Brief communication (Original)

Low antitubercular drug levels in newly infected normal hosts

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Background: Low antitubercular drug level is a risk factor for treatment failures. Antitubercular drug level determination has been suggested for complicated tuberculosis patients, but there has been interest in performing such studies in normal hosts.
Objective: To identify whether there are advantages of routine antitubercular drug level determination.
Patients and Methods: We determined drug levels in 15 new normal host Thai tuberculosis patients by using published methods. All patients received the Directly Observed Treatment Short-course including pyrazinamide, rifampicin, and isoniazid.
Results: We started with 15 patients of whom 27% (4 patients) were mycobacteria smear-positive, 33% (5 patients) had low blood levels of pyrazinamide and 87% had low levels of rifampicin. The drug levels in the smear-positive group were lower than in the smear-negative group. All smear-positive patients had a rifampicin levels lower than the therapeutic range.
Conclusion: Antitubercular drug level determination has a potential to identify patients who may be at risk of poor treatment results.

Keywords: Anti-tubercular drug levels, standard short course regimen, Thailand

Tuberculosis (TB) is a major public health problem worldwide causing 1.7 million deaths in 2010 [1]. In order to manage TB, a Directly Observed Treatment Short-course (DOTS) combined with a standard regimen is used as an effective strategy [2]. The main purpose of DOTS is to confirm antiTB drug ingestion and sustain a serum therapeutic range of antiTB drugs. However, because of patient complication and HIV infection, some TB patients show low antiTB drug levels and developed multidrug resistance or delayed responses [3-7]. It has been suggested that blood levels of antiTB drugs should be determined during treatment [8].

Thailand was ranked 17th on the list of 22 "high-burden" TB countries with 91,374 new TB cases occurring in 2005 [9]. The prevalence of multidrug resistance TB was 1% and 20% among new and previously treated patients [9]. DOTS has been applied in the treatment of TB patients since 1996,

and countrywide DOTS coverage was achieved in 2001 [10, 11]. However, the successful treatment rate for TB in Thailand was approximately 76% in 2003, slightly lower than the WHO target cure rate of 85% [10]. Causes of treatment failure are probably multiple and low antiTB drug levels are one concern [12, 13]. This study was performed to determine advantages of performing serum antiTB drug level determinations in new immunologically normal host Thais.

Materials and methods
Population and blood collection
Fifteen normal host laboratory documented newly TB infected patients were enrolled at the Office of 10 Disease Prevention and Control, Anti-*TB* Association Thailand: Chiang Mai (*ATAT*-Chiang Mai) and Department of Internal Medicine, Faculty of Medicine, Chiang Mai University between October 2008 and September 2010.

Patients were treated using DOTS. They received antiTB drugs according to The Thai Guidelines for TB Control [11] (**Table 1**) which included isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and

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ethambutol for 2 months (initial phase), then INH and RIF for another 4 months (continuation phase). Serum drug level determination was conducted at the end of the initial phase. At the same time, sputum smear microscopy was performed using the Ziehl–Neelsen method. Patients were grouped as smear positive and smear negative. Smear-positivity was used to indicate slow response or unsuccessful treatment. Final treatment results were revealed at the end of the 4-months continuation phase.

The study protocol was approved by the Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand.

Anti-TB drug level determination

At the last day of the initial phase, patients were asked to fast overnight. Venipuncture blood was drawn 2 hours after antiTB drug intake. Each plasma sample was separated by centrifugation and kept at –70°C for further analysis.

Levels of INH, RIF, and PZA were quantified by high-performance liquid chromatography (HPLC) using external standards. Sample pretreatment was modified from Smith et al. [14] and Unsalan et al. [15] methods by using Bondelut C18 extraction cartridges. An HP model 1100 isocratic reversed phase HPLC system (Hewlett–Packard, Palo Alto, Calf., USA) fitted with a C8 column and UV–VIS detector was employed. The mobile phase for RIF determination was 80% acetonitrile with 0.1% trifluoroacetic acid whereas for the INH and PZA determinations was 3% acetonitrile with 0.06% trifluoroacetic acid.

Low levels of antiTB drugs were identified when the antiTB drug level was lower than the therapeutic range (INH < 3 µg/ml, PZA < 20 µg/ml, and RIF < 8 µg/ml.)

Data analysis

All values were arithmetic. Weight adjusted antiTB values were calculated individually before any group mean calculation. Mean comparisons between sex and sputum smear result groups were performed using a Mann–Whitney U test. Fisher's exact test was used to determine the relationship between antiTB drug levels and a sputum smear result.

Results

There were 15 normal hosts newly infected and enrolled in this study. The mean age was 34.80 years in men and 43.67 years in women (**Table 2**). Women had a mean weight lower than men (44.33 vs 47.30 kg).

There were 4 patients who showed positive smears at the end of the initial phase (**Table 3**). INH mean levels from the smear-negative group were lower than those from the smear-positive group (8.16 vs 9.16 µg/ml); however, the weight adjusted INH level of the smear-negative group was significantly higher than in the smear-positive group (0.18 vs 0.16 µg/ml/kg body weight). Similarly, PZA and RIF levels of the smear-negative group were higher than the smear-positive group.

