How to Cite:
Naseem, R., Israr, M., Khan, S., Aarab, M., Yousuf, M., & Abid, H. Z. U. (2023). Investigation of variations of pyrazinamide resistance and gene Xpert-based molecular typing in tuberculosis patients. *International Journal of Health Sciences*, *7*(S1), 2865–2874.
https://doi.org/10.53730/ijhs.v7nS1.14599

Investigation of variations of pyrazinamide resistance and gene Xpert-based molecular typing in tuberculosis patients

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***Abstract*---**The morbidity, death, and medication resistance caused by Mycobacterium tuberculosis are global health concern. The Gene Xpert is employed to diagnose TB early and find pyrazinamide (PYR) resistance at the same time. Our goal was to examine the clinical TB situation at Faisalabad's tertiary care institutions and to use Gene Xpert to determine the prevalence of TB and the drug resistance pattern. In this research, 135 samples from probable TB patients were included, and Gene Xpert identified 127 samples as positive. The gender, age group (50 years), sample type (sputum and pleural), and number of M. tuberculosis by ct value (cycle threshold) were all taken into consideration when categorizing the samples. The findings of the current investigation revealed a significant positive frequency of TB in male patients and in the 31-50 years age ranges. Patients with TB had a high prevalence of M. tuberculosis in the low and medium categories. 16 of the 127 TB patients who tested positive for the

disease had pyrazinamide resistance. Our study concluded that Gene Xpert is a useful method for quickly diagnosing and treating TB since it can quickly detect M. tuberculosis and pyrazinamide resistance.

***Keywords*---**Mycobacterium tuberculosis, Gene Xpert assay, Pleural tuberculosis, pyrazinamide resistance.

Introduction

M. tuberculosis, which causes TB, is a severe public health issue that affects people all over the world. TB most frequently affects the lungs' tissues, which is referred to as pulmonary TB, but it can also affect any other body tissue and is referred to as extra-pulmonary tuberculosis (EPTB) (Liang et al., 2019). Pleural TB is the most typical manifestation of EPTB. In several nations, pleural effusion is frequently brought on by tuberculous pleurisy (Shaw et al., 2019). TB is a major global health problem, especially in developing nations. In Pakistan, there were 5.8% instances of TB, and extra-pulmonary TB (EPTB) accounts for 20% of all TB cases, according to the WHO. It is the ninth most common cause of death worldwide from infectious diseases (Sinshaw et al., 2019, Ullah et al., 2021). Pakistan is now ranked fourth among nations with the highest burden of drug-resistant (DR) TB and fifth overall among nations with the highest burden of tuberculosis (TB) (Ullah et al., 2021). Pakistan contributes significantly to the global TB burden, with about 61% of cases found in the WHO's Eastern Mediterranean region (WHO, 2021). Baluchistan had the greatest number of TB patients (79.4%), followed by Khyber Pakhtunkhwa (68.7%), and Punjab (42.8%). (Ullah et al., 2021). In the entire world, 1.6 million deaths and about 10.1 million cases are reported each year (Phillips, 2018). According to estimates, TB will cause over 10 million illnesses and 1.4 million deaths worldwide in 2019. The number of TB and HIV co-infected people who died globally in 2019 was approximately 208000. (MacLean et al., 2020; WHO, 2021). About one-fifth of reported cases of TB are EPTB. Up to 30% of TB patients develop pleural tuberculosis, the second most typical extrapulmonary site of involvement. The most frequent cause of a lymphocytic pleural effusion in HIV-positive individuals is pleural TB (Mustafa et al., 2020). Drug resistance is shown by the resistance to the antibiotic pyrazinamide. Nearly 90% of isolates that are PYR resistant also exhibit isoniazid (INH) resistance (Atashi et al., 2017). The emergence of drug resistance is a tiresome issue during anti-tuberculous therapy. There are numerous different types of resistance, such as extended drug resistance or multidrug resistance (MDR TB) (XDR TB). MDR TB is a kind of TB in which two of the most effective first-line anti-tuberculosis medications, INH and PYR, are no longer effective (Masenga et al., 2017). The type of MDR-TB known as XDR-TB occurs when there is resistance to more than two antituberculosis medications (Matabane et al., 2015). Patients may get MDR-TB as a result of exposure to the resistant strain or deliberate selection of the resistant strain as a result of ineffective therapy (Mulu et al., 2017). In low-income nations, MDR-TB treatment is extremely challenging. The available TB treatment options are expensive and few. MDR-TB, which has significant mortality rates, can develop in around 3.3% of newly diagnosed TB patients and about 20% of those who have already had treatment for the disease (Matabane et al., 2015). M. tuberculosis's resistance

