Identification of *Mycobacterium tuberculosis* in Pulmonary and Extrapulmonary Specimens of Iranian Hospitalized Patients During 2017–2021

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Abstract

Background: Tuberculosis (TB) is one of the most serious public health problems worldwide which is a chronic infectious disease and is still one of the major challenges for developing countries. This study was undertaken to identify *Mycobacterium TB* (MTB) in clinical specimens in hospitalized patients. **Methods:** The study was carried out on specimens from pulmonary and extrapulmonary suspected TB patients that were admitted to one of the largest tertiary hospitals located in Tehran, Iran from 2017 to 2021. The GeneXpert MTB/rifampin (RIF) method was applied to detect MTB and RIF resistance. Characteristics of demography, clinical features, and lifestyle were obtained from medical case records registered in the hospital. **Results:** Of 957 specimens, 92 (9.61%) were found positive for TB by GeneXpert assay. Of positive samples, 72 (78.26%) were considered pulmonary TB, and 20 (21.73%) of them are associated with extrapulmonary involvement. Four (4.3%) positive TB cases were categorized as rifampicin-resistant. **Conclusion:** This study showed a relatively high incidence rate of TB in distinct types of specimens in Iranian hospitalized patients but a low level of RIF resistance.

Keywords: Extrapulmonary, GeneXpert *Mycobacterium*/rifampin, multidrug resistant, *Mycobacterium tuberculosis*, pulmonary

INTRODUCTION

Tuberculosis (TB) is caused by one of the most important infectious agents, *Mycobacterium* TB (MTB), and it is among the top 10 leading causes of death globally.[1] The possible route of TB transmission from person to person is through the spreading of MTB into the air by an infected person through aerosol droplets.[2] TB mainly affects the pulmonary system (pulmonary TB [PTB]) and also organs of the body other than the lungs (Extra PTB [EPTB]), such as the brain, spine, skin, and lymph nodes. EPTB comprises about 15% to 20% of all incidences of TB in patients and it can be composed of up to 50% of TB cases in immunocompromised individuals.[3] This type of TB infection remains a diagnostic challenge not only due to the low number of bacteria but also due to invasive procedures often required for sample collection.[4] If there is any delay in the initiation of disease detection and treatment, the chance of transmissibility of MTB will be increased, dramatically.[5]

According to the World Health Organization (WHO) Global report, in 2018, an estimated 10 million (range: 9.0–11.1 million) new active TB disease cases were found and about 1.4 million deaths have occurred.[6] In addition, based on the WHO report in 2015, the incidence rate of TB was 16 cases per 100,000 population in Iran. This disease can affect about 16,000 Iranian people and killed about 2000 individuals each year.[7]

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Rifampicin is considered the most effective first-line drug in the treatment of this bacterium.[8] Furthermore, it is believed that 484,000 (417,000–556,000) people developed TB that was resistant to rifampicin, and of these, nearly 78% (187,000) were multidrug-resistant (MDR)-TB cases (which is defined as infection with MTB strain resistant at least to rifampicin and isoniazid) that are much more difficult to treat in comparison with drug-susceptible TB.[9]

At present, one of the major problems in managing TB is the lack of an accurate and rapid diagnostic test for MTB.[10] In Iran, TB diagnosis usually relies on acid-fast bacilli (AFB) staining and the conventional Lowenstein–Jensen (LJ) culture method in conjunction with an assessment of clinical symptoms and radiographic evidence to demonstrate TB.[11] Although AFB staining is a rapid and inexpensive method, it suffered from poor sensitivity. In addition, LJ culture is the gold standard technique for the detection of TB, but it is slow and may take at least 8 weeks.[12] Therefore, some of the cutting-edge technologies such as nucleic acid-based instruments are used for the detection of TB with very high sensitivity and specificity, and they can overcome the problems associated with the classical standard laboratory methods.[13]

Therefore, the present study was designed with the aim to detect the frequency of TB in clinical specimens of patients admitted to the hospital and suspected of having PTB and EPTB.

METHODS

Settings

This cross-sectional study was conducted from August 2017 to July 2021 at Imam Khomeini Hospital Complex (IKHC), Tehran, Iran. This hospital is the largest hospital in the Middle Eastern region, and it is a major tertiary referral hospital, especially in infectious diseases prevention, control, and treatment with more than 1000 active beds that admit patients from other hospitals throughout Iran and also provide primary care for local patients. We went to the electronic hospital registry system and recorded all the patients for whom samples were sent for MTB polymerase chain reaction (PCR).

Medical information of the patients with positive MTB PCR, such as age, sex, occupation, address, and clinical signs and symptoms, were collected from the data recorded in the medical forms of IKHC patients' records archive. No ethical permission for TB data was required for this study.

