Epidemiology and Clinical Characteristic of Mycobacterial Infections in Human Immunodeficiency Virus-Infected Patients in Siriraj Hospital

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Background: *Mycobacterium tuberculosis (MTB) and non-tuberculous mycobacteria (NTM) infections are major health problems in Human Immunodeficiency Virus (HIV)-infected patients. Most previous studies focused mainly on tuberculosis (TB) rather than NTM infections.*

Objective: *To determine clinical features of mycobacterial infections, from both MTB and NTM, in HIV-infected patients in Siriraj Hospital.*

Material and Method: *A retrospective study of adult HIV-infected patients in Infectious Disease Clinic, Siriraj Hospital, was conducted. Clinical characteristics and factors associated with mycobacterial infections were analyzed.*

Results: *Of 253 patients enrolled, 65 (25.7%) developed mycobacterial infections, in which 56 patients (86%) were tuberculosis (TB), whereas NTM was diagnosed in 9 (14%). Of these 65 patients, 45 (69.2%) were culture-proven, 14 (21.6%) were diagnosed TB by positive acid-fast bacilli smears and 6 (9.2%) were diagnosed TB by clinical response to anti-tuberculosis treatment only. Among culture-positive patients, MTB was found in 36 (80%) and NTM in 9 (20%), in which Mycobacterium avium complex (MAC) was the most common among NTM isolates (n = 5), followed by unidentifiable slowly-growing mycobacteria (n = 3) and M. fortuitum (n = 1). Among patients with MTB infection, 58.3% were disseminated. The most affected organ in patients with mycobacterial infections was lung (75%), followed by lymph node (66.7%). Factors associated with mycobacterial infections included male gender (64.6% vs. 54.3%; p = 0.026), higher HIV Viral load ($1.04x10^6$ vs. $0.3x10^6$; p = 0.004), lower hematocrit (32.7% vs. 35.3%; p = 0.032) and higher alkaline phosphatase (ALP) (146 U/L vs. 107 U/L; p = 0.032). In contrast, Pneumocystis pneumonia (PCP) was negatively associated with mycobacterial infections in HIV-infected individuals (28.8% vs. 10.9%; p = 0.004). Favorable treatment response was 86.1% and 77.8% for MTB and NTM infection, respectively, and the 6-month mortality rates were 2.78% and 11.1% for MTB and NTM infection, respectively. In patients who received treatment for TB, 22.2% had hepatitis, 13.9% had drug allergy and 8.3% had immune reconstitution inflammatory syndrome.*

Conclusion: *Disseminated infection is the most common form of mycobacterial infection in HIV-infected patients, resulting in anemia and high ALP levels. PCP was negatively associated with mycobacterial infection. MAC is the most common of the NTM isolates in HIV-infected patients.*

Keywords: *HIV infection, Mycobactria, Tuberculosis, AIDS*

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Mycobacterium tuberculosis (MTB) infection is one of the most important medical problems in Thailand. Prevalence of tuberculosis in Thailand in 2010 is 189 per 100,000 persons of population, which ranked 7 out of 22 countries around the world that had highest incidence of tuberculosis[1]. Previous reports found that 25-30% of Human Immunodeficiency Virus (HIV)-infected patients had tuberculosis and exhibited a higher rate of multi-drug resistant tuberculosis (MDR-TB) compared with those without HIV[2]. Not only tuberculosis, but also non-tuberculous mycobacterial (NTM) diseases are also one of the difficult-to-treat opportunistic infections in HIV-infected patients. A study of NTM infections in Thailand during 2000-2003 revealed that 69% of patients with NTM infections had HIV disease and the most common pathogen was *Mycobacterium avium* complex or MAC (48%), followed by *Mycobacterium kansasii* (19%) and rapidly growing mycobacteria (16%). Almost all of cases were localized infections

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that were confined in the lungs and had a high mortality rate (about 45%) in HIV patient, which was four-fold higher than in those without HIV infection[3]. Although the rate of NTM isolation in patients suspected TB was low (1.2-7%), we should be aware when the patients do not respond to anti-tuberculosis treatment[4-6]. Moreover, a study in 2011 from China reported an incredibly high prevalence of TB and NTM infection rate in HIV patient, which was 53% and 47%, respectively[7]. Studies of the prevalence and characteristics of MTB and NTM among HIV-infected patients in Thailand are limited[8]. The present study, thus, aimed to determine the epidemiology, clinical manifestations, treatment, and outcomes in HIV-infected patients who had MTB or NTM infections in a tertiary care university hospital in Thailand.

