ORIGINAL ARTICLE

Yield of Gene Expert and ZN Staing in diagnosis of Pulmonary and Extra Pulmonary Tuberculosis

NABI RAHMAN[1], MUHAMMAD HAROON TAJ[2], AJMAAL JAMI[3], ANEELA QURESHI[4], M ZAHID SHAH[5], NOOR KHALIQ JAN[6]

[1]Assistant Professor Department of Medicine and Allied Mardan Medical Complex /BKMC
[2]Assistant Professor (Pulmonology), KMU Institute of Medical Sciences (KIMS), Kohat.
[3]Assistant Professor Dept of Medicine IsraMedical University Karachi.
[4]Professor Dept of Pathology IsraMedical University, Karachi.
[5]MBBS MD Internal Medicine Assistant Professor BaqaiMedical University Karachi.
[6]Senior Registrar, Head of Chest Unit Govt Naseer Ullah Khan Babar Memorial Teaching Hospital Kohat Road Peshawer
Correspondence to: Dr Nabi Rahman, Email Address: nabirahman07@gmail.com, Cell No: +923339352516

ABSTRACT

Objective: To determine the prevalence of pulmonary and multi drug resistant TB in patients visited outpatients for respiratory problems.

Study Design: Descriptive, Cross-Sectional

Place and Duration: Conducted at Medicine Department of Mardan Medical complex, Bacha Khan Medical College, Mardan for duration of one year from 15st January 2019 to 14 January, 2020.

Methods: Total 4600 patients were included in this study. Patients were divided into two groups. Group A had 1100 patients and treated by Gene Xpert technique and the group B had 3500 patients checked by Zhiel Neelsen (ZN) Staining. Patients detailed demographics were age, sex were recorded after taking written consent. Complete data was analyzed by SPSS 24.0 version.

Results: Out of 1100 patients in group A 715 (665%) were males and 385 (35%) patients were females, and MTB was detected in 320 (29.09%) patients. In group B 2400 (68.6%)patients were males and 1100 (31.4%) were females, out of these 430 (12.3%) cases showed smear positive. Frequency of rifampicin was 20 (6.25%) out of 320 patients of MTB.

Conclusion: We concluded in this study that gene Xpert is effective for diagnosis of tuberculosis and drug resistant TB while smear positive had many other advantages.

Keywords: Multidrug resistant TB, ZN Staining, Gene Xpert, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a major infectious disease, causing high morbidity and mortality worldwide. Tuberculosis is one of the top ten worldwide causes of death.[1] Pakistan ranks 5th among high burden countries. The prevention and management of tuberculosis is an extremely severe problem with drug resistance. In this form of tuberculosis Pakistan, regrettably, is at the sixth high burden[2]. Approximately 510,000 people, including children, receive an infection in Pakistan every year which is liable to kill over 70,000 children as a result of this disease. A type of tuberculosis isolate MDR resistant to isoniazid[3] and rifampicin in Mycobacterium tuberculosis is a type of tuberculosis.

In 2017 surveillance study by the World Health Organization suggests that about 600,000 patients suffer from TB rifampicin resistance and around 490,000 grow MDR patients. 47 percent of these patients are from India, China, Russia and Pakistan. 518,000 new TB cases with an incidence of 268/100,000 in Pakistan in 2016 and 23/ 100,000 deaths were recorded. in Pakistan. There have been cases of 3.5/100000 and 3 14/100 000 TB & MDR TB patients co-infected with human immunodeficiency virus, respectively. New patients of TB have up to 4.2 % 4% and 16 % of MDR and rifampicin resistance among those who have previously been treated. There is also an advanced form of MDR TB in which MTB also has resistance to flouriquinolones, which is at least one of the three injectable second line antituberculosis medicines i.e. Capreopmycin, Amikacin and Kanamycin.

