IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 1 Ser.19 (January. 2020), PP 60-64
www.iosrjournals.org

Rapid Detection of *Mycobacterium tuberculosis* and Rifampicin Resistance in Sputum by Use of Genexpert Mtb/Rif

Mr. Neeraj Sharma[1], Mr. Rishikeshav Acharya[2]
Mrs. Bimala Dhakal Sharma, Mrs. Jyoti Chhetri
Dr. Rupak Kandel[3]

[1]*Medical Microbiologist and Laboratory Incharge at Nepal Cancer Hospital and Research Center, Harisiddhi, Lalitpur, Nepal*
[2] *Medical Microbiologist at LifeCare Diagnostics and Research Centre Pokhara Pvt Ltd, Pokhara, Nepal*
[3]*Karnali Province Hospital, Surkhet, Nepal*
Corresponding Author: Mr. Neeraj Sharma

Abstract
BACKGROUND*: Tuberculosis remains a major health problem affecting one third of the population. The effective control is based on immediate detection of Mycobacterium tuberculosis. Though conventional method of TB detection exists; Genexpert MTB/RIF assay represents a perfect method in the diagnosis of TB and MDR TB by simultaneous detection of M. tuberculosis and rifampicin resistance. However, MTB/RIF assay is limited to few places in the country.*
METHODS: *A total of 157 expectorated sputum samples from patients with signs and symptoms of suggestive of tuberculosis at Midwestern Regional hospital Surkhet, Nepal were tested directly by ZN staining and GeneXpert MTB/RIF.*
RESULT*: Overall 27.8% (44/157) samples were positive for MTB by GeneXpert. 15.2% (24/158) were males and 12.6% (20/157) were females. 55.7% (34/158) were new suspected cases and 6.3% (10/158) were previously treated cases. Among 27.8% (44/158) positive cases detected by the GeneXpert MTB/RIF 23.4% (37/158) were positive to smear microscopy. 6.8% (3/44) of rifampicin resistance was detected among the positive cases.*
CONCLUSION*: GeneXpert test led to increased TB case detection as compared to smear microscopy. In addition, it was able to diagnose the rifampicin resistance in the Mycobacterium tuberculosis in a single test.*
Key words: Mycobacterium tuberculosis, GeneXpert MTB/RIF assay, Smear microscopy.

Date of Submission: 17-01-2020 Date of Acceptance: 04-02-2020

I. Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, known to have devastating disease of humankind. It affects all ages and prominently reported among the most productive age population (Nepali et al 2013).

Tuberculosis (TB) remains a major public health problem, accounting for more than 9.4 million incident cases and 1.3 million deaths every year, worldwide (Ioannidis P et al 2011). South East Asia Region carries the highest burden of tuberculosis (TB) cases accounting for one third of all cases from five countries, namely Bangladesh, India, Indonesia, Myanmar and Thailand account for 95 per cent of these cases. Nonetheless, tuberculosis is one of the major public health problems in Nepal. About 45 per cent of the total population in Nepal is estimated to be infected with TB, out of which 60 percent are in adult age group. Every year, 40,000 people develop active TB, of them 20,000 have infectious pulmonary disease and 5,000-7,000 people still die per year from TB in Nepal (Nepali et al 2013).

Multi-drug resistant tuberculosis (MDR-TB) is defined as disease caused by *Mycobacterium tuberculosis* with resistance to at least two anti-tubercular drugs isoniazid and rifampicin. Recent surveillance data have revealed that prevalence of the drug resistant tuberculosis has risen to the highest rate ever recorded in the history. The most powerful predictor of the presence of MDR-TB is a history of treatment of TB. Shortage of drugs has been one of the most common reasons for the inadequacy of the initial anti-TB regimen, especially in resource poor setting (Marahatta et al 2011).

The emergence and spread of multidrug resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* complex (MTBC) strains poses significant challenges to disease control (Ioannidis et al 2011). The rapid detection of *Mycobacterium tuberculosis* and rifampin (RIF) resistance in infected

patients is essential for disease management, because of the high risk of transmission from person to person and emergence of MDR-TB and extensively drug resistant tuberculosis (Zeka et al 2011).

