

Original Article



Identification of *Mycobacterium Tuberculosis* Complex in Clinical Specimens in Shahrekord County in Southwest Iran by Real-Time PCR

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Abstract

Introduction: According to the annual reports of the World Health Organization, infection with *Mycobacterium tuberculosis* complex (MTC) is a global and serious health threat. Therefore, this study aimed to identify MTC in various clinical specimens using the real-time polymerase chain reaction (RT-PCR) method.

Methods: This cross-sectional study investigated the presence of MTC in 178 clinical samples collected from different wards of Hajar and Kashani Hospitals in Shahrekord. The attendance of MTC was investigated after nucleic acid extraction and complementary DNA production using RT-PCR according to the manufacturer's instructions.

Results: Out of 178 participants, 92 (51.7%) were female, 86 (48.3%) were male, and most were older than 60. Moreover, the majority of specimens were bronchoalveolar lavage samples. Finally, MTC-positive cases were most frequently observed in participants under 40.

Conclusion: The findings of this study revealed a high frequency of MTC in patients under 40. Nonetheless, further studies and resources are needed to improve preventive measures against the transmission of tuberculosis infection.

Keywords: *Mycobacterium tuberculosis*, Clinical specimens, Real-time PCR, Shahrekord, Iran

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Introduction

Mycobacterium tuberculosis is among the deadliest pathogens that affect humans and are capable of evading innate and adaptive immune responses, leading to increased vulnerability to opportunistic infections (1, 2). The mycobacterial strains categorized in the *M. tuberculosis* complex (MTC) exhibit 99.9% similarity in their 16S rRNA sequences (3). In addition, the MTC comprises several species, including *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, and other genetically related strains (4, 5). While these species demonstrate a broad range of hosts, they are thought to have evolved from a shared ancestor (6). *M. tuberculosis* primarily infects humans, whereas *M. bovis* has a wider host range, particularly involving bovine sources. Other species, such as *M. africanum*, have also been isolated from patients in Africa and can lead to human tuberculosis (TB) (7). Currently, TB represents a global health crisis, with roughly 2 billion individuals (about one-third of the worldwide population) infected with *M. tuberculosis*. According to the World Health Organization (WHO), among the 10.4 million individuals affected by TB, 1.8 million succumbed to the disease in 2015 (8, 9). This

statistic highlights TB as one of the leading causes of mortality globally, particularly affecting low-income nations. Prompt identification of the organism is crucial for TB diagnosis, which can be performed using various molecular and traditional techniques. Lately, molecular assays have become a common approach for detecting *Mycobacterium* species in diverse samples, with the real-time polymerase chain reaction (RT-PCR) assay being noted for its accuracy and frequent application in laboratories. Compared to conventional PCR, the RT-PCR method boasts superior performance and sensitivity and can be directly applied to clinical samples (10). Consequently, it eliminates the need for organism culture growth, making it practical for identifying *Mycobacteria* that typically have a slow growth rate, frequently requiring an incubation period of 4–6 weeks. Reports indicate that although the incidence of TB in Iran decreased in 2010 relative to 1990, it has remained a significant public health concern. According to estimates for 2013, the infection rate in Iran was 21 cases per 100,000 individuals (11). Given Iran's geographical proximity to Afghanistan and Pakistan, regions with high TB prevalence, the disease poses particular challenges in this area (12). Hence, this

study aims to identify strains of the MTC in clinical samples collected from Kashani and Hajar Hospitals in Shahrekord, utilizing the RT-PCR method.

Materials and Methods

Processing of Clinical Specimens

This cross-sectional study was performed on 178 clinical samples of patients referred to Hajar and Kashani Hospitals in Shahrekord, Chaharmahal and Bakhtiari province (from November 2016 to November 2017). Clinical samples, including abscess, bronchoalveolar lavage (BAL), ascitic fluid, bone marrow, cerebral spinal fluid, peripheral blood, pericardial fluid, pleural fluid, serum, sputum, synovial fluid, and urine, were collected from different hospital wards.

Sampling and Handling

The handbook Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics was used to prepare clinical samples before molecular testing. This book was developed by the Federal Budget Institute of Science's Central Research Institute for Epidemiology of the Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow,.