The XDR TB rate among MDR-TB cases is 6.2 percent according to WHO data from 91 countries.[4] Early detection and early treatment is the only key to effective TB management. Many diagnostic approaches are available, but the gold standard is to demonstrate MTB by means of microscopy, culture and Gene Xpert MTB/Rif assays recently established. LPAs have been certified for the diagnosis of M by the World Health Organisation (WHO). tuberculosis and RIF resistance in smear-positive tuberculosis in 2008[5], guided by a systematic review evaluating two first-generation LPAs: INNO-LiPARif. TB assay (Innogenetics, Ghent, Belgium) and Genotype MTBDR assay (Hain Lifescience GmbH, Nehren, Germany)[6], both of which assays are no longer used in clinical practice. New LPA technology versions were developed[7-9] and further studies were conducted.

MATERIAL AND METHODS

The research was conducted in Medicine Department of Mardan Medical complex, Bacha Khan Medical College, Mardan for duration of one year from 15st January 2019 to 14 January, 2020and in this study, we included 4600 patients. Patients detailed demographics including age, sex and body mass index were recorded after taking written consent. Patients with no written consent were excluded from this study. Patients were divided into two groups. Group A had 1100 patients and treated by Gene Xperttechnique and the group B had 3500 patients checked by ZhielNeelsen (ZN) Staining

Sputum samples were obtained from all patients after the informed consent was taken. One sample was collected per patient from patients screened for Gene Xpert, while two samples were collected per patient from patients tested with ZN stain. Demographic data had been identified in alleged patients, including age and sex. Detailed history had also been documented, including prior TB treatment. In

ZheilNeelsen the presence of acid quick bacillis in Smear had been demonstrated and Gen Xpert MTB Rif Assay was used to detect MTB resistance and complex rifampicin. Complete data was analyzed by SPSS 24.0 version.

RESULTS

Out of 1100 patients in group A 715 (665%) were males and 385 (35%) patients were females, and MTB was detected in 320 (29.09%) patients. Among MTB 320 cases, 215 (67.19%) patients were male and the rest of 105(32.81%) patients were females. Frequency of Rifampcin resistant was 20 (6.25%) cases in which 14 (70%) were males and 6 (30%) cases were females. (table 1)

Table 1: Distribution of patients in Gene Xpert

Variables	Male	Female	Total
MTB	215(67.19%)	105(32.81%)	320(29.09%)
Non-MTB	500(64.1%)	280(35.1%)	780(80.91%)
Rifampicin Resistance	14(70%)	6(30%)	20(6.25%)0

In group B 2400 (68.6%) patients were males and 1100 (31.4%) were females, out of these 430 (12.3%) cases showed smear positive. Out 430 cases of smear positive 325(75.9%) cases were males and the rest 105 (24.1%) patients were females. (table 2)

Table 2: Classification of patients by Zn staining

Variables	Male	Female	Total
Smear Positive	325(75.9%)	105 (24.1%)	430(12.3%)
Negative	2075(67.6%)	995(32.41%)	3070(87.7%)

DISCUSSION

While most of the techniques for drug resistance were initially developed in complex isolates of TB, they are still being evaluated to specifically recognise TB complex isolates and to recognise drug resistance allulas in clinical samples (such as sputum). Their potential benefit is that organism development is unnecessary and the outcomes of DST can be calculated in days instead of weeks; evidence indicates that they can be very accurate.[10]

RIF detection is traditionally used as an MDR-TB predictor. Its positive predictive value is dependent on the sensitivity and specificity of MDR and non-MDR resistance testing, which is highest among previously treated cases in settings with high MDR prevalency and low non-MDR RIF resistance.

Our analysis found that Zn stains 12.3% with a proportion between men and women of 75.9% and 24.1% respectively. Gene Xpert positivity was 29.09% and 67.19% with the female and 32.81%.These findings were comparable with previous studies by Ch MK et al.[11] and Buchelli et al[12] presented that 53% Zn stain's smear positive and 82% GeneXpert's positive. However, another analysis by Muniret.al[13] showed a positive diffusion of 67.5% and GeneXpert of 77.4% respectively. Because of the disparity in option criterions, the diagnostic proportions of the above two trials are strong. For smear negative specimens Steingart KR et al compared 44% with the 67% sensitivity of Xpert MTB/RIF when used as an additive measure.[14]

Smear findings of the current study are in line with an incremental production study of the presentation of three sputum smears, which showed a Zn smear positive of 12.3% percent overall, and similar results were found in the earliest studies performed by Migliori GB et al and Saleem S et al.[15-16]

CONCLUSION

In the same sample and at a period of 2 hours Gene Xpert is not only a valuable contribution to the diagnosis of TB, it also offers substantial rifampicin tubercular resistance to drugs. However ZN smear cannot be removed, since it is cheaper, faster and without higher facilities can be achieved.

