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Prevalence of tuberculosis and molecular detection of multidrug resistant-TB in district Peshawar, Pakistan

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Tuberculosis is common in developing countries. There is little information about the molecular detection of tuberculosis and genotyping of multidrug-resistant TB in district Peshawar. Therefore, the current study was planned to study the prevalence of tuberculosis and molecular detection of multidrug resistant-TB through GeneXpert MTB/RIF assay. In the current study a total of 43,400 suspected cases for TB were screened, out of which 4607(0.11%) were diagnosed with TB with a gender distribution of 2347 (53.5%) male and 2040 (46.5%) female patients, with a total of 2659 (60.6%) cases of pulmonary TB and 1728 (39.4%) extrapulmonary TB. Out of 2659 (60.61%) pulmonary samples, 2531 (95.2%) were new cases and 128 (4.8%) were relapse cases. Out of 1728 (39.4%) extrapulmonary TB cases, 1707 (98.78%) were new cases and 21 (1.22%) were relapse cases. A total of 220 (4.78%) Multidrug-resistant tuberculosis (MDR-TB) cases were detected, out of which 60% were male and 40% were female. TB and MDR-TB were detected with a combined gender distribution of 53.8% and 46.2% in male and female patients, respectively. Results of the current study showed that TB infection rate is more prevalent in the combined age group of 0-24 years (i.e., 60%), while multi-drug-resistant tuberculosis is most common in the age group of 25- 34 (i.e., 33.18%). The results also showed that teenagers and youngsters with a combined age group of 0-34 years most commonly suffer from tuberculosis i.e., 73.15% of total infection, which can be related to weak immunity and unhygienic activities. Special initiatives and focus are required from the health department to sensitize this age group to arrest the spread of disease. Tuberculosis infection in Peshawar may also be related to poor hygiene, malnutrition, air pollution, and less awareness about the disease.

Keywords: Molecular detection, Pulmonary, extra-pulmonary, Tuberculosis, Multidrug-resistant tuberculosis, Peshawar

INTRODUCTION

Tuberculosis is an old infectious disease caused by Mycobacterium tuberculosis (gram-positive bacillus) with wide-ranging clinical infections. Mycobacterium usually attacks the lungs, spine, brain, kidney, or other different organs. Proper treatment is required for tuberculosis infection if it is not treated properly it may cause mortality. The top ten worldwide infections which may cause morbidity and mortality include tuberculosis (World Health Organization, 2017).

Tuberculosis is a communicable disease, it can spread directly from one person to another through the air by coughing, sneezing, and speaking in the form of droplets. The survival of this bacterium depends on environmental conditions. Through breathing there is a chance to affect those who are at risk. *M. bovis* can transfer another kind of tuberculosis from unpasteurized milk (Davidson et al. 2017).

Common symptoms associated with

tuberculosis disease include feeling feebleness, swollen lymph organs, sweating at night, weight reduction, fever, and loss of appetite. However, cough for 3 or more than 3 weeks is the utmost widely common symptom of tuberculosis. The patient may be readily identified with chest pain, blood with cough, and sputum or lungs bodily fluid. Different parts of the body like the body's immunological system, kidney, bones rely on the affected organ, and type of attack are indications of tuberculosis (TB) illness (Karakousis et al. 2017). Pulmonary tuberculosis is more prevalent as compared to all other types of tuberculosis (Metanat et al. 2012).

HIV diseases, age, sexual orientation, asthma, smoking propensities, and history of family and contact with tuberculosis-infected people are related to the occurrence and prevalence of tuberculosis. The horizontal measures such as conflicts, vagrancy, poverty, migration, undernourishment, diabetes, kidney problems, alcohol addiction, and societal disorders perform a vital role in escalating tuberculosis statistics which can lower the immune response of the body. Also, poverty, migration, and joblessness may cause an increase in the prevalence and incidence of tuberculosis (TB). Moreover, the prevalence of rifampicin-resistant tuberculosis is on the rise which is a serious health problem and requires urgent remedial work for prevention and control (Babamahmoodi et al. 2015).

