Prevalence of *mycobacterium tuberculosis* and their possible risk factors in suspected *tuberculosis* patients of district mardan khyber pakhtunkhwa, Pakistan

**Abstract**

*Tuberculosis* (TB) is one of the chronic contagious diseases caused by *Mycobacterium Tuberculosis* that affect humans and animals. It is stated that TB is one of the major leading cause of morbidity and mortality throughout the globe. According to the WHO, approximately one-third of people of the world are infected and about 3 million people die annually due to this disease. The present study reported the incidence rate of pulmonary TB and their possible risk factors in suspected TB patients according to age, gender, and location-wise prevalence of the disease in District Mardan, Khyber Pakhtunkhwa, Pakistan. A total of 380 sputum samples were collected from infected Tuberculosis patients and initially screened for acid-fast bacilli through Ziehl Neelson (ZN) microscopy and positive samples were further assayed by Real-time Polymerase Chain Reaction (GeneXpert). Out of these sputum samples 40% were positive for ZN staining and 31.57% were positive by GeneXpert. The prevalence rate was reported high in the age group of above sixty while a lower prevalence rate was found in the age group of 5-20 years. Male to female ratio was recorded at 37.83% and 41.37% respectively. Geographically, rural populations were highly infected by *M. Tuberculosis* which showed 45.19% as compared to the urban population showed 26.16% prevalence rate. All the relative risk factors are statistically significant having p-value (<0.05). It was concluded from our finding that TB was considerably increased in the general population of District Mardan, and proper supervision, diagnosis, treatment, and awareness of rapid prophylactic measures are needed to eradicate the risk.

**Keywords:** tuberculosis, zeihl neelson staining, PCR, genexpert

**Abbreviations:** TB, tuberculosis; ZN, zeihl neelson; PCR, polymerase chain reaction; DM, diabetes mellitus; WHO, world health organization

**Introduction**

*Tuberculosis* (TB) is one of the chronic contagious diseases caused by *M.tuberculosis* that affects humans and animals and one of the leading causes of morbidity and mortality throughout the globe. It primarily causes pulmonary tuberculosis by affecting lungs of the patients but can also infects bones, meninges, joints, intestines, skin, lymph glands, kidneys, and body’s further organs during its second phase of infection and cause extra pulmonary tuberculosis. At the initial phase of infection, most of the individuals show symptomless tuberculosis while most of the patients developed imprecise symptoms like mild cough, wheeze, weight loss, evening pyrexia, night sweats, increased weight loss, lethargy, and general ill-health. However specific symptoms may be determined by the site of infection. *M. tuberculosis* is frequently spread from infected tuberculosis patients to other persons by aerosolized droplet nuclei which are generated during coughing, speaking, laughing sneezing and shouting. The droplets nuclei may persist in the air for some time and dry quickly, and when these nuclei breathe in by the individual it can achieve direct entrance to the terminal air passages. The infected person may be producing more than three thousand contagious particles per cough. It concerns a major health issue and cause serious illness among millions of people annually and considered the second leading cause of death after HIV infection.

Several studies showed that lack of education, non-availability of qualified staff, insufficient medical coverage, malnourished individuals, people suffered from HIV/AIDS, people infected with cancer. Those living with somebody who has active tuberculosis, health care labores in hospitals are in high risks to get tuberculosis. Several scientists demonstrated that tobacco smoking and TB has strongly correlated and considered as the main threat to the development of tuberculosis. Indirect smoking was also contributed to being a major risk factor, and control of smoking could have a significant impact on the incidence and prevalence rate of tuberculosis. Approximately, 1.3 billion citizens in underdeveloped or developing countries use tobacco and lead to a higher tuberculosis rate throughout the world. Diabetes mellitus (DM) was also considered the key factor for the development of tuberculosis from latent to the active stage and about 15% of *tuberculosis* cases might be associated with diabetes mellitus globally. In 2014, World Health Organization (WHO) reported that one-third of the global population was infected with tuberculosis and data collected from 2013 showed that approximately 9 million new cases of positive tuberculosis were reported worldwide. *Tuberculosis* has been prevalent in Pakistan and contributed 44% of the TB disease globally. Pakistan is ranked 8th in the prevalence of...
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TB with an estimate of 1.5 million tuberculosis patients.16 Aim of the current study was to find out the prevalence of tuberculosis and their associated risk factors in suspected TB patients of district Mardan Khyber Pakhtunkhwa, Pakistan.

