The application of gene xpert for the diagnosis of mycobacterium tuberculosis and MDR TB in Kota region

Verma D[1], Sharma R[2], Chand A.E[3], Saxena A[4]

[1]Dr. Dinesh Verma, Assistant Professor, Department of Microbiology, [2]Dr. Rashmi Sharma, Senior Resident, Department of Microbiology, Goverment Medical College Kota. [3]Dr. Anita E Chand, Senior Professor, Department of Microbiology, Government Medical College Kota. [4]Dr. Anil Saxena, Professor, Department of Chest and TB, Goverment Medical College, Kota, Rajasthan, India

Address for Correspondence: Dr. Rashmi Sharma, Email: drrashmikota@gmail.com

....

Abstract

Background: Improved diagnosis of tuberculosis is a global priority for tuberculosis control which requires early case-detection. MDR-TB poses formidable challenges due to the complex requirements for diagnosis and treatment and so enhancement in the capacity to diagnose MDR TB is required. The objective of the study is to assess the performance of GeneXpert MTB/RIF as an initial diagnostic tool for diagnosis of *Mycobacterium tuberculosis* and MDR TB in kota region. **Methods:** Total 330 patients of all age groups having either pulmonary or extrapulmonary tuberculosis were included in the study with study period from February 2016 to July 2016 and samples collected were subjected to Gene Xpert. **Results:** *Mycobacterium tuberculosis* was reported in 102 (30.90%) cases by Gene Xpert and Rifampicin Resistance was detected in 23 (22.54%) out of these 102 cases. **Conclusion:** The implementation of the Xpert MTB/RIF assay could dramatically improve the rapid diagnosis of tuberculosis, especially in cases with suspicion of MDR and smear negative TB.

Key words: Gene Xpert, Mycobacterium tuberculosis, MDR, Rifampicin Resistance

....

Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. As per WHO Global TB Report (2015) out of the estimated global annual incidence of 9.6 million TB cases, 2.2 million were estimated to have occurred in India. i. e. approximately Twenty three percent of global annual TB incidents occur in India making it highest Tuberculosis burden country [1].

In the global tuberculosis report (2014), WHO reported that there were 9 million new cases of TB and 1.5 million deaths in 2013. This included upto 15% burden of paediatric cases and 1.1 million cases among people who were HIV positive. At the same time, global burden of multidrug-resistant TB (MDR-TB) was estimated to be 480,000 cases leading to estimated 210,000 deaths [2]. One-fourth of TB cases notified are

Extrapulmonary and incidence is even higher in children and immunocompromised people [3]. Recurrence of active TB after treatment occurs either due to relapse of infection with the same strain or re-infection with a new strain of Mycobacterium tuberculosis (MTB). The proportion of recurrent tuberculosis cases caused by re-infection has varied widely. Yet, retreatment outcomes are often poor, especially in patients with treatment failure or default [4].

Improved diagnosis of tuberculosis is a global priority for tuberculosis control which requires early case-detection particularly in cases of smear-negative disease which are often associated with the HIV co-infection and young age. HIV-associated TB is often misdiagnosed due to the limitations of conventional diagnostic techniques. MDR-TB poses formidable challenges due to the complex requirements for diagnosis and treatment and so enhancement in the

capacity to diagnose MDR TB is required. Alarming increase in MDR-TB incidence, the global emergence of extensively drug-resistant TB (XDR-TB), documented institutional transmission, and rapid mortality in patients with MDR-TB or XDR-TB who are co-infected with HIV have highlighted the urgent need for rapid diagnostic methods.

