

# Isoniazid Blood Levels in Patients with Pulmonary Tuberculosis at a Tuberculosis Referral Center

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## Key Words

Isoniazid · Serum isoniazid · Therapeutic drug monitoring · Tuberculosis

therapeutic drug monitoring may be needed to optimize INH dosage, especially in patients with inadequate clinical response or toxicity to INH. Copyright © 2010 S. Karger AG, Basel

## Abstract

**Background:** Serum concentrations of isoniazid (INH) were evaluated in Iranian patients admitted to the Tuberculosis Ward of Masih Daneshvari Hospital, Tehran, Iran. Factors correlated to plasma INH levels were determined. **Methods:** Blood samples were obtained 2 h after ingestion of 5 mg/kg INH in 82 patients (1 sample/patient) on days 3–15 of treatment. The following variables were investigated: INH plasma level, duration of therapy, age, sex, weight, dose of INH administered and smoking status. **Results:** The average ( $\pm$ SD) age and weight of patients were  $60.68 \pm 18.53$  years and  $74.96 \pm 7.15$  kg, respectively. INH concentrations were low in 14.63% and high in 23.17% of the patients (INH reference range: 3–5  $\mu$ g/ml). Plasma INH was statistically correlated with duration of INH administration (Kendall's rank correlation,  $r = 0.66$ ,  $p < 0.001$ ) but not with other variables. **Conclusion:** Based on the result of this study, plasma INH concentrations were not within the therapeutic range for 37.80% of the patients on conventional therapy. Therefore

## Introduction

Around two billion of the world's people, mostly those living in developing countries, are infected with *Mycobacterium tuberculosis* [1]. Patients with drug-susceptible strains of tuberculosis (TB) have high cure rates and low toxicity with standard doses of the first-line medications [2]. However, treatment success of new sputum smear-positive TB cases in Iran was 83% in 2006 [3]. This relatively low cure rate may be related to low serum concentrations of anti-TB drugs.

A low serum drug level not only leads to treatment failure, but may also cause drug resistance, thus rendering TB treatment more complex if only half of the multi-drug-resistant (MDR) TB patients would turn out to be smear negative [4]. In 2007, MDR-TB was found to be 48% in previously treated TB cases in Iran [3]. Although Ramazanzadeh et al. [5] reported that MDR-TB is much

higher for bacteria isolated from Afghan TB patients residing in Iran, the prevalence of MDR-TB is increased in previously treated Iranian and Afghan patients [6]. Treatment failure and the high number of MDR-TB patients is a danger for public health [7].

Toxicity related with anti-TB drugs is also problematic. Standard doses of anti-TB drugs may result in toxicity in the patients, especially in developing countries [8].

Therapeutic drug monitoring (TDM) is employed to determine the blood level of medications and to adjust the dose subsequently in order to optimize the therapeutic benefit and minimize the risk of toxicity [2]. There is a lack of published data regarding plasma concentrations of anti-TB drugs in Iranian patients. Since treatment failure, drug resistance and drug toxicity remain ongoing challenges in TB patients in our country [3, 8, 9], TDM may be a rational approach to optimize anti-TB drug dose.

In this study, we measured serum concentrations of INH to investigate the proportion of patients with therapeutic serum concentrations of INH. The variables correlated to serum INH levels in pulmonary TB patients were also studied to determine factors that could affect serum concentrations.

## Patients and Methods

### Study Subjects

This observational study was conducted from May 2006 to March 2007 in the National Research Institute of Tuberculosis and Lung Diseases, Masih Daneshvari Hospital, which is affiliated to Shahid Beheshti University, and approved by the Medical Ethics Committee of the University.

Treatment-naïve patients who were newly diagnosed with pulmonary TB and received daily doses of a four-drug, standard regimen including INH (5 mg/kg), rifampin (10 mg/kg), pyrazinamide (25 mg/kg) and ethambutol (15 mg/kg) [10] for at least 3 days were included in the study.