Nucleic Acid Extraction, Complementary Deoxyribonucleic Acid Synthesis, and Real-Time Polymerase Chain Reaction

After DNA extraction of the samples using the RIBO-prep nucleic acid extraction kit (AmpliSens, Russia), the Nanodrop 2000 Spectrophotometer (Thermo Scientific, MA) measured the DNA concentration. Next, complementary DNA was synthesized by purified DNA. Finally, MTC nucleic acid was detected using AmpliSens® MTC-diff-FRT PCR Kit (InterLabService Ltd., Moscow, Russia), conforming to the manufacturer's instructions.

This kit is an in vitro nucleic acid amplification test for the differentiation of the DNA of MTC, including the human (*M. tuberculosis*), the bovine (*M. bovis*), and the vaccine (*M. bovis* BCG) strains in the clinical material. The transcription levels of STI-87 and 2- $\Delta\Delta C_t$ methods were utilized for internal control and determination of gene expression ratio, respectively. Ultimately, Rotor-Gene 6000 (Corbett Research, Sydney) was employed to perform RT-PCRs.

Statistical Analysis

Descriptive statistics were reported as numbers (%), and the association between each variable and positive pulmonary TB was examined using the chi-square or Fisher's exact test, where appropriate. All statistical analyses were performed using SPSS (version 21), and $P < 0.05$ was considered statistically significant.

Results

Sample Description

Of the 178 participants, 92 (51.7%) were female and 86 (48.3%) were male. The largest proportion of participants (42.7%) was older than 60, followed by 15.2% in the 51–60-year age group. Considering the type of sample, 127 (71.3%), 18 (10.1%), and 14 (7.9%) samples were BAL, pleural fluid, and cerebral spinal fluid, respectively (Table 1).

Mycobacterium Tuberculosis Complex and Characteristics

Of 178 screened subjects, only 20 (11.2%) were positively diagnosed with MTC. About 45% of the screened samples from subjects aged ≤ 40 years were positively diagnosed, significantly higher than the other age categories ($P = 0.004$). Chi-square test showed no significant association between gender and infection of TB, as the

Table 1. Sociodemographic Characteristics of Study Participants

Variable	Sub Group	N (%)	Variable	Sub Group	N (%)
Age	≤ 40	54 (30.3)	Gender	Male	86 (48.3)
	41-50	21 (11.8)		Female	92 (51.7)
	51-60	27 (15.2)	Test	Negative	158 (88.8)
	> 60	76 (42.7)		Positive	20 (11.2)
Sample type	Abscess	1 (0.6)	Hospital wards	Emergency	8 (4.5)
	Ascitic fluid	1 (0.6)		Heart	11 (6.2)
	Bronchoalveolar lavage	127 (71.3)		Infectious	14 (7.9)
	Bone marrow	2 (1.1)		ICU	7 (3.9)
	Peripheral blood	6 (3.4)		Medical emergency	6 (3.4)
	Cerebral spinal fluid	14 (7.9)		Men's internal	50 (28.1)
	Pericardial fluid	2 (1.1)		Orthopedic	1 (0.6)
	Pleural fluid	18 (10.1)		Men's surgery	3 (1.7)
	Serum	1 (0.6)		Men's urology	1 (0.6)
	Sputum	1 (0.6)		Nerves internal	3 (1.7)
	Synovial fluid	2 (1.1)		Pediatrics	2 (1.1)
	Urine	3 (1.7)		Women's internal	72 (40.4)

Note. ICU: Intensive care unit.

proportions of prevalence for males and females were 60% and 40%, respectively ($P=0.38$). Moreover, Fisher's exact test demonstrated no remarkable association between the sample type and hospital ward with TB infection ($P=0.95$, $P=0.17$, respectively, Table 2).