The main mutations that confer (RIF) resistance are located in the *rpoB* gene, specifically, in the well-defined 81bp core region (Ramaswamy and Musser 1998 and Rattan et al 1998). About 95% of RMP resistant strains have a mutation in this region, which facilitates the rapid development of approaches for the detection of resistance to this drug. However, the molecular basis of resistance to INH is more complex because it involves mutations in more than one gene or gene complex such as the katG, inhA, and kasA genes and the intergenic region of the oxyRahpC complex (Ramaswamy and Musser 1998 and Somoskovi et al 2001).

Culture is the "gold standard" for final determination, but it is slow and may take up to 2 to 8 weeks. Although smear microscopy for acid fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques (Zeka et al 2011). Drug susceptibility testing (DST) of *Mycobacterium tuberculosis* in clinical specimens is time-consuming. Isoniazid and rifampicin are crucial elements of the standard treatment regimen of tuberculosis and resistance to these drugs requires extension of therapy (Blanc et al 2003).

Recently, a real-time PCR assay for *Mycobacterium tuberculosis* that simultaneously detects rifampicin resistance was developed on the GeneXpert platform (Cepheid, Sunnyvale, CA, USA), which integrates sample processing and greatly simplifies testing (Boehme et al 2011).

The GeneXpert MTB/RIF is a cartridge-based, automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification technique (NAAT). It was co-developed by the laboratory of Professor David Alland at the University of Medicine and Dentistry of New Jersey (UMDNJ). Cepheid Inc. and "Foundation Innovative New Diagnosis", with additional financial support from the US National institute of Health (NIH) (David 2013).

In the present study, the performance of the GeneXpert assay for detection of *Mycobacterium tuberculosis* was evaluated and the level of rifampicin resistance was determined on the sputum samples received in MWRH Surkhet. This study also revealed the prevalence of MDR tuberculosis among the people and also was helpful in management of the MDR cases

II. Objectives:

- To study the rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance in sputum by use of GeneXpert MTB/RIF.
- To estimate the prevalence of multi drug resistant tuberculosis**.**
- To compare the technique of GeneXpert MTB/RIF against microscopic smear examination.
- To find out the incidence of Multi Drug Resistant Tuberculosis (MDR-TB) in new suspects

III. Methodology

A Cross-sectional study was conducted on patients with suspected of pulmonary TB to evaluate the performance of GeneXpert MTB/RIF assay for detection of MTBC at Mid-Western Regional Hospital, Surkhet, Nepal. This study was conducted in the laboratory of Mid-Western Regional Hospital Surkhet Nepal, 582 Km west from Kathmandu the capital city of Nepal. Mid-western regional hospital is one of the largest hospitals having highest number of patient flow in Surkhet district. The study was done in the patients visiting MWRH suspected to have Tuberculosis and drug resistant tuberculosis on the basis of the clinical history and the clinical symptoms. The study was performed from August 2014 to March 2015. Sputum examination from the referred patients was carried out for smear microscopy and GeneXpert assay during this period. All the patients; male, female of all age group suspected of having tuberculosis and drug resistant tuberculosis referred by physicians and other specialists were included. The extra pulmonary samples were excluded. Sample of choice for the study was early morning sputum. Patients were given two separate containers, a small sterile glass vial for the smear microscopy and a large sterile plastic container for GeneXpert assay. Clear instruction on aseptic technique for sample collection to prevent contamination was given to the patients. They were also requested to bring at least of 2 ml sputum sample in large container and about 1 ml in glass vial provided to them. The sample containers were labeled with the patient's lab number and the date of collection. The patient's details including name, age, clinical history etc. were registered. The containers were sterile wide mouthed and screw capped. The sample container for GeneXpert assay was large with tapering end. All statistical analyze were performed using SPSS 16.0 version. The prevalence of tuberculosis was reported in percentage proportions and the 95% confidence interval (95% CI) of the prevalence of data was estimated from the standard error. A p value < 0.05 was considered statistically significant. Patients were given the sample containers the previous day and requested to bring early morning sputum sample. Samples were processed every day. The sample in the glass vial was used for microscopy and the large plastic container was used for GeneXpert assay. Sputum

smears were microscopically examined by AFB staining method. The organisms appeared red against bluish background.