The following treatment outcome (6–9 months for treatment) definitions are usually used in diagnosed TB patients; Treated: a patient whose sputum smear or culture was negative in the last month of treatment. Recurrence: a patient whose sputum smear or culture is positive after treatment. Expired: A patient who died during treatment.

Classification of pulmonary and extrapulmonary samples

It is well documented that direct extension of the disease from lung tissue leads to other organs' involvement in TB. Consequently, in this study, different samples such as sputum, bronchoalveolar lavage (BAL), pleural fluid, cerebrospinal fluid (CSF), plasma, ascites, abscess, secretions, skin, etc., were collected from suspected cases of TB infection based on clinical criteria (cough of 2 weeks and fever, weight loss of >3 kg or dyspnea, and having radiographic imaging features of TB).

Patients with intrathoracic involvement related to the lung parenchyma and pleural and intrathoracic lymph nodes were considered as PTB. On the other hand, patients with extension of TB to organs or tissues outside the thorax, including those patients who had pulmonary involvement, and also tuberculous intrathoracic lymphadenitis or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of EPTB.

GeneXpert *Mycobacterium tuberculosis*/rifampin assay

The GeneXpert assay was performed on all specimens and the obtained results were interpreted according to the manufacturer's instructions. Briefly, 2 mL of GeneXpert MTB/rifampin (RIF) sample reagent was added to 1 mL of the specimen using a sterile pipette and the specimen was allowed to liquefy for 15 min and then 2 mL of the mixture was transferred into the GeneXpert cartridge. This cartridge contained the required reagents for nucleic acid amplification and RIF drug resistance detection. Results were displayed by the GeneXpert system automatically within about 2 h.

Statistical analysis

All results were statistically analyzed by SPSS software (SPSS Inc., Chicago, IL, USA) version 26, using t-test analysis. Statistical significance was $P < 0.05$ level for all analyses. Demographic characteristics, lifestyle factors, and clinical variables were compared between PTB and EPTB groups.

RESULTS

Of the 957 cases of suspected TB, 92 (9.61%) tested positive (males: 62 [67.3%] and females: 30 [32.7%]) for TB by GeneXpert. The overall male-to-female ratio of TB-positive patients was 2 (62/30). For pulmonary cases, the male-to-female ratio was 2.2 (50/22), but for extrapulmonary patients was 1.5 (12/8) which was statistically significant ($P < 0.05$). The overall mean age of the patients was 46.89 ± 19.49 years and the average hospital stay among our patients was 15.36 ± 13.16 days. The number of samples collected from patients and TB-positive cases was summarized in Figure 1. Among TB-positive patients, the yield of isolation of TB was: 6 (6.5%) trace, 20 (21.7%) very low, 24 (26%) low, 23 (25%) medium, and 27 (29.3%) high, respectively. Of those, only 4 (4.3%) were categorized as rifampicin resistant and 88 (95.7%) as sensitive to rifampicin.

According to different body sites for TB-positive patients, 70 (76%) samples were taken from sputum, 7 (7.6%) from BAL, 4 (4.3%) from pleural fluid, 3 (3.2%) from CSF, 3 (3.2%) from plasma, 2 (2.1%) from the ascites, 1 (1%) from knee secretions, 1 (1%) from lymph node secretions,
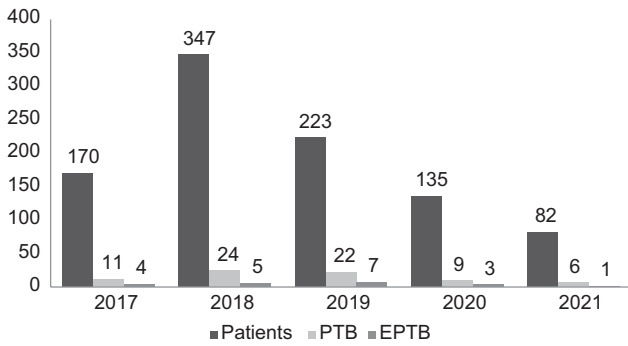
Figure 1: The number of totals, PTB, and EPTB samples from 2017 to 2021. PTB = Pulmonary tuberculosis, EPTB = Extrapulmonary tuberculosis

and 1 (1%) from the skin. Therefore, based on the pulmonary and extrapulmonary criteria mentioned before, 72 (78.26%) samples were considered pulmonary and 20 (21.73%) of them were categorized as an EPTB. Of the 20 cases of EPTB, 8 (40%) had concurrent PTB. The clinical and radiographical features and lifestyle factors associated with the patients who had PTB and EPTB in this study were shown in Table 1. The outcome of treatment showed that 82.2% of the patients were completely treated, 9.59% of the patients were confronted with recurrence of TB and 8.22% of them expired.