Material and methods

Study area

The current study was conducted at the regional tuberculosis Control Program, Mardan Medical complex & Teaching hospital (MMC&TH) Mardan, Khyber Pakhtunkhwa, Pakistan. Sputum samples were randomly collected from the tuberculosis-infected patients along with the history in the main laboratory of Mardan Medical Complex & Teaching Hospital Mardan KpK, Pakistan.

Sample size

A total of 380 Participants were included in the current study. Participants belonged to other districts than district Mardan and having age limit less than 05 years were excluded from this study.

Methodology

Sputum samples were collected in sterile plastic bottles in triplicate from all the enrolled participants and stored at 4°C in the laboratory. A complete informative and designed questionnaire was used for the detailed history of each patient and to report the potential threat features for the infection of M. tuberculosis. All collected samples were liquefied and decontaminated using a standard procedure using sodium hydroxide and isopropanol for the digestion and decontamination of sputum specimens and processed through Ziehl Neelsen staining microscopy and real-time PCR (Gene Xpert).

Ziehl neelsen (ZN) staining microscopy for the detection of acid fast bacilli

Sputum smear was prepared by spreading the sputum on a glass slide and fix it with a flame. Covered the smear with sufficient carbol-fuchsin stain and heat it with the help of Bunsen burner until to evaporate and leave the slide for 5 minutes. The smear was completely decolorized with 3% acid-alcohol for five minutes that become visible to the naked eye. Washed the smear carefully with clean water, drained the slide and covered with the counter-stain (Methylene Blue) for 60-120 seconds. Rinsed the smear with clean water, allowed to dry and examined the smear microscopically, using the 100x oil immersion objective.17 Following staining, identification of AFB was performed under microscope as per the WHO guideline.

GeneXpert approach for the detection of M. tuberculosis

In this method, Sputum samples were treated with sodium hydroxide and isopropanol buffer with the ratio of (2:1) and incubated it for 5 minutes at room temperature for complete detoxification and liquefication. After incubation, 2ml of the eluted sample was transferred into the Cepheid cartridge that contains the wash buffer, reagents for lyophilized DNA extraction and PCR amplification. The reaction cartridge was put in the GeneXpert instrument for filtering, extraction of DNA and PCR amplification. MTB organisms from the sputum sample were first captured on the membrane filter by GeneXpert and the slide and fix it with a flame. Covered the smear with sufficient carbol-fuchsin stain and heat it with the help of Bunsen burner until to evaporate and leave the slide for 5 minutes. The smear was completely decolorized with 3% acid-alcohol for five minutes that become visible to the naked eye. Washed the smear carefully with clean water, drained the slide and covered with the counter-stain (Methylene Blue) for 60-120 seconds. Rinsed the smear with clean water, allowed to dry and examined the smear microscopically, using the 100x oil immersion objective.17 Following staining, identification of AFB was performed under microscope as per the WHO guideline.

GeneXpert Machine.

Statistical analysis

All the data collected from the above mention assays were in a computer and statistically analysis was carried out by Graph Pad prism version 5. Percentages and ratios were calculated for different variables. A P-value (< .05) are considered statistically significant.

Results

A total of 380 samples were received from the infected tuberculosis patients in which 152 samples (40%) were screened positive for tuberculosis by ZN staining while, 120 samples (31.57%) were positive by GeneXpert as shown in (Figure 2).

Age wise distribution of M. tuberculosis prevalence in district Mardan

In the current study a total 380 samples were registered in which the high number of positive cases were reported in the age group of > 60 years which were 64.28% of the total samples, while 39.15% cases were found in the age group of 21 to 40 years which was that
second highest prevalence rate in this study. Furthermore, 37.28% of cases were screened positive in the age group of 41 to 60 years and it was 36.76% for the age group of 5 to 20 years as shown in (Table 1).