Material and Method

Population and study design

A retrospective study was conducted in adult HIV-infected patients followed-up at the Infectious Disease Clinic, Siriraj Hospital, Bangkok, Thailand, between January 2002 and December 2011. Retrospective analysis was performed in all patients followed up at the Infectious Disease Clinic who had sufficient data, by a single investigator who collected the data from medical records using case record forms. Therefore, a total of 253 patients were enrolled.

Data collection

Demographic data, co-morbidity, co-infection and previous opportunistic infection, initial laboratory results were collected. *Mycobacterium* infections were classified as MTB or as NTM infection that was identified by microbiological tests. Each case was reviewed for clinical data which included organ involvement, presenting chest X-ray, laboratory findings on the day of diagnosis, treatment and outcomes.

Microbiological data were reviewed. Infection was defined as isolation of an organism that was associated with symptoms or diseases. In cases of culture-unknown or culture-negative specimens, clinical assessment was justified the definition as MTB based on response to treatment regimens.

A diagnosis of NTM pulmonary infections was based on the American Thoracic Society (ATS) criteria(9). Other NTM infections were defined based on the compatible clinical features accompanying positive culture for NTM from the specimens obtained from the involved organ in the absence of any other isolated pathogens. Disseminated infection was defined as the presence of one of the following: 1) multiple sites of cutaneous abscesses, 2) involvement of two or more non-contiguous extrapulmonary sites, 3) positive blood or bone marrow culture, or 4) clinical evidence of deep infection.

Eligibility criteria

Inclusion criteria were HIV-infected patients aged 18 years or older who were diagnosed mycobacterial infections and followed-up at Siriraj Hospital.

Individuals were excluded if they had incomplete data or medical records, started treatment and/or diagnosed mycobacterial infection at other hospitals, or indicated inconclusive evidence of MTB or NTM infection, such as simultaneous empirical treatment for both MTB and NTM.

Statistical analysis

Continuous data are presented as mean ± SD, whereas categorical data are presented as frequency and percentage. Chi-square test or Fisher's exact test was used for comparison of categorical variables between the two groups. For continuous variables, independent t-test was used for comparison of normal distributions between the two groups. For non-normal distributions, Mann-Whitney test was used for analysis.

Multivariate analysis was performed by the multiple logistic regression using Enter method for all variables with p-value <0.2 in the univariate analysis model. The independency among variables was tested to see if there was a correlation or association among them.

All statistical analyses were performed through the SPSS software (SPSS Statistics package version 16.0). A 2-sided p-value <0.05 was considered to be statistically significant.

Results

Of the 253 patients enrolled, 65 (25.7%) had mycobacterial infection, of which tuberculosis was diagnosed in 56 cases (86%), whereas NTM infection was found in 9 cases (14%). MAC was the most common NTM (n = 5) followed by other slowly growing mycobacteria (n = 3) and *M. fortuitum* (n = 1). Of 65 patients, 45 (69.2%) were culture-proven, of which 36 patients were infected with MTB. Fourteen patients (21.6%) were diagnosed TB based solely on positive acid-fast bacilli, which were responsive to anti-tuberculosis drugs, and 6 patients (9.2%) were

diagnosed by clinical response to anti-tuberculosis treatment only. Demographic data and baseline characteristics of all patients and comparison between those with and without mycobacterial co-infection are shown in Table 1. The mean initial CD_4 count was 93.8 (7.43%) for TB and 56.4 (6.1%) for NTM infection. Factors that were significantly associated with mycobacterial infection included male gender, anemia, higher alkaline phosphatase (ALP) level, and higher HIV viral load. These factors were still statistically significant in multivariate analysis (Table 2). In contrast, previous *Pneumocystis* pneumonia (PCP) was negatively associated with patients who had mycobacterial co-infection.

