

Prevalence of *Mycobacterium tuberculosis* Infection in Suspected Patients in a Teaching Hospital in Northeastern Iran: A Cross-Sectional Study

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Received: July 7, 2017

Accepted: July 21, 2018

Online Published: July 24, 2018

doi:10.5539/jmbr.v8n1p95

URL: <https://doi.org/10.5539/jmbr.v8n1p95>

Abstract

Background: *Mycobacterium tuberculosis* is an infectious agent responsible for major health problems and a large number of mortalities. The prevalence of *Mycobacterium tuberculosis* infection varies across countries. Knowing the infection prevalence can aid in improving public health and reduce the associated costs. The aim of this study was to determine the prevalence of tuberculosis (TB) infection in suspected cases in Mashhad, Iran.

Methods: All the clinical specimens suspected of TB infection were sent to a laboratory for diagnosis during - March 2017 to March 2018. The samples were analyzed microscopically using Ziehl–Neelsen staining, by polymerase chain reaction (PCR) to identify the *Mycobacterium tuberculosis* species using IS6110 primers, and the samples were also grown on Lowenstein–Jensen medium.

Results: Of 2,755 clinical samples analyzed, 153 (5.55%) were identified as *Mycobacterium tuberculosis*-positive, of which 54.9% originated from females and 45.1% from males. The highest rate of infection was observed in spring, especially in May (15%). Most TB cases were found in patients in VIP (43.1%), thorax (17%), and internal (15%) wards. TB infection was mostly detected in bronchial tube (70%) and sputum (23.5%) samples. The most common positive smear was 1+ (36%). Of the 153 cases, (147) 96.1% were culture –positive and 2% were PCR-negative also 84.3% were smear –positive.

Conclusion: The highest rate of infection occurred in spring, when the number of religious tourists entering the city was at its peak. Considering the sensitive location of this city, awareness regarding TB status can lead to improved health in the community and development of basic strategies to control and eliminate the transmission of this infection from Mashhad to other areas.

Keywords: *Mycobacterium tuberculosis*, Prevalence, Religious city

1. Introduction

Tuberculosis (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis*. The disease is a leading cause of mortality worldwide. TB is one of the immediate priorities of the World Health Organization (WHO), especially in countries with a low socioeconomic status (de Oliveira Uehara et al., 2016; Viney et al., 2015).

The Global TB Report by the WHO in 2017 indicated that the TB epidemic remains one of the world's greatest health concerns (Dou, Chen, Kou, & Su, 2015). Although a wide range of efforts have been made to eliminate the disease in accordance with the WHO Plan to Stop Tuberculosis 2015-1998, many countries still report high TB infection rates, particularly African and Asian countries (Organization, 2016a; Wang, Sng, & Tay, 2004). According to the WHO, approximately 20 million people suffer from TB, with about 8 million new cases and 3 million deaths per year. The prevalence of this disease is growing, particularly in individuals with risk factors and

especially in immunocompromised patients (Asgharzadeh & Kafil, 2007; Chaiprasert, Prammananan, Tingtoy, & Na-Ubol, 2006; Wang et al., 2004).

The incidence of TB varies across countries, and according to reports from countries with high infection rates during 1998-2015, 22 countries account for approximately 80% of all global infections (Organization, 2016b). Afghanistan and Uganda were removed from the list in 2017, and India and China constitute about 30% of the total (Organization, 2016b). The prevalence of TB differs not only between countries but can also differ in different provinces of a country. Diverse statistics have been reported for the incidence of TB infection in different regions of Iran, and the infection is especially prevalent in the eastern and northeastern regions, which are near endemic countries listed by the WHO (Organization, 2016a). Religious cities in Iran annually receive a large influx of visitors from neighboring countries including Afghanistan, Pakistan, Iraq, Lebanon, Syria, and Yemen. Mashhad is a major religious tourism city with the annual arrival of many tourists. The study of TB prevalence and the implementation of health surveillance play an essential role in infection control and prevention of transmission to low-incidence TB regions (Tavanaee Sani et al., 2015). We aimed to determine the prevalence of *Mycobacterium tuberculosis* infection in patients suspected of TB in Ghaem Teaching Hospital, Mashhad, Iran.