mechanisms are brought on by the activation of the efflux pump or the acquisition of mutations. INH activator or target Kat G mutations are typically the cause of INH resistance (inhA). The main INH resistance mechanism can either result from a mutation in the inhA gene or its promotor region, which can result in INH inhibition, or it can result from a mutation in the drug kat G's activator, which inhibits INH activation (Vilchèze and Jacobs, 2015). The PYR develops resistance via mutation at 81-bp. PYR's resistance determining region (RRDR) of the rpoB gene, which produces the beta component of bacterial RNA polymerase (Pienaar et al., 2018). The control of TB is not being stopped quickly enough. Age, chronic illnesses, immunosuppressive disorders like diabetes, crowded living situations, alcohol, illicit drug use, and tobacco smoke are only a few of the risk factors that have contributed to the rise in pulmonary and pleural TB cases (Macas et al., 2019). For TB to be eradicated, early, prompt, and effective diagnosis and treatment are required. Ziehl-Neelsen (ZN) staining, a common technique, is quick and affordable for identifying acid-fast bacilli in low-income nations. However, it has a variable sensitivity and a weak positive predictive value (PPV). Culturing is the gold standard way to diagnose TB, however it takes 6–8 weeks, leading to a noticeable delay in diagnosis. The infrastructure, knowledgeable and experienced staff, and specialized labs needed for patient care and outcomes are also in short supply. These elements may make it more difficult to diagnose EPTB (M. Hefzy et al., 2021). The World Health Organization (WHO) recommends a number of molecular techniques for the detection of M. tuberculosis, including next-generation Xpert testing, line probe assays (LPA), PCR-based tests, loop-mediated isothermal amplification (LAMP), Gene Xpert and whole genome sequencing, the Truenat MTB, Truenat MTB Plus, and Truenat MTB-PYR assays, or (MacLean et al., 2020). By using GeneXpert, we wish to screen our population. Gene Xpert is being used more frequently than in previous years. It is a nucleic acid amplification test for M. tuberculosis that is automated and cartridge-based. In less than two hours, this test can identify M. tuberculosis nucleic acid and PYR resistance. This assay was advised by the WHO for the diagnosis of TB, EPTB, and PYR resistance. Additionally, it serves as the first screening test for the detection of MDR-TB (Theron et al., 2014).

Materials and Methods

There was a cross-sectional study done on pleural TB patients with the proper sample. In all, 135 samples (n) of TB patients were gathered from Faisalabad's tertiary care facilities. There were 72 male samples and 55 female samples out of the 135 total samples. The samples were split into three age groups based on their 50-year ages. In the DHQ hospital, the Gene Xpert was used on these samples to analyze the prevalence of TB in various age groups. Patients with pleural TB and ages between 15 to 80 were considered eligible for participation. Patients who were 80 years old, unwilling to participate in the study, or who had previously received anti-tuberculosis therapy were excluded from the study.

Sample collection

Patients presenting to tertiary care institutions in Faisalabad with clinical TB symptoms had samples of pleural fluid and sputum taken from them in order to establish the presence of M. tuberculosis using the Gene Xpert technology.

Classification of patients for gene Xpert

Using age groupings as a basis for classification According to their age groupings and gender, patients were divided into three groups. Group 1 (28 patients), Group 2 (65 patients) and Group 3 (34 patients).

Classification on the basis of type of sample
Two separate types of samples were collected Sputum and pleural samples. About 37 pleural samples and 90 sputum samples were gathered.

