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The influence of acetylation status of tuberculosis patients on the isoniazid serum concentrations and sputum conversion after intensive phase therapy

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ABSTRACT

Isoniazid (INH), one of the major antituberculosis drugs, is metabolized by acetylation. Previously study proved the significant differences of serum INH concentration between subject with fast and slow acetylation status. However, the correlation of acetylation status with treatment outcome after fixed-dose combination antituberculosis therapy (FDC-ATT) was not explained. The aim of this study was to evaluate the influence of acetylation status on the treatment outcome and the serum INH concentrations in the adult tuberculosis patients underwent FDC-ATT. A cross sectional study was carried out on 31 tuberculosis patients. Acetylation status was measured by spectrophotometer and serum INH concentration was measured by high performance liquid chromatography (HPLC). Sputum conversion assay was conducted by Ziehl Nelsen method. t-Test, chi square, Mann-Whitney, and Fisherman were used to analyze the data. The proportion of the fast acetylator was 61.3%, whereas the slow acetylator was 38.7%. The proportion of success and failure sputum conversion were 83.9% and 16.1%, respectively. The mean serum INH concentration in the fast acetylator groups ($1.52 \pm 0.15 \mu\text{g/mL}$) was significantly lower than that in the slow acetylator groups ($3.84 \pm 0.35 \mu\text{g/mL}$). The failure conversion risk of the fast acetylator group was about two folds higher than the slow acetylator group, although it was not significantly different (RR=2.53; 95% CI=0.32-20.00; $p>0.05$). Moreover, the mean serum INH concentration in success ($2.46 \pm 0.31 \mu\text{g/mL}$) and failure ($1.89 \pm 0.20 \mu\text{g/mL}$) sputum conversion was not significantly different ($p>0.05$). In conclusion, the acetylation status does not influence the sputum conversion in adult tuberculosis patients after FDC-ATT although the serum INH concentration on slow acetylation status is higher than that fast acetylation status.

ABSTRAK

Isoniazid (INH) merupakan salah satu obat antituberkulosis utama yang metabolismenya melalui asetilasi. Pada penelitian sebelumnya dilaporkan ada perbedaan kadar INH serum pada orang dengan asetilator cepat dan lambat. Namun demikian hubungan antara status asetilasi dengan keberhasilan terapi penderita tuberkulosis (TB) yang mendapat obat antituberkulosis kombinasi dosis tetap (OAT-KDT) belum dijelaskan secara rinci. Tujuan

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penelitian ini adalah untuk mengkaji pengaruh status asetilasi terhadap keberhasilan terapi dan kadar INH serum penderita TB paru dewasa. Rancangan penelitian adalah potong lintang dengan subjek penelitian sebanyak 31 orang penderita TB. Status asetilasi ditetapkan dengan spektrofotometer dan kadar INH serum dengan *high performance liquid chromatography* (HPLC). Pemeriksaan sputum dilakukan dengan metode Ziehl Nelsen. Analisis statistik dengan uji t, Chi-square, Mann-Whitney, dan Fisherman. Proporsi status asetilasi cepat sebesar 61,3% sedangkan asetilator lambat 38,7%. Proporsi keberhasilan konversi sputum 83,9% dan gagal konversi 16,1%. Rerata kadar INH serum pada asetilator lambat ($3,84 \pm 0,34 \mu\text{g/mL}$) lebih tinggi secara nyata dibandingkan pada asetilator cepat ($1,52 \pm 0,15 \mu\text{g/mL}$) ($p < 0,05$). Risiko gagal konversi pada kelompok asetilator cepat sekitar 2,53 kali dibandingkan pada asetilator lambat, meskipun tidak bermakna secara nyata ($RR = 2,53$; $95\%CI = 0,32-20,00$; $p > 0,05$). Rerata kadar INH serum pada kelompok berhasil konversi yaitu $2,46 \pm 0,31 \mu\text{g/mL}$ tidak berbeda nyata dengan kelompok gagal konversi yaitu $1,89 \pm 0,20 \mu\text{g/mL}$ ($p > 0,05$). Dapat disimpulkan, status asetilasi tidak mempengaruhi konversi sputum pada penderita TB dewasa yang mendapat OAT-KDT meskipun kadar serum INH pada status asetilasi lambat lebih tinggi dari pada status asetilasi cepat.