Clinical manifestations of mycobacterial infections

Most patients of both MTB and NTM infections presented with disseminated infection, 58.7% and 77.8%, respectively. All cases of MAC and *M. fortuitum* infection were disseminated infection, but only one case of slowly growing mycobacterium infection manifested with disseminated form; the other two patients had localized infection in the lungs. Localized infection with MTB (n = 36) mainly involved lungs, which was the most common site of infection (75%), followed by lymph nodes (66.7%), and the central nervous system (8.3%). Fourteen percent of mycobacterial infection cases revealed positive blood culture. All NTM cases had pulmonary

Table 1. Baseline characteristics of HIV-infected patients with and without mycobacterial infections

Parameter	Mycobacterial co-infection		*p*-value
	Yes (n = 65)	No (n = 188)	
Mean age ± SD (year)	39.17±10.9	38.74±10.6	0.946
Male, n (%)	42 (64.6)	102 (54.3)	0.026*
Co-morbidity, n (%)	29 (15.7)	8 (12.7)	0.567
Co-infection, n (%)			
Hepatitis B	9 (5.2)	5 (8.2)	0.397
Hepatitis C	11 (6.7)	7 (11.7)	0.220
Syphilis	7 (4.3)	1 (1.7)	0.362
Opportunistic infections, n (%)			
Pneumocystis pneumonia	7 (10.9)	53 (28.8)	0.004*
Cryptococcosis	6 (9.4)	15 (8.2)	0.771
Salmonellosis	3 (4.7)	3 (1.6)	0.173
Cytomegalovirus diseases	6 (9.4)	14 (7.6)	0.647
Laboratory data (mean)			
CD_4 counts (% CD_4)	93.8 (7.43)	90.7 (6.34)	0.715
HIV viral load (copies/mm^3)	$1.04x10^6$	$0.30x10^6$	0.004*
Hematocrit (%)	32.7	35.3	0.032*
White blood cell counts (cells/mm^3)	5,770	5,881	0.783
Platelet (cells/mm^3)	267,353	259,683	0.613
Aspartate transaminase (U/L)	56	41.7	0.559
Alanine transaminase (U/L)	54.6	29.7	0.091
Alkaline phosphatase (U/L)	146	107	0.032*
Creatinine (mg/dL)	0.815	0.869	0.259

* *p*-value <0.05

Table 2. Multivariate analysis of risk factors associated with mycobacterial infections in HIV-infected patients

Factors	Odd ratio (95% confidence interval)		*p*-value
	Crude	Adjusted	
Low hematocrit (per 1 percent)	1.08 (1.02-1.14)	1.07 (1.01-1.15)	0.032
High alkaline phosphatase (per 1 U/L)	1.00 (1.00-1.01)	1.01 (1.00-1.01)	0.032
Male	1.54 (0.86-2.76)	2.35 (1.11-5.00)	0.026
History of *Pneumocystis* pneumonia	0.03 (0.13-0.71)	0.15 (0.05-0.43)	0.001

infection, followed by lymph node (33%), blood (33%), and CNS infection (11%) as shown in Table 3. Alveolar and reticulonodular infiltrations were the most common findings in both MTB and NTM infections as shown in Table 4. Mean durations of MTB and NTM treatment were 10.2 and 12.8 months, respectively.

Timing of mycobacterial infection and highly active antiretroviral therapy (HAART)

Approximately 80% (n = 45) of MTB infection were diagnosed at the same time of HIV infection, but 67% of patients with NTM infection were diagnosed after HIV diagnosis and the mean time to diagnosis was 36 months. HAART was initiated after treatment for MTB and NTM for 8 weeks and 12 weeks, respectively.

HAART during treatment of mycobacterial infection

Patients with either MTB or NTM co-infected with HIV were mostly treated with non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens and the most common NNRTI used was efavirenz (75.4% and 55.5% in MTB and NTM infection, respectively). The nucleoside reverse transcriptase inhibitor (NRTI) that was mostly used in the regimens was lamivudine (95.4% in MTB group and 89% in NTM group). Others NRTIs used in MTB group were stavudine (44.6%), tenofovir (38.9%) and zidovudine (18.5%), and in the NTM group they were tenofovir (55.6%) and stavudine (55.6%).

