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The relative frequency of *Mycobacterium tuberculosis* and *Mycobacterium avium* infections in HIV positive patients, Ahvaz, Iran

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ABSTRACT

Objective: To estimate the prevalence of *Mycobacterium tuberculosis* (*M. tuberculosis*) and *Mycobacterium avium (M. avium)* infections in HIV-positive patients suspected to have pulmonary and extrapulmonary mycobacterial co-infection using PCR technique. **Methods:** Totally 50 samples comprising sputum, pleural fluid and CSF taken from HIV positive patients suspected to have mycobacterial infection, were processed. The demographic information and results of acid fast staining and culture were recorded for each patient. The PCR for detecting of *M. tuberculosis* comprised of specific primers targeting IS6110 gene sequence. For detecting of *M. avium*, PCR with primers that amplifies the mig gene were used. **Results:** From 50 samples processed, 45 were sputum (90%), 3 pleural fluid (6%) and 2 CSF (4%). In total, 8 (16%) were culture positive, 7 had positive acid fast staining (14 %) and 13 samples (26%) were positive using PCR technique. All the positive samples were sputum and belonged to patients with pulmonary infection. Of these, 9 were positive for *M. tuberculosis* (69.2%) and 4 were identified as *M. avium* (30.8%), which 2 out of 13 positive samples showed mixed infections by both mycobacteria. **Conclusions:** The PCR shows the highest detection rate (26%) of mycobacteria compared with culture and acid fast staining. The majority of infections were with *M. tuberculosis* (18%) and this shows the importance of this mycobacterial co-infection in HIV positive patients in the region of study.

1. Introduction

The genus mycobacteria comprises two well known pathogenic species of *Mycobacterium tuberculosis* (*M. tuberculosis*) (MTB) and *Mycobacterium leprae* (*M. leprae*) and majority of nontuberculous mycobacteria (NTM), some with potential capacity of causing disease such as *Mycobacterium avium* (*M. avium*) complex (MAC).

The conditions lead to immunodeficiency, make the individuals predispose to mycobacterial diseases[1]. The human immunodeficiency virus (HIV) pandemic has increased the burden of tuberculosis (TB), so infections with mycobacteria are frequent in individuals with AIDS[2]. MTB and HIV infections are two major public health problems in many parts of the world, particularly in many developing counties[3,4]. TB is the most common opportunistic disease and cause of the death for those infected with HIV[5]. Similarly, HIV infection is one of the most important risk factors that promotes progression of latent TB infection to active TB disease in people with MTB infection[6,7]. The lifetime risk of tuberculosis in immune-competent persons is 5% to 10%, but in HIV positive individuals, there is a 5% to 15% annual risk of developing active TB disease[6,8]. After AIDS epidemic, NTM especially *M. avium* complex, have increasingly been reported in these severely immunocompromised patients[9,10]. Not infrequently, such patients will also have multiple infections with MTB, *M. avium* and others[11,12]. These infections are usually disseminated and have been shown to contribute

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significantly to the morbidity of AIDS patients[13]. The World Health Organization (WHO) estimates that there are almost 13.7 million people living with tuberculosis and that the disease kills more young people and adults than any other infectious disease in the world. A total of about 1.77 million people died of tuberculosis in 2007 including 456 000 patients infected with HIV[14].

The incidence of TB in patients with HIV infection is nearly 500 times higher than in the general population[12]. Demonstration of acid-fast bacilli (AFB) is the most commonly practiced method of diagnosing TB in the world over. However, except lymph node and pulmonary TB, obtaining an appropriate clinical sample is a difficult task. Moreover, the problem is that a large proportion of patients with pulmonary TB co-infected with HIV may not be excreting AFB in their sputum[15]. So application of more sophisticated techniques such as polymerase chain reaction (PCR) will provide more accurate information of frequency of mycobacterial infections in AIDS patients.

To our knowledge, there are a few retrospective surveys in our country about the prevalence of mycobacterial infections in AIDS patients. These are limited to reports of the AFB results from tuberculosis reference centers and the subject has not been systematically investigated. Therefore, in the present study, we aimed to estimate the prevalence of MTB and *M. avium* infections in HIV-positive patients suspected to have pulmonary and extrapulmonary mycobacterial co-infection using PCR technique. We believe that the results of this study can provide useful information regarding the epidemiology of these infections in the region of study.