Keywords: acetylation status – isoniazid level - success of therapy - conversion sputum - intensive phase treatment - tuberculosis

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). The spread of *M. tuberculosis* occurs from person to person primarily through droplet transmission in air.^{1,2} Tuberculosis is a major health problem in the world, especially in developing countries. Approximately one third of the world's population has been infected with *M. tuberculosis* with the main source of transmission from TB patients basil hold acid (BTA) positif.³

In 2007, World Health Organization (WHO) reported that approximately 9.27 million new cases of TB were reported. This incidence increased from the previous year of 9.24 million new cases in 2006 and 8.3 million new cases in 2000. Mortality due to TB is also reported to be very high. In 2007, 1.3 million deaths from TB or 20 deaths per 100,000 population were reported. Approximately 90% of the total deaths occur in developing countries. According to WHO, 22 countries

are classified as high-burden countries for TB, included Indonesia.⁴

Indonesia ranks third on the list of high-burden tuberculosis countries in the world after India and China with the number of TB patients about 10% of the total number of TB patients in the world. In 2004 there were 539,000 new cases per year and 101,000 deaths occur people from TB in Indonesia. The prevalence rate of smear positive TB in the National was 110 per 100,000 population.³ The prevalence of TB in the Yogyakarta and Bali reported the lowest at 68 per 100,000 people. Pulmonary TB disease morbidity in Yogyakarta was 3.18 per 100,000 penduduk.⁵

Tuberculosis become more serious health problem with the increasing of Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) cases.⁶ Another issue that exacerbates tuberculosis is the emergence of multiple drug resistance TB (MDR-TB), which can lead to cases of TB more difficult to treat.⁷ Resistance to antituberculosis mainly due to the inadequate use of

drugs. Inadequate therapy can be caused by inappropriate management therapy.⁸ Since 1995 TB treatment has been carried out using Directly Observed Treatment Shortcourse (DOTS) which is given in two stages, intensive and continuation phases. At the end of the intensive phase and continuation phase, a patient sputum examination is conducted and evaluated. Successful treatment is considered if the patient sputum smear negative in a microscopic examination is observed.³

Isoniazid (INH) is one of the main TB drugs which is generally given in the form of oral antituberculosis (OAT) drug in combination with others. Isoniazid has bacteriostatic and bactericidal activities. The metabolism of INH is affected by a drug metabolizing enzymes in the liver that is N-acetyltransferase 2 (NAT2) through acetylation reaction. Based on the reaction of NAT2 acetylation by this person can be categorized as fast acetylators or lambat.^{9,10}

Individuals with rapid acetylator phenotypes must take INH doses greater than those with slow acetylator phenotypes.¹¹ Meanwhile another study proved that low serum INH concentrations was observed in individuals with rapid acetylator phenotypes.¹⁰ The mean blood INH concentration 2 hours after INH administration in individuals with slow acetylator phenotypes was two times higher than those with rapid acetylator phenotypes.¹² Other studies also reported an association between INH acetylators status with outcome of TB treatment with once-weekly INH and rifampentine.¹³

NAT2 gene polymorphisms that determine the acetylator phenotypes of INH has been known to vary at different race or ethnicity. In the Indonesian population known to 65.4% of people classified as fast acetylators, and 35.6% are slow acetylators.¹⁴ This study was conducted to evaluate the influence of

acetylator phenotypes of tuberculosis patients on the serum INH concentration and sputum conversion after intensive phase therapy.

