Methodology: Two hundred and twenty-nine patients were randomly selected. The age of patients were more than 15 years of either gender were included. The detail medical history was taken and clinical examination was done. All the recruited patients were advised to collect 4–5 ml of early-morning sputum in a sterilized 50 ml Falcon tube for LPA-GenoType® MTBDR plus.
Results: Mean age of the patients was 49.25±10.64 years. The 119(51.96%) patients were male, while 110(48.03%) patients were females. Most of the patients 137(59.82%) were with age group of 35-60 years. Regarding drug resistance, 7(3.05%) patients were suffering from multidrug resistance tuberculosis, while 9(3.93%), 8(3.49%) patients were respectively suffering from rifampicin mono-resistance and isoniazid mono-resistance. The 202(88.20%) patients were shown sensitivity and susceptibility to all drugs. The 3(1.31%) patient's results were considered as inconclusive and further evaluated by the Lowenstein-Jensen medium, Bactec and Bactec Mgit 960system.
Conclusion: The molecular based detection methods for tuberculosis, such as LPA-GenoType® MTBDR plus is newer and important molecular based technique for early detection of drug-resistant TB. It provides early and effective control of TB and reduces the death rate and TB infection or disease.
Keywords: Multi drug resistant tuberculosis; Ziehl-Neelsen staining; line probe assay (LPA); Baluchistan.

INTRODUCTION

Tuberculosis (TB) is a very common cause of morbidity and mortality throughout the world. It mostly commonly involves the lungs, but can affect any other part of the body. It is caused by slowly growing bacterium called Mycobacterium Tuberculosis (MTB).[1] It mostly spread through the air, but can spread by haematogenous and lymphatic pathway. The prevention, diagnosis and treatment of TB become more complex due to resistance against frequently used anti-tuberculous drugs.[1]

Multi drug resistant TB (MDR-TB) is a type of drug-resistant MTB that does not respond to at least the two main and the most powerful anti-tuberculous medications e.g. rifampicinn (RIF) and isoniazid (INH), most frequently used drugs to treat TB.[2] As a result, this type of the TB disease is very troublesome to treat than typical TB. It treatment is prolong and requires multidrug treatment up to two years. The worldwide load of TB disease, particularly the MDR-TB is increasing and has become an utmost health problem.[1,3]

MDR-TB is very common in those people who don't take their TB medications properly and regularly as told by their health care professionals.[2] MDR-TB is also seen in some people, who suffered from TB disease in the past and completed their anti-TB medicine course, again develop TB disease mostly MDR-TB, due to immunocompromised conditions.[2,3] MDR-TB is also very common in those people coming from of the world, where it is common. It is also noted in those peoples having history of contact with MDR-TB patients.[2,4]

The Ziehl–Neelsen (ZN) staining and microscopy are still very commonly used investigation for the diagnosis of tuberculosis, especially pulmonary tuberculosis, particularly in those countries such as Pakistan, where resources are very limited and burden of TB is very high. This technique has low sensitivity, but higher specificity and is highly observer dependent.[5]

Regardless of massive struggle to increase the diagnosis of TB, still up to 1/3rd of new TB cases are missed due to non-availability of quick, relatively inexpensive and right diagnostic investigation in those countries, where TB is very high. The diagnosis of MTB is difficult and time consuming.[1,6] The various Efforts have been made to improve and develop rapid diagnostic tools and drug susceptibility testing (DST) for TB. The World Health Organization (WHO) had issued policy statements for improving diagnosis of TB by implementation of molecular tests e.g. the Gene Xpert and line probe assay (LPA).[7]

LPA is a rapid technique based on polymerase chain reaction (PCR) that is used to detect MTB as well as drug

sensitivity and susceptibility. The LPA have ability to detect mono, multi and extended drug resistant TB.[7,8]

The first molecular test approved and suggested by World Health Organization (WHO) in 2008 was a commercial line probe assay LPA-Geno Type® MTBDR plus, manufactured by Hain Life science, Nehren, Germany, for detection of resistance to rifampicin.[9] This was followed by Xpert MTB/RIF, in 2010 for simultaneous detection of TB and rifampicin resistance and further updated in 2013 with more significant recommendations.[8] In May 2016, WHO issued new policy regarding LPA for the diagnosis of resistance to second-line anti-TB drugs among those patients diagnosed with rifampicin-resistant TB or MDR-TB.[10] This SL-LPA also detects additional resistance to fluoroquinolones such as moxifloxacin or levofloxacin and injectable anti-TB drugs such as kanamycin, capreomycin, amikacin. The new WHO recommendation applies to use the commercial Genotype® MTBDRsl assay, manufactured by Hain Lifescience, Nehren, Germany SL-LPA only for detection of MDR and XDr-TB.[8,10,11]

MATERIALS AND METHODS

This randomized cross-sectional study was carried at Medicine Department BMCH, with collaboration Pathology Department, to diagnose drug-resistant TB from 1st January 2019 to 29th February 2020.