Antimycobacterial drug susceptibility

Almost all *M. tuberculosis* isolates were susceptible to all first-line drugs with only one case exhibiting multi-drug resistance. Isoniazid had a highest rate of resistance (11.1%). NTM tended to be more resistant to rifampicin (80%), ethambutol (60%), amikacin (66.7%) and ciprofloxacin (83.3%), but only 1 case showed resistance to clarithromycin.

Outcomes of treatment

Clinical response in MTB and NTM infection was 86.1% and 77.8%, respectively. The 6-months mortality was 2.8% and 11.1% for MTB and NTM infection, respectively. In patients who received treatment for TB, 22.2% had hepatitis (elevation of AST or ALT more than 5 times), 13.9% had drug allergy and 8.3% had immune reconstitution inflammatory syndrome (IRIS), which occurred around 7 weeks after initiation of antiretroviral treatment.

Table 3. Organ involvement of mycobacterial infections

Organ involvement	Mycobacterial infection, n (%)	
	MTB* (n = 36)	NTM (n = 9)
Pulmonary	27 (75.0)	9 (100)
Lymph node	24 (66.7)	3 (33.0)
Blood	5 (13.8)	3 (33.0)
Central nervous system	3 (8.3)	1 (11.0)
Genitourinary tract	3 (8.3)	0
Colon	2 (5.6)	0
Liver	1 (2.8)	0
Bone and joints	1 (2.8)	0
Pericardium	1 (2.8)	0
Bone marrow	1 (2.8)	0

MTB = *Mycobacterium tuberculosis*; NTM = non-tuberculous mycobacteria

* Include only culture positive MTB infection

Table 4. Radiological findings in mycobacterial pulmonary infection

Chest X-ray findings	Mycobacterial infection, n (%)	
	MTB* (n = 33)	NTM (n = 9)
Alveolar	15 (45.5)	3 (33.3)
Reticulonodular	13 (39.4)	3 (33.3)
Normal	3 (9.1)	1 (11.1)
Pleural effusion	2 (6.1)	1 (11.1)
Cavitary lesion	0	1 (11.1)

* Include only pulmonary MTB infection

There was only one case of NTM that had hepatitis and it eventually had a mortality outcome.

Discussion

The overall rate of mycobacterial infection in our study was 25.7% with the rate of NTM infection in HIV-infected patients of 3.6%, which was similar to recent studies of NTM infections in Southeast Asia[2,10]. These findings were in contrast to a previous study from China that had very high prevalence of MTB and NTM infections in AIDS patients, 53% and 47%, respectively[7]. The difference between these studies may be based on the fact that the Chinese population had a prevalence of TB 367 per 100,000 persons, which was 2-fold higher than that of Thailand. NTM infection rates vary from countries to countries and this could be due to the difference in epidemiological data of each country. However, the differences in epidemiology in these reports may be

due to different definitions in diagnosis, HIV care and follow-up period. Most of patients who had mycobacterium/HIV co-infection were middle-aged males without co-morbidity, similar to other reports[7,8]. Patients with mycobacterial infection were likely to have anemia and high levels of ALP. This may be indicated of bone marrow and hepatic infiltrative lesions representative of disseminated infection, which was mostly seen in MAC infection[9,10]. In the present study, approximately 80% of TB patients were newly diagnosed HIV infection with a low CD_4 count (less than 100 cells/mm^3). Once the patients received TB and HIV care, co-trimoxazole prophylaxis for PCP was routinely given to all patients. Therefore, as revealed in our results, PCP was shown to be negatively associated with mycobacterial infections. The negative association between PCP and TB in HIV-infected patients was unlikely to be caused by the effect of HAART as the CD_4 counts of patients with and without mycobacterial infections are similar. Radiographic findings between NTM and MTB were indifferent. The most common presentations were alveolar and reticulonodular patterns that were consistent with previous study[8]. The onset of infection in MTB and NTM was different. MTB infection was mostly developed and diagnosed at the same time as HIV, but NTM was detected about 3 years after the diagnosis of HIV. These findings provided a clue for diagnosis when mycobacterial infection is suspected. The response rate of mycobacterial treatment in our study was about 80-90% and only one patient had MDR-TB, which was inconsistent with previous studies that showed a very high mortality rate of 58.7% and higher MDR-TB[7,8]. The higher mortality in the previous study in 2002 in Thailand may be due to the limited access of HIV-infected patients had to receive HAART during that time. The major complication after anti-mycobacterial treatment that was of high concern were drug-induced hepatitis and IRIS. Our study demonstrated that 22% of MTB infection had drug-induced hepatitis and the earliest period of elevated AST and ALT was at about 2 weeks (data not shown). The onset of TB IRIS was about 7 weeks after ARV treatment. This information may guide clinicians to greater awareness of these complications within that period.