MATERIALS AND METHODS

This was an observational study using cross sectional design involving 31 subjects who met the inclusion and exclusion criteria. The inclusion criteria were adult patients with pulmonary TB (aged over 18 years) smear positive TB new cases, received TB treatment category 1 (2HRZE / 4H3R3), and willing to participate in the study. The exclusion criteria were subjects with diabetes mellitus, suffer from liver dysfunction, had a history of allergy to sulfa drugs, could not communicate well, not willing to follow the course of TB treatment with DOTS strategy. Protocol of the study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Protocol of Study

Acetylation status determination

The acetylators phenotypes were determined using sulphadimidine according to the method developed by Rao *et al.*¹⁵ Patients were given sulphadimidine 500 mg orally early in the morning on empty stomach. They were not allowed to take anything for the next two hours. Urine sample were collected before and 6 hours after sulphadimidine administration. Free and total sulphadimidine in the urine were determined by using spectrophotometer (Spectronic 20D + Thermo Fisher Scientific) at a λ of 540 nm. Acetylation status was determined based on the ability to produce the acetylated sulphadimidine (total minus free sulphadimidine) and expressed as a percentage of the total sulphadimidine. Patient was considered as rapid acetylator if

the acetylated sulphadimidine was >70% and as slow acetylators if it was <70%.

Measurement of serum INH concentration

Blood sample was taken before (day 0) and at the end of the intensive of treatment (day 56 of treatment). Three mL blood sample was taken from the median cubital vein two hours after OAT administration. The blood sample was then centrifuged at 3000 rpm, serum was taken and stored at -80 °C until analysed. Serum INH concentration was determined by using High Performance Liquid Chromatography (HPLC) in the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta. A simple extraction was conducted by protein precipitation with 150 µL of trichloroacetic acid 15% in 300 µL serum. The mixture was then vortexed for two min and centrifuged at 3000 rpm for 10 min. The supernatant was collected and 20 µL was injected into the HPLC system. The stationary phase was Novapak® C18 column (150 x 3.9 mm, 3 microns). The mobile phase consisted of 0.05 M sodium dihydrogen phosphate and acetonitrile (97: 3) and set at a flow rate of 1 mL/min at room temperature. The detection was performed at λ of 280 nm.

Sputum examination

Sputum was spread evenly over the central area of the slide which was air dried for about 30 min and heat fixed. The sputum slide was then flooded with Ziehl Neelsen

(ZN) stain which was then heated to dry, kept for 5 min and washed with aquadest. The slide was then flooded with 3 mL of concentrated hydrochloric acid and 97 mL of ethanol 95% to decolourise the ZN stain and washed with aquadest. Thereafter, the slide was flooded with 0.3 g of methylene blue and 100 mL of aquadest for 2 min. The slide was air dried at room temperature and viewed on a microscope under oil immersion for presence of acid-fast bacilli. The bacilli were stained red and the background material was stained blue.

Statistical analysis

Data were presented as mean \pm standard deviation (SD) or percentage and analyzed using SPSS software version 16. Numerical data were compared by using t-test or Mann-Whitney U test, whereas categorical data were compared by using Chi-square test or Fisher exact test. To determine the relative risk (RR) conversion failures in the rapid and slow acetylators group, 2 x 2 table relative risk analysis (Epi Info Program) at the 95% confidence interval was performed.

RESULTS

Among 34 subjects who met the inclusion criteria, only 31 patients completed the study. One subject can not be included until the end of the study period due to suffer from liver dysfunction as characterized by high levels of serum transaminases in early therapy and two subjects do not abide taking the drugs. The characteristics of subjects are presented in TABLE 1.

TABLE 1. Patients characteristic (n=31)

Characteristic	Value
Age (mean ± SD year)	44.2 ± 13.7
Sex [n (%)]	
Male	24 (77.4)
Female	7 (22.6)
Weight (mean ± SD kg)	49.5 ± 5.8
Height (mean ± SD cm)	164 ± 6
Body Mass Index (mean ± SD)	18.4 ± 2.0
Nutritional Status (%)	
Normal	13 (41.9)
Low	18 (58.1)
Smoker [n (%)]	
Yes	20 (64.5)
No	11 (35.5)
INH dose [n (%)]	
225 (3 FDC-ATT)	24 (77.4)
300 (4 FDC-ATT)	7 (22.6)

The proportion of subjects with fast acetylators phenotype status was 61.3%, and 38.7% was people with slow acetylators

status. Characteristics of patients in the fast or slow acetylators did not show any statistically significant difference (TABLE 2).