The participants in the study were selected from medical outpatient and inpatient. The patients age ranged from 35 to 60 years of either gender were included. The medical history of participants was taken in detail and clinical examination was done according to performa, which was made specially for this study. Regarding Specimen collection and processing, the patients were advised to collect 4–5 ml of early-morning sputum in a sterilized 50 ml Falcon tube. All the patients were further advised to wash and clean their mouth and teeth with water before collection of sputum to avoid contamination with food and other particles.

The collected early morning sputum specimens were sent to well-equipped medical laboratory for LPA-GenoType® MTBDR plus for molecular testing to diagnose MDR-TB. The collected early morning sputum specimens were sent to well-equipped medical laboratory for LPA-GenoType® MTBDR plus for molecular testing to diagnose MDR-TB.

In this study only those patients were included, having age more than 15 years, which have sputum smear positive on microscopy by Ziehl–Neelsen staining are randomly selected. The patients unwilling to participate in this study were excluded from study. The diagnosed patients of MDR-TB were also excluded from study.

In this study patients were categorized into MDR-TB, Rifampicin resistant MTB, INH resistant MTB and pan-susceptible MTB according to LPA-GenoType® MTBDR plus report. Some patient's reports were categorized into inconclusive, because no MTB were detected in these samples.

These patients are treated with collaboration of the National TB program (NTP), WHO, and global fund. The treatment is started according laboratory results. All the data was recorded, which was specially made for MDR-TB. All the collected data was entered and analysed with SPSS 27. The P-value < 0.05 was statistically considered significant.

RESULTS

The data of 229 patients were randomly selected. The male patients were 119 (51.96%), while females were 110 (48.03%). The average age of the populated study was 49.25±10.64 years. Most of the patients 137(59.82%) were with age group of 35-60 years (Table 1).

Regarding drug resistance, 7(3.05%) patients were suffering from multidrug resistance tuberculosis, while 9(3.93%), 8(3.49%) patients were respectively suffering from rifampicin mono-drug-resistance and isoniazid mono-drug-resistance. The 202(88.20%) were pan-susceptible. Three (1.31%) of the samples gave invalid results, which were considered as inconclusive and further evaluated by the Lowenstein-Jensen medium, Bactec and Bactec Mgit 960 system (Tables 1-2).

When compared the effect of age, gender, residence, occupation, drug addiction, behavioural status and Previous TB treatment status according to LPA report significant (P<0.05) relationship was observed (Table 1).

DISCUSSION

Molecular test, LPA has been approved and recommended by the WHO. This tests have significantly reduced the need for a primary culture of sputum samples and subsequent drug sensitivity testing (DST) of the mycobacterial isolates.[12] However, it has been pointed out in the WHO policy statement that the LPA is not a complete substitution for standard culture and sensitivity test, and acid-fast bacillus (AFB) culture and sensitivity is still required in those patients in which sputum smear is negative.[13] This LPA shown positive impact regarding the diagnosis and treatment of TB patients.[12,13] It helps in detection of MTB, and also plays a role in DST. In this way LPA have ability to begin correct treatment immediately, as described by other studies in different countries, where prevalence of MDR-TB is very high.[14]

The early diagnosis and immediate treatment MDR-TB will reduce the spread and number of patients in community.[15] The accurate diagnosis and early treatment of MDR-TB is highly required, because it interrupts further transmission of the disease and creation of extensively drug-resistant TB (XDR-TB).[16] The early diagnosis and treatment of TB reduces the unnecessary cost of drugs, which are prescribed for MDR and XDR-TB. It also reduces the serious side effects and of second-line anti-TB drugs.[17]

The standard culture and drug sensitivity / susceptibility testing (DST) on solid media is a prolong process.[18] It has been augmented with automated liquid culture systems e.g. Bactec and Bactec Mgit 960 in many diagnostic laboratories, which decreased the detection time with better sensitivity.[19] The rapid and immediate results can be achieved by molecular methods, such as Gene Xpert and LPA, which detects MTB and as well as drug resistance within 1–2 days.[20] The rapid detection of Rifampicin and INH drug-resistant MTB is extremely important for proper treatment and control of MDR-TB.[21]

Without the LPA, the average time for health care professionals to detect the MTB by culture and sensitivity method is two to seven weeks.[18,19] The duration of culture and DST on conventional solid medium, such as the Lowenstein-Jensen medium is from three to six weeks.[18] In solid medium, growth time of the MTB is two to three weeks, and further three weeks are required for DST. In liquid medium two types of culture and DST ate available e.g. Bactec and Bactec Mgit 960 system. In Bactec, the duration of culture and DST is ten days to twenty onedays. In this test ten days for growth of the MTB and further ten to eleven days required for DST. In Bactec Mgit 960 system, the duration of culture and DST is seven to ten days. In this test one week is required for growth of the MTB and further three days are required for DST.[19-21]