Pakistan is among 22 countries having the highest burden of tuberculosis. In 2015, about 510,000 cases were reported, where most of the cases belonged to the age group of 15 to 34 years, and tuberculosis prevalence was estimated at 270 per 100,000 annually (Tavakoli et al. 2017). In the high burden of tuberculosis, Pakistan is ranked 5^{th} globally, 4^{th} in rifampicin-resistant tuberculosis, and ranked 4^{th} for mortality associated with tuberculosis (34 deaths per 100,000 population) (Turk et al. 2013), where every year, about 420,000 fresh cases of tuberculosis (TB) are reported. In 2010, tuberculosis prevalence was 350 cases per 100,000 population. Due to tuberculosis, 60,000 people died in 2009. In 2013 the new cases of tuberculosis were 275 per 100,000 population (Fatima et al. 2015).

Through chest radiograph, it is possible to diagnose active tuberculosis when the findings for respiration are abnormal, mostly in pulmonary tuberculosis. The typical outcomes of permeates with cavitation in the upper and middle parts of the lungs. (Thrupp et al. 2004).

To diagnose active tuberculosis, the first test is used to examine abnormal findings of phlegm smear for the presence of acid-fast bacilli on chest radiograph (Snyders et al. 2017). For detection of tuberculosis, three sputum specimens are collected on subsequent days. Recently researchers have questioned the need for three specimens. Leonard and his fellows suggest that the examination of two specimens is just as sensitive (Leonard et al. 2005). For the acid-fast bacilli (AFB) test, phlegm is smeared on a slide, dyed, and dried out, and then washed away with alcohol. The bacilli will retain the red color as it will not destain. AFB smear test gives the same results for Mycobacteria because of which this test is not a confirmation for tuberculosis, however for respiratory problems this test is a quick diagnostic method where results are usually available within 24 hours (Snyders et al. 2017).

A sputum sample from a patient with a persistent cough is mostly used for the diagnosis of *tuberculosis*. Identification of *M*. *tuberculosis* (TB) in culture is a definitive diagnosis of tuberculosis. To detect mycobacteria, it may take three to six weeks for measurable growth on culture media. Liquid chromatography is an alternative for culturing solid media. It is used to differentiate and isolate the cell walls of mycolic acid that confirms the disease in 4 to 14 days (Snyders et al. 2017).

For faster detection of *M. tuberculosis*, innovative technologies are constantly invented like nucleic acid amplification test; a molecular test where DNA and RNA are amplified through molecular methods and microorganism is readily detected. Another method is polymerase chain reaction assay (PCR), which provides results within hours and helps in the differentiation of *M. tuberculosis* (TB) from other bacteria based on genetic information. For positive acid-fast bacilli sputum specimen, it provides rapid confirmation of *M*. *tuberculosis*. Some of its limitations include high price, low sensitivity, and low availability. When both PCR and specimen smear are positive, it indicates true tuberculosis. But when polymerase chain reaction assay is positive and specimen smear is negative, it should be considered carefully along with clinical symptoms (Michos et al. 2006).

MATERIALS AND METHODS

Location of the Study

The current study was conducted in collaboration with the Provincial TB Control Program, funded by the global fund, in different localities and health facilities of district Peshawar, KPK.

Inclusion and exclusion criteria

Suspected cases from the Peshawar region were included in this study and patients from other regions were excluded.

Sampling and Collection of Data

Suspected patients referred by qualified Physicians were screened for the current study.

Demographic Data

Demographic data was collected through a questionnaire/proforma.

Study tools

Molecular detection of MDR-TB was carried out through GeneXpert.

Study Period

The current study was conducted from 28th January 2018 to 28th July 2018 in which 43,400 suspected cases were screened for diagnostic of TB.

Ethical approval

This study was approved by the ethical review board of Lady Reading Hospital, Medical Teaching Institution, Peshawar.

Source of Fund

This study was funded by a global fund in collaboration with the government of Pakistan.

GeneXpert MTB/RIF assay

Principle

GeneXpert is an automated nucleic acid amplification-based test. A single disposable GeneXpert cartridge is used that contains PCR reagents in segregated compartments. The bidirectional sequencing is carried out on the 81-bp *rpoB* core area of isolates in all strains. Each test is operated in a closed and separate cartridge system; therefore, the chances of contamination are negligible. For the differentiation of wild-type sequence and the identification of core regions that are associated with rifampicin-resistant mutation, different probes are also used (Getahun et al. 2015).