Informed consent was obtained from all study participants. In this regimen, INH was administered based on body weight (maximum total daily dose 300 mg) early in the morning after fasting for at least 6 h. Exclusion criteria were HIV positivity, heavy smoking (>1 pack per day), regular alcohol consumption, pregnancy, MDR-TB, heart failure, end-stage renal or hepatic disorder, burn, cystic fibrosis, hypoalbuminemia and treatment with any medication that could affect INH serum concentrations (e.g. antacids).

Presence of at least one of the following criteria was defined as drug-induced hepatotoxicity: (1) a rise of 5 times the upper limit of normal levels of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT); (2) a rise in the level of serum total bilirubin >1.5 mg/dl, and (3) any increase in AST and/or ALT above pretreatment levels combined with hepatitis symptoms [11].

### Sample Preparation and Plasma Drug Concentrations

Blood samples were collected 2 h after drug ingestion (1 sample/patient) in an EDTA tube, and centrifuged as recommended by Li et al. [2]. The supernatants were separated and immediately stored at  $-70^{\circ}\text{C}$  (wrapped in foil) and transferred to the reference laboratory on dry ice. Using AÏt Moussa's method, samples were deproteinized by 10% trichloroacetic acid and centrifuged for 10 min at 10,000 rpm; 500  $\mu\text{l}$  of supernatant were separated and diluted with 500  $\mu\text{l}$  of ammonium acetate buffer (0.5 M, pH 8.20) and 100  $\mu\text{l}$  of this solution were injected into a high-performance liquid chromatography system (Shimadzu, Japan) with an LC10AC pump, DGU-14 degasser, SPD-20A UV detector and a VP-ODS (250  $\times$  4.6 mm)  $\text{C}_{18}$  column [12]. The isocratic phase was a mixture of ammonium acetate buffer (0.05 M, pH 6) and acetonitrile (99:1, v/v). Samples were eluted at a flow rate of 1.2 ml/min. INH was detected at 275 nm. The limit of quantification was 0.2  $\mu\text{g/ml}$ , interassay precision (relative standard deviation) was 4.2% and overall accuracy was 94–102%.

Drug concentrations were compared with an INH reference range (3–5  $\mu\text{g/ml}$ ) obtained 2 h after a daily dose of 5 mg/kg in healthy volunteers [13].

### Statistical Analysis

Statistical analysis was performed using SPSS 12.0 for Windows (SPSS, USA). We reported the descriptive results as means  $\pm$  SD. Correlations between plasma INH and plasma INH ( $\mu\text{g/ml}$ ) 2 h after ingestion, duration of therapy (day), age (years), weight (kg), drug dose administered (mg/kg), smoking status (yes/no) and sex (male/female) were assessed. The Mann-Whitney test was used to compare INH plasma levels between hepatitis and non-hepatitis patients. Data were interpreted using 95% confidence intervals. A  $p < 0.05$  was considered statistically significant.

Data were analyzed using a 3-step approach. In the first step, appropriate preliminary analyses (univariate) were based on the nature of the predictor variables (ordinal, categorical/nominal, interval or ratio). Predictors showing a significant association with INH levels ( $p < 0.1$ ) were considered for multivariate analysis leading to a model building. In the second step, the interaction between these predictors was going to be investigated and significant interactions would be included in the final model building (as a new variable made of their multiplication). In the last step, predictors from the first step and their significant interactions were included in multivariate regression analysis. However, in the first preliminary analyses, only 'length of INH treatment (day)' could show a significant relationship with INH level, with no other predictor showing a significant relationship with the INH level at  $p < 0.1$ ; therefore they were highly unlikely to be correlated with the dependent variable (INH levels) and consequently were not entered into the second and third steps of data analysis (data analysis was fulfilled in the first step).

## Results

In total, 82 patients (33 men and 49 women) were included in the study. Average ( $\pm$ SD) age and weight were  $60.68 \pm 18.53$  years and  $74.96 \pm 7.15$  kg, respectively;

**Table 1.** Plasma INH concentrations in the study patients with respect to the duration of therapy

Days on INH	Total (%)	Patients, n (%)		Concentration $\mu\text{g/ml}$ (mean $\pm$ SD)
		<3 $\mu\text{g/dl}$	>5 $\mu\text{g/dl}$	
3-4	12 (14.63)	8 (66.67)	1 (8.33)	2.57 $\pm$ 1.78
5-9	55 (67.07)	4 (7.27)	8 (14.55)	4.53 $\pm$ 1.73
10-15	15 (18.29)	0	10 (66.67)	9.97 $\pm$ 4.25

