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To Compare Result of CBNAAT With Line Probe Assay for Detection of Rifampicin Monoresistant Mycobacterium Tuberculosis.

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Abstract:

Background&Method: The study was carried out in the department of Respiratory Medicine in Index Medical College, Indore with an aim to compare result of CBNAAT with Line Probe Assay for detection of Rifampicin monoresistant mycobacterium tuberculosis. All patients >14 years of age admitted in Chest ward of Index Medical College, Indore with features of TB between May 2020 to April 2021. Study was conducted on 50 patients from the Department of TB and CHEST at Index Medical College, Research centre and Hospital, Indore and sample was sent to Intermediate Reference Laboratory, M.R.T.B. Hospital/Chest Centre, Indore and all the tests was performed with due permission from the Institutional Ethical Committee and informed consent from the subjects or their legal relatives.

Result: The Rifampicin resistance by CBNAAT test. This test shows that on CBNAAT test out of 50 participants 33 were tested positive and 17 were tested negative. The Rifampicin resistance by LPA test. This test shows that on LPA test out of 50 participants 35 were tested positive and 15 were negative. The above table depicts the sensitivity of CBNAAT/Xpert MTB/Rif Assay and Line Probe Assay as 91.4% (CI= 76.9%-98.2%) and 96.9% (CI= 84.2%-99.9%) respectively, while the specificity of both the tests were 93% (CI= 68%-99.8%) and 82.3% (CI=56.7%-96.2%) respectively. Positive predictive value and negative predictive value of CBNAAT are 96.9% and 82.3% respectively while that of LPA are 91.4% and 93% respectively.

Conclusion: According to the present study, as compared to CBNAAT, LPA detects 96.9% of true positives i.e. those who truly have Rifampicin resistance. The test has accuracy of 92%. Accordingly, the positive predictive value of LPA is 91.4% implying that 91.4% of subjects actually have Rifampicin resistance tuberculosis given that the subjects have a positive test results for rifampicin resistance.

Keywords: CBNAAT, Rifampicin, monoresistant& mycobacterium tuberculosis.

Study Designed: Observational Study.

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1. INTRODUCTION

Tuberculosis (TB) remains a significant cause for death worldwide and is the leading cause of death from one infective agent, ranking on top of human immunodeficiency virus (HIV). Globally, in 2018, about ten million people were affected by TB, resulting in 1.3 million deaths among HIV-negative individuals[1]. Drug-resistant TB poses an additional intense challenge, with 484,000 incident cases of multidrug- resistant tuberculosis (MDR-TB). Globally, India along with China and Russian-Federation, contributed about 50% of total MDR/rifampicin- resistant (RR)-TB cases[2].

Early diagnosis of TB, and universal drug susceptibility testing (DST), is the vital element within the machinery of TB control, and thereby very important fact of End TB Strategy. Acid-fast bacilli (AFB) smear microscopy is the most commonly used diagnostic tool for the detection of TB in high burden countries. However, its sensitivity2 was only 46% to 63% when compared against culture, and further decreases in patients with HIV co-infection[3]. A conventional culture- based (phenotypic) approach is still considered as „gold standard," but it is time-consuming and takes around two to three months for the identification and DST of Mycobacterium tuberculosis (Mtb). To unravel these challenges, a serious push has been given to rapid nucleic acid amplification test (NAAT) based molecular tests like line probe assay (LPA) (GenoTypeMTBDRplus, Hain Life science, Nehren, Germany) and Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA). The LPA (version 1) used to detect Mtb and at the same time gave status of rifampicin (RIF) and isoniazid (INH) resistance in sputum samples[4&5]. The test is appropriate for rapid screening of MDR-TB patients but it is recommended in only AFB smear-positive sputum sample because unacceptable performance negated the utilization of this assay in AFB smear-negative samples.

Now, a new modified version (Geno Type MTBDR plus version 2) of the LPA has been introduced which boasts of its increased performance but there are limited studies regarding its comparative performance with other NAAT-based test[6&7]. The Xpert MTB/RIF assay was also recommended for rapid and simultaneous detection of Mtb and RIF susceptibility; however, optimal detection of AFB smear- negative patient still remains challenging."

2. MATERIAL & METHOD

The study was carried out in the department of Respiratory Medicine in Index Medical College Hospital & Research Centre, Indore under approval of the Institutional Ethics Committee .(Ref No.- IMCHRC/IEC/2020/88)

All patients >14 years of age admitted in Chest ward of Index Medical College, Indore with features of TB between May 2020 to April 2021. Study was conducted on 50 patients from the Department of TB and CHEST at Index Medical College, Research centre and Hospital, Indore and sample was sent to Intermediate Reference Laboratory, M.R.T.B. Hospital/Chest Centre, Indore and all the tests was performed with due permission from the Institutional Ethical Committee and informed consent from the subjects or their legal relatives.