Procedure

For the current study specimens were collected in a leak-proof collection container. The cartridge was labeled with sample lab ID and sample reagents in a 2:1 (v/v) ratio were added to the sample and shaken vigorously for 10-20 seconds. The samples were placed for 15 minutes at room temperature and vigorously shaken three to four times during incubation. The sterile transfer pipette was used to aspirate the liquefied sample (2ml) and then transferred to the open port of the cartridge to perform the GeneXpert MTB/RIF assay. The remaining process and test were performed within 30 minutes of adding the sample to the cartridge, following the manufacturer's instruction (Ulrich et al. 2006).

After diagnostic tests, results were collected for tuberculosis and MDR-TB. Patients with positive Tuberculosis were referred to nearest Basic Health Units (BHUs) while patients with MDR-TB were referred to respective units and patients from Peshawar were referred to Lady Reading Hospital, Peshawar for treatment.

RESULTS

Population distribution of tuberculosis

In the current study 43,400 suspected cases referred by the general physician were screened, out of which a total of 4607 cases were confirmed for TB and MDR-TB infection which is 10.6% of suspected cases and 0.11 % of the total population of district Peshawar i.e., 108 cases per 100,000 population (PBS, 2017). 53.5% of patients among confirmed cases were male and 46.5% were female (Fig-1-2). The highest infection rate was observed in the age group of 0-14 years (i.e., 38.6%) with a gender distribution of 59% male and 41% female (Fig-3), followed by a 22.45% infection rate in the age group of 15-24 years, while the least infection rate was recorded in the age group of above 65 years with an infection rate of 6.2% (Fig- 3).

4.2 Gender distribution of tuberculosis

In the current study, the combined gender distribution of 53.8% and 46.2% infection rates was observed in the male and female populations, respectively (Fig-4).

4.3 New and relapse cases of tuberculosis

Out of 2659 (60.61%) pulmonary cases, 2531 (57.69%) were new cases and 128 (2.91%) were relapse cases. Out of 1728 (39.38%) extra pulmonary TB cases, 1707 (38.91%) were new

cases and 21 (0.47%) were relapse cases (Fig-5).

4.4 Multi drug resistant-tuberculosis

A total of 220 Multidrug-resistant tuberculosis (MDR-TB) cases were detected in the current study with a population distribution of 4.78% of MDR-TB among total TB cases detected in the region (Fig-6) with a gender distribution of 60% male and 40% female patients (Fig-7). Among 220 MDR TB patients, 36% were single and 64% were married (Fig-8).

Out of all MDR-TB cases, the highest infection rate of 33.18% was recorded in the age group of 25-34 years, followed by 20% of patients in the age group of 0-14 years, while the least infection rate was recorded in the age group of 45-54 years with an infection rate of 6.36% closely followed by the age group of 15-24 years and above 65 years with an infection rate of 6.81% each (Fig-9).