Prompt and accurate TB diagnosis are prerequisite for early and effective treatment thereby reducing tuberculosis burden and disease transmission but no single diagnostic test currently satisfies all the demands of "rapid", "affordable", and "easy". Conventional diagnostic methods for MTB are slow and lack sensitivity [5]. Sputum smear microscopy is inefficient due to its variable sensitivity particularly in patients with Sputum Smear-Negative TB, Extrapulmonary TB, HIV infected cases and Drug Resistant TB [6,7,8]. Lowenstein-Jensen (LJ) method, "the gold standard test", takes several weeks to produce result causing delayed onset of treatment [8]. Scaling up conventional culture and DST services is still slow and expensive, compounded by huge demands on laboratory infrastructure and human resources and also there are biosafety concerns. The World Health Organization (WHO) has endorsed the use of commercially available liquid culture systems and molecular line probe assays (LPAs) to rapidly detect MDR-TB; however, due to the tests' complexity and cost, as well as the need for sophisticated laboratory infrastructure and trained personnel, uptake has been limited in many resource-constrained settings. [6]. Since the development in the early 1980s of the polymerase chain reaction (PCR), molecular diagnostics have had a major impact on clinical medicine. However, despite several theoretical advantages, the use of molecular tests for TB has been limited, largely due to the complexities of DNA extraction, amplification and detection, and the biosafety concerns related to manipulating *Mycobacterium tuberculosis*. Therefore GeneXpert system was launched in 2004 as it simplifies molecular testing by fully integrating and automating the three processes required for real-time PCR-based molecular testing (ie. specimen preparation, amplification and detection).

Gene Xpert MTB/ RIF assay is an automated closed-cartridge system, easy to operate and user friendly with consistently better sensitivity than sputum microscopy. It is based on a hemi nested real time PCR assay utilizing five molecular beacon technology each labelled with a differentially coloured fluorophore spanning the rpoB gene 81-bp rifampicin resistance determining region (RRDR) of *M. Tuberculosis*. The test concurrently determines MTB and rifampicin susceptibility, which can be used as a surrogate marker for multidrug resistance (MDR–TB) [9]. Thus it can detect TB along with rifampicin resistance in less than two hours, directly from untreated sputum samples [3,10].

Therefore in December 2010, WHO recommended use of a new Cartridge Based Nucleic Acid Amplification test (CB-NAAT), named Gene Xpert system for diagnosis of TB. The October 2013 WHO guidelines include a conditional recommendation for Xpert MTB/RIF as the initial diagnostic test in all adults with suspected tuberculosis and MDR depending upon the available resources. The impact of Xpert MTB/RIF will, however, depend on the system in which it is used and countries will need evidence about patient, programme, and cost effectiveness outcomes to inform policy recommendations for programmatic implementation of the test in their settings [3].

Revised National TB Control Programme (RNTCP) is also currently using Xpert MTB/RIF to diagnose pulmonary TB, paediatric TB, extrapulmonary TB and rifampicin resistance and Multi Drug Resistance Tuberculosis in high risk populations like HIV positive as recommended by WHO under 2013 policy recommendations [2,3,10].

Aims Of The Study- The aim of the present study is to assess the performance of GeneXpert MTB/RIF as an initial diagnostic tool for diagnosis of TB in presumptive TB and MDR cases, and for the detection of Rifampicin Resistance in a tertiary level patient care setting.

Material and Methods

The present study was carried out at District Tuberculosis Centre associated with Government Medical College Kota. The study period was February 2016 to July 2016. The ethical clearance was taken from ethical committee of Govt Medical College Kota for study purpose.

Total 330 patients of all age groups having either pulmonary or extrapulmonary tuberculosis were included in the study if they fulfilled the inclusion criteria.

Inclusion Criteria

1. Presumptive TB case
  *All smear negative cases
  *All Paediatric cases
  *All HIV positive cases
  *All ExtraPulmonary cases

2. Presumptive MDR TB case according to the following criteria
  *Smear positive and Smear negative retreatment case.
  *MDR contact, Pulmonary TB.
  *TB-HIV Co-infected Cases.

Exclusion Criteria
Sputum sample which are either blood stained or contain food particles.

Procedure

All the samples were collected in well labelled falcon tubes. In pulmonary cases two sputum samples were collected: one early morning and other supervised spot specimen. Smears of both the sputum samples were made, stained by Zeihl Neelsen procedure, examined under light microscope and results were noted.

Thereafter only one sample was further processed: if the samples were positive for AFB, more positive sample otherwise good quality or early morning sample was used for Gene Xpert. In paediatric cases gastric lavage was collected in place of sputum. Where invasive techniques were required for sample collection only one sample was collected. In extrapulmonary cases the samples collected were CSF, lymph node fluid, pleural fluid and ascitic fluid.

The Xpert assay was performed according to the manufacturer's instructions [CEPHEID, Sunnyvale, CA, USA].