Discussion

Infection with MTC is a global health threat and remains a serious health problem according to the annual reports of the WHO. TB infects one-third of the world's population, and accurate diagnosis is decisive for preventing mortality and morbidity (13). It should be noted that rapid and accurate diagnostic methods can have an impressive impact on controlling and managing TB infection cases (14). Based on the results of a cross-sectional study conducted by Hassan Zadeh et al (15), of all assessed TB cases, 1.4% were wrongly diagnosed as TB patients, reaching 22.7% in the study of Lin et al (16). Furthermore, differentiating MTC from nontuberculous mycobacteria

(NTM) is a priority for infection control and antibiotic therapy (16).

Molecular nucleic acid amplification tests were recommended by the WHO to diagnose TB due to their greater accuracy than smear microscopy, especially in patients co-infected with human immunodeficiency virus (17). Despite extensive advances in molecular diagnostics, the ability to quickly detect MTB infection by PCR remains insufficient, and there is a possibility of false-negative reactions (18). Among the nucleic acid amplification tests, RT-PCR has several superiorities, such as better specificity and sensitivity, high speed, and low contamination risk, compared to conventional PCR (13, 14, 19). This study focused on identifying MTC in different suspected clinical specimens of Hajar and Kashani Hospitals, the centre of Chaharmahal and Bakhtiari province, using the RT-PCR method.

Based on our findings, the frequency of MTC cases in the study population was 11.2%, which conforms to the

Table 2. Association Between Participant Characteristics and TB Infection

Variable	Sub Group	Pulmonary Tuberculosis Status		P-Value
		Positive, n (%)	Negative, n (%)	
Age	≤40	9 (45.0)	45 (28.5)	0.004
	41-50	6 (30.0)	15 (9.5)	
	51-60	0 (0)	27 (17.1)	
	>60	5 (25.0)	71 (44.9)	
Gender	Male	12 (60.0)	74 (46.8)	0.38
	Female	8 (40.0)	84 (53.2)	
Sample type	Abscess	0 (0)	1 (0.006)	---
	Ascitic fluid	0 (0)	1 (0.006)	
	Bronchoalveolar lavage	18 (90.0)	109 (69.0)	
	Bone marrow	0 (0)	2 (1.3)	
	Peripheral blood	0 (0)	6 (3.8)	
	Cerebral spinal fluid	1 (5.0)	13 (8.2)	
	Pericardial fluid	0 (0)	2 (1.3)	
	Pleural fluid	1 (5.0)	17 (10.8)	
	Serum	0 (0)	1 (0.006)	
	Sputum	0 (0)	1 (0.006)	
	Synovial fluid	0 (0)	2 (1.3)	
	Urine	0 (0)	3 (1.9)	
Hospital wards	Emergency	0 (0)	8 (5.1)	---
	Heart	3 (15.0)	8 (5.1)	
	Infectious	3 (15.0)	11 (7.0)	
	ICU	0 (0)	7 (4.4)	
	Medical emergency	0 (0)	6 (3.8)	
	Men's internal	6 (30.0)	44 (27.8)	
	Orthopedic	0 (0)	1 (0.006)	
	Men's surgery	2 (10.0)	1 (0.006)	
	Men's urology	0 (0)	1 (0.006)	
	Nerves internal	0 (0)	3 (1.9)	
	Pediatrics	0 (0)	2 (1.3)	
	Women's internal	6 (30.0)	66 (41.8)	

Note. TB: Tuberculosis; ICU: Intensive care unit.

results of a study in Ethiopia. In this cross-sectional study, 275 samples were investigated using the culture method, and the results revealed that the overall magnitude of the MTC was 9.5% (20). In other studies conducted in Iran and Morocco, the frequency of MTC was reported to be 4.6% and 32%, respectively. In our research, most positive cases were reported from BAL (Table 2), while in the study by Fakhreddine et al, most positive cases were reported from bronchial aspiration samples (21, 22). This discrepancy may be due to differences in the study design and the applied methods (20).

In this study, most subjects were over 60 years of age (Table 1). However, the results showed that 45% of subjects under 40 tested positive (Table 2). Although there seems to be a relation between age and TB infection, no specific age range has been determined (23). In a 10-year survey in northern Iran on patients with extrapulmonary TB, being younger was introduced as an important risk factor for the disease (24). The results of studies in Iran demonstrated the highest number of TB patients in Shiraz, Yazd, and Gorgan for the age ranges of 51–75, 26–50, and 15–24 years, respectively (25). Discrepancies in the results of studies may be due to differences in patients' age grouping (23).