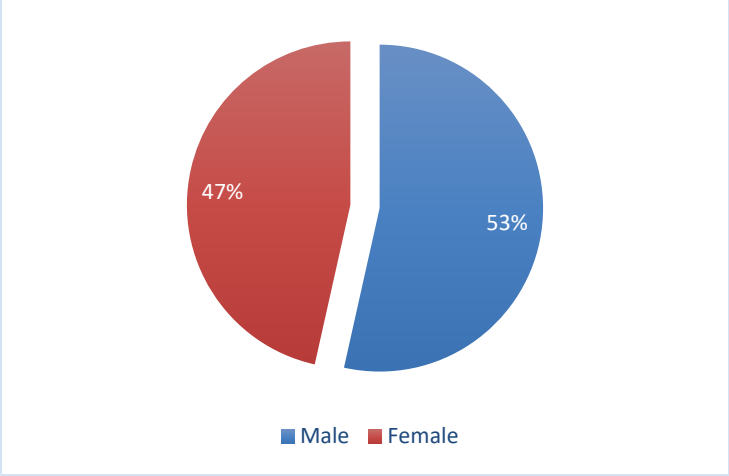

Figure 1: Gender distribution of TB Patients

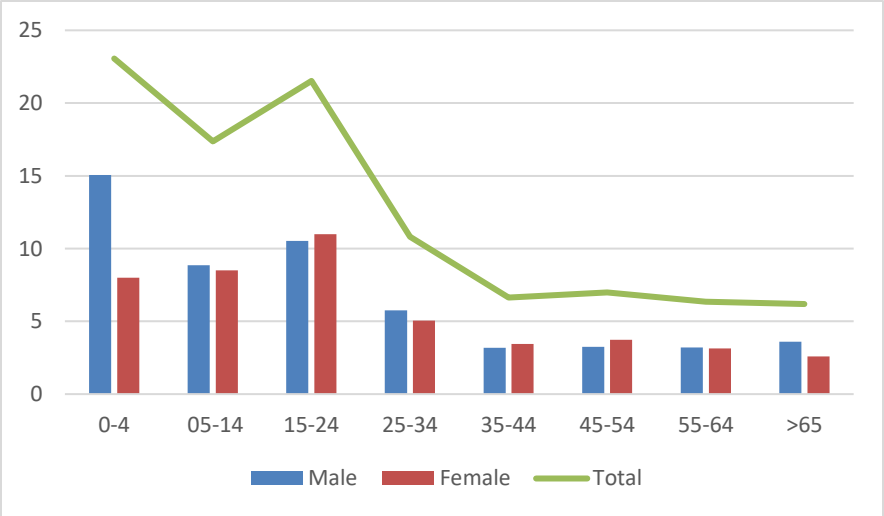

Figure 2: Demographic and Gender distribution of TB patients

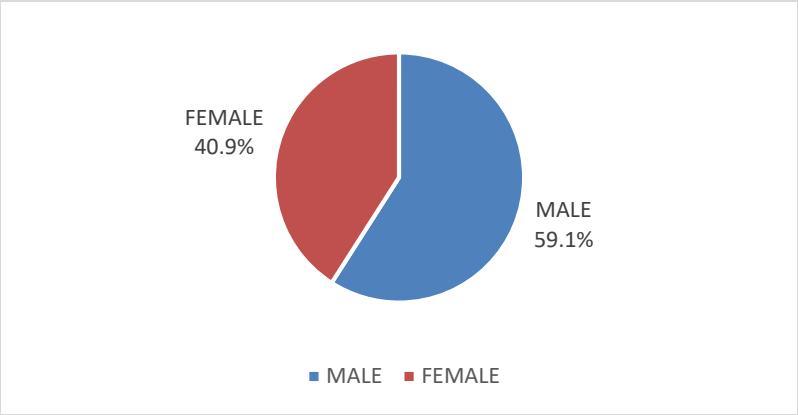

Figure 3: Gender distribution of TB patients in the age group of 0-14 years

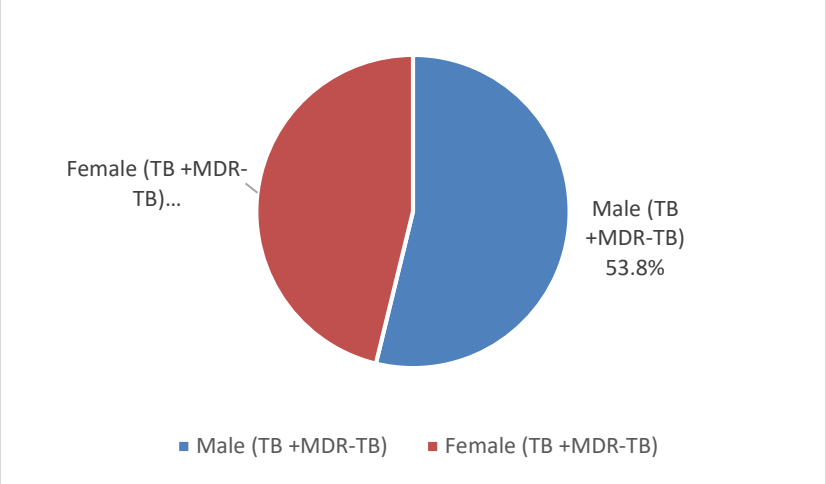

Figure 4: Gender distribution of TB and MDR-TB patients

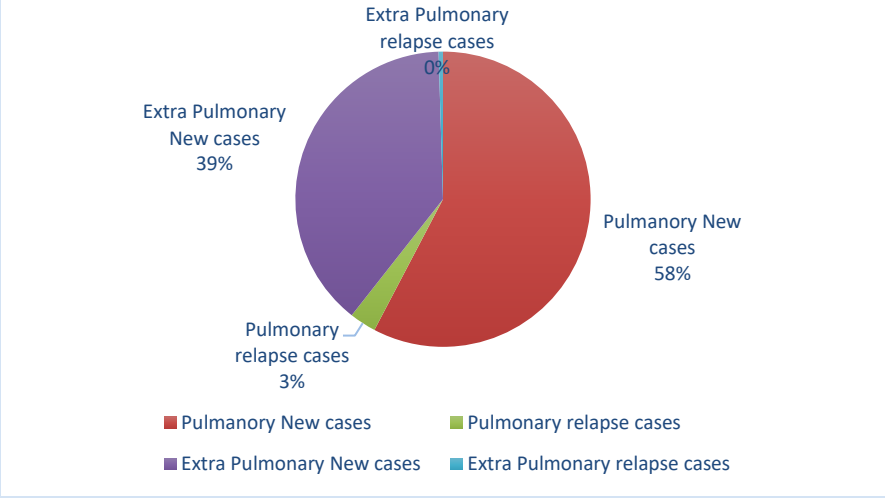

Figure-5: Pulmonary & Extra-Pulmonary TB cases

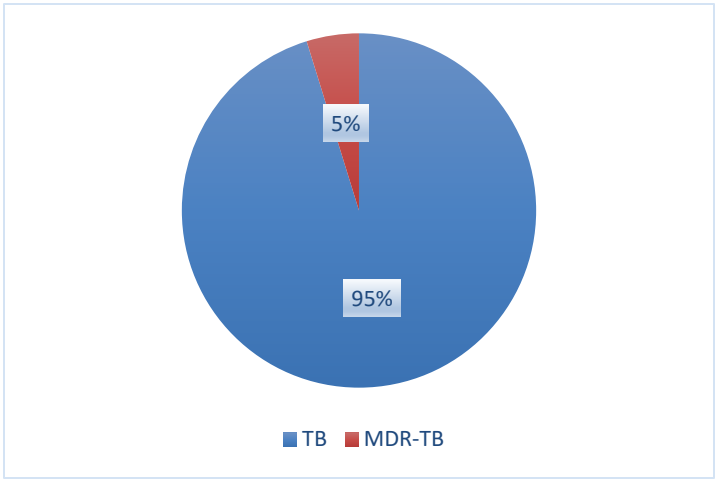

Figure 6: Population distribution of TB and MDR-TB patients

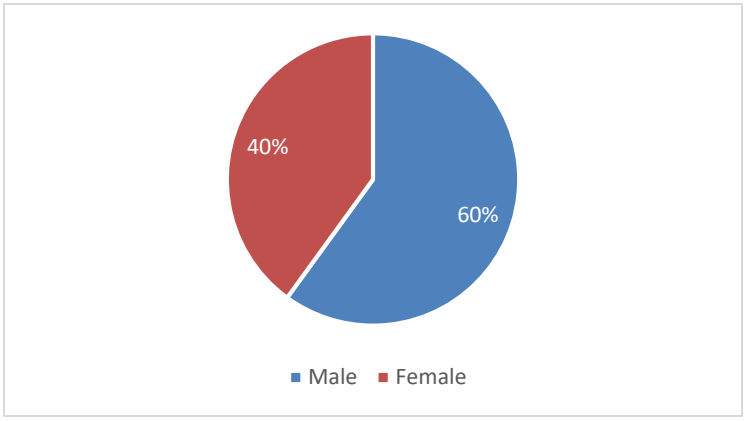

Figure7: Gender distribution of MDR TB

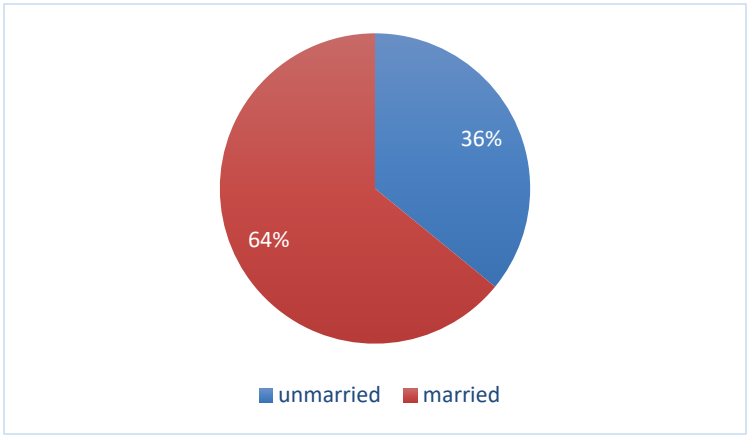

Figure 8: Marital status of MDR-TB patients

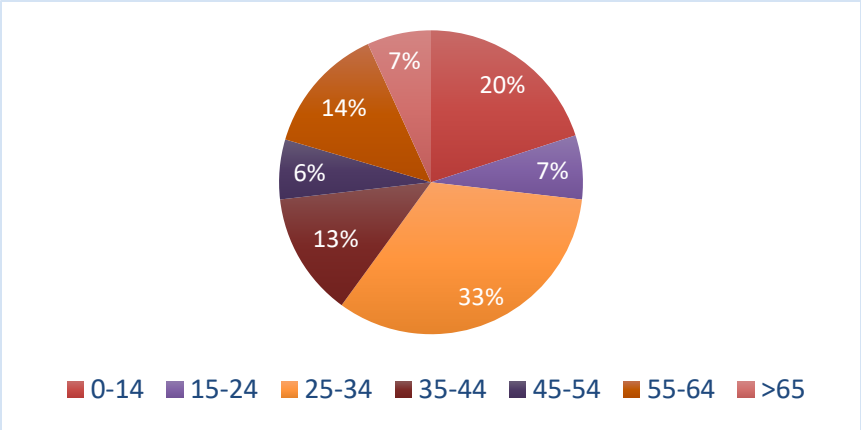

Figure 9: Demographic distribution of MDR-TB Patients

DISCUSSION

In the present study suspected patients were analyzed for detection of tuberculosis in district Peshawar of Khyber Pakhtunkhwa (KP) with a total of 4,269,079 inhabitants (PBS, 2017). Among the diagnosed patients, Multidrug-resistant tuberculosis was also detected. The common and most frequent techniques used for the diagnosis of *M. Tuberculosis* are light microscopy and GeneXpert Assay.