The numbers of low antiTB drug level patients was 1 (7%) for INH, 5 (33%) for PZA and 13 (87%) for RIF. The prevalence of smear-positive patients at the end of initial phase was 27%. When subjects were classified according to antiTB drug level and sputum testing result, the prevalence of PZA level < 20 µg/ml and RIF level < 8 µg/ml in the smear-positive group was greater than in the smear-negative group (75% vs 18% and 100% vs 82%, respectively), but this relationship was not significant (Fisher' exact test $p = 0.077$ and 0.524, respectively) as shown in **Table 3**.

Table 1. Thailand category I tuberculosis drug regimen

Patient weight (kg)	Dose (mg)			
	Isoniazid (INH)	Pyrazinamide (PZA)	Rifampicin (RIF)	Ethambutol (E)
<40	300	1,000	300	800
40–50	300	1,500	450	1,000
>50	300	1,500-2,000	600	1,200

Table 2. Mean comparisons of age, weight, TB drug level between men and women of new TB patients

	Men (n = 12)		Women (n = 3)		*p*
	Mean	SD	Mean	SD	
Age (year)	34.80	9.98	43.67	17.10	0.310
Weight (kg)	47.30	6.55	44.33	4.73	0.394
INH (µg/ml)	9.05	3.55	5.90	0.35	0.083
PZA(µg/ml)	22.54	14.21	29.36	13.59	0.563
RIF(µg/ml)	3.95	6.22	3.15	1.54	0.149

Mean comparison was done by Mann-Whitney U test

Table 3. Relations between treatment drugs level and treatment result among new TB patients

		Sputum conversion result n (%)			Fisher's exact *p*
		Convert	Delay convert	Total	
INH	<3 µg/ml	1 (9)	0 (0)	1 (7)	0.733
	>3 µg/ml	10 (91)	4 (100)	14 (93)	
PZA	<20 µg/ml	2 (18)	3 (75)	5 (33)	0.077
	>20 µg/ml	9 (82)	1 (25)	10 (67)	
RIF	<8 µg/ml	9 (82)	4 (100)	13 (87)	0.524
	>8 µg/ml	2 (18)	0 (0)	2 (13)	
Total		11 (73)	4 (27)	15 (100)	

Convert was identified in patient whose sputum acid-fast bacilli was negative whereas delay convert was identified in patient whose sputum acid-fast bacilli was positive by using Ziehl–Neelsen method.

The results of this study showed that smear-positivity was found in four patients at the end of the initial phase treatment. Their INH levels were above the therapeutic range (>3 µg/ml) whereas the RIF levels were low (<8 µg/ml) (**Table 3**). Smear-positivity appeared after 6 months of treatment in only one patient, whose serum levels of PZA and RIF were lower than in the therapeutic range (<1 and 0.70 µg/ml), but the INH level was higher than in the therapeutic range (9.26 µg/ml).

Discussion and conclusions

Unsuccessful TB treatment with low serum antiTB drug levels was reported among a subset of TB patients when the standard Thai short course regimen was used. Thailand has a strategy to increasing use of the standard short course regimen [3, 5] and thus to maximizing cost-benefit advantages [4, 16]. AntiTB drug determination is a sophisticated and costly procedure, requiring a skillful worker and consumes time. It has not yet been included in TB treatment guidelines [3]. TB treatment costs US$100–

US$1,000 worldwide and US$750 in Thailand [1]. Cost variation depends on the stage of the disease and will increase for MDR-TB or relapse cases [1]. In 2011, funding for TB treatment worldwide was US$5,000 million [1] and US$648 (12.96%) million was expanded for MDR-TB treatment. TB treatment costs increase as MDR-TB and unsuccessful cases increase. AntiTB drug levels determination is the one proposed strategy used for MDR-TB and to prevent treatment failures caused by low antiTB drug levels. This strategy would cost $80 per case and would represent only 10.67% of Thailand's TB treatment costs.

The advantage of antiTB drug level determination was documented among normal host TB patients in Kimerling et al. [17]. These patients received DOTS as our subjects and they showed smear-positive sputum within 12 weeks of treatment. The INH level was low in 36% (5/14) of patient and 51% (8/14) of patients showed low levels of RIF, differing from our results. The INH level in our smear-positive sputum subjects was normal (>3 g/ml) and all smear-positive

sputum subjects showed RIF lower than the therapeutic range (**Table 3**). Kimerling et al. suggested that the early identification of low level antiTB patients will help to adjust drug regimen and prevent unsuccessful treatment. Therefore, factors that should be of clinical importance are; optimal time to collect blood, the necessity of fasting state, and the effects of other drugs, and alcohol on absorption and metabolism [17]. These factors should be reviewed with care to get optimal conditions for antiTB drug determination.

The limitations of our preliminary study are drug resistant determination in low responders and the small number of subjects. There is need for a larger study and drug resistant determination on low responders. Patients showing low antiTB drug levels need to be carefully screened concerning life style and other risk factors that may interfere with the absorption and metabolism of drugs.

Acknowledgement

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