Our machine contains 4 cartriges so 4 samples were processed for each run (figure1). According to standard operating procedure the sampling reagent (containing NAOH and isopropanol) was added at 2:1 ratio to the sample and kept for 15 minutes at room temperature with intermittent shaking. 3ml of this treated sample was transferred to the cartridge and the cartridge was inserted in the module of CBNAAT machine. An automatic process completed the remaining assay steps and the results were displayed on the monitor attached to Gene Xpert after 1hr and 50 minutes.

Results

Total 330 cases were included in the present study. Of these 86 cases were in the Paediatric age group (0-10 years) and the youngest patient was 3 months old. 244 cases were above 10 years of age and the oldest patient was 80 years old. There was no significant sex difference. Out of the total 330 cases 125 were presumptive TB cases and 205 were presumptive MDR cases. Of all the total 86 paediatric cases there were 63 presumptive pulmonary cases, 22 presumptive extrapulmonary cases and 1 MDR contact case. 45 gastric lavage samples and 18 smear negative sputum samples were included for pulmonary cases and MTB was detected in 6 gastric lavage and 1 smear negative sputum samples. 14 CSF samples, 6 pleural fluid samples and 2 were ascitic fluid samples were included for extrapulmonary cases and MTB was detected in 1 CSF sample. No MTB was detected in MDR contact sputum sample. So total 8 paediatric cases were MTB positive and no case was reported to be MDR. (Table 2).

Of all the total 125 presumptive TB cases there were 84 pulmonary cases and 41 extrapulmonary cases. 50 gastric lavage samples and 34 sputum samples were included for pulmonary cases and MTB was detected in 3 smear negative sputum samples and 8 gastric lavage samples. No MTB was detected in PLHIV sample. So out of total 84 pulmonary cases 11 cases (13.09%) were detected MTB positive and out of these 11 cases Rifampicin Resistance was reported in 2 (18.18%) cases. 17 CSF samples, 15 pleural fluid samples, 6 lymph node aspirates and 3 were ascitic fluid samples were included for Extrapulmonary cases and MTB was detected in 6 lymph node aspirates, 2 pleural fluid samples and 1 CSF sample. So out of total 41 Extrapulmonary samples MTB was detected in 9 samples and out of these 9 samples Rifampicin Resistance was reported in 3 (33.33%) samples. So overall in total 125 presumptive TB cases MTB was detected in 20 (16%) cases and out of these 5 (25%) cases were detected MDR. (Table 3).

Of all the total 205 Presumptive MDR cases 140 were smear negative retreatment cases of which 35 were detected MTB positive, 50 were smear positive retreatment cases of which 43 were detected MTB positive, 10 were TB-HIV co infected cases of which 3 were detected MTB positive and 5 were MDR Contact cases of which 2 were detected MTB positive. So total 82 (40%) cases were reported MTB positive out of which 18 (45%) cases were reported MDR. (table4).

Table-1: Distribution of patients.

Age group (years)	No. of patients
0-10	86
>10	244

Table-2: Paediatric cases (0-10 years)

Type of samples		Total samples	M.TB detected	Rifampicin Resistant (MDR)
PULMONARY	Gastric lavage	45	06	00
	Smear negative sputum	18	01	00
EXTRAPULM-ONARY	CSF	14	01	00
	Ascitic fluid	02	00	00
	Pleural fluid	06	00	00
MDR CONTACT		01	00	00
TOTAL SAMPLES		86	08 (9.3%)	00

Table-3: Presumptive TB Cases.

	Type Of Sample	Total samples	M.TB Detected	Rifampicin Resistant (MDR)
PULMONARY (Total cases = 84)	Smear negative sputum	33	03	02
	Gastric lavage	50	08	00
	PLHIV sputum	01	00	00
EXTRAPULMONARY (Total cases = 41)	CSF	17	01	01
	Lymph node aspirate	06	06	00
	Pleural fluid	15	02	02
	Ascitic fluid	03	00	00
TOTAL CASES		125	20 (16%)	05 (25%)

Table-4: Presumptive MDR Cases.