According to the 2015 Global report of WHO, in Iran, the frequency of tuberculosis was 16 cases out of 100,000 people (Torkaman et al. 2014). In 2014 Russia; situated in the North of Iran, reported 83 cases of tuberculosis per 100,000, which declined to 80 in 2015 (World Health Organization, 2017). In Kazakhstan, 89 cases of tuberculosis were reported per 100,000 during 2015 (Yablonskii et al. 2015). In Turkmenistan, the rate of tuberculosis was 70 per 100,000 in 2016, which increased from 69 per 100,000 cases in 2014 (Durdyeva et al. 2015). In Armenia, in 2002 the rate of tuberculosis was 77 cases per 100,000 population (Tavakoli et al. 2017). In 2014, the prevalence of tuberculosis in Afghanistan decreased to 189 per 100,000 population from 333 per 100,000 population in 2006 (Seddiq et al. 2014). In Iraq, the frequency of tuberculosis in 2011 and 2014 were 45 and 43 per 100,000 population respectively (Ali et al. 2013).

In the current study, the prevalence rate recorded for tuberculosis in district Peshawar was 108 cases per 100,000 population while in Pakistan it was reported to be 273 cases per 100,000 which showed that the prevalence rate of TB in Peshawar is lower in comparison to the national TB prevalence rate and neighboring countries Afghanistan. While the prevalence rate in Peshawar is higher when compared to Russia, Iran, Iraq, Armenia, Turkmenistan, and Kazakhstan.

For the current study, a total of 43,400 suspected samples were collected and screened, out of which 4607 were confirmed positive for TB and MDR-TB. Out of these diagnosed cases, 2479 (i.e., 53.8%) patients were male and 2128 (i.e., 46.2%) were female, while MDR-TB was prevalent in the male population as compared to females with a gender distribution of 60% and 40%, respectively. MDR-TB was more prevalent in married than unmarried patients. Formerly reported prevalence in males and females was 44.19% and 55.80%, respectively in Swat (Akhtar et al. 2014), 52% male and 48% female in Pakistan (Gilani et al. 2012) and 57% male and 43% female in Peshawar (Ayaz et al. 2012). The prevalence rate in Peshawar may be attributed to the reason that people of the city are living in joint families and overcrowded places besides poor sanitization.

In the present study, it was observed that the most vulnerable age group was 0-14 (38.6%) who may contract the disease from affected parents/siblings. 21.38% of teenagers and youngsters belonging to the age group of 15-24 comprised the second most affected age group suffering from tuberculosis which may be due to more socializing in this group as compared to others and may be responsible for the spread of disease within the age group.