TABLE 2. Patient characteristics based acetylation status (n=31)

Characteristics	Acetylation status		P
	Slow (n=12)	Fast (n=19)	
Age (mean ± SD year)	42.1 ± 13.2	45.5 ± 14.3	0.518
Sex (%)			
Male	9 (75.0)	15 (78.9)	1.00
Female	3 (25.0)	4 (21.1)	
Weight (mean ± SD kg)	49.3 ± 4.9	49.6 ± 6.5	0.884
Height (mean ± SD cm)	165 ± 7	163 ± 6	0.251
Body Mass Index (mean ± SD)	18.0 ± 2.1	18.7 ± 1.9	0.224
Nutritional Status (%)			
Normal	3 (25)	9 (47.4)	0.129
Low	9 (75)	10 (52.6)	
Smoker (%)			
Yes	8 (66.7)	12 (63.2)	0.842
No	4 (33.3)	7 (36.8)	

Most patients had successful sputum conversion (83.9%), whereas only 16.1% had failure sputum conversion. Characteristics of

patients based on the success and failure of sputum conversion are presented in TABLE 3.

TABLE 3. Patient characteristics based on sputum conversion (n=31)

Characteristics	Acetylation status		p
	Success (n=26)	Failure (n=5)	
Age (mean ± SD year)	42.3 ± 13.1	54.0 ± 14.0	0.083
Sex [n (%)]			
Male	21 (80.8)	3 (60.2)	0.562
Female	5 (19.2)	2 (40.0)	
Weight (mean ± SD kg)	49.9 ± 6.0	47.2 ± 5.2	0.646
Height (mean ± SD cm)	164 ± 6	161 ± 6	0.333
Body Mass Index (mean ± SD)	17.7 ± 2.0	18.2 ± 2.1	0.774
Nutritional Status [n (%)]			
Normal	11 (42.3)	3 (60.0)	1.000
Low	15 (57.7)	2 (40.0)	
Smoker [n (%)]			
Yes	18 (69.2)	2 (40.0)	0.317
No	8 (30.8)	3 (60.0)	
Acetylation status [n (%)]			
Fast	15 (57.7)	4 (80)	0.624
Slow	11 (42.3)	1 (20)	
INH concentration (mean±SEM µg/mL)	22.88±0.51	1.89±0.26	0.519
INH dose [n (%)]			
225 mg (3 FDC-ATT)	19 (73.1)	5 (100)	0.562
300 mg (4 FDC-ATT)	7 (26.9)	0 (0)	

Zero in serum INH concentration was observed before treatment begin. After complete treatment (day 56), the mean serum INH concentration was 2.72 µg/mL (ranged from 0.53 to 13.32 µg/mL). Most individuals who failed sputum conversions (80%) had a fast acetylators phenotype, whereas in the group succeeded only 57.7% sputum

conversion. But this was not statistically significant (Fisher’s exact test, $p > 0.05$). Based on the analysis of the 2x2 table (Epi Info Program) obtained RR = 2.53 (95% CI: 0.32 to 20.00). Although the risk of conversion failure in fast acetylators group seen 2 times greater than the slow acetylators, but not statistically significant (TABLE 4).

TABLE 4. Conversion failure risk based on acetylation status (n=31)

Acetylation status	Sputum conversion		Total	x ² , p	RR (95% CI)
	Success	Failure			
Fast	4	15	12	0.342	0.32-20.00
Slow	1	11	19		
Total	5	26	31		

The mean serum INH concentration in the slow acetylators patients (4.63 µg/mL) was much higher than fast acetylators (1.52 µg/mL). But in the group of slow acetylators, one subject had extreme INH concentration until 13.32 µg/mL. After extreme data excluded,

the mean INH concentration of the slow acetylators (3.84 ± 0.34 µg/mL) was two times higher than that the rapid acetylators (1.52 ± 0.15 µg /mL) (p <0.05) as shown on TABLE 5.