IV. Result

A total of 158 samples were enrolled during the study period. Acid fast staining (AFB) and GeneXpert assay were performed with each sample in mid-western Regional Hospital Surkhet, Nepal. Out of 158 samples collected 44 were detected tuberculosis positive and 114 were detected negative. Among 44 positive cases, 3 were detected as rifampicin resistant and 41 were sensitive to all the antibiotics in DOTS therapy. A total of 158 patients were enrolled. Among them 97 (61.4%) were males and 61 (38.6%) were females. Among 158 enrolled patients 86 (54.4%) were previously DOTS treated cases, suspected of relapse, failure and treatment after default. 72 (44.6%) were new suspects thought to have MDR TB, who were in close contact with the MDR suspects and had history of TB among the family member.

Among 97 males; 24 (15.2%) were detected positive and 73 (46.2%) were detected negative, similarly 20 (12.7%) out of 61 females were detected positive and 41 (25.9%) were detected tuberculosis negative by GeneXpert. Among total positive cases 54.5% were male and 45.5% were female. There was no significant association seen between the tuberculosis positive case and the gender (p= 0.180).

Out of 44 positive cases it was seen that 34 (77.3%) were new suspects and 10 (22.7%) were previously DOTS treated cases. MTB positivity was higher in new suspected case than the previously treated positive cases. Among 34 new suspected positive case16 (36.4%) were male and 18 (40.9%) were female. Likewise, among 10 previously treated positive cases 8 (18.2%) were male and 2 (4.5%) were female. The insignificant association was found between the two types of cases and gender (p=0.069). It was seen that new tuberculosis cases in female was slightly higher than in the males and reactivation of tuberculosis was much higher in males as compared to females.

GeneXpert test detected more positive cases than the sputum smear microscopy did, that was 37 out of 158 (23.4%), while GeneXpert tested positive for 44 out of 158 (27.8%).Among 44 (27.8%) positive results obtained from GeneXpert MTB/RIF assay, rifampicin resistance was detected in 6.8% (3/44) of positive specimens which was 1.9% of the total study population.

Table 1Tuberculosis among the new cases and previously treated cases

MTB Positive	Case Type		Total	
	New Suspected	Previously treated		
Male	16 (36.4%)	8 (18.2%)	24 (54.6%)	
Female	18 (40.9%)	2 (4.5%)	20 (45.4%)	P=0.069
TOTAL	**34 (77.3%)**	**10 (22.7%)**	**44 (100%)**	

Table 2Comparison and association of smear microscopy and GeneXpert

Smear Microscopy	GeneXpert		Total	
	Negative	Positive		
Negative	114 (72.2%)	7 (4.4%)	121 (76.6%)	
Positive	0 0.00%	37 (23.4%)	37 (23.4%)	P=0.000
TOTAL	**114 (72.2%)**	**44 (27.8%)**	**158 (100%)**	

V. Discussion

Mycobacterium tuberculosis has become a serious public health threat worldwide. The most significant step in control of tuberculosis is early and accurate diagnosis which is helpful in earlier treatment and faster disease control. Conventional laboratory methods like ZN smear microscopy is less sensitive as compared to the culture technique and culture and sensitivity technique requires equipped biosafety setup as well as well trained and skilled laboratory manpower, moreover it is time consuming requires 3 to 5 weeks. Recently; attention has been devoted to latest nucleic acid amplification diagnostic systems due to their speed and accuracy. GeneXpert MTB/RIF assay is a rapid molecular biology/gene based assay that can be used close to the point of care by operators with minimal technical GeneXpertise. The technique enables diagnosis of TB and simultaneous assessment of Rifampicin resistance to be completed within two hours. (WHO 2011 and Raviglione et al 2012).