Pulmonary tuberculosis cases detected in the current study (i.e., 60.61%) were higher than extra pulmonary TB (i.e., 39.39%). Relapse cases of pulmonary TB were 4 times higher than relapse cases of extrapulmonary TB. MDR-TB was highest in the age group of 25-34 years. 73.8%

TB infection rate was recorded in the combined age group of 0-34 years, which makes this age group most vulnerable for TB.

In our present study, the gender distribution of TB was recorded as 53.5% in males and 46.5% in the female population. However, interestingly the gender distribution recorded for TB in the age group of 0-14 years was 59% in males and 41% in females, which is closely similar to the MDR-TB infection rate, while in the rest of the age groups, the difference recorded in gender distribution was less conspicuous.

For control and elimination of TB special focus is required on the combined age groups of 0-34 comprising of infants and young adults. Within this age group, nearly 40% of total TB patients belonged to the age group of 5-24 years. A major chunk of these patients may belong to the school, Madaris, and college-going children besides daily wagers. Therefore, special care and preventive measures may be placed in these institutions and workplaces to arrest the spread of tuberculosis and eliminate the disease.

According to the National Nutrition Survey 2018 (UNICEF, 2018), 40.2% of Pakistani children belonging to the age group of 0-5 years have stunted growth and 17.7% of this age group suffer from wasting. In KP province the prevalence of stunted growth recorded was 40% and 15% wasting. The combined frequency of 55% of KP children suffering from malnutrition resulting in stunted growth and wasting may be a major contributor to the disease burden of 38.6% of TB patients in the age group of 0-14 years in district Peshawar.

According to World Economic Forum (2021), Peshawar is ranked amongst the top polluted cities in the world with a PM10 particulate concentration of 540 μgm^{-3}. Ambient air pollution is closely associated with TB (Xiang et al 2021), where Epidemiological research suggests an association between air pollution especially indoor air pollution in homes with TB infection (Rutgers Global Health Institute, 2021).

Poor physical health with stunting in the first two years including conception has been linked to having an association with poor cognitive and educational performance (WHO, 2015). Fifty-five percent of KP children and 57.9% of Pakistan are suffering from malnutrition resulting in stunted growth and wasting. Stunting and wasting in children coupled with high air pollution to which they are exposed consequently may be linked to poor physical and mental health, which may have devastating effects on the future growth and development of the region due to a poorly skilled population. Emergency corrective measures are required to be put into effect by the government to address malnutrition in Pakistani children and the air pollution to which they are exposed. This will not only check the spread of TB but also create healthy minds and bodies for the creation of a knowledge economy for future growth and development of the region already exposed to the "War on terror" and suffering from continuously deteriorating law and order situation.

CONCLUSION

Based on the results of the current study it is recommended that to control the spread of TB amongst the children and adult population, federal and provincial government needs to take emergency corrective measures. Awareness programs may be started to sensitize the general population and especially parents of the severely affected age groups of 0-24 years. Federal and provincial health and nutrition departments need to devise nutritional guidelines not only for adults with TB but for "the mother and child" in the age group of 0-5 years exposed to malnutrition, to help develop and boost their immune system. Nutritional supplements may be provided to the school and Madaris-going children besides daily wagers in the age group of 0-14 years. Hence nutritional support in the form of a high protein diet should be part of the TB control program or emphasis should be given to provide nutritional support to children and adults with TB.

As TB is seen more in patients with low socioeconomic populations; living in overcrowded populations with poor ventilation/poor housing systems, which highly contributes to the spread of the disease, therefore government planners should work to improve the living conditions of the population at risk. Air pollution also contributes to the spread of the disease by compromising the immune as well as the respiratory system of the exposed population. The federal and provincial environmental protection agencies should actively play their role in controlling pollution especially air pollution in Peshawar city; ranked highly in the most polluted cities of the world.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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We would like to thank the National Tuberculosis Control program, local TB control Units, and Pulmonology unit of Lady Reading Hospital, Peshawar for their help and support. The role of these organizations is monumental in reducing the TB prevalence rate in Peshawar by more than 50% of the National TB prevalence rate.

AUTHOR CONTRIBUTIONS

SA collected experimental data and wrote the manuscript. AB generated experimental data and reviewed the manuscript. FA designed the study, analyzed the data, reviewed and edited the manuscript. MF formatted and edited the manuscript.

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