TYPE OF CASES		Total Cases	MTB Positive Cases	Rifampicin Resistant cases (MDR)
RETREATMENT CASES	Smear Positive	50	42	09
	Smear Negative	140	35	07
MDR CONTACT CASES		05	02	01
TB-HIV CO-INFECTED CASES		10	03	01
TOTAL CASES		205	82 (40%)	18 (45%)

Discussion

Early and improved diagnosis of Mycobacterium tuberculosis and MDR in presumptive cases is essential for National Control Program, nevertheless till recently it was often confronted by the limitation of availability of single diagnostic test which satisfies all demands of being rapid, affordable and easy. In December 2010, WHO endorsed the use of Gene Xpert system to full fill all these requirements. In the present study Xpert MTB/RIF has been utilized as the initial diagnostic method to diagnose paediatric TB, pulmonary TB,

extrapulmonary TB and Rifampicin Resistant Tuberculosis in high risk populations as recommended by WHO under 2013 policy recommendations [11].

In the present study 86 patients were from the paediatric age group (0-10yrs) from which samples obtained were gastric lavage (45), smear negative sputum samples (18), CSF (14), pleural fluid (6) and ascitic fluid (2). Application of Xpert assay detected MTB in 9.3% of these cases which is approximately 8% increase over the smear microscopy. Our study correlated with the study done by Do Chau Giang et al in 2013 in which Xpert detected MTB in 20.6% in clinically diagnosed children which was 11% increase over smear. TB in children account for 15% of total TB burden. About 74,000 children die of TB every year and there are around half a million new cases annually [12]. Like in adults, the majority (70–80 %) of child TB cases present with pulmonary tuberculosis (PTB). Tuberculosis in children has been relatively neglected, mainly due to challenges in the availability of effective diagnostic tools [13]. Diagnosis of childhood TB is difficult and microbiological confirmation by smear is rare. Children typically are unable to expectorate sputum or produce small quantities. So gastric lavage is the preferred sample for the diagnosis of pulmonary TB in children. Few bacilli are present in the respiratory secretions and smear has a limit of detection of approximately 5,000-10,000 acid fast bacilli (AFB)/ml [14]. Extrapulmonary TB is also high in children which is much more difficult to diagnose by microscopy. So in October 2013 WHO recommended that Xpert assay should be used as initial diagnostic for the diagnosis of paediatric TB [11].

In the present study there were 125 presumptive (clinically suspected) TB cases among which MTB was detected in 20 (16%) cases and rifampicin resistance was detected in 5 (25%) out of these 20 cases by Xpert Assay. Out of these 125 cases 84 were pulmonary and 41 extrapulmonary cases in which MTB was detected in 11 pulmonary and 9 extrapulmonary cases respectively and Rifampicin Resistance was reported in 2 pulmonary and 3 extrapulmonary cases respectively. Among the 84 presumptive pulmonary cases 33 cases were sputum smear negative among which 3 cases were reported MTB positive by Gene Xpert and rifampicin resistance was detected in 2 (66.66%) out of these 3 cases. Thus applications of Xpert Assay have resulted in approximately 10% additional MTB detection over smear. Sputum microscopy has low sensitivity

especially in HIV patients, MDR cases and extrapulmonary cases. Smear examination may miss about 25% of MTB positive cases. The impact of delayed TB detection is threefold: firstly, morbidity to the individual is increased and in many cases will persist beyond the disease episode in severe permanent lung damage; secondly, undetected smear-negative TB will become progressively more infectious and transmit within the community; and finally the economic impact on the household is magnified by repeated visits to healthcare facilities, differential diagnostic testing and treatments, and loss of earnings due to healthcare seeking and morbidity.

An in vitro study demonstrated a limit of detection as low as 131 CFU/ml of MTB for Xpert Assay [15]. Xpert will divert treatment away from "false cases" to "true" smear-negative TB cases, thereby increasing the accuracy of treatment and cost-effectiveness, while reducing the burdens of toxicity and cost of treatment in patients who do not in fact have TB [16, 17]. Xpert have 72.5% sensitivity for smear negative and culture positive TB in comparison to 99.2% for smear and culture positive TB according to a clinical study.