TABLE 5. Serum INH concentration in the fast and slow acetylation status (n=30)

Acetylation status	n	Serum INH concentration (µg/mL)					p
		Mean	SEM	95% CI	Min-Max		
Fast	19	1.52	0.15	1.20-1.83	0.53-2.54	0.000	
Slow	11	3.84	0.34	3.07-4.61	2.29-5.01		

The mean INH concentration in the subjects with failed in sputum conversions (1.89 ± 0.20 µg/mL) was significantly lower than the subjects with success in sputum conversions (2.88 ± 0.51 µg/mL) (p<0.05). One subject with extreme INH concentration was found in the subjects with success in

sputum conversion. After subjects with extreme INH concentration excluded, the mean INH concentration between the subjects with success in sputum conversion (2.46 ± 0.31 µg/mL) was similar to those with failed in sputum conversion (1.89 ± 0.20 µg/mL) (p>0.05).

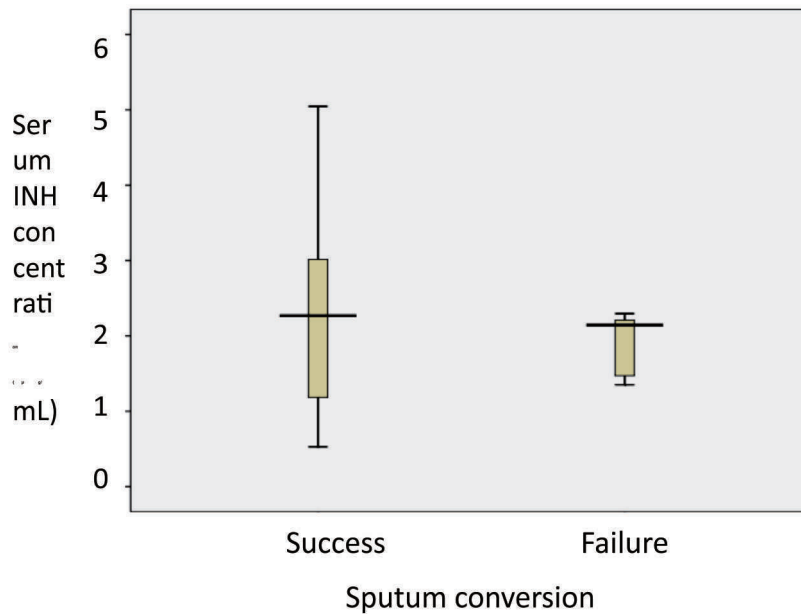


FIGURE 1. Serum INH concentration based on sputum conversion (n=30)

Most subjects (77.4%) received 3 FDC-ATT tablets (225 mg INH), and 22.6% received 4 FDC-ATT tablets (300 mg). The mean serum INH concentration in the group receiving 225 mg or 3 tablets FDC-ATT was

no higher than those receiving 300 mg or 4 tablets FDC-ATT ($2.55 \pm 0.30 \mu\text{g/mL}$ vs. $1.63 \pm 0.31 \mu\text{g/mL}$; Mann-Whitney test $p > 0.05$) (TABLE 6).

TABLE 6. Serum INH concentration based on INH dose (n=30)

Dose of INH	n	Mean	SEM	95% CI	Min-Max	p
225 mg	24	2.55	0.30	1.92-3.18	0.59-5.05	0.147
300 mg	6	1.63	0.31	0.83-2.44	0.53-2.67	

DISCUSSION

The proportion of rapid acetylation status (61.3%) was higher than that slow acetylation status (38.7%) in this study. This proportion was similar with the results reported in the previous study conducted in the Community Health Care and Center for Pulmonary Disease Treatment (BP4) in Yogyakarta which found 64% of patients were rapid acetylation status

and 36% of patients were slow acetylation status.¹⁶ Furthermore, it was estimated that approximately 2/3 (66.67%) of the Indonesian people classified as rapid acetylation status and 1/3 (33.33%) as slow acetylation status.¹⁴