Our results further revealed that the number of studied women was higher than that of men (Table 1), which is consistent with the results of Khosravi et al (26). In our study, more MTC-positive cases were observed in men than women (Table 2). The survey results in 175 suspected TB patients in India (27) are in line with our results (70.8% men vs. 29.1% women). According to the literature, the different prevalence of TB has been described in other populations of males and females, and increasing age exacerbates this issue (28, 29). Based on global reports, the male-to-female ratio of TB diagnosis is estimated to be around 1.6:1 (30). Various reasons have been given to explain this diversity, among which are biological differences (28), smoking, and differences in access to health and care facilities (30). The findings of a study of male and female mice represented that male mice were more susceptible to TB than female mice due to differences in sex hormones (31). Despite all these, no significant relationship was observed between patients' gender and MTC infection, which corroborates the results of Kahasae et al (20). Among the presumptive samples of MTC, the BAL sample had the highest frequency compared to other samples, which matches the results of the study by Arora et al (27). However, in other studies, sputum (32–35) and lymph node (26) samples were reported as predominant specimens. *Returning to the subject*, no statistically significant relationship was found in this study between the type of clinical sample and the hospital ward with TB infection.

Due to the prominent role of healthcare workers, especially nurses, in the treatment and care of patients with infectious diseases such as TB, this group is at a higher risk of contracting TB than the general population (36). Accordingly, examining the prevalence of TB patients in

different hospital wards can provide decision-makers with useful information to implement preventive measures (e.g., installing appropriate ventilation) to prevent the transmission of infection. In this regard, studies on a larger sample size than our study can be useful. In our study, there was no significant difference between the frequency of MTC and gender ($P=0.38$), sample type ($P=0.95$), and hospital wards ($P=0.17$). Conversely, there results (Table 2) revealed a significant difference between the frequency of MTC and patients' age ($P=0.004$).

In addition, investigating the relationship between TB and the age of patients can help design new vaccines in the near future (37). In a study by Sadr et al, direct detection of *M. tuberculosis* using RT-PCR was performed on 32 sputum samples (including 15 smear-positive and 17 smear-negative samples) and 53 sputum samples (29 smear-positive and culture-negative samples and 24 smear-negative and culture-positive samples) from patients suspected of TB from two health centers, respectively. Of the 17 smear-negative and culture-negative samples, 4 were positive by RT-PCR. In 9 samples (of the 15 smear-positive and culture-positive samples), RT-PCR did not detect *M. tuberculosis*. (38). Unlike this study, where phenotypic and genotypic methods were only performed on sputum samples, the culture of samples was not performed in our study. However, in both studies, RT-PCR was used as an effective and rapid method for identifying MTC. In a study by Rahman et al, the frequency of MTB and NTM was investigated in 782 clinical samples from patients suspected of having TB by RT-PCR. Among all the analyzed samples, 49 and 74 were positive for MTB and NTM, respectively (39). In line with our study, which was conducted on various clinical samples, Rahman et al also focused on various clinical samples, such as sputum, bronchial lavages, body fluids, tissues, needle aspirates, and swabs. The present study was not without limitations. Due to limited access to the information of the participants, it was impossible to include other risk factors, such as smoking, alcohol consumption, and human immunodeficiency virus co-infection status in the study.

Conclusion

Our findings revealed that most isolated MTC were from BAL samples. Additionally, a high frequency of MTC was observed in the age group of ≤ 40 years. Our results further indicated that RT-PCR is a highly effective, rapid, and accurate method for diagnosing MTC. Finally, it is recommended that further large-scale studies with larger sample sizes be conducted to increase the validity of the results.

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Authors' Contribution

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Competing Interests

The authors have no conflict of interests to declare regarding the study and the preparation of the article.

Ethical Approval

The study protocols received ethical approval from the Ethics Committee of Shahrekord University of Medical Sciences (ethical code IR.SKUMS.REC.1398.061).

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