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Pattern of rifampicin resistance and gene xpert based molecular typing of tuberculosis patients in tertiary care hospitals

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Abstract: *Mycobacterium tuberculosis* associated morbidity, mortality and drug resistance is a global health issue. The Gene Xpert is used for early diagnosis of TB and simultaneous detection of Rifampicin (RIF) resistance. We aimed to determine situation analysis of clinical TB in tertiary care hospitals of Faisalabad and to find out frequency of TB and drug resistance pattern by Gene Xpert. A total of 220 samples from suspected patients of TB were included in this study and 214 samples were detected as positive by Gene Xpert. Samples were classified on the basis of gender, age group (<30, 30-50 and >50 years), type of sample (sputum and pleural) and number of *M. tuberculosis* by ct value (cycle threshold). The results of present study showed high positive frequency of TB in male patients and in 30-50 years of age groups by Gene Xpert. High number of *M. tuberculosis* was found in low and medium category in TB patients. Out of 214 positive TB patients, rifampicin resistance was detected in 16 patients. In conclusion, our study identified that Gene Xpert is an effective approach for diagnosing TB by detection of *M. tuberculosis* and rifampicin resistance in <2 hours for rapid diagnosis and management of TB.

Keywords: Pleural tuberculosis, Gene Xpert assay, rifampicin resistance.

INTRODUCTION

Tuberculosis (TB) is a serious public health problem worldwide that is caused by *M. tuberculosis*. TB affects most commonly the tissues of the lungs known as pulmonary TB but it can also involve any other tissue of the body which is known as extra-pulmonary tuberculosis (EPTB) (Liang *et al.*, 2019). The most common presentation of EPTB is pleural TB. Tuberculous pleurisy is common cause of pleural effusion in many countries (Shaw *et al.*, 2019). TB is global health issue particularly in developing countries. According to WHO, Pakistan reported 5.8% cases of TB and extra-pulmonary TB (EPTB) contributes 20% of all TB cases. It ranks as the tenth leading cause of death among infectious diseases worldwide (Sinshaw *et al.*, 2019, Ullah *et al*., 2021). Pakistan currently ranks fifth among the countries having highest burden of TB and ranks fourth among the list of countries having highest burden of drug resistant (DR) TB (Ullah *et al*., 2021). Pakistan is one of major contributor to TB burden in all over the world and almost 61% cases of TB were determined in the Eastern Mediterranean WHO region is Pakistan (WHO, 2021). The highest percentage of TB patients was determined in Baluchistan (79.4%) followed by Khyber Pakhtunkhwa (68.7%) and Punjab (42.8%) (Ullah *et al*., 2021). Almost 10.1 million cases are reported each year and 1.6 million deaths are occurred yearly in the whole world (Phillips, 2018).

The estimation of about 10 million people illness is due to TB and around a total of 1.4 million people die from it globally in 2019. About 208000 TB patients co-infected with human immunodeficiency virus (HIV) died worldwide in 2019 (MacLean *et al.*, 2020;WHO, 2021). EPTB accounts for about one-fifth of TB cases are reported. Pleural tuberculosis arises in up to 30% of TB patients, which is second most common site of extra-pulmonary involvement. Pleural TB is the most common source of a lymphocytic pleural effusion in HIV positive people (Mustafa *et al.*, 2020). Rifampicin (RIF's) resistance is used as an indicator for drug resistance. The strains which are RIF resistant almost 90 % of them also show resistance to isoniazid (INH) (Atashi *et al.*, 2017).

Drug resistance development is a wearisome problem in the course of anti-tuberculous therapy. Resistance is of many types either of multi drug resistance (MDR TB) or extensively drug resistance (XDR TB). MDR TB is a type of TB in which resistance is found against two foremost first line anti-tuberculosis drugs such as INH and RIF (Masenga *et al.*, 2017). XDR TB is the form of MDR-TB in which resistance is present to more than two anti-tuberculosis drugs (Matabane *et al.*, 2015). Patients can develop MDR-TB either due to exposure to the resistant strain or selection of resistant strain by inappropriate treatment (Mulu *et al.*, 2017).