Discussion

TB is a serious disease which accounts for a major global public health problem worldwide. MTB has infected nearly one-third of the world's population, and TB remains one of the leading causes of death globally.[14] Although the lung remains the common site of TB infection, there are increasing reports of EPTB, showing the potential spread capacity of MTB to other organs in the body.[15] EPTB occurs in 10% to 42% of adult TB patients depending on race or ethnic background, age, immune status, presence or absence of underlying disease, and genotype of the MTB strain. Sometimes it is accompanied by pulmonary disease and it can affect any organ in the body and has numerous clinical manifestations and thus requires a high index of clinical suspicion.[3]

According to the WHO report, among the Middle Eastern countries, Iran has a high prevalence of MDR-TB with 1.3% in 2015.[16] MDR-TB is defined as TB caused by strains of MTB that are resistant to at least RIF and isoniazid. Mono-resistance to RIF is rare; however, over 90% of RIF-resistant isolates also exhibit resistance to isoniazid. Therefore, the detection of RIF resistance may serve as an alternate indicator for MDR-TB.[17] Although in some studies conducted in Iran resistance to RIF was not reported,[18] we found 4 (4.3%) RIF-resistant TB isolates among the patients. They were isolated from four immunocompromised male patients who were admitted to different wards (intensive care unit [ICU], respiratory, infectious disease, and medical) in our hospital from sputum, BAL, and CSF samples. These results

Table 1: Clinical factors and radiographical findings associated with pulmonary tuberculosis and extrapulmonary tuberculosis patients having positive GeneXpert results in this study

Characteristics	PTB (n=72), (%)	EPTB (n=20), (%)	P
Demographic			
Gender (male/female)	50/22	12/8	0.001
Age (years)	50.3	42.5	0.001
Mean of hospital stay (days)	18.7	12.2	0.037
Symptoms			
Chronic cough	59 (81.9)	7 (35)	0.004
Hemoptizi	15 (20.8)	2 (10)	0.353
Decreased appetite	41 (56.9)	9 (45)	0.190
Decreased weight	48 (66.6)	11 (55)	0.033
Fever	52 (72.2)	10 (50)	0.024
Respiratory distress	44 (61.1)	6 (30)	0.114
Chest pain	23 (31.9)	5 (25)	0.065
Back pain	4 (5.5)	0	1.000
Night sweats	26 (36.1)	8 (40)	0.120
Fatigue	29 (40.2)	9 (45)	0.025
Ascites	5 (6.9)	4 (20)	1.000
Enlarged lymph nodes	5 (6.9)	8 (40)	0.358
Urinary symptoms	7 (9.7)	4 (20)	0.011
Abdominal pain	16 (22.2)	6 (30)	0.951
Headache	3 (4.1)	5 (25)	0.615
Neurological symptoms	1 (1.3)	3 (15)	0.493
History of imprisonment	14 (19.4)	5 (25)	1.000
Living in endemic areas	15 (20.8)	8 (40)	0.818
Injecting addiction	16 (22.2)	2 (10)	0.078
Homeless people	0	0	0
Underlying diseases			
HIV positive	12 (16.6)	4 (20)	0.493
Health-care staffs	2 (2.7)	0	0.931
Smoking	26 (36.1)	9 (45)	0.233
Opium addiction	16 (22.2)	6 (30)	0.959
Diabetes	8 (11.1)	7 (35)	0.152
Recipient of body parts	0	0	0
Chemotherapy	2 (2.7)	0	1.000
Immunosuppressive drugs	5 (6.9)	3 (15)	0.674
Malnutrition	1 (1.3)	0 (0)	0.493
Malignancy	4 (5.5)	1 (5)	0.107
Loss of conscientious	4 (5.5)	2 (10)	1.000
Edema	8 (11.1)	2 (10)	0.711
Chest imaging			
Infusion	10 (13.8)	5 (25)	0.041
Pneumothorax	4 (5.5)	0	1.000
Tree in bud appearance	24 (33.3)	3 (15)	0.002
Consolidation	12 (16.6)	2 (10)	0.188
Collapse	7 (9.7)	1 (5)	1.000
Ground-glass appearance	7 (9.7)	0	1.000
Centri nodular	11 (15.2)	5 (25)	0.092
Lymph node lymphadenopathy	10 (13.8)	4 (20)	1.000
Pulmonary nodules	33 (45.8)	5 (25)	0.080
Bronchiectasis	5 (6.9)	1 (5)	0.199

Contd...