Among 41 extrapulmonary samples MTB was detected in 9 (21.95%) samples and MDR was reported in 3 (33.33%) out of these 9 cases. Maximum number of MTB was detected in lymph node aspirate samples. Diagnosis of extrapulmonary TB remains especially challenging since the number of Mycobacterium tuberculosis bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. Histology is time-consuming to undertake. Tissue microscopy after special staining is often negative and when mycobacteria are seen, it is impossible to distinguish MTB from nontuberculous mycobacterial disease. Therefore WHO has recommended that Xpert may be used as a replacement test for usual practice (including microscopy, culture and histopathology) for diagnosis of extrapulmonary specimens. In study done by Stephen D Lawn et al in 2012 (Diagnosis of extrapulmonary tuberculosis using the Xpert MTB/RIF assay) the sensitivity and specificity of Gene Xpert was 79% and 97.3% respectively as compared to culture [18].

In the present study there were 10 TB-HIV co infected cases and 1 PLHIV case. MTB was detected in 3 (30%) TB-HIV co infected patients and MDR was reported in 1 (33.33%) out of these 3 cases. Tuberculosis (TB) is a

leading cause of morbidity and mortality among patients infected with HIV worldwide. In sub-Saharan Africa, the TB and HIV epidemics are closely related, with more than 70% of all TB cases in South Africa co-infected with HIV [19,20]. Diagnosing TB in patients infected with HIV is challenging because of not only the atypical clinical presentation of TB disease, and the paucibacillary nature of pulmonary TB disease in patients with HIV. The most widely used TB diagnostic test worldwide is sputum smear microscopy, which fails to detect TB in over 60% of cases, particularly in high-HIV prevalence settings [21–24]. Smear-negative TB in persons infected with HIV is associated with poorer outcomes, in part because of delays in TB diagnosis and treatment initiation [25, 26] which results in disease progression and often death among patients co-infected with HIV [27, 28]. Prompt diagnosis and treatment of TB among HIV-negative people is also crucial to reduce TB transmission to people living with HIV. Thus all people living with HIV should be regularly screened for TB. In high-HIV prevalence settings, where WHO approved molecular tests (e.g. Xpert MTB/RIF) are available, they should be the primary diagnostic test for TB in people living with HIV.

In the present study out of the total 102 MTB positive cases 23 (22.54%) cases were reported to be Rifampicin Resistant. Therefore there is increased early detection of MDR TB. It is high in high risk groups (presumptive MDR cases) among which 18 (45%) out of 82 MTB positive cases were reported to be MDR. Prior to application of this assay, patients at high risk for MDR TB would have to be referred to a tertiary setting and wait for 6 to 8 weeks for results of phenotypic drug susceptibility testing resulting in high loss to follow-up and delays in treatment initiation. The line probe assays for MDR diagnosis have also largely been limited to tertiary centres. However, early identification of possible MDR cases is key to reduce community transmission and reducing the incidence of MDR TB. Traditional phenotypic DST is considered the gold standard for TB drug resistance testing; however studies are now showing cases of TB isolates that have rpoB mutation that are not detected by phenotypic assays [29,30,31,32]of such cases. Thus WHO recommends that Xpert MTB/RIF should be used as the initial diagnostic test in individuals (both adults and children) suspected of having TB who are considered to be at risk of harbouring drug-resistant TB bacilli and also includes both adults and children who have been treated with anti-TB drugs and in whom TB has again been diagnosed, that is, all retreatment categories (failure, defaulter and return after relapse).

So major advantage of Gene Xpert is that MTB is detected rapidly and minimal technical training is required to perform the test, also it simultaneously detects Rifampicin Resistance so correct treatment can be started at an early stage of the disease and also detects true TB Negative patients thus contributes to cost saving by avoiding unnecessary treatment. However the assay have several disadvantages also which are as follows:

1. Hetero-resistance defined as the presence of both sensitive and resistant MTB populations is often suggested to be responsible for discordant DST results. Discordance between Xpert and phenotypic testing require confirmation by another molecular assay. Additionally, larger studies are essential to ascertain the clinical significance of such cases. Also it can miss the resistance detection outside RRDR foci.

2. Xpert cannot be used for assessing the emergence of Rifampicin Resistance during treatment. Also it is not suitable to detect INH monoresistance.

3. Inability to differentiate XDR-TB from MDR-TB as it can detect only Rifampicin Resistance. It can detect rifampicin resistance only if rpoB allele is present in at least 65% of DNA present in sample.

4. It is not suitable for monitoring patients' response to treatment and so conventional microscopy and culture are required for monitoring MDR-TB patients during treatment.