**Table 2.** Study variables and their correlation with INH concentrations ( $\mu\text{g/ml}$ )

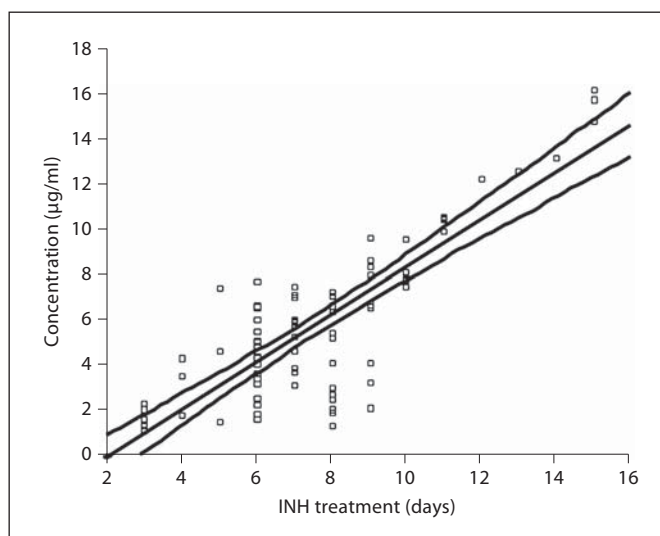
Variable	Means $\pm$ SD or n	Test used	Analysis result
Age, years	60.68 $\pm$ 18.54	SRC	p = 0.67 r = -0.05
Sex, males/females	33/49	MWT	p = 0.44
Weight, kg	74.96 $\pm$ 7.15	SRC	p = 0.94 r = 0.009
Smoking status			
Smoker	28	MWT	p = 0.65
Nonsmoker	58		
INH dose, mg/kg	4.04 $\pm$ 0.42	SRC	p = 0.94 r = -0.009
Length of drug use, days	6.90 $\pm$ 2.96	KRC	p < 0.0001 r = 0.66

SRC = Spearman's rank correlation; MWT = Mann-Whitney test; KRC = Kendall's rank correlation.

34.15% (n = 28) of the patients were smokers, and 10.9% (n = 9) of the patients had hepatitis. Plasma concentration of INH was 5.24  $\pm$  3.33  $\mu\text{g/ml}$ .

Table 1 shows plasma concentrations of INH according to the duration of therapy. In 12 patients (14.63%), INH levels were below the therapeutic range and in 19 patients (23.17%) it was higher. Only 51 (62.19%) had plasma INH levels within the therapeutic range. Of the 15 patients in whom plasma INH was assayed between days 10-15 of therapy, 10 patients had INH concentrations above the therapeutic range, while in 8 of 12 in whom plasma INH was monitored between days 3-5, the target range was not achieved (fig. 1).

Table 2 shows the variables and their correlation with plasma INH concentration. Duration of INH administration showed a statistically significant correlation with



**Fig. 1.** Correlation between days of drug use and plasma concentration of INH.

plasma INH concentration (Kendall's rank correlation, r = 0.66, p < 0.001).

There were no significant correlations between age, sex, weight, smoking status and INH dose (mg/kg) and plasma INH levels. In hepatitis patients, the blood level of INH was significantly higher compared with non-hepatitis patients (11.48  $\pm$  2.92 vs. 4.47  $\pm$  2.47  $\mu\text{g/ml}$ , p < 0.001).

## Discussion

INH is still a cornerstone in the first-line treatment of TB since its introduction in 1952, which, in combination with rifampin, pyrazinamide, and ethambutol, yields satisfactory results following standard 6-month treatment [14].

Subtherapeutic drug levels and toxic plasma concentrations of INH are two important problems in TB treatment, of which either can lead to treatment failure and drug resistance, and the other results in noncompliance and toxic side effects; the former is mostly comprised of rapid acetylators and the latter generally of slow acetylators. Different proportions of rapid and slow acetylators (which affect INH pharmacokinetics) have been reported based on ethnic or geographic population origin [15].