It is very difficult to treat MDR-TB in low income countries. Treatment modalities of TB are costly and limited. Almost 3.3% of new TB patients and about 20 % of earlier treated patients can develop MDR-TB which is responsible to cause high death rates (Matabane *et al.*, 2015).

The resistance mechanisms in *M. tuberculosis* are due to efflux pump activation or procurement of mutation. The

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INH resistance usually rises by mutation in Kat G which is activator of INH or target of INH (inhA). The chief resistance mechanism to INH can be either due to mutation in the activator of drug kat G that leads to inhibition of activation of INH or if there is a mutation in the inhA or its promotor region can lead to inhibition of INH (Vilchèze and Jacobs, 2015). The RIF's resistance happens by mutation in 81-bp RIF's resistance determining region of rpoB gene (RRDR) which encodes beta subunit of RNA polymerase of bacteria (Pienaar *et al.*, 2018).

There is lack of rapid stoppage in the control of TB. There are many risk factors like age, chronic diseases and immunosuppressive conditions such as diabetes, overcrowding, alcohol, drugs and tobacco smoke, which have played an important role in the increased frequency of pulmonary and pleural TB (Macías *et al.*, 2019).

The early, quick, and perfect diagnosis and treatment is necessary for the elimination of TB. In low income countries, a standard technique like Ziehl-Neelsen (ZN) staining is inexpensive and prompt for the identification of acid-fast bacilli. Though, it has poor positive predicative value (PPV) and variable sensitivity. The gold standard method to diagnose TB is culturing but it takes 6-8 weeks, which give rise to prominently delay in diagnosis. Moreover, there is shortage of infrastructure, expert and experienced staff along with specialized lab that is required for patient care and outcomes**.** These factors can lead to aggravate the problem of EPTB diagnosis (M. Hefzy *et al.*, 2021).

There are several molecular methods used for the detection of *M. tuberculosis* which are recommended by WHO such as Line probe assays (LPA), Loop-mediated isothermal amplification (LAMP), Next-generation Xpert testing**,** PCR-based test, Truenat MTB, Truenat MTB Plus, and Truenat MTB-Rif assay or Gene Xpert and whole genome sequencing (MacLean *et al.*, 2020). We want to screen our population by GeneXpert. The use of Gene Xpert has been increased from last several years. It is an automatic, cartridge based nucleic acid amplification test for the detection of *M. tuberculosis.* This test can detect nucleic acid of *M. tuberculosis* and RIF's resistance in less than 2 hours. The WHO recommended this assay to diagnose TB and EPTB along with RIF resistance. It is also used as initial screening test for the diagnosis of MDR-TB (Theron *et al.*, 2014).

MATERIALS AND METHODS

A cross-sectional study with appropriate sampling of pleural TB patients was conducted. A total of 220 samples (n) of TB patients were collected from tertiary care hospitals, Faisalabad. Among 220 samples, there were 138 samples of male and 82 samples of female. The samples were divided into three age groups according to their ages *i.e.*<30 year, 30-50 years and >50 years. The Gene Xpert was performed on these samples in DHQ hospital for the analysis of prevalence of TB in different age groups. In the inclusion criteria: The age of patients of both genders were between 15-80 years and also patients had pleural TB and in exclusion criteria, the patients of age <15 years and >80 years, those who were not willing to participate in the study and those who had already taken anti-tuberculosis therapy were excluded.

Sample collection

Samples of pleural fluid and sputum were collected from patients presented in tertiary care hospitals, Faisalabad with clinical symptoms of TB for confirmation of *M. tuberculosis* by Gene Xpert technique.