REFERENCES

1. WHO TB Key facts sheet. [updated18 September 2018, Cited August 2019] Available from URL:[h tt p s ://www.who. i n t/ n ews -ro o m /f a c tsheets/detail/tuberculosis].
2. Waheed Y, Khan MA, Fatima R, Yaqoob A, Mirza A, Qadeer E, et.al. Infection control in hospitals managing drug-resistant tuberculosis in Pakistan: how are we doing?. Pub Health Act. 2017; 7(1): 26- 31.
3. World Health Organization. Global tuberculosis report, 2016. WHO/HTM/ TB/2016.13. Geneva, Switzerland: WHO, 2016. (Accessed on 19th A u g u s t 2 0 1 9)
4. World Health Organization. Global TB Report 2017. (Accessed on August 2019)
5. World Health Organization. WHO policy statement. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (English and Russian), 2008. www.who.int/tb/features_archive/policy_statement.pdf?ua=1 Date last accessed: December 22, 2016.
6. Ling DI, Zwerling AA, Pai M GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta analysis. EurRespir J 2008; **32**: 1165–1174.
7. Barnard M, Gey van Pittius NC, van Helden PD, et al.The diagnostic performance of the GenoTypeMTBDRplus version 2 line probe assay is equivalent to that of the Xpert MTB/RIF assay. J ClinMicrobiol 2012; **50**: 3712–3716
8. Crudu V, Stratan E, Romancenco E, et al. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances. J ClinMicrobiol 2012; **50**: 1264–1269.
9. Arentz M, Sorensen B, Horne DJ, et al. Systematic review of the performance of rapid rifampicin resistance testing for drug-resistant tuberculosis. PLoS One 2013; **8**: e76533
10. Mitarai S, Kato S, Ogata H, et al. Comprehensive multicenter evaluation of a new line probe assay kit for identification of Mycobacterium species and detection of drug-resistant Mycobacterium tuberculosis. J ClinMicrobiol 2012; **50**: 884–890.
11. Ch MK, Hanif A, Shafiq S, Iqbal R. Diagnosis of Pulmonary and Multidrug Resistant Tuberculosis at DHQ Teaching Hospital Gujranwala. Pak J Chest Med 2019; 25 (2): 64-67.
12. Buchelli Rmirez H L, Gracia-Clemente M M, Alvarez-Alvarez C, Palacio-Gutierrez J J, PandoSandovalA,Gagatek S, AriasGuillen M, Quenzada-Loaiza C A, Casan-Clara. Impact of the Xpert MTB/RIF molecular test on the late diagnosis of pulmonary tuberculosis.Int J Tuberc Lung Dis. 2014;18(4): 435-37.
13. Munir MK, Rehman S, Aasim M, Iqbal R, Saeed S. Comparison of ZiehlNeelsen microscopy with GeneXpert for detection of Mycobacterium tuberculosis. IOSR-JDMS. 2015;14(11):56-60
14. Steingart KR, Schiller I, Horne DJ, et al.Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014; **1**: CD009593.
15. Migliori GB, Matteelli A, Cirillo D, Pai M. Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Current standards and challenges. Can J Infect Dis Med Microbiol. 2008;19(2):169-172. doi:10.1155/2008/857901
16. Saleem S, Shabbir I, Iqbal R, Khan SU. Value of three sputum smears microscopy in diagnosis of pulmonary tuberculosis. Pak J Med Res. 2007;46(4):94-7.