In the current study 54.5% male and 45.5% females were detected positive to MTB by GeneXpert. No significant association was found between the positive cases and the gender (p=0.180). Similar result was obtained by Pinyopornpanish et al in 2013 where 40.4% females and 59.6% males were detected positive. In

this study total 27.85% were found to be positive by GeneXpert whereas only 23.42% were shown positive by ZN smear microscopy. The significant association was seen between GeneXpert assay and positive TB cases (p=0.000). The finding was similar to the study conducted by Ndubuisi et al 2016, reported 15.8% and 25.93% of positive cases by smear microscopy and GeneXpert assay respectively. Similarly, Shah et al 2016 and Boehme et al 2011 reported 62.60%, 59.93% and 14.03%, 10.51% of GeneXpert and ZN smear microscopy positive results respectively. In contrast Guenaoui et al 2016 reported 100% positive case by both smear microscopy and GeneXpert. This result suggests that the sensitivity and specificity of GeneXpert assay was higher in comparison with microscopy.

Among 44 specimens with MTB positive by GeneXpert, rifampicin resistance was detected in 6.8% of positive specimens. This finding was supported by Ndubuisi et al 2016 where the study reported 3.1% of rifampicin resistance among detected TB positive patient whereas Shah et al 2016 reported high prevalence (21.60%) of rifampicin resistance among TB positive cases by GeneXpert assay. Rifampicin resistance is a precursor to the development of multidrug resistant tuberculosis (MDR-TB) and a reliable predictor of multi-drug resistance in settings. This is upsetting as MDR-TB spread on the community could be on-going. Thus, WHO 2015 recommends that if GeneXpert detects rifampicin resistance in patients considered at risk of MDR-TB. In the current study, rifampicin resistance among the newly suspected case was 2.9%. There was no significant association found between rifampicin resistance and new suspected case (p=0.293). This finding was lower than the study conducted by Guled et al 2016 and Sharma et al 2011 which reported 5.2% and 6.8% in newly diagnosed cases. This might be due to the close contact with the patients suffering from MDR TB.

VI. Conclusion

The present study demonstrated *Mycobacterium tuberculosis* along with rifampicin resistance in the population of Surkhet district by GeneXpert assay. It is a very useful method in the diagnosis of tuberculosis which not only detects the presence of *Mycobacterium tuberculosis* in the clinical specimens but also can detect the rifampicin resistance in a single test within a couple of hours. The prevalence of multi drug resistant TB was also seen in the study population which was found higher in the previously treated case in comparison to the new suspects. The study also showed that prevalent of major common symptoms like fever, weight loss and persistent cough for 3 weeks could be one of the diagnostic tool for MTB infection. Although GeneXpert assay is more sensitive than the smear microscopy (AFB) due to some limitation GeneXpert MTB/RIF cannot replace conventional TB diagnostic tools.

VII. Recommendations

Based on the findings, of the study following recommendations have been made;

1. GeneXpert assay can be used in the diagnosis of TB which is more sensitive and specific than the smear microscopy.
2. This can be useful tool in diagnosis of MDR-TB also because it can detect rifampicin resistance within 2 hours.
3. GeneXpert assay can be used for rapid screening of resistant tuberculosis before prescribing any treatment regimen
4. Although *Mycobacterium tuberculosis* culture is considered as a gold standard it requires advanced setup and skilled technician to perform the test GeneXpert can be established easily with simple training to the technician.
5. The government should also give priority to establish GeneXpert in remote places of the country.

References

[1]. Blanc L, Chaulet P, Espinal M, Graham S, Grzemska M, Harries A, Luelmo F, Maher D, O'Brien R, Raviglione M, Rieder H, Starke J, Uplekar M and Wells C (2003) Treatment of tuberculosis, guidelines for national programmes, 3rd ed. World Health Organization, Geneva, Switzerland **313**:11-75.

[2]. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, Gler MT, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiyev R, Raymond L, Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D, Perkins MD (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the GeneXpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicenter implementation study Lancet; **377**: 1495–1505.

[3]. David R. Boulware (2013) Utility of the GeneXpert MTB/RIF Assay for Diagnosis of Tuberculous Meningitis Plos one Med **10**: 1-7.

[4]. Guenaoui K, Harir N, Ouardi A, Zeggai S, Sellam F, Bekri F. Touil SC (2016) Rapid diagnosis of *Mycobacterium tuberculosis* by Gene eGeneXpert MTB/RIF assay in Western Algeria Basic Research Journal of Microbiology ISSN 2354-4082. **3:**12-17.