Classification of patients for gene xpert

Classification on basis of age groups

Patients were classified into three groups on the basis of gender regarding their age groups

Group 1: Included 24 cases (6 males and 18 females) of TB patients with age <30 years.

Group 2: Included 134 cases (87 males and 47 females) of TB patients with age ranged from 30-50 years.

Group 3: Included 62 cases (45 males and 17 females) of TB patients with age >50 years.

Classification on the basis of type of sample

Two types of samples were collected *i.e.* sputum and pleural. The 174 sputum samples and 46 pleural samples were collected.

Protocol of gene xpert for detection of M tuberculosis

The Gene Xpert test is an automatic assay for detection of DNA of *M. tuberculosis* complex and resistance related mutations. It assimilates and systemizes processing of sample, amplification of nucleic acid and recognition of target sequence in samples by means of real time PCR. Sputum sample was added in the bottle containing reagent of Gene Xpert and then mixing was done in vortex mixture and incubated at room temperature for 15 minutes. Same procedure was done for pleural sample. Cartridge of Gene Xpert was labelled and by using pipette, sample from bottle was picked and poured in the cartridge. Lid of cartridge was tightly closed. Then cartridge was loaded in the Gene Xpert system. The result of Gene Xpert was observed by comparing the detection of *M. tuberculosis* with ct range. The result was displayed in numbers of *M. tuberculosis* as High (ct: <16), Medium (ct:16-22), Low (ct: 22-28) or Very Low (ct: >28)(Elbrolosy *et al.*, 2021).

Ethical approval

The study was approved by the Institutional Biosafety Committee (IBC) of University of Agriculture, Faisalabad (935/ ORIC, dated 19/2/2021) and Human research ethic

review committee of tertiary care hospitals, Faisalabad (TUF/2020/161, dated 23/12/2020).

STATISTICAL ANALYSIS

The data of Gene Xpert (*M. tuberculosis* and RIF resistance detection) was analyzed by calculating percentage positivity or frequency of TB patients and comparison of variables by using Graphpad Prism5 software.

RESULTS

In our study, a total of 220 samples were included, out of 220 samples, 214 samples were determined as positive for TB by Gene Xpert. We determined the frequency of TB according to their age, gender, sample type and number of *M. tuberculosis* regarding the ct value. We also detected RIF's resistance in positive TB patients by Gene Xpert. The positive results for *M. tuberculosis* by Gene Xpert are displayed in table 1.

Gender wise occurrence of TB by gene xpert assay

The frequency of TB in male and female patients was detected by Gene Xpert. The confirmed positive male pleural TB patients by Gene Xpert were 133 and positive female TB patients were 81. The higher frequency (62.61%) in male TB patients was observed and lower frequency (37.38%) in female TB patients was determined by Gene Xpert as shown in the fig. 1.

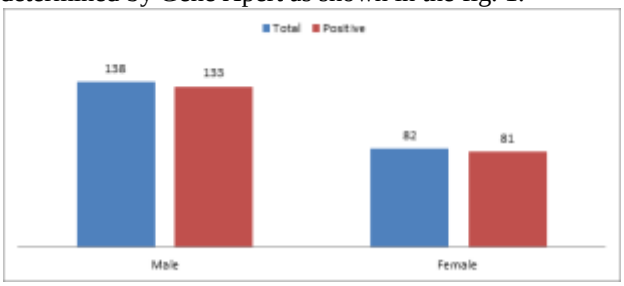

Fig. 1: Frequency of *M. tuberculosis* with respect to Gender by Gene Xpert

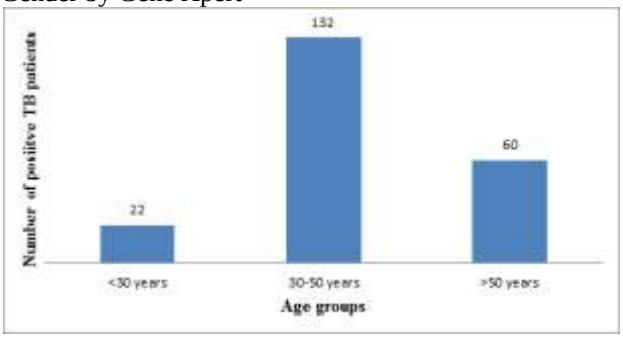

Fig. 2: Frequency of TB positive patients with respect to age groups by Gene Xpert