Protocol of gene Xpert for detection of M tuberculosis

The Gene Xpert test is an automated assay for finding M. tuberculosis complex DNA and mutations linked to resistance. It integrates and systematizes sample processing, nucleic acid amplification, and target sequence detection in samples using real-time PCR. Sputum sample was added to the Gene Xpert reagent container, mixed in a vortex, and then left to sit at room temperature for 15 minutes. The pleural sample was handled using the same method. Gene Xpert cartridge was labelled, and sample from bottle was taken and placed into the cartridge using a pipette. The cartridge's lid was securely fastened. The Gene Xpert system was then loaded with the cartridge. By contrasting the detection of M. tuberculosis with ct range, the Gene Xpert result was seen. The outcome was shown as a High (ct: 28) number of M. tuberculosis cases (Elbrolosy et al., 2021).

Ethical endorsement
The ethical endorsement was taken from Human Research Ethics Review Committee of the research institute.

Analysis of Statistics

Using the SPSS software, the data from Gene Xpert (M. tuberculosis and PYR resistance detection) was examined by calculating the percentage positivity or frequency of TB patients and comparing factors.

Results

A total of 135 samples from which 127 were found to be positive for TB by Gene Xpert were included in our investigation. According to their age, gender, sample type, and number of M. tuberculosis in relation to the ct value, we calculated the frequency of TB. By using Gene Xpert, we were also able to identify PYR resistance in TB patients. Table 1 shows the Gene Xpert results for M. tuberculosis that were favorable.

Gender wise occurrence of TB by gene Xpert assay

A total of 135 samples from which 127 were found to be positive for TB by Gene Xpert were included in our investigation. According to their age, gender, sample type, and number of M. tuberculosis in relation to the ct value, we calculated the frequency of TB. By using Gene Xpert, we were also able to identify PYR

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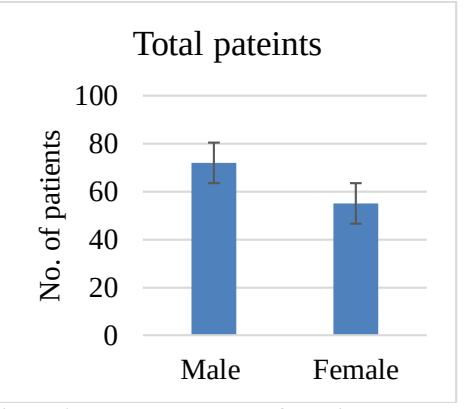

Fig.1: Gender wise occurrence of TB by gene Xpert assay

Occurrence of TB with respect to different age groups

Different age groups' TB prevalence was recorded. According to their ages, the TB patients who tested positive were separated into three groups. In comparison to the other two age categories, 50 years and older, the 30 to 50 age group had the highest number of positive TB patients (65). When the other two groups were evaluated to one another, the group >50 years of age, or 60 as comparative to 50 years of age, showed higher frequency (28), and the group 30 years of age showed the lowest positivity (34), as shown in fig. 2.