In conclusion, the prevalence of mycobacterial infections among HIV infected patients was higher than in normal hosts. MTB and MAC infections usually present with disseminated infection. Factors associated with mycobacterial infections include low CD_4 counts (<50 cell/mm^3), anemia and high ALP.

What is already known on this topic?

Mycobacterial infection is prevalent in HIV-infected individuals and *Mycobacterium avium* complex is the most common causative organism among non-tuberculous mycobacteria.

What this study adds?

Male gender and high HIV viral load were positively associated with mycobacterial infection in Thai HIV-infected patients.

Pneumocystis pneumonia was negatively associated with mycobacterial infection in Thai HIV-infected patients.

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Potential conflicts of interest

None.

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ลักษณะทางคลินิกของการติดเชื้อมัยโคแบคทีเรียในผู้ป่วยติดเชื้อเอชไอวีในโรงพยาบาลศิริราช

เมธี ชยะกุลคีรี, ธีรวัฒน์ นาคสงวน

ภูมิหลัง: *การติดเชื้อวัณโรคและเชื้อกลุ่มมัยโคแบคทีเรียที่ไม่ใช่วัณโรค เป็นปัญหาทางสาธารณสุขที่สำคัญโดยเฉพาะอย่างยิ่งในผู้ป่วยติดเชื้อเอชไอวี การศึกษาก่อนหน้านี้ส่วนใหญ่เป็นการศึกษาเกี่ยวกับเชื้อวัณโรค ไม่ค่อยมีการศึกษาถึงเชื้อกลุ่มมัยโคแบคทีเรียที่ไม่ใช่วัณโรคมากนัก*

วัตถุประสงค์: *เพื่อศึกษาลักษณะทางคลินิกของวัณโรคและการติดเชื้อกลุ่มมัยโคแบคทีเรียที่ไม่ใช่วัณโรค รวมไปถึงผลการรักษาและผลแทรกซ้อนข้างเคียงต่าง ๆ ในผู้ป่วยติดเชื้อเอชไอวีในโรงพยาบาลศิริราช*

วัสดุและวิธีการ: *เป็นการศึกษาแบบย้อนหลังโดยเก็บข้อมูลจากผู้ป่วยติดเชื้อเอชไอวี ในคลินิกโรคติดเชื้อภาควิชา อายุรศาสตร์ โรงพยาบาลศิริราช วิเคราะห์อาการแสดงทางคลินิกและปัจจัยต่าง ๆ ที่มีผลต่อการวินิจฉัยและรักษาวัณโรคและเชื้อมัยโคแบคทีเรียที่ไม่ใช่วัณโรค*

