DETECTION OF RPOB GENE MUTATION ASSOCIATED WITH RIFAMPICIN RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS* (MTB) PATIENTS USING GENE XPERT

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ABSTRACT

Mycobacterium tuberculosis (MTB) is the leading cause of TB infection in humans. Effective diagnosis and treatment are important to reduce the TB burden. WHO recommends GeneXpert test for MTB detection as well as Multi drug resistance (MDR) detection. In the present study, the prevalence of tuberculosis in the population of Kotaddu was investigated. The study was conducted at THQ Hospital, Kot Addu. Eight hundred twenty-five patients that were registered or visiting THQ Kotaddu were enrolled in this study. It was observed that among 825 study participants, MTB was detected in 253 patients (31%). It was also observed that in 247 (30%) patients, MTB was detected in AFB staining. Abnormalities in chest X-rays was seen in 30% of study participants. The accuracy of GeneXpert along with AFB staining and X-ray findings was also analyzed. The difference between GeneXpert and X-Ray analysis was also statistically significant. It was also observed that the sensitivity of GeneXpert was 100% with 99% specificity. It was observed that GeneXpert could diagnose TB with more accuracy as compared to other traditional diagnostic tests. However, traditional tests should also be applied parallel with GeneXpert to verify and strengthen the diagnostic outcomes.

Key Words: Tuberculosis, rifampicin resistance, rpob gene, Gene expert, Tb diagnosis

INTRODUCTION

Tuberculosis is a deadly disease that has been affecting mankind for 4000 years. *Mycobacterium tuberculosis* is the leading cause of TB infection in human. It primarily targets the lungs. However, it can also affect meninges, intestine, bones, joints and other body tissues. The main source of transmission for TB is droplets or aerosols (Khan *et al.*, 2019).

It is the main cause of morbidity worldwide and the leading cause of mortality, particularly in developing countries (Zaman, 2010). According to the World Health Organization, 1.4 million deaths were recorded due to TB in 2019. 95% of deaths due to TB has been reported in developing countries (Goldman and Schafer, 2011). In Pakistan, the incidence and prevalence of tuberculosis are high (Bakuła *et al.*, 2019). Young adults, health care workers and immunocompromised people are more prone to TB infection (Fogel, 2015).

Rifampicin is first-line anti-tuberculosis drug that has a bactericidal effect. It inhibits bacterial DNA-dependent RNA polymerase that is encoded by the rpob gene. Strains become resistant to rifampicin when mutations occur in limited regions of rpob. There is 81bp RIF resistance determining region (RRDR) present in rpob gene. Mutations in this region are majorly responsible for Rifampicin resistance. In some cases, mutations outside the RRDR may also contribute to resistance (Zaw *et al.*, 2018). The strain may become resistant to only rifampicin or isoniazid in addition to rifampicin. GeneXpert MTB/RIF is a WHO-recommended molecular assay that can diagnose TB and rifampicin resistance simultaneously. It is a nucleic acid amplification assay that is done on the GeneXpert instrument system (Iademarco, 2013). This real-time PCR detects the pathogen in the sputum sample and mutations in the beta subunit of rpob gene (RNA polymerase enzyme) (Lawn and Nicol, 2011).

Pakistan is ranked as a hotspot of MDR TB. It is important to assess the prevalence of MDR TB in different regions, leading to efficient TB management. So, in this study, we aim to determine the prevalence of RIF resistant TB in Kot Addu and surrounding areas. In the presented study, we also aim to assess the sensitivity of the GeneXpert assay.

METHODOLOGY

A present cross-sectional study was conducted from May 2020 to October 2020. Data for the research was collected from pathological laboratories of Tehsil Head Quarter (THQ), Kot Addu. The research methodology was

designed considering ethical standards, and samples were collected after the consent of subjects. Demographic details of patients were recorded on a questionnaire, and samples were collected for further testing. Data were collected from 825 patients registered in THQ, Kot Addu. Ethical approval was taken from the ethical committee of THQ, Kot Addu. A questionnaire was designed to gather information like demographic details and Clinical manifestations in study participants. Data were then entered into the Excel sheet for analysis.

Sample Collection and testing

Study participants were asked to provide sputum samples for testing. For this purpose, participants were provided with sterile containers. Acid-fast staining was done following the pre-defined standard operating procedure (Bayot *et al.*, 2020). Hemoglobin level and Total leucocyte counts were analyzed using an automated hematology analyzer. Chest Xray for tuberculosis was done, and CXR findings were evaluated according to the Centers of Disease Control (CDC) guidelines. GeneXpert MTB/RIF test was used to detect the presence of *Mycobacterium tuberculosis* and mutation in Rpob gene simultaneously.