**Sex wise distribution of M. tuberculosis prevalence in district Mardan**

Of the total cases 152 (40%) were positive comprises male to female ratio was 56 (37.83%) and was 96 (41.37%) respectively by ZN staining, while male to female ratio was 42 (28.37%) and 78 (33.62%) by GeneXpert analysis as shown in (Table 2).

**Geographic distribution of M. tuberculosis prevalence in district Mardan**

On the basis of geographic distribution of M. tuberculosis prevalence in district Mardan 94 (45.19%) cases were reported positive from rural population by ZN staining and 85(40.86%) by GeneXpert while from the urban population those cases were 45(26.16%) by ZN staining and 35(20.34%) by GeneXpert as shown in (Table 3).

**Distributions of M. tuberculosis Individuals in risk group**

Here (Table 4) summarized the Distributions of M. tuberculosis Individuals in their relative risk Groups. The maximum percentage of 25% was observed in individuals having previous history of anti-TB medication which is statistically non-significant (P=0.3043). Similarly, other risk group includes history of smoking (14.47%) (P=0.0272), history of diabetes (8.55%) (P=0.004), history of hepatitis (3.94%) (P=0.0411) and previous history of contact with MTB positive patients which were 7.89% (P=0.0035). All the results are statistically significant and p-value less than .05.

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### Table 1 Age wise distribution of M. tuberculosis prevalence in district Mardan

| Age group | Samples | ZN staining (+ve) | ZN staining (%age) | GeneXpert (+ve) | GeneXpert (%age) |
|-----------|---------|------------------|-------------------|----------------|-----------------|
| 20-25     | 68      | 25               | 36.76%            | 22             | 32.35%          |
| 21-40     | 166     | 65               | 39.15%            | 40             | 24%             |
| 41-60     | 118     | 44               | 37.28%            | 45             | 38.13%          |
| > 60      | 28      | 18               | 64.28%            | 13             | 46.42%          |
| Total     | 380     | 152              | 40%               | 120            | 31.57%          |

### Table 2 Sex wise distribution of M. tuberculosis prevalence in district Mardan

| Sex       | Samples | ZN staining (+ve) | ZN staining (%age) | GeneXpert (+ve) | GeneXpert (%age) |
|-----------|---------|------------------|-------------------|----------------|-----------------|
| Male      | 148     | 56               | 37.83%            | 42             | 28.37%          |
| Female    | 232     | 96               | 41.37%            | 78             | 33.62%          |
| Total     | 380     | 152              | 40%               | 120            | 31.57%          |

### Table 3 Geographic distribution of M. tuberculosis prevalence in district Mardan

| Area      | Samples | ZN staining (+ve) | ZN staining (%age) | GeneXpert (+ve) | GeneXpert (%age) |
|-----------|---------|------------------|-------------------|----------------|-----------------|
| Rural     | 208     | 94               | 45.19%            | 85             | 40.86%          |
| Urban     | 172     | 45               | 26.16%            | 35             | 20.34%          |
| Total     | 380     | 152              | 40%               | 120            | 31.57%          |

### Table 4 Distributions of M. tuberculosis individuals in risk group

| S. No | Possible risk factors                  | +ve Cases | %age  | P-value | R. Risk |
|-------|---------------------------------------|-----------|-------|---------|---------|
| 1     | History of Smoking                     | 22        | 14.47%| 0.0272  | 1.480   |
| 2     | History of Diabetes                    | 13        | 8.55% | 0.004   | 1.881   |
| 3     | History of Hepatitis                   | 6         | 3.94% | 0.0411  | 1.911   |
| 4     | History of previous anti-TB medication | 38        | 25%   | 0.3043  | 0.8616  |
| 5     | History of Contact with MTB positive patients | 12 | 7.89% | 0.0035 | 1.950 |
| Total |                                       | 152       | 100%  |         |         |