The mean serum INH concentration on 31 patients two hours after drug administration was $2.72 \pm 0.43 \mu\text{g/mL}$ ranged from 0.53 to 13.32 $\mu\text{g/mL}$ and the median was 2.21 $\mu\text{g/}$

mL in this study. Serum INH concentration on TB patients after treatment has been reported in the previous studies. A therapeutic drug monitoring study on TB patients conducted in Virginia, USA reported that the median of serum INH concentration two hours after drugs administration was 1.9 µg/mL (interquartile range 1.1-3.5 µg/mL).¹⁷ Another study conducted on TB patients in Turkey found the mean serum INH concentration two hours after OAT treatment was 3.83 ± 2.09 µg/mL.¹⁸ Furthermore, study conducted on TB patients in Alberta, Canada found the mean serum INH concentration was 4.13 ± 3.9 µg/mL.¹⁹

The success of TB treatment is proven by the sputum conversion from smear positive to smear negative at the end of the intensive phase of treatment. In this study TB treatment success rate achieved 83.9%, while the failure rate was 16.1%. The TB treatment success rate has been reported from various regions. The Province Health Office in Yogyakarta Special Region reported that the TB treatment success rate achieved 87%.²⁰ A study conducted in UK demonstrated the TB treatment success rate was 87%.²¹ However, in 2013 in America and European regions the TB treatment success rate was 75%, whereas in Malaysia was 67% less than the 85% success target set by the WHO. Globally, the TB treatment success rate for people newly diagnosed with TB was 86% in 2013.^{22, 23}

The TB treatment failure on the rapid acetylator group (80% or 4 people) was higher compared to on the slow acetylation status group (20% or 1 people). The RR of failure in the sputum conversion of fast acetylation status group was two times higher than the slow acetylation status group although it was not significantly different. This results is different from the previous study that showed

a relationship between acetylation status and outcome of TB treatment with once-weekly INH and rifapentine.¹³ The different of dosage regimen and patient compliance may cause the different of these results.

The mean serum INH concentration of slow acetylation status group (3.84 ± 0.34 µg/mL) was higher than that rapid acetylation status (1.52 ± 0.15 µg/mL). The higher serum INH concentration of slow acetylation status compared to that rapid acetylation status was also reported in the previous studies. Schaaf *et al.*¹² reported the serum INH concentration of slow acetylation status (8.6 µg/mL) was two-fold higher than that rapid acetylation status (3.94 µg/mL). Furthermore, Conte *et al.*²⁴ also reported the serum INH concentration of slow acetylation status (1.1 ± 0.8 µg/mL) was higher than that rapid acetylation status (0.5 ± 0.6 µg/mL).

The mean serum INH concentration in the patients with failed in sputum conversions (1.89 ± 0.20 µg/mL) was significantly lower than the subjects with success in sputum conversions (2.46 ± 0.31 µg/mL). However, it was not significantly different ($p > 0.05$). It was reported that effective of tuberculostatic minimal concentration of INH range from 0.025 to 0.05 µg/mL, whereas the therapeutic INH concentration range from 0.5 to 2.0 µg/mL.²⁵⁻²⁷ In this study, the mean serum INH concentration in the both patients with failed and success in sputum conversions met the therapeutic INH concentration that indicated serum INH concentration did not influence the sputum conversion. No different in serum INH concentration in the patients with failed and success in sputum conversions nor in the patients with delays and avoid delay in sputum conversions were also reported in the previous study.²⁵

CONCLUSIONS

Adult pulmonary TB patient with rapid acetylation status has lower serum INH concentrations than slow acetylation status. However, these serum INH concentrations are still within the therapeutic INH concentration therefore the acetylation status does not affect the sputum conversion after the intensive phase of TB treatment.

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