Statistical Analysis

Data were entered in an Excel file and transferred to GraphPad Prism 8 for statistical analysis. Descriptive variables were summarized in the form of frequencies or percentages. Mean differences in the hematological profile of patients suffering from Rifampicin resistant and Rifampicin sensitive strains were determined by applying independent t-test, $p < 0.05$ was considered significant.

RESULTS

Demographic findings

Eight hundred twenty-five study participants were enrolled. Data were collected on a structured questionnaire, and different diagnostic tools were used to check the health status of study participants. Among study participants, 456 (55%) were male, while 369 (45%) were females. All participants were divided into two groups based on age. It was observed that 116 (14%) participants belong to the age group <30 while 254 (31%) belong to the age group >30 years. The age of 455 (55%) patients were not known.

Diagnostic findings of Study participants

GeneXpert was used to detect the presence of Mycobacterium tuberculosis in sputum samples. Mtb was detected in 253 (31%) samples, while in 572 (69%) Mtb was not detected. RIF resistance was observed in 67 (8%) study participants, while 186 participants (Mtb positive) contain RIF sensitive strains. AFB staining was also done to strengthen the GeneXpert results. Among 825 participants, 247 (30%) indicate smears of different grades (1+ to 4+) while 578 (70%) gives negative results in AFB staining. Total Leucocyte counts (TLC) of all study participants indicate that 445 (54%) have 1100-10000 leucocytes while 380 (46%) have 10000-20000 leucocytes. X-Ray findings indicate that the Chest X-ray of 579 (70%) participants was clear and normal, while 246 (30%) have some abnormality in their X-Ray.

GeneXpert results further classified MTB positive patients. Among 253 patients, 231 patients were highly positive for MTB, followed by low positive (16) and intermediate positive (6), respectively. RIF resistant patients were also sub-classified as low positive (23), intermediate positive (21) and high positive (23). X-Ray findings of patients indicate that 91 have bilateral opacity in the supraclavicular region. Sixty-four patients have opacity in the form of the patch in the right lobe of a lung, while 90 patients have opacity on the lung's upper right lobe. AFB staining indicates smears of different grades that includes 1+, 2+, 3+ and 4+. Among these 2+ was in 91 patients followed by 3+ (70), 4+ (51) and 1+ (35) respectively.

Accuracy of GeneXpert over AFB staining and X-Ray findings

Fisher's exact test was applied to check the accuracy of GeneXpert and AFB staining. It was observed that 247 patients were Mtb positive in both Gene Xpert and AFB staining. 572 patients were negative in Gene Xpert and AFB staining. It was observed that six samples that were negative in AFB staining were positive in GeneXpert analysis. There was no Gene Xpert negative sample that was observed Mtb positive in AFB staining. Fisher's exact test was significant as the $p < 0.0001$.

Fisher's exact test was applied to check the accuracy of GeneXpert and X-ray findings. It was observed that 246 patients were Mtb positive in both Gene Xpert and X-Ray findings. 572 patients were negative in Gene Xpert and X-Ray findings. It was observed that seven samples that were normal in X-ray were positive in GeneXpert analysis.

There was no Gene Xpert negative sample that was observed Mtb positive in X-Ray. Fisher's exact test was significant at $p < 0.0001$.

Positive Predictive Value and Negative Predictive Value of GeneXpert

Positive predictive values and negative predictive values were also calculated using QuickCalc (Online GraphPad Prism Calculations). With 31% prevalence of TB in the population of Kot Addu. It was observed that PPV is 97.82%, predicting that in case of a positive result, there is a 97.82% chance that the patient has a disease with 2.18% chances of false-negative results. Negative predictive value was 100%, indicating that is the case of a negative result, there are 100% chances that patients do not have the disease with a zero % chance of false-negative results. Table 1 indicates the predictive values if we test 1000 people from the selected population.

Table 1. PPV and NPV of GeneXpert for TB diagnosis.