Occurrence of TB with respect to different age groups

The frequency of TB in different age groups was observed. The positive TB patients were divided into three groups regarding their age. There was more number of positive TB patients (132) in 30-50 years of age group as compared to other two groups *i.e.* <30 years (22) and >50 years (60). When other two groups were compared with each other, more frequency was found in >50 years of age group *i.e.* 60 as compared to <30 years of age, in which 22 patients were detected as positive. So, the higher positivity (62.14%) was observed in 30-50 years followed by >50 years of age (28.03%) and lowest positivity (9.81%) was detected in <30 years of age group as presented in fig. 2.

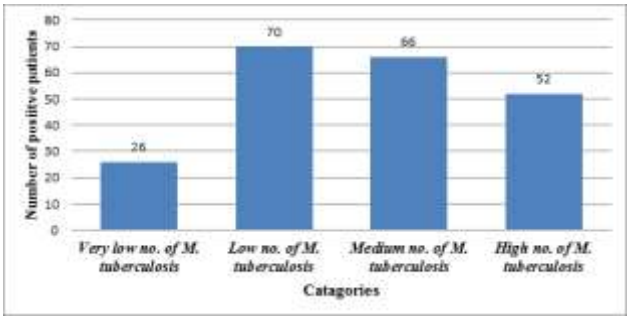

Fig. 3: Detection of positive TB patients in correspondence to ct value of Gene Xpert

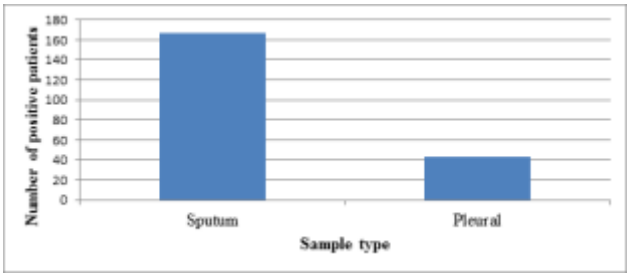

Fig. 4: Frequency of positive TB patients in sputum and pleural samples by RIF assay

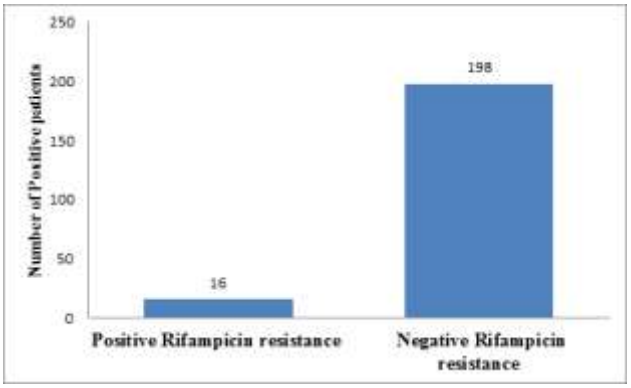

Fig. 5: Detection of rifampicin resistance in positive TB patients by Gene Xpert

Presence of M. tuberculosis in correspondence to the ct value in gene xpert

Based upon the ct value of Gene Xpert, the result can be categorized into very low, low, medium and high number of *M. tuberculosis* and the ct value is inversely proportional to presence of *M. tuberculosis*. In low

Table 1: Classification of positive TB patients for Gene Xpert on the basis of gender regarding age groups.