Decreased and increased plasma levels of INH were found in 14.63 and 23.17% of our patients, respectively. The concentration required to reach a therapeutic response ranges from 3 to 5  $\mu\text{g/ml}$  after a daily dose of INH

[13, 16]. Hepatitis related to plasma INH was noted in 10.9% of our patients.

INH has been indicated as a cause of hepatotoxicity. INH is inactivated by NAT2, resulting in acetylisoniazid, which is hydrolyzed to acetylhydrazine. It has been proposed that acetylhydrazine is oxidized into hepatotoxic intermediates by cytochrome P<sub>450</sub> 2E1. The other metabolic pathway to generate toxic intermediates is the direct hydrolysis of INH to hydrazine, a potent hepatotoxin. Slow acetylators not only acetylate the parent component more slowly, but also acetylhydrazine, the immediate precursor of the toxic intermediates, to the harmless diacetylhydrazine. Therefore, slow acetylation may be a risk factor for the development of anti-TB drug-induced hepatotoxicity [17, 18]. Ray et al. [19] conducted a study in 90 patients receiving anti-TB treatment and noted subtherapeutic plasma INH levels in 48% of their patients, while this level was above the therapeutic range in 29%. Since killing kinetics of INH against *M. tuberculosis* is concentration dependent [20], subtherapeutic drug concentrations could lead to treatment failure and drug resistance. The higher prevalence of high compared to low plasma concentrations of INH and the increased rate of hepatitis patients may be due to acetylation status. The predominance of slow over rapid acetylators has been shown in Iranians [21, 22].

INH toxicity is also a function of treatment duration, i.e. the longer the treatment duration, the higher the odds of drug toxicity and toxic adverse effects to drugs, especially in slow acetylators [23]. In our study, 15-day treatment with INH was correlated with plasma INH, i.e. INH concentration increased with treatment duration. This increase surpassed the therapeutic limit after 6 treatment days: in 10 of 15 patients treated for 10–15 days, drug levels were above the therapeutic range, and thus these patients were prone to drug toxicity.

Since INH has a short elimination half-life, accumulation beyond day 2 of treatment is not expected from data acquired in single-dose pharmacokinetic studies. Thus, a different mechanism must be considered for this phenomenon. As INH is also an enzyme inhibitor, time-dependent changes in INH metabolism may be present. One probable mechanism for INH accumulation is suppression of NAT2 by the parent drug [17, 24]. The results of this study need to be confirmed in future studies involving a larger sample size and considering more sensitive biomarkers as well as demographic and medical history variables.

On the other hand, 8 of 12 patients treated for 3–5 days did not reach the therapeutic level. It could be inferred

that INH level measurements may not be suitable for patients during the early days of treatment since the low plasma level could erroneously lead the clinician to a dose increment possibly resulting in drug toxicity.

Kimerling et al. [4] and Ray et al. [19] reported that due to the discrepancy in interpersonal INH concentrations at similar dosages, TDM is required for efficient treatment. We came to the same conclusion as we did not detect a statistically significant correlation between either dose, or patients' weight and INH blood concentration.

We excluded patients with certain conditions (e.g. end-stage hepatic disease and alcoholism) due to potential confounding effects on outcome. They are the very conditions in which TDM must be considered seriously, but Ray et al. [19] proposed that TDM be used in all patients receiving INH not only because it is an inexpensive test, but also due to the benefit conferred by preventing costly effects on outcome due to drug level alterations [19]. This claim should be investigated in cost-effectiveness studies.

This preliminary study performed on Iranian patients provided an overview of causes of TB treatment failure and drug-related toxicity of anti-TB drugs in as much as it is related to considerations affecting INH administration, and its conclusions could not be further extended. More sophisticated studies are required to investigate further issues such as differences in concentrations 2 and 6 h after drug ingestion, serum concentrations of other anti-TB drugs, TDM especially for patients with comorbidity (e.g. hepatitis) and cost-benefit analysis of TDM in all TB patients.

## Conclusion

Only 62.19% of our patients had plasma INH within the therapeutic range. We suggest TDM to adjust INH dose, especially in TB patients with inadequate treatment response or toxicity.

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