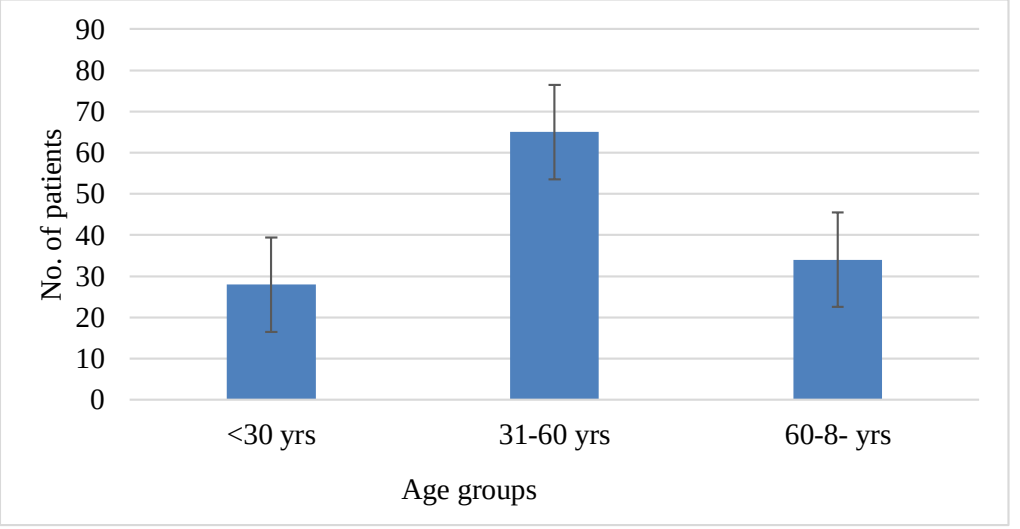

Fig. 2: TB patients with respect to different age groups

Presence and Detection of high number of M. tuberculosis in correspondence to the ct value in gene Xpert

The results of Gene Xpert can be classified into very low, low, medium, and high numbers of M. tuberculosis, and the ct value is inversely proportional to the presence of M. tuberculosis. According to the ct value of Gene Xpert, there were 25 positive patients in the low category and 58 positive patients in the medium category and the high category had fewer positive TB patients (44).

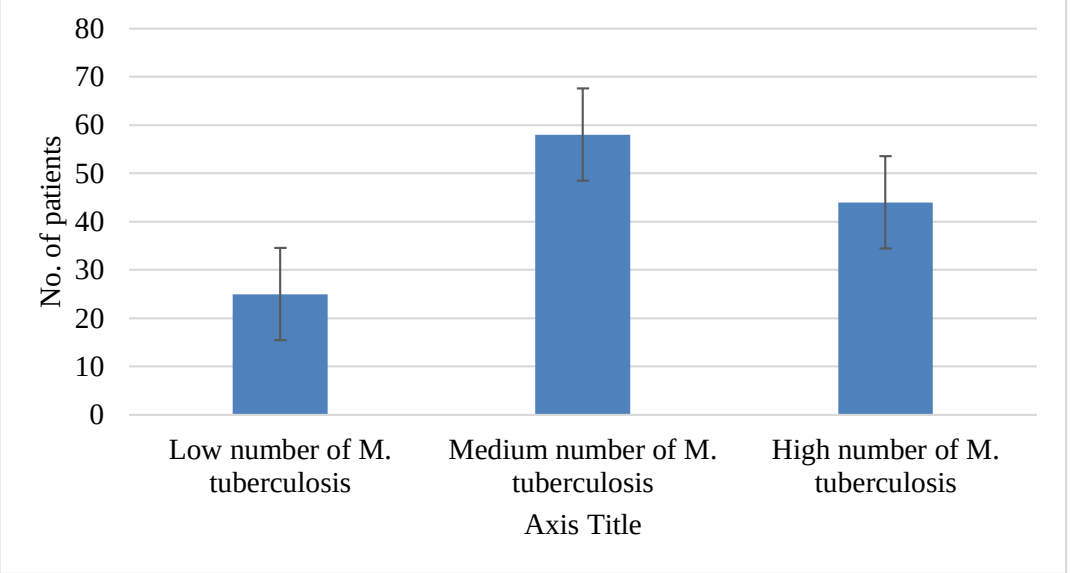

Fig. 3: Detection of high number of M. tuberculosis in correspondence to the ct value in gene xpert

Gene Xpert detects pyrazinamide resistance in tuberculosis patients

PYR resistance was determined in positive pleural TB patients using Gene Xpert. Gene Xpert identified 127 samples as positive for M. tuberculosis out of 135. PYR resistance was found in 15 patients, while 112 patients tested negative for PYR resistance (see fig. 4).