2. Materials and Methods

2.1. Study Design

This cross-sectional study was carried out during March 2017 to March 2018. Samples were analyzed in a comprehensive microbiology laboratory at Ghaem Teaching Hospital, in Mashhad, Khorasan Razavi province, Iran. This study was conducted because Mashhad is a religious tourism hub and millions of religious tourists visit the city annually. All the suspected cases of *Mycobacterium tuberculosis* infection visiting the microbiology laboratory of the hospital were enrolled, and informed consent was obtained from the patients. Broncho-alveolar, sputum, skin, and cerebrospinal fluid samples were obtained from patients in different hospital wards. Samples that were not suitable for transfer or not approved by the center expert were excluded from the study.

2.2 Decontamination, Culture, and Staining

All clinical specimens were decontaminated by N-acetyl-L-cysteine-NaOH in a Class-2 cabin. Following decontamination, LJ medium (Biomérieuxmarcy, France) was inoculated with the samples and incubated at 37°C for 4-8 weeks. During this time, the *Mycobacterium* colonies were counted and results recorded. The decontaminated sediment from the clinical specimens was prepared on a microscopic slide using the Ziehl–Neelsen technique (Gopinath & Singh, 2009; Li et al., 2009).

2.3 Nucleic Acid Extraction

DNA was extracted from clinical specimens with an RTP Mycobacteria Kit (STRATEC Molecular GmbH, Berlin-Buch, Germany) according to the manufacturer's instructions. Briefly, 200 µl of the clinical specimen was mixed with 200 µl of NAC buffer, kept at room temperature for 20 min, and centrifuged at 11,000 x g. The supernatant was then mixed with 400 µl of buffer R and transferred to an extraction tube. Other steps were performed according to the standard protocol. The extracted DNA purity and concentration were determined on a Nanodrop 1000 (Thermo Scientific). Samples were stored at -20°C until further analyses.

2.4 Polymerase Chain Reaction (PCR) Amplification

Conventional PCR was performed using the primers listed in Table 1 on an I-cycle iQ Thermal Cycler (Bio-Rad Laboratories Inc., CA). The 20 µl reactions contained 10 µl of 2X Taq Premix including 15 mM MgCl₂, 1 µl (0.5 pmol/µl) of each primer, 4 µl of template DNA, and 6 µl of nuclease-free water (Applied Biosystems/Ambion, TX). The PCR protocol was as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, amplification at 63°C for 30 sec, and extension at 72°C for 45 sec, followed by a final extension at 72°C for 5 min. The PCR products were electrophoresed on 1.5% agarose gels.

Table 1. IS6110 primer for the detection of *Mycobacterium tuberculosis*

IS6110	Sequences	Length	TM	GC%	Product
Forward primer	CGTGAGGGCATCGAGGTGGC	20	65.61	70.00	245 bp
Reverse primer	GCGTAGGCGTCGGTGACAAA	20	63.35	60.00	

3. Results

Of the 153 positive cases, 69 (45.1%) were male and 84 (54.9%) were female. The prevalence of TB infection from the 2,755 patients was 5.55%. The highest infection rate was in spring (10.5%) and in the month of May

(15%) and the lowest rates were observed in summer and autumn. Of the hospital wards, the highest infection rates occurred in patients in the VIP, thorax, and internal wards with 43.1, 17, and 15%, respectively, while the lowest rates occurred in the ear, nose, and throat, operating room, intensive care unit, and gynecology wards. Of the 153 positive samples, 69.9% were bronchial, 23.5% were sputum, and 0.7% were isolated from urine or abscess samples. 15.7% of the positive PCR samples were negative by microscopy, which could be due to the low sensitivity of the staining method. Of the 153 positive samples, 55 were 1+ (35.9%) by microscopy, 37 were 3+ (24.2%), and 7(4.6%) were 4+. Of the 153 positive samples, 147 (%96.1) were culture-positive and 6 (3.9%) were culture-negative. The PCR results were 98% positive and 2% negative (Figs. 1-5).