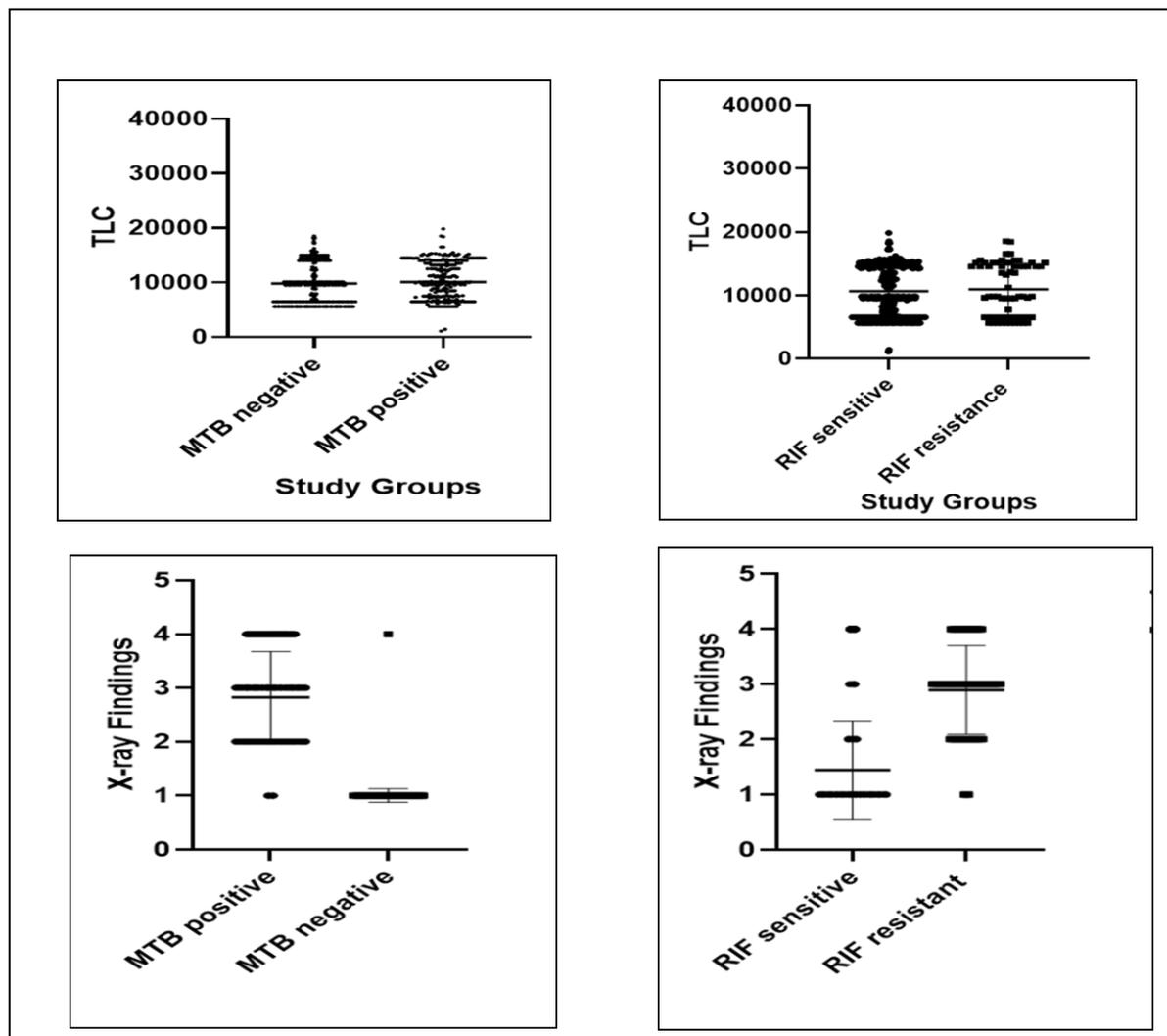|	Disease Present	Disease Absent	Total
Test Positive	310.0	6.9	316.9
Test Negative	0.0	683.1	683.1
Total	310.0	690.0	1000.0

Fig. 1. TLC values and X-Ray findings among different study groups.

TLC among MTB positive and MTB negative patients

TLC among two different groups; MTB negative (MTB not detected) and MTB positive (MTB detected) was compared. Mean with standard error for TLC was 10451.68 ± 169.36 in Patients with MTB negative. Mean with standard error for TLC was 11260.93 ± 344.27 in Patients with MTB positive. It was observed that there is a statistically significant difference between the means of these two groups ($p < 0.0001$) as shown in Fig. 1.

TLC among RIF sensitive and Resistant patients

TLC among two different groups, RIF sensitive and RIF resistant, was compared. Mean with standard error for TLC was 10676.34 ± 166.42 in RIF sensitive. Mean with standard error for TLC was 10970.15 ± 506.42 in RIF resistant. It was observed that there is no statistically significant difference between the means of these two groups ($p < 0.6149$) as illustrated in Fig. 1.

X-Ray finding in MTB positive and negative patients

X-Rays finding in MTB positive, and MTB negative patients were also compared. It was observed that there was a significant difference (P-value <0.0001) between Xray findings of MTB positive and MTB negative patients. MTB negative patients have clear or normal Xray, While MTB positive patients have bilateral opacity in the supraclavicular region, opacity in the form of the patch in the right lobe of the lung or opacity on the upper right lobe of the lung. Significant difference between X-Ray finding of MTB positive and MTB negative indicates that X-Ray is an efficient way to check the presence of MTB infection as shown in Fig. 1.