2. Materials and methods

2.1. Study population and setting

In total 50 samples were taken from HIV positive patients admitted to the Infectious Diseases ward of Razi teaching hospital of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Southwestern Iran, from April 2010 to March 2011. All the patients were suspected of having mycobacterial infection. The study was approved by Institutional Ethics Committee after submission of preliminary proposal of the study.

The demographic information including age, sex and underlying disorders were collected from each patient and HIV infection was diagnosed on the basis of enzyme-linked immunosorbent assay (ELISA) and western blotting. Clinical and radiographic features were the basis of suspicion of tuberculosis in patients. Patients undergone anti mycobacterial therapy prior to admission, were excluded from the study. Various samples including sputum, pleural fluid and CSF were collected and were immediately transferred to the Infectious and Tropical Diseases Research Center of the University, where the initial processing and DNA extraction performed on the samples, using standard extraction and purification kit (Cinnagen Co., Tehran, Iran).

2.2. PCR assay for M. tuberculosis

The amplification was performed as previously described using a set of *Mycobacterium tuberculosis*-specific primers of IS1 and IS2, which amplifies a 123 bp of the IS6110-gene fragment[16]. In brief, the reaction volume (25 μL) composed of 50 mmol KCl, 10 mmol Tris-HCl (pH 8.3), 1.5 mmol $MgCl_2$, 0.2 mmol of each deoxynucleotide triphosphate, 0.5 μmol of each primer, 1 units of *Taq* polymerase (Cinnagen Co., Tehran,Iran), 14 μL of sterile distilled water and 5 μL of processed DNA sample. Amplification was done with a four-stage protocol on a Techgene thermocycler (UK) using the program as below:

An initial denaturation of 94 ℃ for 5 min followed by 30 cycles of 94 ℃ for 1 min, 68 ℃ for 1 min and 72 ℃ for 1 min and final extension of 72 ℃ for 5 min. For analysis of the amplified products, they were loaded on a 2% agarose gel containing 0.5 mg/mL ethidium bromide in electrophoresis and photographed by using the gel documentation system.

2.3. PCR assay for M. avium complex

The primers used were those reported earlier as mig upper, 5′-CCC GTT CAA CGT CAA CTT CC-3′; and mig lower, 5′-GGG CTC GCC GGT CAT CAG GT-3′. These primers amplify a 737 bp fragment within the coding region of the gene[17]. DNA amplification was performed in the same manner as described above for MTB, using a 25 μL reaction mixture containing all the buffer components and enzyme, plus 10 pmol of each primer and 1 ng DNA template. The cycling conditions were as follows: initial denaturation at 95 ℃ for 5 min and 30 cycles of 30 s of denaturation at 95 ℃ and 2 min of annealing and extension at 68 ℃, followed by a 5 min final extension at 72 ℃. The PCR products were loaded on a 2% agarose gel containing 0.5 mg/mL ethidium bromide in electrophoresis, with a 1 kb DNA ladder used for size determination, and photographed by using the gel documentation system.

3. Results

The study patients comprised of 45 male (90%) and 5 female (10%). The patients′ age ranges were 22 to 36 with the mean of 30. The majority of patients (74%), were drug user, with 34 (68%) male and 3 (6%) female and the rest were HIV positive non-drug users (26%). Among the patients, there were 8 HCV positive (16%), 16 HBV positive (32%) and 8 with

HCV and HBV infection simultaneously (16%).

From 50 samples processed, 45 were sputum (90%), 3 pleural fluid (6%) and 2 CSF (4%). In total, 8 (16%) were culture positive, 7 had positive acid fast staining (14 %) and 13 samples (26%) were positive using PCR technique. Figure 1 represents the PCR amplification for samples positive for *M. avium* yieled a 373 bp fragment. All the positive samples were sputum and belonged to patients with pulmonary infection. Of these, 9 were positive for MTB (69.2%) and 4 were identified as *M. avium* (30.8%), which 2 out of 13 positive samples showed mixed infections by both mycobacteria.