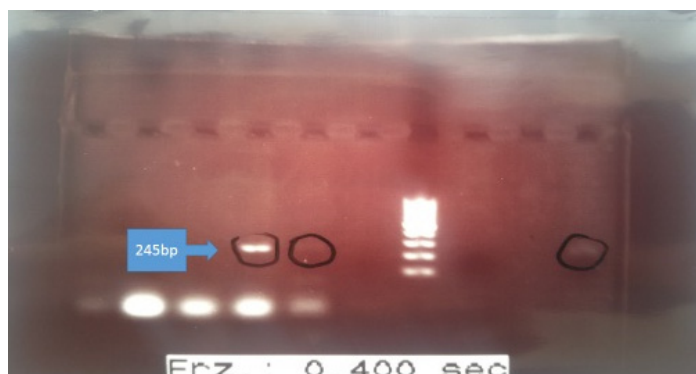


Figure 1. The 245-bp amplified product obtained from *M. tuberculosis*

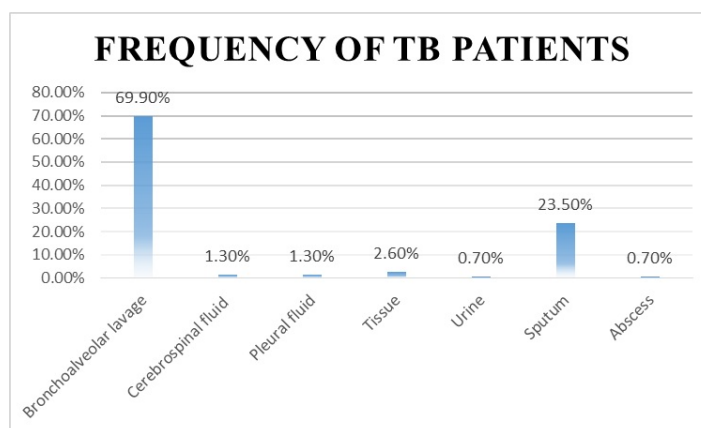


Figure 2. Tuberculosis prevalence by specimen

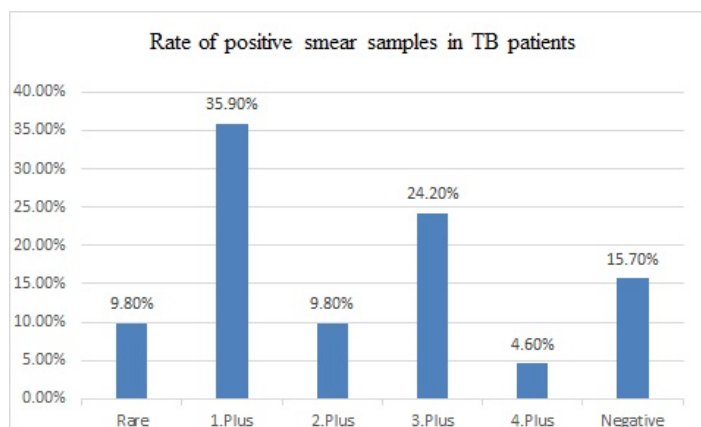


Figure 3. Rates of positive smear samples according to the American Thoracic Association