Age/ sample	Positive male (n)	Positive female (n)	Total
<30 years (n=24)	5	17	22
30-50 years (n=134)	85	47	132
>50 years (n=62)	43	17	60
Total	133	81	214

category, the positive patients were 70 and 66 positive patients were found in medium category in correspondence of ct value of Gene Xpert. Less number of positive TB patients was detected in high category i.e. 52 and 26 positive patients were identified in very low category as shown as fig. 3.

Detection of high number of M. tuberculosis in different samples by gene xpert

The presence of *M. tuberculosis* was detected in different samples such as sputum samples and pleural fluid by Gene Xpert. The high number of *M. tuberculosis* was detected in sputum samples 171 in comparison of pleural samples 43 as shown in fig. 4.

Detection of rifampicin (RIF) resistance in TB patients by gene xpert

RIF resistance by Gene Xpert in positive pleural TB patients was determined. Out of 220 samples, 214 samples were detected as positive for *M. tuberculosis* by Gene Xpert. RIF resistance was detected in 16 patients and 198 patients were negative for RIF resistance as shown in fig. 5.

DISCUSSION

TB is a public health risk and mortality rate is increasing day by day particularly in the low-income settings. To decrease the death rate, it is important to detect *M. tuberculosis* early and to start appropriate treatment and timely diagnosis of TB is considered as a major pillar to control the disease. The diagnosis of EPTB is a significantly severe problem and accuracy of recent tests is inadequate (Silva *et al*., 2021; WHO, 2017). The timely diagnosis and proper treatment of TB can enhance cure rate, decrease rate of transmission, illness and death. The keystones for the diagnosis of TB are smear microscopy by acid fast staining and the culturing. Culturing is considered as gold standard technique but it is time taking. There is need of suitable infrastructure and expert staff for culturing (Dunn *et al.*, 2016).

However acid-fast staining is quick and cheap but it has variable sensitivity and limited specificity. It cannot differentiate between non-tuberculous *Mycobacteria* and *M. tuberculosis*. In contrast, WHO has recommended Gene Xpert which is a fully automated and quick method for the diagnosis of TB (WHO, 2013). The importance of Gene Xpert in diagnosis of *M. tuberculosis* is recognized due to its feasibility and suitability as a reliable, quick and economic test (Metcalf *et al.*, 2018).

Our data evidenced that in 30 to 50 years of age groups, more number of positive TB patients was detected by Gene Xpert than the other two groups. According to Smiljić *et al.* (2019) and Zhang *et al.* (2011), all age group can be effected with TB but more number of TB patients were found in 25-44 years of age groups. Tostmann *et al.* (2008) described in their study the common age group of 15 to 34 years for TB and partially within 25-44 years of age group. Based on Gene Xpert detection of *M. tuberculosis*, more number of patients fall in low and medium category in correspondence to ct value.

The result of Gene Xpert revealed more frequency of male TB patients than female patients. While study by Kabir *et al.* (2021) also observed more ratio of male TB patients as compared to female TB patients and these results also resembles to the studies by Goroh *et al.*(2020), Hernández-Garduño *et al.* (2004) and Linguissi *et al.*(2015), in which males were more effected by *M. tuberculosis* than females.

In sputum sample, more number of positive TB patients was detected as compared to pleural sample by performing Gene Xpert. While study by Mechal *et al.*(2019) found that both pulmonary and extra-pulmonary samples had almost the same sensitivity and specificity by Gene Xpert.

The study found that there is more RIF resistance in sputum sample in contrast to pleural samples. According to Zong *et al.*(2019), detection of RIF by Gene Xpert showed same sensitivity between low and high TB prevalence countries. While study by Rahman *et al.* (2017) who found the 5.2% RIF resistance by Gene Xpert and between treated and untreated TB patients, prevalence of RIF was high.

CONCLUSION

The Gene Xpert is an innovative and useful technique for early detection of *M. tuberculosis*. Gene Xpert is highly sensitive and specific test for diagnosis of *M. tuberculosis* and its resistance.

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