

# DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* DNA AND RIFAMPICIN RESISTANCE FROM EXTRA-PULMONARY SAMPLES BY AUTOMATED NESTED RT-PCR IN POST RENAL TRANSPLANT AND CHRONIC RENAL FAILURE PATIENTS: A SINGLE-CENTER STUDY

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**Abstract**– Patients with kidney diseases particularly on dialysis and post renal transplant patients have a higher tendency to develop MTB infection and its Rifampicin Resistance. The purpose of the study was to determine whether or not patients with chronic kidney disease and those who had undergone a renal transplant had MTB infection and whether or not it was resistant to rifampicin. The Department of Pathology and Lab Medicine, Transfusion services, and Immunohematology conducted this study, which is a retrospective record-based data analysis study carried out in the Department. At the IKDRC-ITS, the research was carried out over the course of approximately two and a half years. The study included a total of fifty cases of presumed tuberculosis that were identified through the use of the Xpert MTB/RIF assay. The extrapulmonary samples that were collected included ascitic fluid, pleural fluid, pericardial fluid, pus, tissue, cerebrospinal fluid, lymph nodes, bone marrow, and urine. In order to identify MTB DNA and Rifampicin resistance, the samples were subjected to automated real-time polymerase chain reaction (Gene X'pert) tests. Among these fifty MTB-positive samples, the maximum number of patients was twenty between the ages of 21 and 40, fourteen between the ages of 41 and 60, eight patients older than sixty, and eight patients younger than twenty years. Rifampicin sensitivity was discovered in 49 of the individual patients. Within the population of patients who have received a renal transplant, the incidence of MTB infection, also known as pulmonary tuberculosis, is quite high. Greater sensitivity in detecting *M. tuberculosis* complex and rifampicin resistance can be achieved through the use of the GeneXpert® test, which is a real-time PCR technique that is both straightforward and quickly performed.

## INTRODUCTION

MTB infection and its complications are more likely to occur in patients who have kidney diseases, particularly those who are undergoing dialysis and those who have recently undergone a renal transplant. Protection against Rifampicin (Lenk and Schroeder, 2001; Singh and Limaye, 2014). Despite the fact that tuberculosis (TB) continues to be a significant obstacle for public health around the world, our capacity to combat this disease has been

severely hampered by the lack of adequate diagnostic laboratory tests. The diagnosis of extrapulmonary tuberculosis (EPTB) continues to be difficult due to the fact that the number of *Mycobacterium tuberculosis* (MTB) bacilli that are present in tissues at disease sites is frequently low, and clinical specimens from deep-seated organs may be difficult to obtain. When it comes to establishing a diagnosis of tuberculosis, histology is both invasive and time-consuming. It is difficult to differentiate between tuberculosis and other

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mycobacterial diseases when tissue microscopy is performed after special staining. When mycobacteria are observed, it is impossible to differentiate between the two. However, the culture, which is the foundation of diagnosis, frequently results in significant delays, which in turn compromises patient care and outcomes (Lawn and Zumla, 2012; Hamdar *et al.*, 2024).

Another significant step forward in the field of rapid molecular tuberculosis diagnostics is the introduction of the automated nested RT-PCR assay. This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR. It is designed to be used by operators with minimal technical expertise, and it enables the diagnosis of tuberculosis (TB) and simultaneous assessment of rifampicin resistance to be completed within two hours (Purohit and Mustafa, 2015; Rozales *et al.*, 2014).

For many decades, it was believed that tuberculosis (TB) could be cured, provided that the medications were taken on a consistent basis and in accordance with the established protocol. It is possible for drug resistance to develop if the treatment protocol is not followed properly. The term “multi drug resistant tuberculosis” (MDR) refers to the condition that occurs when the patient is resistant to both isoniazid and rifampicin. Extensively drug-resistant tuberculosis (XDR-TB) is a more severe form of multidrug-resistant tuberculosis that occurs when there is no safe treatment option. In the first place, the most important step in the process of controlling tuberculosis is early and timely detection. The problem of late detection is not only linked to the spread of the disease, but it is also a significant contributor to a poor prognosis.