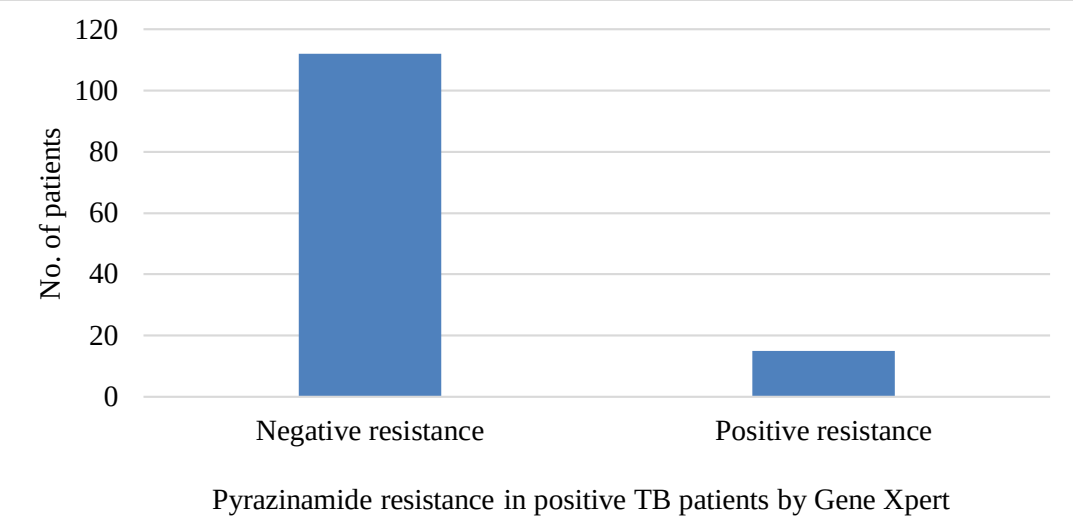

Fig. 4: Gene Xpert detects pyrazinamide resistance in tuberculosis patients

Discussion

TB is a risk to public health and the mortality rate is rising, particularly in low-income countries. To reduce the death rate, it is critical to detect M. tuberculosis early and begin appropriate treatment; timely diagnosis of TB is regarded as a major pillar in disease control. The diagnosis of EPTB is a significant problem, and the accuracy of recent tests is insufficient (Silva et al., 2021; WHO, 2017). Timely diagnosis and treatment of tuberculosis can improve cure rates while decreasing transmission, illness, and death. Smear microscopy with acid fast staining and culturing are the cornerstones of tuberculosis diagnosis. Culturing is regarded as the gold standard technique, but it is time consuming. A suitable infrastructure and expert staff are required for cultivation (Dunn et al., 2016). Although acid-fast staining is fast and inexpensive, it has variable sensitivity and limited specificity. It is unable to distinguish between non-tuberculous Mycobacteria and M. tuberculosis. In contrast, the WHO has recommended Gene Xpert, a fully automated and rapid method for TB diagnosis (WHO, 2013). The significance of Gene Xpert in the diagnosis of Mycobacterium tuberculosis is recognized due to its feasibility and suitability as a reliable, quick, and cost-effective test (Metcalf et al., 2018). Our data showed that more positive TB patients were detected by Gene Xpert in the 30 to 50 years age groups than in the other two groups. According to Smilji et al. (2019) and Zhang et al. (2011), TB can affect people of any age, but the 25-44 age group has the highest number of TB patients. Tostmann et al. (2008) described in their study the common age group for tuberculosis (TB) of 15 to 34 years, and partially within the 25-44 years age group. According to Gene Xpert detection of M. tuberculosis, the majority of patients fall into the low and medium ct value categories. The results of Gene Xpert revealed that male TB patients were more common than female TB patients. Whereas the Kabir et al. (2021) found a higher ratio of male TB patients than female TB patients, their findings are similar to those of Goroh et al. (2020), Hernández-Garduo et al. (2004), and Linguissi et al. (2015), in which males were

more affected by M. tuberculosis than females. By using Gene Xpert, a greater number of positive TB patients were detected in sputum samples than in pleural samples. According to Mechal et al. (2019), both pulmonary and extra-pulmonary samples had nearly the same sensitivity and specificity by Gene Xpert. The study discovered that sputum samples have higher PYR resistance than pleural samples. According to Zong et al. (2019), identification of PYR by Gene Xpert had the same sensitivity in countries with high and low TB prevalence. Rahman et al. (2017) discovered 5.2% PYR resistance by Gene Xpert and between both treated and untreated TB patients, indicating that the prevalence of PYR was high.

Conclusion

The Gene Xpert is a novel and beneficial technique for detecting M. tuberculosis early on. Gene Xpert is a highly specific and sensitive test for detecting M. tuberculosis and resistance to it.

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