Table 1: Demographic information of MDR-TB patients according to LPA report

Characteristics	MDR-TB	Rifampicin resistance	Isoniazid Resistance	Pan-susceptible	Inconclusive	Total No. of patients	P value
Age (years)							
15-34	1 (0.43%)	2 (0.87%)	1 (0.43%)	61 (26.63%)	-	65 (28.38%)	0.001
35-60	4 (1.74%)	6 (2.43%)	5 (2.71%)	120 (52.4%)	2 (0.87%)	137 (59.82%)	
> 60	2 (0.87%)	1 (0.43%)	2 (0.87%)	21 (9.17%)	1 (0.43%)	27 (11.79%)	
Gender							
Male	4 (1.74%)	5 (2.18%)	6 (2.62%)	102 (44.54%)	2 (0.87%)	119 (51.96%)	0.003
Female	3 (1.31%)	4 (1.74%)	2 (0.87%)	100 (43.66%)	1 (0.43%)	110 (48.03%)	
Residence							
Rural	2 (0.87%)	4 (1.74%)	3 (1.31%)	87 (37.99%)	1 (0.43%)	97 (42.53%)	0.004
Urban	5 (2.18%)	5 (2.18%)	5 (2.18%)	115 (50.21%)	2 (0.87%)	132 (57.64%)	
Occupation							
Employed	3 (1.31%)	4 (1.74%)	4 (1.74%)	71 (31%)	1 (0.43%)	83 (36.24%)	0.016
Unemployed	4 (1.74%)	5 (2.18%)	4 (1.74%)	131 (57.2%)	2 (0.87%)	146 (63.75%)	
Drug addiction							
Yes	6 (2.62%)	7 (3.05%)	6 (2.62%)	18 (7.86%)	3 (1.31%)	40 (17.46%)	0.002
No	1 (0.43%)	2 (0.87%)	2 (0.87%)	184 (80.34%)	-	189 (82.53%)	
Smoker							
Yes	5 (2.18%)	6 (2.62%)	4 (1.74%)	86 (37.55%)	2 (0.87%)	103 (44.97%)	0.013
No	2 (0.87%)	3 (1.31%)	4 (1.74%)	116 (50.65%)	1 (0.43%)	126 (55.02%)	
Previous TB treatment status							
Relapse/reinfection	2 (0.87%)	3 (1.31%)	2 (0.87%)	4 (1.74%)	1 (0.43%)	12 (5.24%)	0.023
Default	1 (0.43%)	2 (0.87%)	1 (0.43%)	4 (1.74%)	-	8 (3.49%)	
Treatment failure	2 (0.87%)	3 (1.31%)	4 (1.74%)	5 (2.18%)	1 (0.43%)	15 (6.55%)	
New TB cases	2 (0.87%)	1 (0.43%)	1 (0.43%)	189 (82.53%)	1 (0.43%)	194 (84.71%)	

Table 2: Resistance pattern of rifampicin (R), isoniazid (H) or both (HR) according to LPA report

Resistance pattern of R, H or HR	Relapse/ Reinfection	Default	Treatment failure	New TB case
R	3 (1.31%)	1 (0.43%)	2 (0.87%)	2 (0.87%)
H	2 (0.87%)	2 (0.87%)	3 (1.31%)	1 (0.43%)
HR (MDR-TB)	2 (0.87%)	1 (0.43%)	4 (1.74%)	1 (0.43%)
No resistance	4 (1.74%)	4 (1.74%)	5 (2.18%)	189 (82.53%)
Invalid result	1 (0.43%)	-	1 (0.43%)	1 (0.43%)
Total	12 (5.24%)	8 (3.41%)	15 (6.55%)	194 (84.7%1)

The reasons for invalid or inconclusive LPA report may be due to following reasons, such as improper collection of specimen, and as well as improper sampling and storage of specimen.[22] The other reasons for invalid or inconclusive LPA report may be due to the mishandling of reagents, when they are not placed at proper temperature, improper mixing of reagents with sputum or particular specimen, addition of improper or insufficient amount of reagents within specimen, improper placement of strips in the reagents and improper washing methods.[23,24]

The aim of our study was to determine the utility and role of the LPA for the early diagnosis of MDR-TB. Our study shows the sensitivity and specificity of 98.69 respectively for both rifampicin and isoniazid. According to Meaza[25] et al. study LPA is having both higher sensitivity 77.8% and specificity 97.9%.[25] Tahseen[26] et al. conducted a study in Pakistan which shows, sensitivity of 100% of while specificity of 98.8% for RMP, INH and FQ by LPA.[26] Ruvandhi[27] et al. study shows rifampicin sensitivity of 96.7% and specificity of 98.8%, while isoniazid sensitivity of 90.2% and specificity of 99.2%.[27]

CONCLUSION

LPA is a newer and important molecular based technique for early detection of drug-resistant TB. It can diagnose mono, multi and extended drug resistant TB. It provides early and effective control of drug-resistant TB and reduces the death rate and TB infection or disease. It has been replacing the culture and sensitivity techniques in cases of TB, However not a complete substitution for standard culture and sensitivity test. LPA provides reliable results on smear-positive specimens, however culture and sensitivity techniques are still important in those patients presenting with negative sputum smear and LPA.

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