5. Xpert Assay also have several technical problems, including requirement for stable electricity supply, limited temperature range, availability of maintenance, and bulky consumables.

Conclusion

The results of this study indicate that the implementation of the Xpert MTB/RIF assay could dramatically improve the rapid diagnosis of tuberculosis, especially in cases with suspicion of MDR and smear negative TB. However further research is needed to evaluate the cost effectiveness, patient acceptability and impact on overall out-of-pocket expenditure of Xpert testing provided at a direct cost to the patient and to determine the optimal sustainable use of this technology while maximizing equality of access.

References

1. TB India 2016 Revised National Tuberculosis Control Programme, Annual Status Report.

2. Tuberculosis. WHO Global Tuberculosis Report 2014.http://www.who.int/tb/publications/factsheet_ global. pdf.

3. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/ RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update. Geneva: World Health Organization;Issued date 2013.

4. S teingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014 Jan 21;(1): CD009593.doi: 10.1002/14651858. CD009593. pub3.

5. Small PM, Pai M. Tuberculosis diagnosis--time for a game change. N Engl J Med. 2010 Sep 9;363(11):1070-1. doi: 10.1056/NEJMe1008496. Epub 2010 Sep 1.

6. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol M, Floyd K, Centis R, Cirillo DM, Tortoli E, Gilpin C, de Dieu Iragena J, Falzon D, Raviglione M. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. Eur Respir J. 2013 Jul;42(1):252-71. doi: 10.1183/09031936.00157212. Epub 2012 Nov 22.

7. Xpert MTB/RIF for people living with HIV. World HealthOrganizationOctober2014.http://www.who.int/tb/ challenges/hiv/Xpert_TBHIV_Information_Note_final. Assesed on 13 Nov;2014.

8. Walusimbi S, Bwanga F, De Costa A, Haile M[1], Joloba M, Hoffner S. Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infect Dis. 2013 Oct 30;13:507. doi: 10.1186/1471-2334-13-507.

9. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? Lancet Infect Dis. 2014 Jun;14(6):527-32. doi: 10.1016/S1473-3099 (13)70360-8. Epub 2014 Jan 15.

10. Guidance document for use of Catridge Based-Nucleic Acid Amplification Test (CB- NAAT) under Revised National TB Control Programme (RNTCP) issued central TB division, directorate general of health services September 2013.

11. Geneva: World Health Organization; 2013. WHO Guidelines Approved by the Guidelines Review Committee. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update.

12. World Health Organisation. Guidance for national tuberculosis programmes on the management of tuberculosis in children second edition. Geneva, Switzerland; 2014. http: //apps. who.int/iris/bitstream/ 10665 / 112360/1/9789241548748_eng.pdf. Accessed 8th September 2014

13. Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. Clin Infect Dis. 2010 May 15;50 Suppl 3:S184-94. doi: 10.1086/651490.

14. Kelynack T. Tuberculosis in Infancy and Children. New York: William wood and Co; 1908.

15. "Frequently asked questions on Xpert MTB/RIF assay" Retrieved on 12 June 2012, http://www. medscape. com/ viewarticle/745030_2

16. D. W. Dowdy, A. Cattamanchi, K. R. Steingart, and M. Pai, "Is scale-up worth it? Challenges in economic analysis of diagnostic tests for tuberculosis," PLoS Medicine, vol. 8, no. 7, Article ID e1001063, 2011. Published: July 26, 2011http://dx.doi.org/10. 1371/ journal.pmed.1001063.

17. Vassall A, van Kampen S, Sohn H, Michael JS, John KR, den Boon S, Davis JL, Whitelaw A, Nicol MP, Gler MT, Khaliqov A, Zamudio C, Perkins MD, Boehme CC, Cobelens F. Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. PLoS Med. 2011 Nov;8(11):e1001120. doi: 10.1371/journal. pmed.1001120. Epub 2011 Nov 8.

18. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert(®) MTB/RIF assay. Expert

Rev Anti Infect Ther. 2012 Jun;10(6):631-5. doi: 10. 1586/eri.12.43.

19. Lawn SD, Churchyard G. Epidemiology of HIV-associated tuberculosis. Curr Opin HIV AIDS. 2009 Jul;4(4):325-33. doi: 10.1097/COH.0b013e32832c7d61.