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Discussion

_Tuberculosis_ is one of the most contagious diseases infected those people who live in crowded, ill-ventilated places and those who are ill-fitted to contract _tuberculosis_ in terms of their immune status or whose nutritional level is so low that they easily capitulated to _tuberculosis_. According to the Global TB Report in 2008, the World Health Organization revealed that Pakistan is on 8th positions among the twenty-two high-burden _tuberculosis_ countries worldwide, where approximately 280,000 people develop TB primarily adults in their creative years. Similar to other growing countries, the high-risk groups for _tuberculosis_ infection in Pakistan, may comprise individuals with diabetes, people with hepatitis, HIV/AIDS infected people or people suffered from cancer, those individuals who have been contacting with active _tuberculosis_ patients, residents of homeless shelters, the malnourished, poor and impecunious people and present or former prisoners. According to the WHO recommendation, a new molecular technique, GeneXpert has been used for the rapid detection of _M. tuberculosis_ and can be used to screen those strains which show resistance to Rifampicin mainly in extra pulmonary samples. The result of GeneXpert shows a determined positivity rate which stated that it is an absolute specific and sensitive technique as compared to conventional methods.

In the present study, 152 (40%) out of 380 collected sputum specimens were observed positive under Ziehl Neelson microscopy by oil immersion lens which showed pink, rod-shaped _Mycobacterium_ with a blue background. Similarly, other studies showed 10.6% and 20.0% prevalence rate for acid-fast bacilli by the Ziehl Neelson technique. The results showed that the prevalence of _M. tuberculosis_ was high in the age groups of 60–69 which were (64.28%). Similarly, in the age group of 21–40, (39.15%) and 41–60 (37.28%) while a low prevalence rate was recorded between the age groups 5–20 years which was 36.76%. This result shows that age affects the prevalence of _tuberculosis_.

The higher prevalence rate in the age groups of greater than 60 years may be attributed to the greater contact to the risk factors and prolong _tuberculosis_ infection. Several researchers showed that _tuberculosis_ is more prevalent in old stages mostly in old men due to their suppressive immune system using various drugs, smoking, and alcohol which may lead to disease development at a high rate.

The maximum percentage of 41.37% was reported in female patients while 37.83% of patients were recorded in male. Our study results are similar to other studies that reported a high rate of _tuberculosis_ prevalence in the female population as compared to male. Females are more susceptible to disease development as compared to males because of extra immune deficient and some hormonal changes in their reproductive years which may lead to a higher level of disease progression. In Hong Kong study reported the highest rate of _tuberculosis_ prevalence in women community observed mostly in their reproductive ages and due to missing of anti-TB medication. Geographically, the present study shows the highest prevalence rate of TB in the rural population of district Mardan which is 45.19% while this rate of prevalence is lower in the urban community which is 26.16%. Mainly the rural areas suffered to poverty, poor diagnosis, non-availability of qualified staff, insufficient education rate, and unsatisfactory coverage of immunization.

The distributions of _M. Tuberculosis_ Individuals in their relative risk groups are also discussed with respect to their statistical p-value. The maximum percentage of 25% was observed due to previous history of anti-TB medication (P=0.3043) which is statistically non-significant. Similarly, other risk factor includes history of smoking (14.47%) (P=0.0272), history of diabetes (8.55%) (P=0.004), history of hepatitis (3.94%) (P=0.0411) and previous history of contact with MTB positive patients which were 7.89% (P=0.0035). All the results are statistically significant and p-value less than 0.05. The results of our study are correlated with other previous studies.

Conclusion

The current study showed that most of the people living in rural area were infected by _M. tuberculosis_, and its percentage varies among different age groups, geographically and male to female community. The screening of _M. tuberculosis_ in the local population of district Mardan showed high prevalence rate in female population as compared to male. Moreover, this ratio was also high in the age group of greater than sixty years while low prevalence rate was reported in the age group of 5-20 years. It is suggested to pay full attention on diagnosis and to treat the early phase of _tuberculosis_ infection in Pakistan and to develop an efficient control program for the eradication of this disease.

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Conflicts of interest

The authors of this manuscript do not have any conflict and competing of interest.

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