[5]. Guled AY, Elmi AH, Abdi BM, Rage AMA, Ali FM, Abdinur AH, Ali AA, Ahmed AA, Ibrahim KA, Mohamed SO, Mire FA, Adem OA and Osman AD (2016) Prevalence of Rifampicin Resistance and Associated Risk Factors among Suspected Multidrug Resistant Tuberculosis Cases in TB Centers Mogadishu-Somalia: Descriptive Study Open Journal of Respiratory Diseases. **6**: 15-24.

[6]. http://whqlibdoc. who.int/publications /2011/9789241501545_eng.pdf Accessed: 20th April 2017

[7]. Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagi M, Raftopoulou R, Konstantinidou E, Marinou I, and Kanavaki S (2011) Cepheid GeneXpert MTB/RIF Assay for *Mycobacterium tuberculosis*Detection and Rifampin Resistance Identification in

Patients with Substantial Clinical Indications of Tuberculosis and Smear-Negative Microscopy Results JOURNAL OF CLINICAL MICROBIOLOGY; **49**:3068–3070.
[8]. Marahatta SB, Kaewkungwal J, Ramasoota P, Singhasivanon P, (2011) Risk Factors of Multi-Drug Resistant Tuberculosis (Mdr Tb) In Nepal Epidemiol Community Health **65**: 344-345.
[9]. Ndubuisi NO, Azuonye1 OR, Victor NO, Happiness OA and Daniel OC (2016) Diagnostic Accuracy of GeneXpert MTB/RIF Assay in Diagnosis of Pulmonary Tuberculosis Journal of Infectious Diseases and Treatment ISSN 2472-1093 2016 **2** :01-03.
[10]. Nepali RB and Paneru DP (2013) Compliance to Directly Observed Treatment Short Course (DOTS) Chemotherapy among the Patient of Pulmonary Tuberculosis in Banke District of Nepal JHAS. **3**:17-20.
[11]. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, Supparatpinyo K and Sirisanthana T (2015) Comparison of GeneXpert MTB/RIF Assay and the Conventional Sputum Microscopy in Detecting *Mycobacterium tuberculosis* in Northern Thailand. Hindawi Publishing Corporation Tuberculosis Research and Treatment Volume 2015, Article ID 571782, 01-06.
[12]. Ramaswamy S and Musser JM (1998) Molecular genetic basis of anti-microbial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber Lung Dis **79**:3–29.
[13]. Raviglione M, Marais B, Floyd K, Lonnroth K, Getahun H, Migliori GB, (2012) Scaling up interventions to achieve global tuberculosis control: progress and new developments. Lancet. **379**: 1902–1913.
[14]. Shah RH, Inayat N, Bouk GR, Akhtar M, Khooharo MA and Qayoom S, (2016) Rapid Detection Of Tuberculosis And Rifampicin Resistance With Automated GeneXpert Mtb/Rif Assay PJCM. **22**: 61-64.
[15]. Sharma SK, Kumar S, Saha PK, George N, Arora SK, Gupta D, Singh U, Hanif, M and Vashisht, RP (2011) Prevalence of Multidrug-Resistant Tuberculosis among Category II Pulmonary Tuberculosis Patients. Indian Journal of Medical Research, **133**:312-315.
[16]. World Health Organization (2011) Policy Statement: Automated real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: GeneXpert MTB/RIF System. Geneva, Switzerland:
[17]. World Health Organization (2015) Global Tuberculosis Report 20th edition
[18]. Zeka NA, Tasbakan S, and Cavusoglu C, (2011) Evaluation of the GeneXpert MTB/RIF Assay for Rapid Diagnosis of Tuberculosis and Detection of Rifampin Resistance in Pulmonary and Extrapulmonary Specimens J ClinMicrobiol. **49**: 4138–4141.

Neeraj Sharma, Rishikeshav Acharya, Bimala Dhakal Sharma, Jyoti Chhetri and Dr. Rupak Kandel "Rapid Detection of Mycobacterium Tuberculosis and Rifampicin Resistance in Sputum by Use of Genexpert Mtb/Rif." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(1), 2020, pp. 60-64.