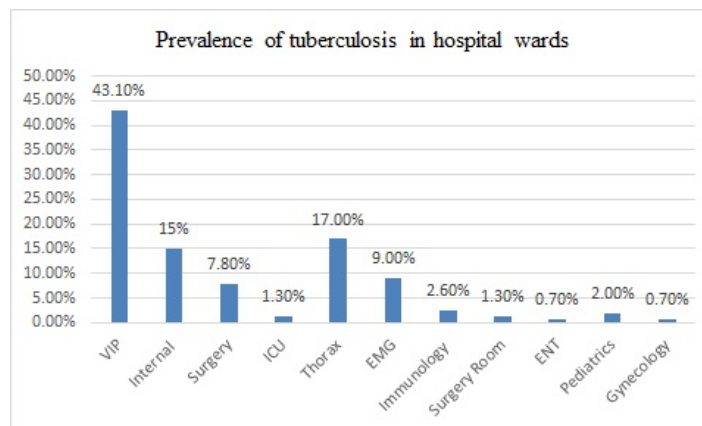


Figure 4. Tuberculosis prevalence by ward

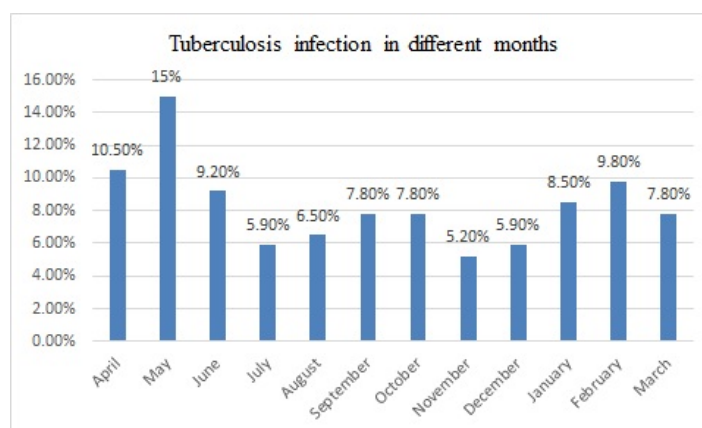


Figure 5. Tuberculosis prevalence by month

4. Discussion

The current study is one of the few large scale investigations, which were carried out on the prevalence determination of TB infection in Mashhad, Iran. We have demonstrated that the prevalence of TB infection was 5.55% (153 /2,755). Also the highest prevalence of TB infection was observed in spring and summer, especially in May, which agrees with the results of other studies conducted in the north of Iran and migrant population entering Iran (Leung et al., 2005; Moosazadeh, Khanjani, & Bahrapour, 2013; Moosazadeh, Khanjani, Bahrapour, & Nasehi, 2014). In previous studies, the infection incidence varied in different months (Douglas, Strachan, & Maxwell, 1996; Leung et al., 2005; Rios, Garcia, Sanchez, & Perez, 2000). Considering that Mashhad is a religious city, the arrival of many religious tourists seems to account for the high prevalence of TB in spring and summer. The arrival of pilgrims at these times, especially from Afghanistan, Pakistan, Iraq, and Lebanon could account for the increased infection rates (RAFIEI, Besharat, JABARI, Golalipour, & NASER, 2009).

Most of the positive samples in this study were obtained from the VIP, thoracic, and internal wards. In addition, 15% of the positive specimens were negative for *Mycobacterium tuberculosis*, due to low sensitivity ranging from 40 to 80%. Specially lower sensitivity is encountered in pediatric TB, extrapulmonary TB and in HIV-infected TB patients, which indicates that TB infection control is essential in countries with high AIDS rates With negative sputum smears (Babady & Wengenack, 2012; Campos, Rocha, Willers, & Silva, 2016; Palomino, 2005).

The remarkable thing is that the TB infection rate was higher in females (54.9%) than in males, which agrees with the findings of other studies (Alavi, Khoshkho, Salmanzadeh, & Egtesad, 2014; Babamahmoodi et al., 2015; Bialvaei, Asgharzadeh, Aghazadeh, Nourazarian, & Kafil, 2017; Dhanaraj et al., 2015; Ghaffari-Fam et al., 2015; Mao et al., 2014). It seems that the greater prevalence of women in family especially crowded families with low socioeconomic status, society and other factors may contribute to this high rate, although in some studies, men

had higher infection rates than women (Babamahmoodi et al., 2015; Bialvaei et al., 2017; Zadeh et al., 2013). Despite the relative ease of access to health care and effective drugs, TB remains a health problem in Iran, especially in the border regions (Alavi et al., 2014; Feyisa et al., 2016). Also target gene in this study was is6110, that the is6110 gene has a variable number of copies ranging from 0 to 25, possibly due to the lack of identification of species without is6110, the result is negatively reported (Xiong, Kong, Yang, Cheng, & Gilbert, 2006) as In our study, of the 153 positive cases, 3 cases were reported negative, although the result of their culture was positive.

Due to its proximity with countries endemic for TB, Iran acts as a bridge for spreading TB to other countries with low TB incidence. Considering that various factors such as smoking, AIDS, drug resistance, advanced age, gender, and neighboring countries led to an increase in the prevalence and spread of TB infection to Iran, a global screening program to examine the existing status and health control policies is necessary. Limitations of the present study included lack of access to Afghan and Arab refugees residing in the province and migrants who travel continuously.

5. Conclusion

Performing epidemiological studies in each country and region, especially in over-crowded and tourist areas, is a strategy to manage the spread of TB. In addition, screening travelers entering these areas is an effective way to control TB transmission. Further studies on the provincial migrants are recommended.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors wish to appreciate the cooperation of the staff of the laboratory of microbiology of Ghaem Hospital in Mashhad, Iran.

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