Characteristics	PTB (n=72), (%)	EPTB (n=20), (%)	P
Emphysema	4 (5.5)	2 (10)	1.000
Atelectazie	5 (6.9)	2 (10)	1.000
Cavitation	19 (26.3)	5 (25)	0.013
Miliary TB	4 (5.5)	8 (40)	1.000
Structural disorders of the lungs	7 (9.7)	3 (15)	0.107
Joint pain	2 (2.7)	2 (10)	1.000

Table 1: Contd...

TB=Tuberculosis, PTB=Pulmonary TB, EPTB=Extra PTB

show the increasing frequency of MDR-TB in developing countries such as Iran, and it could a major threat to the life of human beings and requires the use of highly sensitive and specific techniques for early detection of TB. Of the estimated 74,000 cases of MDR-TB in 2015 in Iran, only 60% were diagnosed (due to limited access to rapid and quality diagnosis) and treated.[19] Therefore, rapid diagnosis of TB prevents the development of drug resistance and improves the outcome of illness, whereas delayed diagnosis increases illness and death, and incorrect diagnosis causes unnecessary treatment.

In our study, the CSF was the most common site of EPTB. All CSF-positive samples were collected from patients admitted to ICUs. Two of these patients were discharged after receiving appropriate therapy but one of them passed away due to TB consequences. It is widely believed that central nervous system infection accounts for about 5% of all cases of TB, although the exact incidence and prevalence of TB meningitis in most parts of the world are not known, precisely. This type of infection is the most severe form of EPTB and remains one of the major global health threats with a high mortality rate.[20]

In general, conventional techniques for detecting TB have low sensitivity in distinct clinical specimens. Modern molecular approaches have changed the field of diagnosis of TB and it contains high sensitivity and specificity results.[20] GeneXpert MTB/RIF uses qualitative nested real-time PCR to detect the specific sequence for MTB as well as that for rifampicin-resistance mutation in the *rpoB* gene and can supplement standard diagnostic tools. This assay is highly rapid, sensitive, and specific in the diagnosis of both PTB and EPTB, and the sensitivity of this test is 95% in smear positive and also 50% to 80% in smear-negative clinical samples, respectively.[21] Although culture is considered the gold standard for the definitive diagnosis of TB, it has low sensitivity for specimens from extrapulmonary sites.[16] This assay was as sensitive for the diagnosis of MDR-TB and simultaneously detected TB and RIF resistance in a short turnaround time of about 2 h. This extremely helpful diagnostic tool should be implemented for screening and management of MDR-TB in TB-endemic countries and can provide accurate results and can allow rapid initiation of MDR-TB treatment.[22]

The most significant clinical features of PTB and EPTB-positive patients in our study were chronic cough (64.1%), fever (56.5%), decreased weight (52.1%), decreased appetite (44.5%), fatigue (31.5%), and night sweats (28.2%) ($P < 0.05$). Furthermore, pulmonary nodules (35.8%) and cavitation (20.6%) ($P < 0.05$) are the major radiographical findings associated with TB-positive patients in this study. In addition, EPTB patients have experienced some clinical factors such as night sweats (40%), ascites (20%), enlarged lymph nodes (40%), urinary symptoms (20%), abdominal pain (30%), headache (25%), and neurological symptoms (15%). These findings are in accordance with the study carried out by Loddenkemper *et al.* revealed that the most frequent symptoms of active disease are fever, anorexia or reduced appetite, weight loss, night sweats, anemia, and persistent cough.[23]

In this study, we analyzed 957 clinical samples using the GeneXpert MTB/RIF test to evaluate different types of specimens of patients who were suspected of having PTB and/or EPTB. Among positive cases, 72 (78.26%) were from pulmonary and 20 (21.73%) were from extrapulmonary samples. The highest number of positive cases was related to sputum in pulmonary and CSF in extrapulmonary samples. Our findings indicate that the GeneXpert MTB/RIF test performs well in detecting EPTB from different samples. In this way, Habous *et al.* found that the GeneXpert MTB/RIF assay has a high sensitivity (82.6%) and specificity (100%) for the detection of PTB and EPTB in nonrespiratory samples.[24] In addition, a study conducted by Mechal *et al.* also showed that the GeneXpert MTB/RIF test has a high sensitivity (78.8%) and specificity (90.3%) for the diagnosis of EPTB samples.[25]

CONCLUSION

The findings of this study illustrated the incidence of PTB and EPTB among patients admitted to the largest hospital complex in Iran. Modern molecular diagnostic techniques such as GeneXpert MTB/RIF are crucial parts of the WHO new TB control strategy. This diagnostic method could improve the management of TB patients through the rapid introduction of anti-TB treatment in a different types of specimens and controlling the disease by early contact tracing.

Ethical clearance
As mentioned in the setting subheading, no ethical permission for TB data was required for this study. Furthermore, the study was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.IKHC.REC.1398.141).

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Conflicts of interest
There are no conflicts of interest.

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