A significant number of cases continue to go undiagnosed because there is no diagnostic test that is both quick and reliable. As a result, the World Health Organisation has suggested that active screening be performed on all patients suspected of having tuberculosis. As the gold standard, the GeneXpert® test has been recommended by the WHO. This method not only has a high degree of accuracy in detecting *M. tuberculosis*, but it also has the capability of simultaneously determining drug resistance against Rifampicin (Pagaduan and Altawallbeh, 2023; Muneer *et al.*, 2019).

With the help of an automated nested real-time polymerase chain reaction (RT-PCR) assay, the purpose of the study was to determine whether or

not patients with chronic kidney disease and those who had recently undergone a renal transplant had an MTB infection and whether or not it was resistant to Rifampicin (Kanaan *et al.*, 2004; Babafemi *et al.*, 2017).

## MATERIALS AND METHODS

The Department of Pathology and Lab Medicine, Transfusion services, and Immunohematology conducted this study, which is a retrospective record-based data analysis study carried out in the department. At the IKDRC-ITS, the research was carried out over the course of approximately two and a half years, beginning in June 2018 and ending in December 2021. Before the participants were allowed to participate in the study, the ethical committee of the institute was provided with comprehensive information regarding the study, and a clearance certificate was obtained. Due to the fact that this is a record-based study and there was no interaction with the participants, a request was made to the EC to waive the requirement for consent.

**Length of the Study:** The duration of the study was approximately three and a half years.

This is a retrospective record-based study, which is the design of the study.

### Setting and location of the study

G.R. Doshi and K.M. Mehta Institute of kidney Diseases and Research Centre, Dr. H.L. Trivedi Institute of Transplantation Science (IKDRC) in Ahmedabad were responsible for the research that was conducted for this publication.

These are the criteria that were used to determine who was included and who was not included in the study:

### Inclusion Criteria

In order to identify *Mycobacterium tuberculosis* and rifampicin resistance, extrapulmonary samples from patients with chronic kidney disease and those who had undergone a renal transplant were analysed between September 2018 and December 2021. These samples included ascitic fluid, pleural fluid, pericardial fluid, pus, tissue, cerebrospinal fluid, lymph nodes, bone marrow, and urine samples drawn from patients.

### Exclusion Criteria

Those patients in the study population who were

diagnosed with pulmonary mycobacterial tuberculosis were not included.

### Protocol for the Study

As part of the current investigation, we examined the records of patients with chronic kidney disease and those who had undergone a renal transplant between the months of June 2018 and December 2021. The extrapulmonary samples that were collected included ascitic fluid, pleural fluid, pericardial fluid, pus, tissue, cerebrospinal fluid, lymph nodes, bone marrow, and urine. In order to identify MTB DNA and Rifampicin resistance, the samples were subjected to automated real-time polymerase chain reaction (Gene X'pert) tests.

For the purpose of enhancing tuberculosis diagnosis and the identification of rifampicin resistance, the World Health Organisation (WHO) suggested in 2011 that countries begin rolling out the Xpert MTB/RIF assay system. As a result, the Xpert MTB/RIF assay has been utilised as the primary diagnostic instrument in our hospital for the purpose of diagnosing tuberculosis and rifampicin resistance among cases of presumed tuberculosis. The individuals who were suspected of having tuberculosis and rifampicin resistance were the ones who provided clinical samples. Following the instructions provided by the manufacturer, the Xpert MTB/RIF assay was carried out on each and every type of sample. In a nutshell, the clinical samples were diluted with sample reagent, vortexed three times, and then stored for fifteen minutes. After that, two millilitres of the mixture was transferred to an Xpert cartridge, and the Xpert machine was loaded with it. For MTB, the results were evaluated and reported as either detected, not detected, or invalid/error. Similar to the previous example, the results of the rifampicin resistance test were reported as susceptible, resistant, or indeterminate.

Tabulation was performed on the descriptive data that was collected, which included information on age, gender, samples, and the status of microscopy. After being cleaned up and double-entered with Epi Data 3.1, the data were then transferred to SPSS version 20 for further analysis. Any Xpert results that were deemed to be "indeterminate" were not included in the document that was used for data extraction. In order to analyse the data, SPSS version 20 was utilised. Descriptive statistics, including percentiles, proportions, and frequency distribution by tabulation, were utilised in order to provide a

summary of the findings. When the P-value was less than 0.05, it was deemed to be statistically significant.