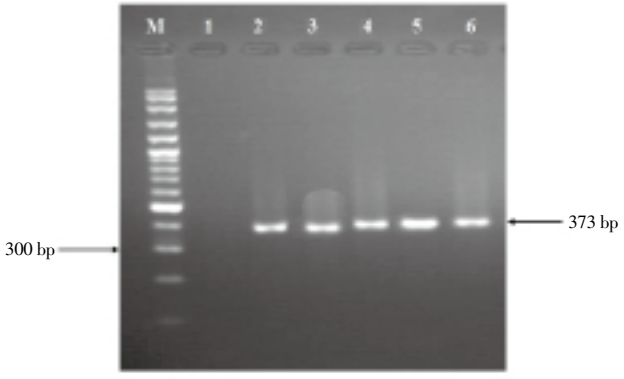

Figure 1. Agarose gel electrophoresis of PCR assay for the identification of mig gene of *M. avium*.
M: DNA size marker; 1: negative control; 2: Positive control; 3-6 positive isolates.

All the patients infected with MTB were male drug users (18%), 4 were positive for HCV (8%), 1 was HBV positive (2%), and 2 were simultanous HCV and HBV positive (4%). Among 4 patients positive for *M. avium*, 2 were drug user (50%), 1 was infected with HCV (25%) and 1 was infected with both HCV and HBV (25%). The other 2 patients with mixed mycobacterial infections were nor drug user, neither had viral infection.

4. Discussion

The increasing rate of HIV infection in many countries has had an impact on TB epidemiology. While TB prevalence has remained stable, TB incidence continues to rise, especially in countries most severely affected by the HIV epidemic as well as those facing political turmoil, migration, poverty and unemployment and where intravenous drug abuse is rampant[7].

In our study the rate of mycobacterial infection among HIV positive patients was 26% by PCR technique. This rate was reported as 38.5% in one study from India, a country with high burden of AIDS[18]. Obviously we could expect a higher rate of HIV-TB co-infection in such studies[12].

The majority (90%) of patients entered our study were male. The findings were in agreement with other studies showing the men as predominance of HIV positive patients[12,19]. However in one study, the male to female ratio was reported as 1:1.2 among 120 HIV-infected patients in northern Tanzania[20-26]. This reflects that the rate of gender-dependant HIV infection is different in various geographical parts. Besides, our HIV cases were predominantly drug users and this shows the high prevalence of HIV among drug users, as previously reported from other parts of the world[1,13,27].

Most of our suspected HIV-positive patients had pulmonary involvement, comprised all of positive patients for mycobacterial infections. Of course the rate of isolated mycobacterial infection was lower compared to reports from other parts of the world[20]. Despite the selection of preferably HIV-positive patients before antibiotic therapy, a few included patients, probably had taken some medications with no clear history of antibiotic therapy in their first admission. This may explains the lower rate of mycobacterial isolation in culture and disagreement of PCR results (26%) with culture (16%) and acid fast staining (14%) in present study. Besides, the lower rate of AFB by staining, may be due to other respiratory tract infections common in HIV positive patients as has been explained in a recent study[28]. Even in the study of Palmieri *et al*, they reported negative AFB staining among their HIV positive patients co-infected with TB[29].

We could not find any extrapulmonary mycobacterial infection among the screened HIV positive patients. The lower sample size in present study due to restriction of duration of sampling and difficulties in sample collection from patients, may be an explanation for lack of positive extrapulmonay co-infection in present study.

MTB was identified in the rate of 69.2% among positive samples and this shows the higher involvement of MTB in such patients compared to MAC infection (30.8%) in our study. For being ensure that all *M. avium* cases are identified by PCR, we used primers based on the mig gene. The mig gene is the only well-characterized virulence factor identified in MAC as reported earlier[17]. In a similar study, various molecular methods were used to evaluate organisms in the MAC, in patients who are immune-compromised, such as those with AIDS. By using PCR assay, which amplifies the mig gene, they showed that 85% of the isolates from HIV-positive patients were *M. avium*. They identified 92 *M. avium* isolates, 75 of which were from HIV-positive patients[11].

There were some patients with infections of HBV and HCV in our study which is common in HIV-positive patients as earlier stated[28]. However due to low rate of such co-infection, we could not find a significant correlation between these viral infections and mycobacterial disease in present study.

In conclusion, the PCR showed the highest detection rate (26%) of mycobacteria compared with culture and acid fast staining. The majority of infections were with MTB (18%) and this shows the importance of this mycobacterial co-infection in HIV positive patients in the region of study.

Conflict of interest statement

We declare that we have no conflict of interest.

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