X-Ray finding in RIF sensitive and RIF resistant patients

X-Rays finding in RIF sensitive, and RIF resistant patients were also compared. It was observed that there was a significant difference ($p < 0.0001$) between Xray findings of RIF sensitive and RIF resistant patients. The ligand on Y-axis describes different representations of disease which include 1 (Chest X-Ray clear), 2 (Opacity on upper right lobe of lung), 3 (Bilateral opacity in supraclavicular region) and 4 (Opacity in the form of patch in the right lobe of lung) as described in Fig. 1. Most of the RIF Sensitive patients have clear or normal Xray, While RIF resistant patients have bilateral opacity in the supraclavicular region, opacity in the form of the patch in the right lobe of the lung or opacity on the upper right lobe of the lung.

Correlation between AFB smear grades and Hb

The correlation was checked between AFB smear grades and Hb level. Different AFB smear grades 1+, 2+, 3+ and 4+ were analyzed for their correlation with Hb levels in study participants. It was observed that there is no statistically significant correlation ($p < 0.5218$) between AFB smear grades and level of Hb in Tb patients. Which indicates that Hb levels remains same in MTB negative participants as well as patients with different AFB smear grades.

DISCUSSION

TB is an infectious disease that is affecting the human population worldwide. In a low-income country like Pakistan, Lack of education and poor health conditions enhance the risk of tb infection and transmission. In the presented study, we aim to access the prevalence of tuberculosis in the population of Kot Addu.

Eight hundred twenty-five patients that were registered or visiting THQ Kotaddu were enrolled in this study. Collected data indicates that among the study population, 55% were male while females were 45%. This shows that the prevalence of Tb was high among the male population as compared to the female population. Previous studies also represent a high prevalence of TB among the male population as compared to females. It was also reported that in low-middle income countries, prevalence is high among men (Horton, MacPherson, Houben, White, & Corbett, 2016). However, the difference between both groups varies in a different region of the world (Marçôa *et al.*, 2018; Thorson *et al.*, 2007).

It was observed that the prevalence of TB was 31% in the study population. Rifampicin resistance was observed in 26% of Mtb positive patients. A comparative study-National antituberculosis drug-resistant survey in Pakistan-reported that 3.7% of new cases and 18.1% of previously treated patients have Multiple drug resistance (Tahseen *et al.*, 2016).

Accuracy of GeneXpert along with AFB staining and X-ray findings was also evaluated. Fisher's exact test to compare the accuracy of different diagnostic tests. It was observed that there is a significant difference between the positive relation of GeneXpert and AFB staining results. The difference between GeneXpert and X-Ray analysis

was also statistically significant. Our results for accuracy check were consistent with the study done by Panday and Yadav (2019) who reported that GeneXpert has high sensitivity and specificity as compared to other diagnostic tests.

The accuracy of clinical test can be assessed using the Positive predictive test and Negative predictive tests. Previous studies have also reported high accuracy, sensitivity and specificity of GeneXpert compared to other diagnostic methods (Lawn and Nicol, 2011; Pandey and Yadav, 2019; Steingart *et al.*, 2013).

Leukocytosis and leukocytopenia are two conditions that is commonly found in tb patients. We compared total leucocyte count in Mtb positive and Mtb negative patients. Mtb positive patients exhibit elevated TLC as compared to negative patients. There was a statistically significant difference between the means of these two groups P < 0.0001. Another study reported the same TLC variation among patients suffering from pulmonary tb (Abdelkareem *et al.*, 2015). It was also observed that there was no significant difference between the TLC of RIF resistant and sensitive patients. This means the present of resistant strains don't have any effect on reduction or elevation in WBCs count. We also compared the X-ray findings of Mtb positive and Mtb negative patients. There was a significant difference between Xray findings of Mtb positive and negative patients. Mtb negative patients have clear chest Xray, and no abnormality was found in their X-ray. However, Opacities were seen in chest Xray of Mtb positive patients. A significant difference was also observed between the chest X-ray of RIF resistant and sensitive Mtb positive patients. RIF sensitive patients have clear X-ray as compared to RIF resistant patients. Correlation between AFB smear grades and HB level in Mtb positive patients was also accessed. The correlation between smear grades and Hb level was not significant. However, many studies have previously reported tuberculosis associated anaemia (Gil-Santana *et al.*, 2019).

CONCLUSION

The present study indicates that GeneXpert is a novel diagnostic tool for the detection of TB and drug resistance. The major aim of the present work was to find out the prevalence of TB and RIF resistance among Kot Addu population. It was observed that GeneXpert could diagnose tuberculosis with more accuracy as compared to other traditional diagnostic tests. However, traditional tests should also be applied parallel to GeneXpert to verify and strengthens the diagnostic outcomes.

Author's Contribution. All authors contributed equally.

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