Subjects were included on the basis of their diagnosis of TB as per NTEP guidelines.1

A) CBNAAT Material Required:

- Falcon tube.
- Sample (sputum, gastric aspirate, pus, pleural fluid, ascitic fluid, incision & drainage sample, CSF, aspiration & biopsy material, mid stream urine).
- **Sample collection:**
- Collect 1 -4 ml specimen.

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- Collect the sample in the tube called FALCON tube.
- Specimen should be held at 2-8C wherever possible.
- Do not leave the specimen at room temperature for more than 3 days.

B) Line Probe Assay (LPA) Sample required:

1. AFB smear microscopy positive specimens
2. Culture isolate

Amount of sample-

1. Direct patient materials-500 mic. lit.
2. Isolates from liquid media-1000 mic.lit.
3. Isolates from solid media-300 mic.lit.

Inclusion criteria:

- All presumptive case of MDR-TB.
- Willing to participate and willing to give written consent.
- Age >14 year.

Exclusion criteria:

- Sputum smear negative
- Patient refusing to participate in the study
- Age <14.

3. RESULTS

Table 01: Rifampicin Monoresistance by CBNAAT

Rifampicin Monoresistance by CBNAAT		
	Frequency	Percent
POSITIVE	33	66.0
NEGATIVE	17	34.0
Total	**50**	**100.0**

The Rifampicin resistance by CBNAAT test. This test shows that on CBNAAT test out of 50 participants 33 were tested positive and 17 were tested negative.

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Table 02: Rifampicin Monoresistance by LPA

Rifampicin Monoresistance by LPA		
	Frequency	**Percent**
POSITIVE	35	70.0
NEGATIVE	15	30.0
Total	**50**	**100.0**

The Rifampicin resistance by LPA test. This test shows that on LPA test out of 50 participants 35 were tested positive and 15 were negative.

Table 03: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of CBNAAT and LPA for the smear positive samples

Tests	**Sensitivity (%)**	**Specificity (%)**	**Predictive values (%)**	
			Positive	**Negative**
CBNAAT	91.4% (76.9%- 98.2%)	93% (68%-99.8%)	96.9%	82.3%
LPA	96.9% (84.2%-99.9%)	82.3% (56.7%-96.2%)	91.4%	93%

The above table depicts the sensitivity of CBNAAT/Xpert MTB/Rif Assay and Line

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Probe Assay as 91.4% (CI= 76.9%-98.2%) and 96.9% (CI= 84.2%-99.9%) respectively, while the specificity of both the tests were 93% (CI= 68%-99.8%) and 82.3% (CI=56.7%-96.2%)respectively.Positive predictive value and negative predictive value of CBNAAT are 96.9% and 82.3% respectively while that of LPA are 91.4% and 93%respectively.

4. DISCUSSION

In the present study, the performance of NAAT-based Xpert MTB/RIF assay and Line Probe Assay were compared for early detection of Rifampicin mono-resistance tuberculosis[8].According to the inclusion criteria, fifty smear positive cases were tested for Rif resistance. Of these smears, 66% (n=33) and 70% (n=35) smear were identified as Rif resistant by CBNAAT and LPA respectively, remaining were sensitive for rifampicin.When CBNAAT and LPA were compared, it was found that LPA has higher sensitivity than that of CBNAAT i.e. 96.9% and 91.4%respectively[9]. Means 96.9% of tested smear samples was be identified as true Rif resistant by using Line probe assay while only 91.4% smear samples can be identified as true when tested using CBNAAT. The results are in accordance with the study done by Arichaet al13 where sensitivity of LPA is higher than that of CBNAAT. In this study, sensitivity of LPA is similar to the study done in Ethiopia14 on high risk MDR-TB population[10].

Specificity of a test i.e. the ability to find the true negative, in this study it's the ability to find those which are rifampicin sensitive. Specificity of CBNAAT is higher than that of LPA i.e. 93% and 82.3% respectively[11]. This shows that CBNAAT is better in identifying those samples that are not Rif resistant which is not in accordance with the studies done on smear positive samples of South African and South American population specificity of LPA is higher than Xpert MTB/Rif Assay(4).

Predictive values of CBNAAT were observed as- PPV 96.9% and NPV 82.3% while the predictive values of LPA were observed as PPV 91.4% and NPV 93%. Predictive values of both the tests were observed to be higher thus a better diagnostic tool for RIF resistance diagnosis[12].

The results are different from the studies done in Kenya(3) where the tests have low predictive values.The variation in results of the study can be attributed to the very low sample size, different geographical features of the sampling locations and difference in sampling methods.

5. CONCLUSION

According to the present study, as compared to CBNAAT, LPA detects 96.9% of true positives i.e. those who truly have Rifampicin resistance. The test has accuracy of 92%. Accordingly, the positive predictive value of LPA is 91.4% implying that 91.4% of subjects actually have Rifampicin resistance tuberculosis given that the subjects have a positive test results for rifampicin resistance.

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