20. Abdool Karim SS, Churchyard GJ, Abdool KQ, Lawn SD. HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response. Lancet 2009;374:921–933. doi: 10.1016/ S0140-6736 (09)60916-8. Epub 2009 Aug 24.

21. Reid MJ, Shah NS. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. Lancet Infect Dis. 2009 Mar;9(3):173-84. doi: 10.1016/S1473-3099(09)70043-X.

22. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. Lancet. 2007 Jun 16;369 (9578):2042-9.

23. Hargreaves NJ, Kadzakumanja O, Whitty CJ, Salaniponi FM, Harries AD, Squire SB. Smear-negative pulmonary tuberculosis in a DOTS programme: poor outcomes in an area of high HIV seroprevalence. Int J Tuberc Lung Dis 2001;5:847–854.

24. Banda H, Kang'ombe C, Harries AD, Nyangulu DS, Whitty CJ, Wirima JJ, Salanipon FM, Maher D, Nunn P. Mortality rates and recurrent rates of tuberculosis in patient with smear-negative pulmonary tuberculosis and tuberculous pleural effusion who have completed treatment.Int J Tuberc Lung Dis.2000Oct;4(10):968-74.

25. Palmieri F, Girardi E, Pellicelli AM, Rianda A, Bordi E, Rizzi EB, Petrosillo N, Ippolito G. Pulmonary tuberculosis in HIV-infected patients presenting with normal chest radiograph and negative sputum smear. Infection. 2002 Apr;30(2):68-74.

26. Pablos-Méndez A, Sterling TR, Frieden TR. The relationship between delayed or incomplete treatment and all-cause mortality in patients with tuberculosis. JAMA. 1996 Oct 16;276(15):1223-8.

27. Gandhi NR, Shah NS, Andrews JR, Vella V, Moll AP, Scott M, Weissman D, Marra C,Lalloo UG, Friedland GH. HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. Am J Respir Crit Care Med 2010 Jan 1;181(1):80-6.doi:10.1164/rccm.200907-0989OC. Epub 2009 Oct 15.

28. Meintjes G, Rangaka MX, Maartens G, Rebe K, Morroni C, Pepper DJ, Wilkinson KA, Wilkinson RJ. Novel relationship between tuberculosis immune reconstitution. inflammatory syndrome and anti-tubercular drug resistance. Clin Infect Dis 2009 Mar 1;48(5):667-76. doi: 10.1086/596764.

29. Van Deun A, Barrera L, Bastian I, Fattorini L, Hoffmann H, Kam KM, Rigouts L, Rüsch-Gerdes S, Wright A. Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results. J Clin Microbiol. 2009 Nov; 47(11):3501-6. doi: 10. 1128/JCM.01209-09. Epub 2009 Sep 16.

30. Ho J, Jelfs P, Sintchencko V. Phenotypically occult multidrug-resistant Mycobacterium tuberculosis: dilemmas in diagnosis and treatment. J Antimicrob Chemother. 2013 Dec;68(12):2915-20. doi: 10.1093/ jac/ dkt284. Epub 2013 Jul 9.

31. Somoskovi A, Deggim V, Ciardo D, Bloemberg GV. Diagnostic implications of inconsistent results obtained with the Xpert MTB/Rif assay in detection of Mycobacterium tuberculosis isolates with an rpoB mutation associated with low-level rifampin resistance. J Clin Microbiol. 2013 Sep; 51(9):3127-9. doi: 10. 1128 /JCM.01377-13. Epub 2013 Jul 12.

32. Williamson DA, Roberts SA, Bower JE, Vaughan R, Newton S, Lowe O, Lewis CA, Freeman JT. Clinical failures associated with rpoB mutations in phenotypically occult multidrug-resistant Myco-bacterium tuberculosis. Int J Tuberc Lung Dis. 2012 Feb;16(2):216-20. doi: 10.5588/ijtld.11.0178.

…………….....................

How to cite this article?

Verma D, Sharma R, Chand A.E, Saxena A. The application of gene xpert for the diagnosis of mycobacterium tuberculosis and MDR TB in Kota region. *Int J Med Res Rev* 2016;4(10):1863-1870.doi:10.17511/ijmrr. 2016.i10.25.
…….