## RESULTS

The study included a total of fifty cases of presumed tuberculosis that were identified through the use of the Xpert MTB/RIF assay. 54.8% of the participants were males, which is significantly higher than the percentage of females. The proportion of females to males who participated in the study was 1:1.2. Half of the participants were people who lived in urban areas.

Samples taken from the participants in our research project include urine, tissue, pus, tissue, FNAC, synovial fluid, cerebrospinal fluid, lymph node fluid, and ascitic fluid. A quarter of the participants in our research groups were between the ages of 25 and 50. In the fifty clinical samples that were examined using the Xpert MTB/RIF assay, each and every one of the samples tested positive for MTB. It was found that one of the MTB positive cases was resistant to the antibiotic rifampicin. Rifampicin sensitivity was discovered in 49 of the individual patients.

**Table 1.** Different sources of sample collection

Sr. No.	Source of sample	No. of cases
1.	Ascitic fluid	1
2.	Pus	5
3.	Lymph node	1
4.	Urine	27
5.	Tissue	8
6.	Other fluid	3
7.	FNAC	3
8.	Synovial fluid	1
9.	CSF	1
10.	Total	50

Among these fifty MTB-positive samples, the maximum number of patients was twenty between the ages of 21 and 40, fourteen between the ages of 41 and 60, eight patients older than sixty, and eight patients younger than twenty years. There was one sample that showed rifampicin resistance (RR) out of fifty MTB positive samples. The distribution of high, medium, low, and very low detection levels among the fifty samples that tested positive for MTB was as follows: three, eleven, sixteen, and twenty samples, respectively (Table 2).

**Table 2.** CBNNAT (MTB-RIF Result) in different number of cases

Sr. No.	CBNNAT (MTB-RIF Result)	Nop. of cases
1.	High	3
2.	Medium	11
3.	Low	16
4.	Very low	20

## DISCUSSION

An infection with *Mycobacterium tuberculosis* is a significant opportunistic infection that is associated with an increased risk of mortality after organ transplantation. Depending on the endemicity of the region, the prevalence of tuberculosis infection can range anywhere from 0.5 percent to 15 percent. In their study, Marouane and colleagues discovered that 34.6% of 153 samples tested positive for tuberculosis using GeneXpert®MTB/RIF, and all of the samples were susceptible to rifampicin. However, only ten of the 283 people who received renal transplants tested positive for tuberculosis, with extra pulmonary tuberculosis being the more prevalent form of the disease in this population. Comparatively, the prevalence of tuberculosis in renal transplant recipients in developed Western countries ranges from 1% to 4%, while in developing countries, the prevalence can reach up to 11.5%. (Singh and Paterson, 1998; Soroohan *et al.*, 2022; Hyun *et al.*, 2024).

MTB was found to be present in 1.3% of patients who had received solid organ transplants, according to a study that was carried out. Using Zeihl Neelsen (ZN) microscopy, BACTEC MGIT liquid culture, and Gene Xpert assay, a study was conducted in Pakistan on 259 clinically suspected patients.<sup>14</sup> The results showed that GeneXpert was the most reliable method for detecting tuberculosis. Our research revealed that the prevalence of MTB infection in renal transplant recipients from different samples were least resistant to rifampicin. This finding is in line with the findings of previous studies. There was a combination of probes DE that had the highest frequency of rpoB mutations, followed by probes A, B, and C. Probe E had the highest frequency of rpoB mutations. We reported one sample with RR.

## CONCLUSION

Within the population of patients who have received a renal transplant, the incidence of MTB infection,

also known as pulmonary tuberculosis, is quite high. Greater sensitivity in detecting *M. tuberculosis* complex and rifampicin resistance can be achieved through the use of the GeneXpert® test, which is a real-time PCR technique that is both straightforward and quickly performed. If additional testing were performed with a greater number of extrapulmonary samples, the prevalence of MTB infection in these patients would be more accurately determined.

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