ผลการศึกษา: *ผู้ป่วยทั้งหมดที่ได้รับการเก็บข้อมูลจำนวน 253 ราย พบว่ามีการเชื้อมัยโคแบคทีเรีย 65 ราย (ร้อยละ 25.7) ในจำนวนนี้เป็นเชื้อวัณโรค 56 ราย (ร้อยละ 86) และเชื้อกลุ่มมัยโคแบคทีเรียที่ไม่ใช่วัณโรค 9 ราย (ร้อยละ 14) ในกลุ่มนี้วินิจฉัยจากการเพาะเชื้อมัยโคแบคทีเรีย 45 ราย (ร้อยละ 69.2) ซึ่ง 36 ราย เป็นผู้ป่วยวัณโรค มีผู้ป่วย 14 ราย (ร้อยละ 21.6) วินิจฉัยจากการย้อมเชื้อ และอีก 6 ราย (ร้อยละ 9.2) วินิจฉัยจากการตอบสนองจากการรักษาวัณโรค ผู้ป่วยทั้งหมดที่ติดเชื้อกลุ่มมัยโคแบคทีเรียที่ไม่ใช่วัณโรควินิจฉัยจากการเพาะเชื้อ โดยเชื้อที่พบได้บ่อยที่สุดเป็น Mycobacterium avium complex (MAC) (5 ราย) รองลงมาเป็นเชื้อมัยโคแบคทีเรียชนิดโตช้า (3 ราย) และ Mycobacterium fortuitum (1 ราย) ในกลุ่มเชื้อวัณโรคนั้นพบว่าส่วนใหญ่ติดเชื้อแบบแพร่กระจายหลายอวัยวะ ประมาณร้อยละ 58.3 และในกลุ่มมัยโคแบคทีเรียที่ไม่ใช่วัณโรคนั้นพบว่ามีการติดเชื้อแบบแพร่กระจายทุกราย อวัยวะที่ติดเชื้อบ่อยที่สุด 2 อันดับแรกของทั้งสองกลุ่มเชื้อ คือ ปอด (ร้อยละ 75) และต่อมน้ำเหลือง (ร้อยละ 66.7) ปัจจัยที่มีผลต่ออาการติดเชื้อมัยโคแบคทีเรียคือ เพศชาย (ร้อยละ 64.6% และร้อยละ 54.3, p 0.026) จำนวนเชื้อไวรัสเอชไอวีในเลือดขนาดสูง ($1.04x10^6$ และ $0.3x10^6$, p 0.004) การมีภาวะซีด (ร้อยละ 32.7 และร้อยละ 35.3, p 0.032) และการมี alkaline phosphatase (ALP) ขึ้นสูง (146 U/L และ 107 U/L, p 0.032) และปัจจัยเชิงผกผันของการติดเชื้อมัยโคแบคทีเรีย คือการติดเชื้อ Pneumocystis ที่ปอด ซึ่งพบว่าในผู้ป่วยติดเชื้อมัยโคแบคทีเรียมีสัดส่วนของผู้ป่วยที่มีการติดเชื้อ Pneumocystis ที่ปอดน้อยกว่าผู้ป่วยเอดส์โดยทั่วไป (ร้อยละ 28.8 และร้อยละ 10.9, p 0.004) ผลการรักษาวัณโรคและโรคติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่วัณโรคนั้น พบว่ามีการตอบสนองต่อการรักษาที่ค่อนข้างดี คิดเป็นร้อยละ 86.1 และร้อยละ 77.8 ตามลำดับ โดยพบว่าอัตราตายคือ ร้อยละ 2.78 ในผู้ป่วยวัณโรค และร้อยละ 11.1 ในผู้ป่วยโรคติดเชื้อมัยโคแบคทีเรียที่ไม่ใช่วัณโรค ผลข้างเคียงของการรักษาที่พบได้คือ ภาวะตับอักเสบ (ร้อยละ 22.2) แพ้ยา (ร้อยละ 13.9) และการเกิด immune reconstitution inflammatory syndrome ภายหลังการรักษาเอดส์และวัณโรค (ร้อยละ 8.3)*

สรุป: *การติดเชื้อหลายอวัยวะแบบแพร่กระจายนั้นพบได้บ่อยทั้งในวัณโรคและเชื้อก่อโรคมัยโคแบคทีเรียที่ไม่ใช่วัณโรคในผู้ป่วยที่มีการติดเชื้อเอชไอวีร่วมด้วย ผู้ป่วยที่อาจมีการติดเชื้อดังกล่าวคือผู้ป่วยที่มีภาวะซีด การมีเชื้อไวรัสเอชไอวีในเลือดปริมาณสูง และมี ALP สูง สำหรับการติดเชื้อ Pneumocystis นั้นมีความสัมพันธ์เชิงผกผันกับการติดเชื้อวัณโรค เชื้อ MAC เป็นเชื้อก่อโรคมัยโคแบคทีเรียที่ไม่ใช่วัณโรคที่พบได้บ่อยที่สุดในผู้ป่วยเอดส์*